PLLA scaffold with delivery system of bioactive agents for anti-inflammation and re-endothelialization

Dr. Dong Keun Han¹, Mr. Seung-Woon Baek¹,², Dr. Da-Seul Kim¹, Dr. Chun Gwon Park²
¹CHA University, Seongnam-si, South Korea, ²Sungkyunkwan University, Suwon-si, South Korea

Introduction: A vascular poly(L-lactic acid) stent for treating atherosclerotic cardiovascular diseases still requires some improvement, such as anti-inflammation and re-endothelialization. Magnesium hydroxide (MH) inhibits inflammation response by neutralizing the acidic byproducts of PLLA. Polydeoxyribonucleotide (PDRN) upregulates the expression of VEGF as one of the A2A receptor agonists and L-arginine (Arg, R), synthesized into nitric oxide by intracellular eNOS, induces endothelialization. Mesenchymal stem cell-derived extracellular vesicles (EVs), composed of a lipid bilayer and transfer bioactive materials such as protein and nucleic acid, regulate homeostasis in blood vessels.

Methods: In this study, we developed a coating system to deliver hydrophilic bioactive agents using nanoemulsion and drop-casting methods to BVS. The MH-incorporated PLLA scaffold (PM) was prepared with hot-pressing methods. Then, the surface of PM was coated using a biodegradable polymer solution in which PDRN and Arg were dispersed using Span 80 (PMP and PMPR). As a final process, its surface was coated with polyethyleneimine (PEI), and then MSC-derived EVs were immobilized on its surface (PMPR-EV).

Results: Representative gene expression of inflammatory cytokine decreased in the PMP, PMPR, and PMPR-EV. Tube-forming assay to investigate the re-endothelialization effect demonstrated that total tube length and the number of branch points significantly increase in the order of PM, PMP, PMPR, and PMPR-EV.

Discussion and Conclusion: Taken together, the synergistic interaction of PDRN, Arg, and EVs alleviated inflammation and facilitated angiogenesis. When this coating system is applied to a biodegradable vascular scaffold (BVS), it is expected that it can address to overcome the side effects such as inflammation, thrombosis, neointima formation, and restenosis and become a new treatment technology for cardiovascular disease.
Biocompatible And 3D-printable Ionic liquid/collagen Based Hydrogel With Tunable Conductivity And Mechanical Properties For Peripheral Nerve Regeneration

Mr. Jiarui Zhou\textsuperscript{1}, Dr. Vijayavenkataraman Sanjairaj\textsuperscript{2}

\textsuperscript{1}New York University, New York, United States, \textsuperscript{2}New York University Abu Dhabi, Abu Dhabi, United Arab Emirates

Introduction: The function of nerve is realized through the transmission of electrical signals within the cells and the electrical signals converted into chemical signals through neurotransmitters across the cells. Appropriate electrical conductivity is one of the important criteria for preserving the integrity of nerve function. Many natural biofriendly materials are not inherently conductive and not ideal for use in nerve tissue repair, therefore it is necessary to add conductive materials/fillers to regulate electrical conductivity, promote signal exchange between nerves, and accelerate the nerve repair process.

Methods: In this work, we formulated a biocompatible and self-crosslinkable ionic liquid (IL) based-bioink made with fish skin-derived collagen, alginate, choline bicarbonate and acrylic acid. The bioinks are characterized to evaluate their mechanical and rheological properties, and conductivity. Furthermore, printability and biocompatibility of these bioinks were examined by bioprinting with primary human mesenchymal stem cells (hMSCs).

Results: The conductivity and Young’s modulus of collagen-IL hydrogel increased linearly with the increase of IL concentration without affecting biocompatibility. The conductivity of collagen-IL hydrogel increased by 171.4% from 0.56 S/mm to 1.52 S/mm and Young’s modulus increased by 27.4% from 13.5 kPa to 17.2 kPa when IL concentration increased from 0% to 15%. In all IL hydrogels, the live to dead cell ratio was greater than 70% on Day 7 and the stained cells showed an increased number of green fluorescent cells on day 7 compared to day 1 and day 3 of culture, indicating good cell compatibility and viability. The viscosity of the bioink, which directly relates to printability, can be easily adjusted by varying the IL crosslinking time as it is a photosensitive material and does not require any additional crosslinkers. The shear storage modulus of the polymerized IL increased with the increase of crosslinking time from 0h to 48h and no significant decrease was found until day 7.

Discussion and Conclusion: In conclusion, we formulated and 3D bioprinted a hMSCs-laden 3D collagen-IL conductive bioink, which has the potential to be used as a conductive filler in the nerve guidance conduit to promote cell proliferation and repair long peripheral nerve injuries. We demonstrated that the novel collagen-IL hydrogel can achieve the appropriate Young’s modulus, conductivity, and viscosity, making it a suitable candidate for bioprinting. The 3D cell culture analysis with hMSCs further verified the biocompatibility of the collagen-IL hydrogel.

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Stage-specific and location-specific cartilage calcification in osteoarthritis development

Dr. Xiaozhao Wang1,2,3,4, Qin Wu3, Ru Zhang1,2,4, Hongwei Ouyang1,2,3,4

1Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative Medicine, and Department of Orthopedic Surgery of the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, 2Department of Sports Medicine, Zhejiang University School of Medicine, Hangzhou, China, 3Zhejiang University-University of Edinburgh Institute, Zhejiang University School of Medicine, and Key Laboratory of Tissue Engineering and Regenerative Medicine of Zhejiang Province, Zhejiang University School of Medicine, Hangzhou, China, 4China Orthopedic Regenerative Medicine Group (CORMed), Hangzhou, China

Authors: Xiaozhao Wang1,2,3,4, Qin Wu3, Ru Zhang1,2,4, Hongwei Ouyang1,2,3,4,*

Affiliations: 1. Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative Medicine, and Department of Orthopedic Surgery of the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310058, China. 2. Department of Sports Medicine, Zhejiang University School of Medicine, Hangzhou, 310058, China. 3. Zhejiang University-University of Edinburgh Institute, Zhejiang University School of Medicine, and Key Laboratory of Tissue Engineering and Regenerative Medicine of Zhejiang Province, Zhejiang University School of Medicine, Hangzhou, 314400, China. 4. China Orthopedic Regenerative Medicine Group (CORMed), Hangzhou, 310058, China.

Background: Osteoarthritis (OA) involves pathological cartilage calcification, its progress is not fully understood. This study investigated the stage- and location-specific deposition and characteristics of minerals in human OA cartilages via multiple nano-analytical technologies.

Methods: Normal and OA cartilages were serially sectioned for micro-computed tomography, scanning electron microscopy with energy dispersive X-ray spectroscopy, micro-Raman spectroscopy, focused ion beam scanning electron microscopy, high-resolution electron energy loss spectrometry with transmission electron microscopy, nanoindentation, and atomic force microscopy to analyze the structural, compositional, and mechanical properties of cartilage in OA progression.

Results: We found that OA progressed by both top-down calcification at the joint surface and bottom-up calcification at the osteochondral interface (figure 1). The top-down calcification process started with spherical mineral particle formation in the joint surface during early-stage OA (OA-E), followed by fiber formation and densely packed material transformation deep into the cartilage during advanced-stage OA (OA-A). The bottom-up calcification in OA-E started when an excessive layer of calcified tissue formed above the original calcified cartilage, exhibiting a calcified sandwich structure. Over time, the original and upper layers of calcified cartilage fused, which thickened the calcified cartilage region and disrupted the cartilage structure. During OA-E, the calcified cartilage was hypermineralized, containing stiffer carbonated hydroxyapatite (HAp). During OA-A, it was hypomineralized and contained softer HAp. This discrepancy may be attributed to matrix vesicle nucleation during OA-E and carbonate cores during OA-A.

Discussion and Conclusion: This work refines our current understanding of the mechanism underlying OA progression and provides the foothold for potential therapeutic targeting strategies once the location-specific cartilage calcification features in OA are established.

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Mg-containing Biodegradable Polymer Bone Repair Material

Mrs. Yuxiao Meng¹, Mr. Yufan Wu¹, Mr. Bin Zhou¹, Mrs. Xueyan Jiang¹, Mr. Ming Zhang¹
¹Shenzhen Zhongke Jingcheng Medical Technology Co., Ltd., Shenzhen, China
Authors: Yuxiao Meng¹, Yufan Wu¹, Bin Zhou¹, Xueyan Jiang¹, Ming Zhang¹,*

Affiliations: 1,*[Ming Zhang, Shenzhen Zhongke Jingcheng Medical Technology Co., Songgang Street, Bao'an District, Shenzhen, Guangdong Province, 518105, China. Email:zhangming1307@163.com. (*Correspondence)]

Category: Tissue Engineering and Regeneration

Background: In this study, magnesium metal was innovatively added to a novel scaffold composited with poly(lactic-co-glycolic acid) (PLGA), tricalcium phosphate (TCP), which was fabricated by low-temperature rapid prototyping technology. We aim to verify the effectiveness and safety of the novel scaffold in repairing non-load-bearing bone defects of limbs, which composited is compared with contain β-TCP bioceramics in clinical application.

Methods: Subjects selected the patients with non-load-bearing bone defects in the extremities who needed bone grafting. The clinical trial was carried out in 8 clinical trial centers in different regions. All subjects meeting the inclusion criteria were randomly divided into experimental group and control group. The experimental group was treated with Mg-containing biodegradable polymer bone repair material, while the control group was treated with β-TCP bioceramics for treatment. The subjects were followed up three times at the 12th, 24th and 36th weeks after bone grafting. And collect treatment, observation and evaluation data to evaluate the safety and effectiveness of experimental products.

Results: Scaffolds had high porosity and perfect connectivity. Massive micropores distributed on the pore wall of the scaffold which ranged from 2.5 to 90 μm. The enrollment of subjects is ongoing. A total of 140 subjects were recruited, accounting for 80.0% of the total recruitment at so far. No serious adverse events occurred in all subjects. There were significant differences in the treatment results between the experimental group and the control group at the 12th, 24th and 36th weeks after bone grafting. The overall state of the subjects in the experimental group is better than that in the control group.

Discussion and Conclusion:: Study showed the novel scaffold composited can be degraded and absorbed in vivo, and has a promoting effect on the formation of new bone. It can be used to fill the bone defect caused by trauma, disease, surgery, etc., and promote the bone regeneration at the bone defect site.

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Introduction: Shoulder stiffness is a common musculoskeletal disorder characterized by thickening of the joint capsule and restricted range of motion, affecting 2-5% of the general population and over 20% of diabetic patients. Fibrosis of the joint capsule, resulting from activation of fibroblasts, is the key pathological feature. Activated fibroblasts have proliferative and contractile properties, producing excessive collagen. Despite its high incidence, effective anti-fibrotic therapies, particularly those targeting fibroblasts, are still lacking.

Materials and methods: In this study, microRNA-122 was first identified as a potential therapeutic agent for combating fibroblast activation in shoulder stiffness. For efficient drug delivery, Agomir-122 was then loaded into poly(lactic-co-glycolic acid) (PLGA) nanoparticles (Agomir-122@NP) - a biocompatible carrier with good properties. Furthermore, considering the homologous targeting effect, we extracted cell membranes from activated fibroblasts and wrapped them around Agomir-122@NP (Agomir-122@MNP) to inhibit fibroblast proliferation, contraction, and collagen synthesis.

Results: After confirming the targeting effect of Agomir-122@MNP on fibroblasts, we observed that Agomir-122@MNP could effectively inhibit fibroblast activation and improve shoulder joint capsule fibrosis in both preventative and therapeutic animal models, restoring joint mobility.

Conclusion: This study developed an efficient targeted delivery method with the potential for clinical translation in treating shoulder joint stiffness.
Regeneration effects of injectable hydrogel with anti-apoptosis, anti-inflammatory, and pro-angiogenesis properties on severe skeletal muscle defects

Dr. Zhiwen Luo¹, Cheng Hu², Shiyi Chen¹
¹Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai, China, ²School of Bioengineering, Sichuan University, Chengdu, China

Regeneration effects of injectable hydrogel with anti-apoptosis, anti-inflammatory, and pro-angiogenesis properties on severe skeletal muscle defects

Zhiwen Luo1, Cheng Hu2, Shiyi Chen1,*
1 Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China
2 School of Bioengineering, Sichuan University, Chengdu, China, 610065
Email: zhiwen.luo_fudan@hotmail.com

Introduction: Skeletal muscle injuries caused by trauma, tumor resection, and ischemia-reperfusion affect body posture, functional movement, and ultimately limit daily activities and reduce quality of life. Severe skeletal muscle injuries require therapeutic intervention to support functional regeneration. Immune modulation to promote skeletal muscle regeneration is an emerging therapeutic strategy, and preclinical studies have shown promising results with immune-regulatory substances filling the injury site.

Materials and methods: Here, we synthesized an injectable hydrogel that responds to the inflammatory microenvironment of skeletal muscle injury, which provides controlled release of the drug curcumin (Cur) and customized recombinant humanized collagen type III (RhCol III) for cardiac repair. The hydrogel was comprehensively evaluated using molecular, behavioral, and histological approaches both in vitro and in vivo.

Results: Cur-hydrogel has excellent antioxidant and anti-inflammatory effects, effectively reducing ROS levels and cell apoptosis after skeletal muscle injury and inhibiting inflammatory responses. Customized RhCol III promotes cell proliferation, migration, and angiogenesis. The therapeutic hydrogel improves the microenvironment of local skeletal muscle healing by inhibiting M1-type macrophages and promoting M2-type macrophages. Behavioral experiments confirm that the therapeutic hydrogel restores mouse motor function to normal levels.

Conclusion: In vitro and in vivo data suggest that a combined therapy strategy with anti-apoptosis, anti-inflammatory, and pro-angiogenesis properties is a promising strategy for treating severe skeletal muscle defects and has important clinical application value. Additionally, this work demonstrates the enormous potential of customized smart hydrogels in promoting myocardial infarction repair and regeneration.
Injectable remodeling hydrogels derived from alendronate-tethered alginate calcium complex for enhanced osteogenesis

Mr. Han-Sem Kim

1Dankook University, , South Korea

Introduction: Combinations of hydrogel materials and therapeutic agents have been actively reported to facilitate bone defect healing. However, conventionally hydrogels using cross-linker would result in low stability of the hydrogel itself, loss of agents during cross-linking, and complexity of use.

Subjects and Methods: In this study, alendronate was tethered to an AlA to improve bone healing and drug-loading stability. AlA was further functionalized with Ca2+ (AlACa). A mixture of AlACa and alginate formed AlAA hydrogel.

Results: The longer gelation time of AlAA hydrogels than that of the control CaAlg group makes AlAA suitable for injecting into the defect site. AlAA hydrogel is a remodeling hydrogel that is biodegradable through reversible ionic bonding based on natural polymers. Although the stiffness and strength further increased with increasing AlACa content, the flexibility and stress relaxation properties of the hydrogel were not reduced owing to the remodeling of the cross-linking in the hydrogel network, and the stable differentiation and mineralization ability of hMSCs were confirmed in the hydrogel until day 21.

Discussion and Conclusion: Hence, this injectable AlAA hydrogel showed the best mechanical stiffness with appropriate stress-relaxation and cellular behavior, indicating that it would be beneficial as a scaffold in the bone tissue engineering field.
Wharton’s Jelly MSC Exosomes Loaded with Apelin: A Promising Therapy for Insulin Resistance Reversal

Mr. jing cui1,2, Mr. Mingkun Wang1,2, Mr. Mingkun Wang2,1, Ms. Yan Zhang2,1, Mr. Ningkun Zhang2,1, Dr. Yu Chen2,1

1Navy Clinical College, The Fifth School of Clinical Medicine, Anhui Medical University, He’fei, China, 2Department of Cardiology, The Sixth Medical Center of Chinese People’s Liberation Army General Hospital, Beijing, China

Background: Diabetes results from β-cell dysfunction and insulin resistance. Apelin is a peptide hormone that can improve insulin resistance, but it is hindered by poor targeting and a short half-life. To address these limitations, we engineered exosomes derived from Wharton’s Jelly-derived MSCs (WJMSCs), containing Apelin. Our study demonstrates that Apelin-containing stem cell-engineered exosomes offer a novel option for reversing insulin resistance in diabetes, overcoming the aforementioned drawbacks.

Methods: In this study, we employed a mouse model of type 2 diabetes induced by a high-fat diet and low-dose streptozotocin (STZ) injection. After identifying and isolating human Wharton’s Jelly-derived MSCs, apelin-expressing lentiviral particles were used to transduce the cells and obtain mice containing WJMSCs that expressed apelin, which was verified by flow cytometry, western blotting, and qPCR analysis. Apelin-WJMSCs-exos were obtained through size-exclusion assay and characterized accordingly. For in vivo experiments, type 2 diabetic mice were injected with 1×10^10 pellets of Apelin-WJMSCs-exos or an equivalent dose of WJMSCs-exos via tail vein injection 7 days after STZ injection. Plasma glucose levels were monitored, and glucose tolerance and insulin tolerance tests (OGTT and IPITT, respectively) were conducted. Confocal microscopy and immunocytochemical analysis were used to assess the therapeutic effect of each infusion group and quantify islet beta cells. Additionally, we employed insulin resistance models of 3T3-L1 adipocytes to detect GLUT4 expression in each group using protein blots, and the pathways affected by such engineered exosomes were determined by measuring changes in AKT, AMPK, and their phosphorylation.

Results: Mice with type 2 diabetes infused with Apelin-WJMSCs-exos exhibited significantly lower blood glucose levels and increased proliferation of endogenous pancreatic β-cells. Additionally, continuous infusion of Apelin-WJMSCs-exos resulted in increased plasma levels of insulin and C-peptide. Apelin-WJMSCs-exos influenced AKT, eNOS, and AMPK activity in adipose tissue of mice through phosphorylation.

Discussion and Conclusion: In this study, we demonstrated the potential of exosomes loaded with WJMSC-derived Apelin to ameliorate insulin resistance in type 2 diabetic mice. Our results indicate that exosomes loaded with Apelin exhibit superior efficacy compared to those loaded solely with WJMSCs. These findings hold promise for the development of a clinically viable treatment option for type 2 diabetes.

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Characterization of subpopulation of multipotent mesenchymal stromal cells with antifibrotic properties

Prof. Vsevolod Tkachuk¹,², Dr. Natalia BASALOVA², Dr. Mikhail ARBATSKY¹, Dr. Maksim VIGOVSKY², Dr. Maria KULEBYAKINA¹, Dr. Anastasiya EFIMENKO¹,²
¹Department of Biochemistry and Regenerative Biomedicine, Faculty of Medicine, Lomonosov Moscow State University, Moscow, Russia, ²Institute for Regenerative Medicine, Lomonosov Moscow State University, Moscow, Russia

Authors: Vsevolod A. Tkachuk1,2, Natalia A. Basalova2, Mikhail S. Arbatsky1, Maksim A. Vigovsky2, Maria A. Kulebyakina1, Anastasia Y. Efimenko1,2

Affiliations: 1Department of Biochemistry and Regenerative Biomedicine, Faculty of Medicine, Lomonosov Moscow State University, Moscow, Russia; 2Institute for Regenerative Medicine, Lomonosov Moscow State University, Moscow, Russia.

Introduction. Multipotent mesenchymal stromal cells (MSCs) are a promising tool for use in regenerative medicine since these postnatal stem cells play a key role in repair and regeneration processes. MSCs regulate the balance between slow recovery of native tissue structure (regeneration) and rapid tissue repair by formation of a fibrotic scar. In the latter process MSCs can contribute to a myofibroblast pool promoting the progression of fibrosis but they also could suppress fibrosis by producing anti-fibrotic cytokines and microRNAs. Therefore, MSC could contain distinct subpopulations which respond differently to fibrotic stimuli.

Aims and Methods: Here we studied the potential mechanisms of the pro- and antifibrotic activity of MSCs. We used multipotent mesenchymal stromal cells isolation from human subcutaneous adipose tissue, single-cell RNaseq approach, contractile functional tests, and proteomic analysis of MSC secretome.

Results: Using the single-cell RNaseq approach we described two functionally distinct MSCs subpopulations that are formed under the established profibrotic stimuli including a key profibrotic factor TGFb and fibrotic-like decellularized extracellular matrix (ECM) produced by fibroblasts. Most MSCs differentiated into aSMA+ myofibroblasts with a profibrotic effect on nearby cells. However, some MSCs did not differentiate into myofibroblasts, and these cells were characterized by the expression of PDGFR-alpha and lack of aSMA expression. We demonstrated that PDGFR-alpha+/aSMA- MCSs have less contractile ability, but more invasive in ECM remodeling. To characterize antifibrotic effects of PDGFR-alpha+/aSMA- MCSs on nearby cells, we analyzed the secreted protein factors and microRNAs produced by them. We have shown that the antifibrotic effect of these cells was mediated by the secretion of proteins that interact with components of TGFb, Notch, Wnt and IGF signaling cascades, as well as by several microRNAs. We also identified 10 proteins in secretome of total population of MSCs that activate pro-inflammatory NF-kB signaling and potentially mediate the attenuated antifibrotic effects on TGFb-induced fibroblast differentiation. On the contrary, fractioning of the conditioned medium into the extracellular vesicles and a soluble fraction led to the depletion of profibrotic factors and an enrichment by the antifibrotic components of MSC secretome. The addition of separate secretome fractions to the fibroblasts in the profibrotic conditions significantly reduced their differentiation into myofibroblasts.

Discussion and Conclusion: Thus, we elucidated the mechanisms of the pro- and antifibrotic action of MSCs and proposed an approach to enhance the antifibrotic properties of the MSCs secretome.

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The Classification of Histological Images of Decellularized Corneal Scaffolds through Deep learning

Xinyu Wang1,2, Abdulrahman Aly Awad1, Vincent Chan1, Sayel Daoud5, Aamna Mohammed Al Shehhi1, Dr. Peter Corridon1,2,4

1Biomedical Engineering and Healthcare Engineering Innovation Center, Khalifa University, Abu Dhabi, United Arab Emirates, 2Department of Immunology and Physiology, College of Medicine and Health Sciences, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates, 4Center for Biotechnology, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates, 5Anatomical Pathology Laboratory, Cleveland Clinic Abu Dhabi, Abu Dhabi, United Arab Emirates

†Xinyu Wang1,2, †Abdulrahman Aly Awad1, Vincent Chan1, Sayel Daoud5, *Aamna Mohammed Al Shehhi1, *Peter R. Corridon1,2,4

1Biomedical Engineering and Healthcare Engineering Innovation Center, Khalifa University, Abu Dhabi, United Arab Emirates
2Department of Immunology and Physiology, College of Medicine and Health Sciences, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates
4Center for Biotechnology, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates
5Anatomical Pathology Laboratory, Cleveland Clinic Abu Dhabi, Abu Dhabi, United Arab Emirates

†Co-first Authors:
Xinyu Wang and Abdulrahman Aly Awad contributed equally to this paper

* Correspondence:
Aamna Mohammed Al Shehhi
aamna.alshehhi@ku.ac.ae
Peter R. Corridon
peter.corridon@ku.ac.ae

Presenting author: Xinyu Wang (student)

Introduction: The worldwide donor supply for corneal transplantation cannot meet the existing and projected demands. Such scarcity has promoted the development of keratoprostheses (corneal alternatives). Decellularized keratoprostheses are considered promising cornea replacements with a high potential for somatic growth, reduced complications, and much fewer immune rejections. Therefore, characterizing decellularized corneal scaffolds prior to in vivo transplantation is essential. Histology visualization is a common way to obtain the structural characteristics of corneal substitutes. Currently, the analysis of histological images is not limited by the interpretation of pathologists and scientific researchers but by deep learning (DP) techniques with high accuracy. Various models have been applied in medical or pathological image processing, such as convolution neural networks (CNN), recurrent neural networks (RNN), generative adversarial nets (GANs), U-Net, and transfer learning models. Those models are widely applied in image processing for a distinct purpose. Especially for the transfer learning model, it is highly recommended for microscopic image analysis when the image amount is limited.

Methods: This study focused on roughly 450 histological images obtained from native and decellularized corneal segments counterstained with hematoxylin and eosin using a 20X brightfield microscopy. The images were resized and analyzed via transfer learning models, including EfficientNetB0, B1, B2, B3, S772, and S368, and fine-tuning to classify the corneal scaffolds with different decellularization treatments.
Results: Regarding the transfer learning models we used, they all showed over 87% accuracy in classifying histology images with different decellularizing treatments before fine-tuning. As expected, the accuracies of all the models were improved above 89%. More specifically, model S772 reached the highest accuracy with 95% before fine-tuning and 96% after fine-tuning.

Discussion and Conclusions: The results exhibited that the transfer learning models we applied can categorize the corneal structures with various treatments. This approach improves the efficiency of structural characterization of corneal scaffolds and provides future studies with foresight on using transfer learning models to manage and analyze more intricate images.
Exosomes purified from miRNA-223 overexpressed MSCs show enhanced anti-inflammatory ability

Ms. Ka Man Li¹, Ms. Smriti Arya¹, Dr. Eiji Kobayashi¹,², Ms. Hikari Kurogi¹,²
¹Rohto Advanced Research Hong Kong Ltd, Hong Kong, China, ²Regenerative Medicine Research and Planning Division, ROHTO Pharmaceutical Co., Ltd, Kyoto, Japan

Background: Mesenchymal Stem Cells (MSCs) and its exosome-based therapy has been widely recognized to show its potential against treating numerous diseases. However, it still reserves rooms for improvement, including its low harvest yield and limited efficacy. Hence, we aimed to enhance the therapeutical effects of MSCs/exosomes by overexpressing miRNA-223.

Methods: In this study, synthesised miRNA-223 was transfected to MSCs and exosomes were obtained. MSCs and exosomes were further characterized and tested under various in vitro assays including anti-apoptosis, anti-inflammatory as well as cell proliferation. Inhouse formulated clinical grade serum free MSC medium were used in this study.

Results: Elevated miRNA-233 expression level was successfully observed in both transfected MSCs and isolated exosomes. The miR223-MSCs and miR223-exosomes both showed higher anti-apoptotic and anti-inflammatory effect. Furthermore, MSC derived-exosomes were observed to present enhanced anti-inflammatory effect than the modified MSCs itself.

Discussion and conclusion: In this study, we demonstrated miR-223 can be successfully overexpressed using our inhouse culture system using clinical-grade, serum-free MSC medium. Importantly, our derived exosome is superior to MSCs itself in anti-inflammatory aspect. Since cell free therapy has better immunogenicity and longer shelf life than cell-based therapy, these results provide insights into the development of genetically modified exosome-based cell-free therapy.

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In situ conductive bioglu on for utilization of bioelectronics in living organism

Mr. Hyun Tack Woo
Pohang University of Science and Technology, , South Korea

Background: There are increasing demands for conductive materials in the biophysical environment, especially for bioelectronics device applications including neural and cardiac stimulation and recording, electroceuticals, and neural prostheses. In order to overcome the mismatch caused by the difference in physical properties and charge carriers between conductive materials and biological tissues, there have been trials to bridge the interfaces.

Methods: In the present work, we newly developed double-layered biocompatible nanoparticles with a eutectic gallium-indium alloy (EGaIn) core-shell. Bulk EGaIn is dispersed evenly via ultrasonic in solution which contains hyaluronic acid (HA) molecules. Nanoparticles made coacervate state with bioengineered mussel adhesive protein (MAP).

Results: EGaIn, which is used as a core, is liquid phase at body temperature for its low melting point, is far less toxic, and possesses a negligible vapor pressure. EGaIn nanoparticles have been successfully formed with HA whose net charge is negative in neutral pH conditions. HA is a widely used biocompatible, biodegradable polysaccharide. The thickness of coating has been regulated through the concentration of HA solution. Coacervated conductive bioglu has been formed with the mixing of MAP and nanoparticles. MAP contains rich DOPA residues that play a critical role in adhesive and cohesive effect in mussel byssus and abundant lysine residues that make a protein positive net charge.

We confirmed that the successful formation of conductive bioglu originated from the electrostatical force between HA and MAP. Our novel material can be applied as interface between electrode and tissues through utilizing various residues in MAP, doing critical role for bioelectronics utilization.

Discussion and Conclusion: We demonstrated that the HA-BP hydrogel can support preosteoclast differentiation and its associated activity but inhibits subsequent osteoclast maturation from primary macrophages, thereby resulting in enhanced in-situ bone regeneration in vivo. The current work demonstrated a promising strategy to design biomimetic biomaterial scaffolds capable of regulating bone resorption and formation to promote bone regeneration.
High-efficient engineering of osteo-callus organoids for rapid bone regeneration within one month

Ms. Chang Xie

Zhejiang University, China

Introduction: Large bone defects that cannot form a callus tissue are often faced with long-time recovery. Developmental engineering-based strategies with mesenchymal stem cell (MSC) aggregates have shown enhanced potential for bone regeneration. However, MSC aggregates are different from the physiological callus tissues, which limited the further endogenous osteogenesis.

Subjects and Methods: This study aims to achieve engineering of osteo-callus organoids for rapid bone regeneration in cooperation with bone marrow-derived stem cell (BMSC)-loaded hydrogel microspheres (MSs) by digital light-processing (DLP) printing technology and stepwise-induction. To deeply gain insight into the cell differentiation in MSs system, bulk-RNA sequencing and data analysis were performed and we also compared the bulk-RNA data of cultured BMSCs in MSs at varied time points with the single-cell RNA Seq data of the clinical phalange tissue samples in early childhood. Finally, we then implanted the osteo-callus organoids into the distant femur defects in rabbits and analyzed the regenerated tissues by micro-computed tomograms (micro-CT), histological staining, immunofluorescent staining and transmission electron microscope (TEM).

Results: The printed MSC-loaded MSs aggregated into osteo-callus organoids after chondrogenic induction and showed much higher chondrogenic efficiency than that of traditional MSC pellets. Moreover, the osteo-callus organoids exhibited stage-specific gene expression pattern that recapitulated endochondral ossification process, as well as a synchronized state of cell proliferation and differentiation, which highly resembled the diverse cell compositions and behaviors of developmentally endochondral ossification. Lastly, the osteo-callus organoids efficiently led to rapid bone regeneration within only 4 weeks in a large bone defect in rabbits which need 2-3 months in previous tissue engineering studies.

Discussion and Conclusion: The findings suggested that in vitro engineering of osteo-callus organoids with developmentally osteogenic properties is a promising strategy for rapid bone defect regeneration and recovery.
Novel Immortalized MSC cell line for study of adipogenic differentiation and insulin signaling.

Dr. Konstantin Kulebyakin1, Mr. Nikita VOLOSHIN1, Dr. Pyotr TYURIN-KUZMIN1, Mr. Vadim CHECHEKHIN1, Dr. Maxim KARAGYAN1, Ms. Alexandra PRIMAK1, Ms. Mariya SKRYABINA1

1Lomonosov Moscow State University, Moscow, Russia

Introduction. Modern biomedical science still experiences a significant need for easy and reliable sources of human cells. They are used to investigate pathological processes underlying disease, pharmacological studies, and eventually as a therapeutic product in regenerative medicine. For decades adult pool of mesenchymal stem / stromal cells (MSCs) remains a promising source of stem and progenitor cells. Their isolation is more feasible than most other stem cells from human donors, yet they have a fair share of disadvantages. The latter includes significant variability between donors, loss of potency, and transformation during long-term culture, which may impact the efficacy and reproducibility of research. One possible solution is a derivation of immortalized MSCs lines which receive a broader use in many medical and biological studies. Recently in our work, we demonstrated that in the most widely commercially available hTERT immortalized MSCs cell line ASC52telo, sensitivity to hormonal stimuli was reduced (1), affecting their differentiation efficacy. Furthermore, we found that immortalized MSCs have impaired insulin-dependent and cAMP-dependent signaling, which impairs their adipogenic, but not osteogenic or chondrogenic potential in experimental conditions (2). In this regard we decided to create an immortalized cell line with nonimpaired insulin sensitivity to provide a reliable platform for study of insulin signaling in MSC.

Methods. Human MSCs derived from adipose tissue of a healthy donor (male, 28 years old, inguinal hernia) were obtained from the biobank of the Institute for Regenerative Medicine, Lomonosov MSU, collection ID: MSU_MSC_AD (https://human.depo.msu.ru) and cultured in the medium supporting the growth of undifferentiated mesenchymal progenitor cells (Advance Stem Cell Basal Medium, HyClone) containing 10% of a growth factor supplement (Advance Stem Cell Growth Supplement, HyClone), 100 U/ml of penicillin/streptomycin (Gibco). To immortalize the cell culture, we used a genetic construct, based on pVLT vector (Evrogen, Moscow). The vector contained the cDNA sequence encoding human telomerase-reverse transcriptase (forward primer - 5’-CCACCGAAATCGCCACCATGCCGGCGCTCCCGCTGGCCAGCCGTGCGCT; reverse primer - 5’-GGTCGTCGACTCAGTCCAGGATGGTCTTGAAGTCTGAGGGCAGTGCCGGGTG) and the puromycin resistance gene for selection.

Results. We denoted obtained immortalized cell line as hTERT-MSC195. These cells can maintain their proliferative and differentiation potential up to 30+ passages, while primary MSC significantly alter their properties after 7-8 passages. We found that in experimental conditions hTERT-MSC195 demonstrate adipogenic and osteogenic differentiation potential similar to the primary MSC from healthy donor. Moreover, we found that hTERT-MSC195 doesn’t demonstrate significant changes in insulin sensitivity comparing with primary culture.

Conclusion. hTERT-MSC195 cell line looks like very promising tool in study of MSC biology, combining easier culturing approaches with preserved differentiation potential and hormonal sensitivity.


Development of a decellularized liver matrix-based nanocarrier for liver regeneration after partial hepatectomy

Mr. Yong-Heng Lin¹, Mr. Yu-Chuan Chiu¹, Mr, Yung-Te Hou¹
¹National Taiwan University, Taiwan

Background: The liver plays an indispensable role in the human body, with important functions such as protein synthesis, urea metabolism, and detoxification. Despite this, chronic hepatitis is one of the top 10 most common causes of adult deaths worldwide. Silymarin has long been used as an ideal reagent for comparing hepatoprotective bioactive components. However, oral absorption of silymarin is low, resulting in poor bioavailability. Additionally, liver dysfunction puts liver-diseased patients at increased risk of morbidity and mortality during surgical operations. Therefore, there is an urgent need to develop a drug to improve liver regeneration in these patients, in order to expand the pool of patients who are eligible for surgical intervention and improve their success rates.

Methods: We developed a novel nanomedicine that employs the decellularized liver matrix (DLM) and TA to accelerate the recovery of injured liver following partial hepatectomy (PH). DLM is highly biocompatible and can establish a favorable microenvironment for hepatocytes in the liver. To enhance hepatic function after PH, we devised a TA-loaded, mPEG-modified decellularized liver matrix (TA-mPEG-DLM) and characterized its properties. First, DLM was subjected to PEGylation, and then we employed the ultrasonic emulsion technique to produce novel nano-sized DLM. Next, we examined the cell viability and hepatic functions of the TA-mPEG-DLM in vitro. Finally, we evaluated the effectiveness of TA-mPEG-DLM in promoting the regeneration of injured hepatocytes following PH in mice in vivo.

Results: On day 1, 3, and 5, the albumin synthesis increased by 34.6%, 38%, and 32.2%, respectively, in the 0.001 mg/mL TA-mPEG-DLM group, demonstrating the positive effects of TA-mPEG-DLM group on enhancing hepatic functions without causing hepatotoxicity (Fig. 1B). Additionally, the AST levels in the TA-mPEG-DLM group on day 1, 3 and 7 were 11.3%, 28.2%, 8.6% lower those in the control group, indicating the inhibition of severe liver inflammation caused by PH (Fig. 1C). Furthermore, in Fig. 1D, the ALB/GLO levels in the TA-mPEG-DLM group on day 1 (1.24 ± 0.02) and day 3 (1.28 ± 0.1) were 5%, 18.5% higher than those in the control group (1.18 ± 0.05 and 1.08 ± 0.1, respectively), suggesting that TA-mPEG-DLM may promote liver regeneration and reduce the inflammatory response caused by PH, leading to a positive effect on maintaining the ALB/GLO ratio.

Discussion and Conclusion: In this study, we successfully developed TA-mPEG-DLM as a novel targeted drug delivery system (TDDS) for sustained promotion of hepatic functions and reduction of liver inflammation. One of the major advantages of TA-mPEG-DLM is its excellent performance in loading and releasing TA for liver regeneration. Our results provide the foundation for future investigations on the clinical application of TA-mPEG-DLM as a potential TDDS for targeting the liver.
Stretchable Ferrogel Prepared with Hyaluronic Acid for Biomedical Applications

Mr. Minhyung Kong¹, Hyun Seung Kim¹, Kuen Yong Lee¹
¹Department of Bioengineering, Hanyang University, Seoul, South Korea

Title: Stretchable Ferrogel Prepared with Hyaluronic Acid for Biomedical Applications

Authors: Minhyung Kong, Hyun Seung Kim, Kuen Yong Lee

Category: Design and Application of Biomaterials

Affiliations: Department of Bioengineering, Hanyang University, Seoul 04763, Republic of Korea

Introduction: Ferrogel is a smart hydrogel system that contains magnetic nanoparticles and is capable of responding to changes in magnetic fields. It has shown promise in various biomedical applications, including tissue engineering. The goal of this study was to develop a 3D-printable ferrogel with improved stretchability using hyaluronic acid (HA) and superparamagnetic iron oxide nanoparticles (SPION).

Subjects and Methods: Hydrogel was prepared from hydrazide-modified HA and oxidized HA in the presence of sodium alginate and calcium ions. SPION were integrated into the hydrogel to form ferrogel. The viscoelastic properties of the ferrogel were investigated using a rotational viscometer, and its printability was evaluated using an extrusion-based 3D printer.

Results: Ferrogel with enhanced toughness and self-healing ability was prepared. Ferrogel displayed notable stretchability and increased in size by more than double its original dimensions. Double cross-linking in the ferrogel significantly improved its mechanical properties, including stiffness and toughness. Additionally, the ferrogel demonstrated the remarkable ability to be 3D-printed and maintained its shape post-printing without the use of a frame material.

Discussion and Conclusion: HA-based ferrogel with excellent toughness and 3D printability was successfully prepared, which may have a potential to be utilized in biomedical applications, including tissue engineering. The incorporation of magnetic nanoparticles enabled the ferrogel to respond to the application of magnetic fields. This system could be useful for the creation of active scaffolding systems for tissue engineering applications.
Three-dimensional Bioprinting Of Self-healing Hydrogel With Enhanced Toughness

Mr. hyunseung kim¹, Mr. Kuen Yong Lee¹

¹Department of Bioengineering, Hanyang University, Seoul, South Korea
Title : Three-dimensional bioprinting of self-healing hydrogel with enhanced toughness

Authors : Hyun Seung Kim, Kuen Yong Lee

Affiliations : Department of Bioengineering, Hanyang University, Seoul 04763, Republic of Korea

Category : Tissue Engineering and Regeneration

Introduction : Three-dimensional (3D) bioprinting enables to fabricate complex 3D structures that can mimic the microenvironment of living cells. Hydrogels should overcome the shear force generated during extrusion and be sufficiently elastic to build 3D interconnected structures when used as bioinks. However, polysaccharide-based hydrogels have inherently limited mechanical properties and printability.

Subjects and Methods : Hyaluronic acid (HA) was modified with diol-containing molecules, followed by oxidation. Oxidized HA and hydrazide-conjugated HA were used to form self-healing hydrogels. Alginate was also added to the hydrogels and cross-linked with calcium ions. Various characteristics of 3D-printed constructs were investigated as scaffolds for cartilage regeneration.

Results : HA-based hydrogels showed long-term stability, self-healing ability, and enhanced toughness compared to other polysaccharide-based hydrogels. These hydrogels also showed excellent self-standing ability after 3D printing without using a frame material. Chondrogenic differentiation of ATDC5 cells cultured within the 3D-printed constructs was confirmed in vitro.

Discussion and Conclusion : It was demonstrated that HA-based hydrogels with self-healing and self-standing ability could be used as 3D bioink. Prominent chondrogenic differentiation of ATDC5 cells was observed when the cells were cultured within the 3D-printed constructs in vitro. This approach may provide a promising strategy to design and fabricate HA-based scaffolds for tissue regeneration, including cartilage regeneration.

Acknowledgement : This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MIST) (NRF-2020R1A2C1012199).
Retinal Degeneration Treatment By Subretinal Transplantation Of Several Types Of Mesenchymal Stem Cells And Human Pluripotent Stem Cell-derived Retinal Pigment Epithelium Into Retinal Degeneration Model Rats

Ms. Qian Liu¹, Jun Liu¹, Minmei Guo¹, Guoping Fan², Akon Higuchi¹,³

¹State Key Laboratory of Ophthalmology, Optometry and Visual Science, Eye Hospital, Wenzhou Medical University, Wenzhou, China, ²Department of Human Genetics, David Geffen School of Medicine, Los Angeles, USA, ³Department of Chemical and Materials Engineering, National Central University, Taoyuan, China

Authors: Qian Liu¹, Jun Liu¹, Minmei Guo¹, Guoping Fan², Akon Higuchi¹,³ *

Affiliations: ¹State Key Laboratory of Ophthalmology, Optometry and Visual Science, Eye Hospital, Wenzhou Medical University, Xueyuan Road, Wenzhou, Zhejiang, China. ²Department of Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA 90095 USA. ³Department of Chemical and Materials Engineering, National Central University, Jhongda Rd., Jhongli, Taoyuan, Taiwan China.

Introduction: Retinal degeneration (RD) is one of the diseases characterized by irreversible and progressive vision loss, where the loss of retinal cells is difficult to be regenerated. Transplantation of retinal pigment epithelium (RPE) cells, stem cells and/or photoreceptor cells seems to be a promising treatment for RD patients. Several types of stem cells have been used for clinical trials and treatment of RD animal models. However, there have been rare studies, which deal with the comparison of subretinal transplantation of several types of stem cells (hAFSCs, hDPSC, hADSCs, hBMSCs, and, hiPSCs) as well as hPSC-derived RPE cells on the protection effects, paracrine effects and treatment efficiency of RD on animal models of RD (Royal college of surgeon, RCS, rats). Therefore, we investigated and compared the subretinal transplantation of several types of stem cells as well as hPSC-derived RPE cells for the protection effects, paracrine effects and treatment efficiency of RD on RCS rats.

Subjects and Methods: Stem cell or hPSC-derived RPE cell suspension (1x10⁵ cells/2μL) labelled with CellTracker Green was carefully delivered into the subretinal space of 3-week-old RCS rats. The control group received subretinal PBS injection or non-injection. After the injection, fundus photography (FP) was conducted immediately on the eye of each RCS rat to evaluate the site of the cell injection. Optomotor response (OMR) and electroretinography (ERG) were conducted on each RCS rat at week 1-4 and week 4 and 8 respectively, to evaluate the improvement of visual function of each RCS rat. Retinal section of paraffin embedding and subsequently haematoxylin-eosin (HE) staining were performed to evaluate the retinal structures and thickness for the comparison of each RCS rat transplanted with several stem cells (Fig. 1).

Results: Each stem cell, hPSC-derived RPE cell or PBS (blank experiment) was successfully transplanted into at least six RCS rats subretinally. Compared with control groups, RCS rats, which received stem cells transplantation subretinally, showed higher ERG waves, which indicated better visual functions than those of control group at 4 weeks of post-injection. At 8 weeks of post-injection, only RCS rats transplanted with hPSC-derived RPE cells maintained their visual function. The observation of histological section and the section stained with H&E indicated that the retinal thickness of outer nuclear layer (ONL) of RCS rats were thicker in hPSC-derived RPE transplantation group compared with control groups.

Discussion and Conclusion: hPSC-derived RPE transplantation on RCS rats showed the best improvement of visual function evaluated from both ERG amplitude and time of ERG wave maitanence. hMSCs, such as hDPSCs, showed temporal improvement of visual function at 4 weeks of post-injection but not at 8 weeks of post-injection. Our study investigated a protective effect of
vision structure and function from RD by each stem cell on RCS retina, which may support short-time treatment of RD by subretinal transplantation of each hMSC.
Long-term stability and transfection efficacy of the lyophilized lipid nanoparticles entrapped with mRNA

Dr. Ting WANG1, Mr. Tao YU1, Ms. Wan Qi Li1, Dr. Tzu-Cheng SUNG1, Dr. Akon HIGUCHI1,2
1State Key Laboratory of Ophthalmology, Optometry and Visual Science, Eye Hospital, Wenzhou Medical University, Wenzhou, China, 2Department of Chemical and Materials Engineering, National Central University, Taiwan, China

Background: Lipid nanoparticles (LNPs) are promising carriers for gene therapeutics. They have been widely used in different kinds of disease treatment and for delivering targeted agents, such as antisense oligonucleotides, small interfering RNA (siRNA), mRNA, and DNA inhibitors. The successful application of siRNA drugs (Onpattro) as well as two COVID-19 mRNA vaccines (Comirnaty® and Spikevax) have opened a golden era for the development of LNPs-mediated gene drugs. However, stability of LNPs-mRNA is a bottleneck, which is highly recommended to be deeply studied, as the cold-chain storage and transportation condition of two LNPs encapsulated mRNA for COVID-19 protection is harsh and highly costly.

Methods: The factors that influence long-term stability of LNPs-mRNA were investigated in this study. The cryoprotectants (sucrose) concentration, lipids types as well as the molar ratio of 4 lipids contained in LNPs were optimized by the design of orthogonal experiment (DOE). The long-term storage stability of screened optimal formulation was evaluated in vitro and in vivo. The physicochemical properties of LNPs-mRNA were determined by the DLS instrument and mRNA assay using RiboGreen reagents. The HEK-293T cell line was used to evaluate the cellular uptake profile or transfection efficiency of LNPs-mRNA by detecting the expression of eGFP with fluorescence microscopy and flow cytometry. The C57LB/6 mice were introduced to evaluate the in vivo efficiency and distribution of mRNA expression by using the IVIS image system.

Results: Lyophilized LNPs entrapped with eGFP/luciferase labeled mRNA were prepared, of which compositions are mimicking the 4 types of lipids contained in Comirnaty® and Spikevax vaccines. Meanwhile, the main distinct parameters between these 2 vaccines, such as sucrose concentration, molar ratio of 4 type lipids, N/P ratio and lipids types, were optimized, in order to find their contribution to maintain stability during the lyophilization process. We demonstrated that 8.7% (w/v) sucrose is the optimal sucrose concentration to maintain stability of lyophilized mRNA-LNPs mimicking Comirnaty® vaccines. The influence of molar ratio of 4 type lipids was investigated by DOE, and one optimal formulation with high ability to maintain stability after lyophilization process was selected for long-term storage stability study in vitro and in vivo.

Discussion and Conclusion: The current work demonstrated that lyophilization is a promising strategy to overcome the bottleneck of instability of mRNA-LNPs. The compositions of LNPs have an essential effect on maintaining stability of mRNA-LNPs, and further studies are needed to dig into the understanding of the data and the consideration of mechanism.

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Different profiles of PTH1R-dependent calcium signal determine the switch between pro- or anti-osteogenic effect of PTH on MSC.

Dr. Konstantin Kulebyakin¹, Dr. Pyotr TYURIN-KUZMIN¹, Mr. Nikita VOLOSHIN¹, Mr. Vladimir USACHEV¹, Mr. Mikhail NIKOLAEV¹, Mr. Vadim CHECHEKHIN¹, Dr. Maria VORONTSOVA¹

¹Lomonosov Moscow State University, Moscow, Russia

Introduction. Bone tissue is a dynamic structure that is intensively renewed throughout an individual’s life. The dynamic balance between resorption and synthesis plays a key role in the renewal and remodeling of bone tissue. Parathyroid hormone (PTH) is one of the key regulators of calcium and phosphate metabolism in the body, controlling bone metabolism and ion excretion by the kidneys. At present, attempts to use PTH as a therapeutic agent have been associated with side effects, the nature of which is not always clear and predictable (1). In addition, it is known that in vivo impairment of PTH post-receptor signaling is associated with atypical differentiation behavior not only of bone cells but also of connective tissues, including adipose tissue. In this work, we studied the functional responses of multipotent mesenchymal stromal cells (MSCs) to the action of PTH at the level of single cells (2).

Methods. We used MSCs isolated from the periosteum and subcutaneous adipose tissue to compare characteristics of cell responses to PTH. To negate donor to donor variability, we obtained periosteum and subcutaneous adipose from each donor to use it in comparative studies. Different profiles of calcium response were obtained using single-cell calcium imaging.

Results. In our work, we discovered that MSC both from the periosteum and adipose tissue carry only PTH type 1 receptors on their surface. However, we found that activation of this receptor by PTH can result in three distinctive calcium responses: single transients of calcium, calcium oscillations, and hormone-activated smooth increase of intracellular calcium. These types of calcium responses led to principally different cellular responses of MSCs. cAMP-dependent smooth increase of intracellular calcium was associated with the pro-osteogenic action of PTH, whereas phospholipase C-dependent calcium oscillations led to a decrease in osteogenic differentiation intensity. Different variants of calcium responses are in dynamic equilibrium. Suppression of one type of response leads to increased activation of another type and, accordingly, to a change in the effect of PTH on cell differentiation.

Conclusion. The discovery of a dynamic balance between the pro- or anti-osteogenic effect of PTH opens prospects for streamlining its action on osteogenesis. As a result, that will help to overcome the problems of using PTH for bone regeneration and the treatment of osteoporosis, associated with the multiplicity of its effects on resident bone tissue stem cells.


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Decellularized Extracellular Matrix Particle-based Biomaterials for Cartilage Repair Applications

Jiangyao XU1, Peng GUO1, Mauro ALINI1, Andrea VERNENGO1, Sibylle GRAD1, Jeroen GEURTS2, Zhen LI1
1AO Research Institute Davos, Davos, Switzerland, 2Lausanne University Hospital, Lausanne, Switzerland

Introduction: Decellularized extracellular matrix (dECM) materials derived from cartilage have demonstrated promising regenerative capabilities for cartilage repair due to their chondrogenic bioactivity [1]. However, conventional decellularization processes have limited retention of ECM components and impair the integrity of functional ECM molecules, resulting in compromised biomimetic properties of these materials. Therefore, the current research aims to fabricate biomimetic materials encapsulating cartilage dECM particles with intact molecular structures and natural components for cartilage repair.

Methods: A detergent-free decellularization strategy was developed to prepare dECM particles from bovine cartilage tissue which were then incorporated into hyaluronic acid-tyramine (THA) hydrogels [2]. Bovine cartilage tissue fragments were subjected to five cycles of freeze/thaw followed by pulverization in a liquid nitrogen Mixer Mill at 25 Hz for 3 minutes. The resulting material was then rinsed and treated with DNase I and protease inhibitor cocktail for 8 hours to remove cellular DNA. The decellularized tissue was then lyophilized and re-homogenized via pulverization in a liquid nitrogen Mixer Mill at 25 Hz for 15 minutes to generate dECM particles. THA was synthesized by attaching tyramine to hyaluronic acid via amide bond formation in water. After mixing THA with dECM particles at different concentrations, enzymatic cross-linking was initiated by adding H2O2 and incubating at 37°C for 30 minutes. Additional light cross-linking was performed using green light and eosin Y as the photoinitiator. The THA-dECM hydrogels were subsequently subjected to swelling, stability, and compression tests to characterize their properties.

Results: The results demonstrated that the decellularization strategy maintained intact proteoglycans and collagens with high retention rate and adequately removed DNA (Fig. 1 A-C). Hydrogels containing dECM particles demonstrated long-term stability and high retention of glycosaminoglycans and collagens after up to 14 days of incubation in medium at 37 °C. Moreover, the addition of 20% dECM particles enhanced the compressive modulus of THA hydrogels (Fig. 1 E), bringing it closer to the mechanical properties of native cartilage. Conclusions: Our decellularization method successfully preserved functional and intact cartilage components at high yield. Hydrogels containing dECM particles possess long-term stability and enhanced mechanical properties, making them promising biomaterials for cartilage repair.

References:

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Universal human pluripotent stem cells: Generation and differentiation

Dr. Tzu-cheng Sung

1State Key Laboratory of Ophthalmology, Optometry and Visual Science, Eye Hospital, Wenzhou Medical University, Wenzhou, China, 2Department of Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, USA, 3Department of Chemical and Materials Engineering, Central University, Jhongli, Taiwan, China

Background: The requirement of organs and tissue transplantation becomes more and more urgent because of accidents, birth defects, and diseases. Human pluripotent stem cells (hPSCs) are an attractive source of tissue regeneration. However, human leukocyte antigens (HLAs) matching on the surface of transplanted cells with patient organs and cells is a major barrier for the clinical application of hPSCs, which limits the transplantation being available between HLA compatible typing of transplanted cells and patient organs or cells.

Methods: In this study, we generated universal human induced pluripotent stem cells (UhiPSCs) without gene modification or editing. The generation of UhiPSCs is performed from mixing allogenic multi-donors of human amniotic fluids (hAFs). Subsequently, human amniotic fluid stem cells (hAFSCs) were established by culturing hAFs on tissue culture polystyrene dishes. hAFSCs were reprogrammed into hiPSCs using non-integrating method of Sendai virus vector.

Results: The cells expressed less HLA class Ia (HLA-A, -B, and -C) and class II after reprogramming of hAFSCs derived from multi-donor of human amniotic fluids. The cells (UhiPSCs) expressed less HLA class Ia and II even after differentiation into mesenchymal stem cells (MSCs), cardiomyocyte and embryonic bodies (EBs). The differentiated cardiomyocyte from UhiPSCs could keep beating even after the treatment of allogeneic mononuclear cells derived from different hAF donors.

Discussion and Conclusion: We will continue to evaluate immunocompatibility of UhiPSCs and their differentiated cells by using humanized mice, which express human natural killer (NK) cells, and cytotoxic CD8+ T cells to mimic the human immune response in mice. We expect that our UhiPSCs has the potential to overcome the HLA compatibility barrier of tissue transplantation.

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Efficient differentiation of human pluripotent stem cells into cardiomyocytes on cell sorting thermoresponsive surface

Dr. Tzu-cheng Sung

Background: The human pluripotent stem cells (hPSCs) are a stable and limitless cell source in regenerative medicine, because hPSCs can differentiate into any types of the cells in our human body. However, current differentiation process of hPSCs into cardiomyocytes to enhance their purity requires some purification processes, such as Fluorescence-activated cell sorting (FACS) or Magnetic-activated cell sorting (MACS), which are laborious and expensive.

Methods: In this study, we developed cell sorting plates, which were prepared from immobilization of thermoresponsive poly(N-isopropylacrylamide) and extracellular matrix proteins on the cell culture plates. After hPSCs were induced into cardiomyocytes on the thermoresponsive surface coated with laminin-521 for 15 days, the temperature of the cell culture plates was decreased to 8-9 °C to detach the cells partially from the thermoresponsive surface.

Results: The detached cells exhibited a higher cardiomyocyte marker of cTnT than the remaining cells on the thermoresponsive surface as well as the cardiomyocytes after purification using conventional cell selection. The detached cells expressed several cardiomyocyte markers, such as α-actinin, MLC2a, and NKX2.5.

Discussion and Conclusion: This study suggests that the purification of hPSC-derived cardiomyocytes using cell sorting plates having the thermoresponsive surface is a promising method for the purification of hPSC-derived cardiomyocytes with inexpensive and easy operational way compared to conventional laborious purification processes.

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Impact of the Operator on Qualitative and Quantitative Parameters of Non-Activated Platelet-Rich Plasma: Practical Implications for Regenerative Medicine Applications

Dr. Sharun Khan, Dr. S. Amitha Banu, Dr. K. M. Manjusha, Dr. Rohit Kumar, Dr. Obli Rajendran Vinodhkumar, Dr. Ujjwal Kumar De, Dr. A. M. Pawde, Dr. Kuldeep Dhama, Dr. Amarpal

Background: Platelet-rich plasma (PRP) has broad therapeutic applications in veterinary regenerative medicine. However, several variables have been found to affect the therapeutic potential of PRP. The present study evaluated the impact of the operator on the qualitative and quantitative parameters of non-activated PRP derived from canine whole blood.

Methods: Blood samples were collected from twelve clinically healthy adult dogs to prepare PRP using a double spin method. Both operators prepared the PRP from the pooled individual blood samples using the same protocol and equipment. The prepared PRP was then characterized, classified, and coded using the previously-established minimum reporting requirements. Reliability analysis with intra-class correlation (95% confidence intervals) was calculated in a random mixed effect model with absolute agreement using R statistical package software with irr library. In addition, Cronbach’s alpha was calculated using the ltm library of the R statistical package.

Results: Variables such as WBC concentration, relative WBC composition, and mean platelet volume showed poor reliability, indicating a difference between the two operators evaluated. In addition, WBC concentration and mean platelet volume showed unacceptable internal consistency. Furthermore, the qualitative characteristics of the PRP prepared by the two operators differed, indicating a difference in variables such as the factor increase in platelet and platelet concentration.

Discussion and Conclusion: Our findings suggest that the operator significantly affects the qualitative and quantitative characteristics of PRP prepared by double centrifugation protocol, even when the same protocol is used. The conclusions of this study further support the call for developing a consensus/guideline for reporting the variables affecting PRP preparation and composition. Our findings have direct implications for regenerative medicine and reiterate the need for establishing minimum reporting requirements for the therapeutic use of PRP.
Hepatoprotective Effect of Mesenchymal Stem Cells with Platelet-Rich Plasma and Hepatocyte Growth Factor in Bile Duct Ligation Induced Liver Cirrhosis Model in Rat

Dr Shivaraju Shiavarmu, Dr Swapan Kumar Maiti, Dr. Amitha Banu S, Dr E Kalaiselvan, Dr Divya Mohan

1Division of Surgery, ICAR-Indian Veterinary Research Institute, Bareilly, India

Title: Hepatoprotective Effect of Mesenchymal Stem Cells with Platelet-Rich Plasma and Hepatocyte Growth Factor in Bile Duct Ligation Induced Liver Cirrhosis Model in Rat.

Authors: Shivaraju S, Maiti S K, Amitha Banu S*, Kalaiselvan E, Divya M

Affiliations: Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India (*Correspondence).

Category: Stem Cells and Cell-Based Therapies

Background: Liver cirrhosis is a global concern with high prevalence and mortality. The limitations associated with the current therapeutic options include high cost and immune-mediated rejection. This paved the way for exploring novel strategies to promote hepatic self-rehabilitation ability and reversing the insidious mechanism.

Methods: The hepatoprotective activity of rat adipose-derived mesenchymal stem cells (ADMSCs) in conjunction with platelet-rich plasma (PRP) and recombinant human hepatocyte growth factor (rhHGF) on the liver fibrosis/cirrhosis model in rats was evaluated separately and in combination.

Results: Treatment with PRP or rhHGF alone does not result in significant hepatoprotection in the rat bile duct ligation (BDL) model of liver cirrhosis. However, transplantation of ADMSCs alone has the potential to alleviate impaired liver conditions. The combination of PRP and rhHGF may provide a better ameliorative effect than either PRP or rhHGF alone for the treatment of biliary cirrhosis. When compared to ADMSC, PRP, and rhHGF alone or the combination of PRP and rhHGF, the combination of ADMSC + PRP or ADMSC + rhHGF may significantly improve hepatoprotective capacity.

Discussion and Conclusion: Overall, the injection of ADMSC through the tail vein reduces inflammation, hepatocyte damage and collagen deposition, resulting in the overall improvement of liver function. These improvements in hepatic metabolism are better when ADMSC is given with PRP and rhHGF compared to monotherapy. However, the underlying mechanisms by which ADMSCs repair hepatic injury and fibrosis remain unclear. It was reported that mesenchymal stem cells (MSCs) have anti-fibrotic effects by inhibiting the proliferation of hepatic stellate cells; inhibiting collagen synthesis, induction of apoptosis of hepatic stellate cells and having immune-modulated effects. In addition, MSCs transdifferentiates into hepatic progenitor cells and secrete trophic factors, cytokines and chemokines, which promote the regeneration of the impaired liver. This study was able to demonstrate an apparent arrest in the progress of liver fibrosis in the treated animals.

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Hepatic Patch for Liver Regeneration after CCl4 Poisoning

Ms. Ting Yi Wu 1, Mr. Yi-Cheng Hsieh 2, Dr. Yung-Te Hou 2
1 National Taiwan University, Taiwan

Background: The liver plays an indispensable role in the human body with important functions such as protein synthesis, urea metabolism, and detoxification. However, due to drug abuse and the change of diet habits, cases of liver fibrosis/cirrhosis have gradually become more common. As of today, the main clinical treatment for liver cirrhosis is liver transplantation. However, patients suffering from liver cirrhosis face problems such as donor shortage, surgical risks, and the possibility of infections and complications in the bile duct. Thus, finding an alternative treatment for liver transplantation becomes an important task. The aim of this research is to provide a new therapeutic treatment for fibrotic livers by creating a liver patch that is made from decellularized liver matrix combined with hepatocyte growth factor/heparin–complex (HGF/heparin-DLM).

Methods: The process of decellularization was performed by perfusion through the hepatic portal vein. Then, we dissolved the decellularized liver in HCl and then placed in the 37°C incubator overnight to finally obtain our decellularized liver matrix (DLM). Acetic acid and pepsin were then used to dissolve the DLM (adjusted pH to 7.4). Then, the DLM solution was mixed with 10× PBS in a 9:1 proportion and placed it in the 37°C for gelation. After the EDC/NHS cross-linking procedure was done, the DLM foam can be picked up by a tweezer. Lastly, we modified the DLM with heparin and HGF to complete our HGF/heparin-DLM.

Results: The MTT assay and Albumin secretion were first tested to verify the recovery effects of the HGF/heparin-DLM patch on primary hepatocytes that were seeded before they were intoxicated. In Fig. 1A, the relative cell viability of the positive group remained the highest initially but decreased over time. No significant difference was observed between the negative group, DLM group, and hep-DLM group on days 1, 3, and 5. However, the HGF/heparin-DLM group exhibited a higher relative cell viability than the other groups. Fig. 1B shows that the albumin secretion of the HGF/heparin-DLM group, on day 1, was around 50% of the positive group, and was also four times larger than the negative group. On day 3 and 5, the albumin secretion of the HGF/heparin-DLM group still remained higher than the negative group, and was about 60% and 50% of the positive group consecutively. Fig. 1C displays the results of the MTT analysis performed on day 10 onto small hepatocytes that were pre-intoxicated before seeding to confirm the therapeutic effects of the HGF/heparin-DLM patch. The negative group exhibited a lower relative cell viability compared to the control group. However, either DLM or hep-DLM increased the relative cell viability, but the HGF/heparin-DLM patch resulted in a relative cell viability increase of approximately two-fold compared to the positive group.

Discussion and Conclusion: Our results confirm that the toxicity response of hepatocytes with CCl4–induced hepatocytes can be delayed and their functions and viability can be improved in HGF/heparin-DLM in vitro. We have also confirmed that the HGF/heparin-DLM patch contains therapeutic effects when it is applied onto CCl4-induced hepatocytes.
Functional analysis of miRNAs encapsulated in small extracellular vesicles related to the efficacy of polydactyly-derived chondrocyte sheets

Mrs. Miki Maehara\textsuperscript{1,2}, Mrs. Eriko Toyoda\textsuperscript{1,2}, Mr. Tadashi Akamatsu\textsuperscript{3}, Mr. Masahiko Watanabe\textsuperscript{1,2}, Mr. Masato Sato\textsuperscript{1,2}

\textsuperscript{1}Department of Orthopaedic Surgery, Surgical Science Tokai University School of Medicine, Isehara, Japan, \textsuperscript{2}Center for Musculoskeletal innovative Research and Advancement (C-MiRA), Isehara, Japan, \textsuperscript{3}Department of Plastic Surgery, Surgical Science Tokai University School of Medicine, Isehara, Japan

Background: We conducted a clinical study involving the allogeneic transplantation of juvenile polydactyly-derived chondrocyte sheets (PD sheets) to establish a definitive therapy for osteoarthritis of the knee. A total of ten patients were enrolled in the study and followed up for more than three years. Positive results such as improvement in clinical symptoms and cartilage structure have been obtained, and such improvements may be the result of the paracrine effect, which is one of the potential action mechanisms of PD sheets. PD sheets release small extracellular vesicles (sEVs) as anabolic humoral factors, and the miRNAs contained in sEVs (sEV-miRs) are involved in the therapeutic efficacy of PD sheets (Fig. 1). Our aim in this study was to investigate the mechanism of cartilage regeneration by sEV-miRs released by PD sheets, which we had previously confirmed to have a high potential to regenerate hyaline cartilage.

Methods: PD sheets were prepared from 8 donors' cells with predicted efficacy based on in vivo and in vitro studies. A comparative analysis of miRNA expression was conducted via small RNA sequencing, using two different approaches. First, miRNAs that were expressed significantly higher in sEVs released into the culture supernatant than in PD sheets themselves were identified. Second, miRNAs that were expressed significantly higher by PD sheets with high efficacy were identified. Subsequently, the sEV-miRs identified as potentially involved in efficacy were examined for their effects on catabolic genes in therapeutic target cells.

Results: The results of the comparative analysis by small miRNA sequencing revealed two sEV-miRs that were common to both experiments: miR-141-5p and miR-4700-5p. These two miRNAs were then transfected into chondrocytes and synovial cells that had been stimulated by IL-1b. In chondrocytes, miR-141-5p and miR-4700-5p suppressed Col1 expression. Additionally, miR-141-5p enhanced the gene expression of Sox9. In synovial cells, both miR-141-5p and miR-4700-5p suppressed the gene expression of ADAMTS5 and VEGFA. Moreover, miR-4700-5p also suppressed the expression of MMP3, MMP13, and IL-6 in synovial cells.

Conclusion: Our study identifies miR-141-5p and miR-4700-5p as sEV-miRs that are associated with the effectiveness of PD sheet treatment for cartilage repair. These miRNAs are capable of suppressing cartilage degeneration and intra-articular inflammation, contributing to the regenerative mechanism of PD sheet therapy. It will be important to investigate the target genes of these miRNAs and to further elucidate their mechanisms of action. Ultimately, our findings suggest that sEVs and sEV-miRs have potential to be used in minimally invasive, cell-free joint therapies for cartilage repair.

Delivery of antisense oligonucleotide to lung cell using mixed peptide micelle

Ms. Minji Kang

1Department of Bioengineering, Seoul, South Korea

Background: Acute lung injury is an inflammatory phenomenon characterized by respiratory failure, lung infiltration, hypoxia, and edema. Most of them occur when severe alveolar damage occurs due to increased barrier permeability between the alveoli and capillaries due to pneumonia or sepsis, triggers an explosive inflammatory response. In addition, the infiltration of neutrophil into lung tissue increases, resulting in an immune response.

Methods: In this work, we combined two micelles into one micelle form using R3V6 peptide with high gene delivery efficiency and glycyrrhizic acid (GL) with anti-inflammatory effect. The antagonist sequence of microRNA155, which is expressed in the inflammation of acute lung injury, was bound to cholesterol and allowed to bind with RG micelles.

Results: RG micelle, which was made by sonication of R3V6 and GL, is incubated with cholesterol-attached antagonist miRNA155 (A155c) to prepare A155c-RG complex. The optimal combination ratio of the three materials was found to be 1:10:10 through flow cytometry. The following complex was found to lower the cytokine levels of TNF-α and IL-6 in LPS-induced RAW264.7 cells.

Discussion and Conclusion: We improved the delivery efficiency of A155c by using RG micelle and enhanced its anti-inflammatory effect. This work demonstrated a promising strategy to combine two micelles into one to maximize their respective strengths.

Acknowledgement: This work was partly supported by the BK21 FOUR (Fostering Outstanding Universities for Research) program through the National Research Foundation (NRF) funded by the Ministry of Education of Korea.
Adult spinal cord tissue transplantation combined with local tacrolimus sustained-release collagen hydrogel promotes complete spinal cord injury repair

**Prof. He Shen**

1Suzhou Institute of NanoTech and NanoBionics, Chinese Academy of Sciences, Suzhou, China

Title: Adult spinal cord tissue transplantation combined with local tacrolimus sustained-release collagen hydrogel promotes complete spinal cord injury repair

Authors: He Shen1,*

Affiliations: 1Suzhou Institute of NanoTech and NanoBionics, Chinese Academy of Sciences, Suzhou, 215123, China (* Correspondence)

Category: Tissue Engineering and Regeneration

Background: The strategy of replacing a completely damaged spinal cord with allogenic adult spinal cord tissues (aSCs) can potentially repair complete spinal cord injury (SCI) in combination with immunosuppressive drugs, such as tacrolimus (Tac), which suppress transplant rejection and improve graft survival. However, daily systemic administration of immunosuppressive agents may cause harsh side effects. Herein, a localized, sustained Tac-release collagen hydrogel (Col/Tac) was developed to maximize the immune regulatory efficacy but minimize the side effects of Tac after aSC transplantation in complete SCI recipients.

Methods: Thoracic aSCs of rat donors were transplanted into the complete thoracic spinal cord transection rat recipients, after which Col/Tac hydrogel was implanted.

Results: The Tac-encapsulated collagen hydrogel exhibited suitable mechanical properties and long-term sustained Tac release behavior. After Col/Tac hydrogel implantation in SCI rats with aSC transplantation, the recipients’ survival rate significantly improved and the side effects on tissues were reduced compared with those with conventional Tac medication. Moreover, treatment with the Col/Tac hydrogel exhibited similarly reduced immune rejection levels by regulating immune responses and promoted neurogenesis compared to daily Tac injections, and thus improved functional restoration.

Conclusions: Localized delivery of immunosuppressive agents by the Col/Tac hydrogel may be a promising strategy for overcoming immune rejection of transplants, with significant potential for clinical application in the future.

Acknowledgement: This work was supported by grants from the National Natural Science Foundation of China (81891002, 81971178), and the Youth Innovation Promotion Association CAS (No. 2021319).
Adipocytolytic Effect of Carbon Dioxide Gas-Generating Polymer Micelles

Mr. Yunchan Lee¹, Chunggoo Kim, Kuen-Yong Lee¹
¹Department of Bioengineering, Hanyang University, Seoul, South Korea

Authors: Chunggoo Kim, Yun-chan Lee, Kuen-Yong Lee

Affiliations: Department of Bioengineering, Hanyang University, Seoul, Republic of Korea (*leeky@hanyang.ac.kr)

Category: Design and Application of Biomaterials

Introduction: Obesity is a critical global health issue with chronic implications, and the demand for safe and effective fat reduction methods is rapidly increasing. However, current approaches such as liposuction often come with side effects and may not be suitable for all patients. To address these challenges, we propose the use of polymer micelles capable of generating carbon dioxide gas within adipocytes to induce adipocytolysis, resulting in localized fat reduction.

Subjects and Methods: Amphiphilic polymers containing carbonate linkages were synthesized through an acyl halide reaction between methoxypoly(ethylene glycol) (mPEG) and octyl chloroformate (OC). Specifically, mPEG-mono-arm-OC (mPEG-OC) and mPEG-tri-arm-OC (mPEG-T-OC) were synthesized, and their characteristics were investigated. Using the amphiphilic polymers, micelles were formed in distilled water through a solvent evaporation method. Carbon dioxide gas generation from the micelles was confirmed using ultrasound imaging and ¹H NMR spectroscopy. To enhance the uptake of the micelles by adipocytes, nona-arginine (r9) peptide was conjugated to O-(2-aminoethyl) PEG via carbodiimide chemistry, and mixed micelles were formed with mPEG-OC or mPEG-T-OC. We evaluated the in vitro cytotoxicity of the gas-generating micelles using differentiated 3T3-L1 adipocytes by an MTS assay. Furthermore, we assessed the efficacy of the micelles for reducing adipose tissue in vivo using a diet-induced obese mouse model.

Results: We confirmed the formation of carbonate linkages in amphiphiles using FT-IR and ¹H NMR spectroscopy. The mean sizes of micelles were found to be 156 ± 52 nm and 147 ± 59 nm for mPEG-OC and mPEG-T-OC, respectively. Carbon dioxide generation from the micelles lasted for 12 h, and the amount of gas generated increased with the number of arms in the PEG derivative. Micelles containing r9 peptides demonstrated enhanced adipocytolytic effects compared to unmodified micelles. Notably, mPEG-T-OC micelles exhibited superior cytotoxicity compared to mPEG-OC micelles. In vivo studies demonstrated that the administration of r9-mPEG-T-OC micelles into the fat pad resulted in significant reduction of local fat without any significant side effects.

Discussion and Conclusion: In our study, we prepared PEG-based micelles capable of generating carbon dioxide gas and evaluated their potential as an adipocytolytic agent for reducing fat tissue. We found that micelles prepared from amphiphilic polymers with multiple carbonate linkages enhanced gas generation, and the addition of r9 peptide to the micelles further improved their uptake by adipocytes. In vivo experiments also demonstrated the significant fat tissue reduction. Overall, our findings suggest that the development of gas-generating polymer micelles is a promising strategy for localized fat reduction with potential applications in the field of biomedical engineering.
Constructing regenerative microenvironments with functional biomaterials for spinal cord injury repair

**Prof. He Shen**

1Suzhou Institute of NanoTech and NanoBionics, Chinese Academy of Sciences, Suzhou, China

Title: Constructing regenerative microenvironments with functional biomaterials for spinal cord injury repair

Authors: He Shen

Affiliations: Suzhou Institute of NanoTech and NanoBionics, Chinese Academy of Sciences, Suzhou, 215123, China

Category: Design and Application of Biomaterials

Background: Spinal cord injury (SCI) often leads to the loss of motor and sensory functions and is a major challenge in neurological clinical practice. The pathophysiological changes after SCI forming an inhibitory microenvironment significantly impair neural regeneration.

Methods: To resolve the inhibitory effects of SCI microenvironments on neural regeneration, the implantation of specifically functionalized biomaterials in the lesion area has been developed to help promote axon regeneration and facilitate neuronal circuit generation by remolding SCI microenvironments. Moreover, structural and functional restoration of the spinal cord through the transplantation of naïve spinal cord tissue grafts from adult donors will open up new avenues for SCI treatment.

Results: The functional biomaterial-based SCI repair strategies regulated the immune, neurovascular and neuroregenerative microenvironment to reduce secondary injury and scar formation and enhance neurogenesis and angiogenesis, and axonal regrowth, and thus restored neural network and motor function. The therapeutic efficiency and biocompatibility of the spinal cord microenvironment reconstruction strategies have also been investigated in preclinical large animal experiments.

Discussion and Conclusion: Various microenvironment reconstruction strategies have been developed based on functional biomaterials, specific cell types, and key factors to regulated molecule balance and cell fate after SCI, and consequently to promote neural regeneration and functional recovery. The biomaterial-based approaches are promising and have great potential for SCI clinical therapies in future translational research.

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Cell membrane nanoparticles for therapy of inflammatory lung disease

Ms. Chuanyu Zhuang

1Department of Bioengineering, College of Engineering, Hanyang University, Seoul, South of Korea

Background: Acute lung injury (ALI) is an inflammatory disease with a high mortality rate caused by trauma, near-drowning and viral infection. Treatments for ALI include mechanical ventilation, fluid management and anti-inflammatory drugs. Gene therapy has been suggested as a novel therapy for ALI and HO-1 is an anti-inflammatory enzyme. Polymers have emerged as non-viral gene carriers and cell membranous organelles have been studied as vesicles for drug delivery.

Methods: In this work, dexamethasone-conjugated PEI (DP) was synthesized and evaluated as a plasmid DNA carrier and an anti-inflammatory drug. pHO-1 nanoparticles were prepared with DP and cell membrane (CM) for enhanced gene delivery into the lungs. The triple nanoparticles were subjected to in vitro transfection assays and evaluated in a BALB/c mouse model for anti-inflammatory therapy.

Results: The in vitro luciferase assays showed that the DP/CM/pLuc nanoparticles exhibited a higher transfection efficiency than PD/pLuc. And DP/CM/pDNA nanoparticles showed lower toxicity evaluated using MTT assays. Furthermore, DP/CM/pLuc nanoparticles reduced the TNF-a level more efficiently than DP/pLuc nanoparticles evaluated by ELISA assays. The in vivo H&E evaluation in BALB/c mouse model showed that the DP/CM/pHO-1 nanoparticles reduced pulmonary hemorrhage.

Discussion and Conclusion: DP/CM/pDNA triple nanoparticles were investigated as therapeutic gene carriers into an ALI mouse model by intratracheal injection. The results of the in vitro and in vivo experiments showed that DP/CM/pDNA triple nanoparticles increased the efficiency of pDNA delivery into the lung and enhanced anti-inflammatory therapeutic effects. This work demonstrated that DP/CM/ pHO-1 nanoparticles may be a promising strategy for ALI therapy.

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Anisotropic conductive matrices promote cardiac maturation and post-infarction functions by restoring electrical integrity

Prof. Xiaohui Zhang¹
¹Xi'an Jiaotong University, Xi'an, China

Background: Myocardial infarction (MI) remains the leading cause of death globally, often leading to impaired cardiac function and pathological myocardial microenvironment. Electrical conduction abnormalities of the infarcted myocardium not only induce adverse myocardial remodeling but also prevent tissue repair. Restoring the myocardial electrical integrity, particularly the anisotropic electrical signal propagation within the injured area after infarction is crucial for an effective function recovery.

Methods: In this work, we developed a reduced graphene oxide (rGO) functionalized electrospun silk fibroin (rGO/silk) biomaterial which presented anisotropic conductivity, and investigated its effects on cardiac maturation along with external electrical stimulation in vitro, and its potential as cardiac patches in improving the post-MI myocardial function of rat models in vivo.

Results: The in vitro studies showed that the anisotropic rGO/silk matrix coupled with external electrical stimulation promoted the cardiac tissue formation and maturation in vitro with increased expressions of cardiac-specific proteins, the formation of sarcomeric structures and gap junctions, and tissue contraction. The in vivo studies showed that the anisotropic conductive rGO/silk patches exhibited remarkable therapeutic effect on repairing the infarcted myocardium compared to the nonconductive silk and isotropic conductive rGO/silk patches with enhanced pumping function, reduced susceptibility to arrhythmias, thickened left ventricular walls and improved survival of functional cardiomyocytes. It also demonstrated to promote the angiogenesis of capillaries in the infarcted myocardium.

Discussion and Conclusion: We demonstrated the great potential of reduced graphene oxide functionalized silk biomaterials in recapitulating the anisotropic electrical microenvironment and promoting the maturation of the regenerated cardiac tissues in vitro. When implanted in vivo as a cardiac patch, the anisotropic conductive rGO/silk biomaterials can effectively and biomimetically reconstruct the electrical myocardial microenvironment, thus promoting the repair of infarcted myocardium.

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An Algorithmic Design Approach to Generate Lymph Node-Inspired 3D Microprinted Cell Scaffolds

Dr. Matthew (Ho Wai) Chin¹, Dr. Barry Reid¹, Veronika Lachina², Dr. Sophie Acton², Prof. Marc-Olivier Coppens¹

¹EPSRC "Frontier Engineering" Centre for Nature Inspired Engineering (CNIÉ) & Chemical Engineering, University College London, London, United Kingdom, ²Stromal Immunology Group, MRC Laboratory for Molecular Cell Biology, University College London, London, United Kingdom

Authors: Matthew H. W. Chin (1), Barry Reid (1), Veronika Lachina (2), Sophie E. Acton (2), Marc-Olivier Coppens (1)*

Affiliations: (1) EPSRC “Frontier Engineering” Centre for Nature Inspired Engineering & Department of Chemical Engineering, University College London, Torrington Place, London WC1H 0AJ, United Kingdom. (2) Stromal Immunology Group, MRC Laboratory for Molecular Cell Biology, University College London, London WC1E 6BT, United Kingdom. (*Correspondence)

Category: Design and Application of Biomaterials; SYIS

Background: Immune cells routinely interact with in vivo physical networks, such as those formed by fibroblastic reticular cells (FRCs) in lymph nodes. 3D cell scaffolds recapitulating the structural complexity of FRC networks are therefore a valuable tool in deciphering how physical networks influence the migration and communication of immune cells, such as T cells. However, conventional biomaterial fabrication methods are limited in their control over the microarchitecture. In this study, we combined algorithmic design and two-photon polymerisation-based direct laser writing (2PP DLW) to create complex 3D networks at the microscale.

Methods: Driven by the nature-inspired solutions (NIS) methodology [1–3], we developed an algorithmic design pipeline to generate scaffold models based on the small-world topology of FRC networks. For fabrication, we optimised 2PP DLW parameters to 3D print scaffolds at the microscale.

Results: Network analysis confirmed that our generated scaffold models possessed small-world characteristics comparable to FRC networks. We tackled the challenges of floating islands and intersecting structures with a simple heuristic that imposed spatial constraints on our network generating model. Confocal microscopy showed that scaffolds supported the adhesion and growth of FRCs.

Discussion and Conclusion: In this work, we demonstrated the feasibility of algorithmically generating 3D printable cell scaffolds. Combined with 2PP DLW, we were able to control the network topology of porous scaffolds with microscale precision. This serves as a promising tool for future studies on the role of physical networks in regulating T cell migration, as well as lymph node tissue engineering.


Bacterial polyester – poly(3-hydroxyoctanoate) (P(3HO)) as a versatile material for medical applications

Mrs. Katarzyna Harazna¹,²,³*, Mrs. Annabelle T. Fricker³, PhD Caroline S. Taylor³, PhD Rafał Konefał⁴, Mrs. Aneta Medaj⁵, PhD Sonia Bujok¹, PhD Małgorzata Zimowska¹, PhD Bartosz Leszczyński⁶, PhD Andrzej Wróbel⁶, Professor Andrzej J. Bojarski⁷, Professor Ipsita Roy³, Professor Maciej Guzik¹

¹Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, Krakow, Poland, ²Krakow Institute of Technology, Lukasiewicz Research Network, Krakow, Poland, ³The University of Sheffield, Sheffield, United Kingdom, ⁴Institute of Macromolecular Chemistry Czech Academy of Sciences, Prague, Czech Republic, ⁵Faculty of Chemistry, Jagiellonian University, Krakow, Poland, ⁶Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University, Krakow, Poland, ⁷Jerzy Maj Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland

Authors: Katarzyna Harazna¹,²,³*, Annabelle T. Fricker³, Caroline S. Taylor³, Rafał Konefał⁴, Aneta Medaj⁵, Sonia Bujok¹, Małgorzata Zimowska¹, Bartosz Leszczyński⁶, Andrzej Wróbel⁶, Andrzej J. Bojarski⁷, Ipsita Roy³, Maciej Guzik¹

Affiliations:
¹Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, Krakow, Poland.
²Krakow Institute of Technology, Lukasiewicz Research Network, Krakow, Poland.
³The University of Sheffield, Sheffield, United Kingdom, ⁴Institute of Macromolecular Chemistry Czech Academy of Sciences, Prague, Czech Republic.
⁵Faculty of Chemistry, Jagiellonian University, Krakow, Poland.
⁶Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University, Krakow, Poland.
⁷Jerzy Maj Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland.

(Correspondence: katarzyna.harazna@lukasiewicz.kit.gov.pl)

Category: Tissue Engineering and Regeneration

Background: Bacterial polymers - polyhydroxyalkanoates (PHAs), belong to a group of biodegradable biopolymesters that can be accumulated by numerous bacteria in the form of intracellular granules. These polymers are synthesised under unfavourable conditions for bacteria from various carbon sources, including cellulotic biomass, post-production waste, and traditional fatty acids. Due to their lack of toxicity when in contact with mammalian cells, tissues, and body fluids, these polymers are completely biocompatible. As a result, they can find applications in various branches of medicine. One of the representatives of medium-chain polyhydroxyalkanoates (mcl-PHA) is poly(3-hydroxyoctanoate) (P(3HO)), a polymer with elastomeric properties.

Methods: To obtain P(3HO), the environmental bacterial strain - Pseudomonas putida KT2440 and octanoic acid (carbon source) were used. The controlled feeding process was carried out in a 5-liter fermenter to obtain significant amounts of biomass containing the polymer. P(3HO) was isolated from the dried bacteria and purified using chemical methods. Three-dimensional polymeric materials were obtained by combining solvent casting and particulate leaching (SCPL). The obtained materials were subjected to thorough physicochemical, structural, and biological analysis. In vitro tests were performed for skin (HaCaT), muscle (C2C12), and neuronal (NG108-15) cell lines and indirect cytotoxicity of the materials was assessed according to ISO10993-5. Cell adhesion, morphology, and proliferation were then evaluated. Additionally, cell nuclei and cytoskeleton staining confirmed cell migration into prepared porous structures. This work also intends to demonstrate the feasibility of producing a polymeric carrier for the active substance. To modify P(3HO) with a non-steroidal, anti-inflammatory drug, namely diclofenac (DIC), a single-stage, solvent-free synthesis method (catalyst: p-toluenosulfonic acid) was applied. Diclofenac release studies were carried out in PBS at pH 5 and
7.4. lipase-containing medium. The quantities of the released drug were calculated based on NMR spectra.

Results: The resulting polymer is flexible, with a tensile strength of 102.95 ± 7.53 %. In addition, the determined melting point of 53 ⁰C confirms that the polymer is favourable to the process. Moreover, the prepared soft foams are hydrophobic (106 ± 2⁰) and have more than 75% porosity. The obtained materials have an adequate roughness of 193.33 ± 53.46 nm, which allow cell adhesion to the biomaterial surface. Indirect cytotoxicity studies carried out in tests based on resazurin reduction and involving live/dead staining showed that the obtained materials are non-toxic. Direct proliferation tests showed a change in cell number on the surface of the tested materials at the appropriate time intervals. This confirms that the materials are completely biocompatible. The SEM microscopy observations revealed that the cells colonise the surface of the prepared biomaterials. In addition, the staining of cell nuclei with DAPI dye and their visualisation using confocal microscopy shows that the cells also penetrate deep into the materials due to the porous structure. In addition, the release studies that were carried out show that the release kinetics of diclofenac from the prepared carriers is not dependent on the pH of the medium.

Discussion and Conclusion: The conducted experiments have shown that P(3HO), which represents the polyhydroxyalkanoate family, is a versatile polymer. Due to its suitable physicochemical and mechanical properties, it is easy to process, which makes it suitable for many applications. Furthermore, its biocompatibility makes it a good material for medical applications. This study presents the possibility of using this polymer to construct drug carriers, personalised wound dressings, and materials for regenerating nerve tissue and blood vessels.

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Bioprinted anisotropic scaffolds with fast stress relaxation bioink for engineering 3D skeletal muscle and repairing volumetric muscle loss

Ms. Ting Li
1
1Southern Medical University, China

Introduction
Owing to faster stress relaxation which promotes the organization of the cellular microenvironment, viscoelastic hydrogels can enhance 3D cell migration and proliferation [1]. However, the majority of synthetic photocurable hydrogels utilized as 3D bioprinting bioink materials are typically elastic [2]. It would be advantageous for 3D bioprinting tailored 3D skeletal muscles in vitro and mending volumetric muscle loss (VML) in vivo to develop a photocurable hydrogel bioink with fast stress relaxation, however this remains a tough challenge.

Subjects and Methods
Gelatin methacryloyl (GelMA) and fibrinogen are combined in our study to create an interpenetrating network (IPN) hydrogel with tunable stress relaxation. These IPN hydrogels in particular demonstrated greater 3D cellular proliferation and improved differentiation. Also, using IPN hydrogel as bioink materials and the printing gel-in-gel technique, we bioprinted an anisotropic biomimetic scaffold that was used for in vivo VML repair and in vitro 3D skeletal muscle tissue models.

Results
These IPN hydrogels with faster stress relaxation showed higher 3D cellular proliferation and better differentiation. A 3D anisotropic biomimetic scaffold was further developed via a printing gel-in-gel strategy, where the extrusion printing of cell-laden viscoelastic FG hydrogel within Carbopol supported gel. The 3D engineered skeletal muscle tissue was further developed via 3D aligned myotube formation and contraction. Furthermore, the cell-free 3D printed scaffold was implanted into a rat VML model, and both the short and long-term repair results demonstrated its ability to enhance functional skeletal muscle tissue regeneration.

Discussion and conclusion
These data suggest that such viscoelastic hydrogel provided a suitable 3D microenvironment for enhancing 3D myogenic differentiation, and the 3D bioprinted anisotropic structure provided a 3D macroenvironment for myotube organization, which indicated the potential in skeletal muscle engineering and VML regeneration.

References
An Injectable Asymmetric-Adhesive Hydrogel as a GATA6 + Cavity Macrophage Trap for Preventing the Formation of Postoperative Adhesions after Minimally Invasive Surgery

Mr. Wu Xiaoqi¹, Ling Wang, Yaobing Wu
¹ Department of Anatomy, School of Basic Medical Sciences, Southern Medical University, Guangzhou, China

• Authors: Xiaoqi Wu1, Ling Wang1, Yaobing Wu*

• Affiliations: Department of Anatomy, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, China; 1 co-first authors *, correspondence author.

• Category: Design and Application of Biomaterials

Background:
Antiadhesive hydrogels have been developed for preventing the formation of postoperative adhesions. However, it is challenging to design an injectable hydrogel with superior tissue retention properties that can be easily administered during minimally invasive surgical procedures to prevent the development of postsurgical adhesions. In this study, an injectable asymmetric-adhesive hydrogel is fabricated using photocurable catechol-grafted hyaluronic acid (HAD) for use during minimally invasive procedures to prevent the development of postoperative adhesions. The hydrogel exhibits superior tissue retention properties and favorably inhibits postoperative adhesion formation. This is the first time that an injectable hydrogel is designed via photocrosslinking to control asymmetric-adhesive capability. The results show that laparoscopically delivered HAD precursor acts as a wet adhesive on the injured cecum, while its outward-facing side is nonadherent after photocrosslinking. Intriguingly, the HAD acts as a physical barrier and polyanion trap to neutralize scavenger receptors, thereby inhibiting collagen deposition and uncontrolled recruitment of GATA6 + cavity macrophages. Furthermore, the HAD significantly downregulates the expression of fibrosis-related and proinflammatory cytokines and promotes macrophage polarization. These results demonstrate that injection of the hydrogel can be readily integrated into laparoscopic surgery. Moreover, the HAD may be suitable for preventing adhesion formation after minimally invasive surgical procedures.

Subjects and Methods:
Methods: ① First, The HAD precursor was fabricated by grafting 3,4-dihydroxy-phenylalanine (DA) and 2-aminoethyl methacrylate (AEMA) on HA chain. Then the characterization of HAD precursors and hydrogels were conducted. ② Subsequently, the asymmetrical adhesion properties of HAD hydrogels were tested through an in vitro fibrinogen adhesion test. ③ To evaluate the biocompatibility of these hydrogels, human gastric mucosal epithelial cells were treated with the HAD precursor solution at different concentrations in cytotoxicity assays (CCK-8 test). ④ To verify the antiadhesion efficiency of the injectable hydrogels in vivo, a rat sidewall defect-cecum abrasion model was established. The extent of adhesion formation in each group was also scored according to the standard scoring system. ⑤ To further explore the clinical translational potential of HAD formulation in minimally invasive surgery, we created artificial incisions on the cecum of adult male rabbits by laparoscopy. Anesthesia was maintained with an inhalational anesthetic. Moreover, the mechanism of preventing adhesions through inhibiting GATA6+ macrophages was verified in an immunofluorescence study.

Results: We designed an injectable asymmetric adhesion HAD hydrogel for preventing adhesions after minimally invasive surgery. Then we evaluated the asymmetric adhesiveness of HAD hydrogels by
the qualitative and quantitative tests. Before applying HAD hydrogels into animal experiments, we verified the in vitro and in vivo biocompatibility. Subsequently, a rat sidewall defect-cecum abrasion model was established to prove the HAD hydrogel exhibited a good antiadhesion efficiency. Moreover, the application of HAD Hydrogels for preventing adhesions in minimally invasive surgeries also proved the laparoscopic feasibility and good barrier efficiency. The HAD hydrogels not only played an important role in acting as a physical barrier, but also functioned as a polyanion trap for capturing the GATA 6+ macrophages, thus leading to the inhabitation of abdominal adhesion.

• Discussion and Conclusion:
In this work, we successfully designed and prepared an injectable photocurable hydrogel with asymmetric-adhesive properties. Furthermore, injection of the hydrogel was easily integrated into the laparoscopic surgical procedures to prevent postsurgical adhesion in rabbits. We further demonstrated that this HAD polyanion formulation can act as an MSR antagonist to inhibit superaggregation of GATA6 + cavity macrophages, thereby preventing collagen deposition and peritoneal adhesion formation. In addition, the HAD formulation promoted M1 to M2 phase transition of marrow-derived macrophages, which is beneficial for wound healing.

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Safety and Efficacy of Implanted Self-expandable Metallic Stent-mediated Radiofrequency Ablation to Manage Stent-induced Tissue Hyperplasia in Rat Gastric Outlet Model.

Mr. Dong-sung Won

1Asan Medical Center, South Korea

Background: Progressive tissue through the stent meshes is still considered significant obstacles to successful stent placement in unresectable malignant gastric outlet obstruction (GOO). The radiofrequency ablation (RFA) prolonged stent patency by inducing coagulative necrosis in the proliferated tissues. Application of RFA, guide wire with RFA electrodes must be passed through the occluded stent. However, technical difficulties may be encountered in the case of high-grade, progressive tumor growth with severe stenosis and tortuous anatomical parameters. This study aimed to investigate the safety and efficacy of self-expandable metallic stent (SEMS)-mediated RFA for treating stent-induced tissue hyperplasia by grasping the implanted SEMS using a monopolar SB Knife in a rat GOO model.

Subjects and Methods: The animals were randomly divided into four groups of 10 rats as follow: group A, SEMS placement only; group B, SEMS-mediated RFA at 4 weeks; group C, SEMS-mediated RFA at 4 weeks and was housed until 8 weeks, and group D, SEMS-mediated RFA both at 4 and 8 weeks. SEMS-mediated RFA was used for 180 s at 15 W while maintaining a temperature of 60°C. Endoscopic and fluoroscopic examinations, and histological and immunohistochemistry (IHC) analysis was performed to evaluate mucosal changes and stent patency.

Results: SEMS placement and SEMS-mediated RFA were technically successful. There is no evidence of perforation, and the luminal patency was wider immediately after the RFA in fluoroscopic findings. Endoscopic findings confirmed that group D significantly lower level of granularity than groups B and C (all p < 0.05). The mean thickness of submucosal fibrosis, percentages of tissue hyperplasia area, collagen deposition, HSP 70-positive deposition, TUNEL-positive deposition, and α-SMA-positive deposition were all significantly different among the groups (all variables; p < 0.001).

Discussion and Conclusion: We In the rat GOO model, SEMS-mediated RFA successfully managed stent-induced tissue hyperplasia after SEMS placement. Uniform circumferential coagulative necrosis was achieved by simply grasping the tip of stent to deliver RFA energy to tissue. SEMS-mediated RFA could be a future strategy to reduce intimal proliferation and treat various endoluminal tumors that compress after SMES placement.
The distribution of bone void in thoracolumbar spine in adult Chinese with and without osteoporosis: a prospective, multi-center study based on 464 vertebrae

Mr. Junyu Lin

The University Of Hong Kong, , Hong Kong SAR

Background: Bone void is a novel intuitive morphological indicator to assess the bone quality but has not been described in vertebrae. Our previous developed phantom-less technology assured that BMD of any interested area in the vertebra could be detected.

Methods: This study aimed to investigate the distribution of bone void in thoracolumbar spine in adult Chinese with quantitative computed tomography (QCT) from a prospective, multi-center study. The bone void was defined as the trabecular net region with extremely low bone mineral density (BMD) (<40mg/cm³), detected by an algorithm based on phantom-less technology. A total of 464 vertebrae from 153 patients (51.9±13.3 years old) were included. The vertebral trabecular bone was divided into eight sections by middle sagittal, coronal and horizontal planes. Bone void of whole vertebra and each section were compared between normal, osteopenia and osteoporosis groups and between spine levels. Correlation between bone void and BMD was analyzed. ROC curves were drawn to observe the optimum cutoff points of void volume between three groups.

Results: The void volume of whole vertebra was 131.5±243.1mm³, 1304.0±1084.0mm³ and 5783.0±3549.0mm³ in normal, osteopenia and osteoporosis groups, respectively. The detection rate of vertebra with bone void and the standardized void volume were larger in lumbar than thoracic vertebrae. The L3 presented the largest void (2193.0±3391.0 mm³), while the T12 had the smallest void (465.7±712.0mm³). The bone void was mainly located in superior-posterior-right section (42.2%). Additionally, the bone void correlated negatively with BMD in all groups. The void volume increased most obviously in inferior-anterior-right section but least obviously in inferior-posterior-left section as aging. The cutoff points were 261.2mm³ between normal and osteopenia (sensitivity=0.878, specificity=0.915) and 1639.4mm³ between osteopenia and osteoporosis (sensitivity=0.974, specificity=0.876).

Discussion and Conclusion: This is the first study to demonstrate the bone void distribution in vertebrae using clinical QCT data. It provides a new aspect of description on bone quality and may serve as guidance for clinical practice. Based on phantom-less technology, the bone void may be used to screen the people who are at risk of vertebral compression fracture, but their BMD are normal or slightly decrease. It is a complementary option in osteoporosis screening.

Keywords: bone void, vertebra, thoracolumbar spine, osteoporosis, section
Mg-containing Hybrid Interference Screw Promotes The Healing of ACL Reconstruction

Mr. Yuantao Zhang¹, Prof. Jiankun Xu¹
¹The Chinese University of Hong Kong, Hong Kong SAR

Introduction:
Anterior cruciate ligament (ACL) is the most prone to injury during sports and exercise. ACL reconstruction is commonly used to restore the function of knee after ACL tear. However, ACL reconstruction has a failure rate of 5% in the first year after operation and a significant number of patients require a revision surgery. The poor osteointegration of tendon graft in bone tunnels is the major cause contributing to unsatisfactory clinical outcomes. Magnesium (Mg) and alloys have been recognized as potential biodegradable materials in orthopedics due to promising effects on osteogenesis, angiogenesis, and tissue regeneration [1-3]. However, one of the obstacles limiting the application of pure magnesium implant in clinic is that mechanical support is impaired at the rapid degradation phase after implanted in vivo. We innovatively designed a Mg-containing hybrid interference screw to maintain the mechanical strength while making use of the biological effects of Mg. In this study we tested the feasibility of our innovative hybrid interference screw in ACL reconstruction in goats, which is essential for broadening clinical applications.

Subjects and Methods: Fig.1A shows the assembly of Mg-containing hybrid interference screw. The outside layer was a hollow titanium (Ti) screw with drilling holes. The magnesium rod was inserted into the titanium screw along its longitudinal axis. Following the protocol approved by the institutional Research Ethics Committee, 24 skeletally mature Chinese mountain goats were divided into control group and Mg group, which were fixed with conventional titanium interference screw and Mg-containing hybrid interference screw to fix the graft in bone tunnel in ACL reconstruction, respectively. At pre-op, 4 weeks, 8 weeks and 16 weeks after operation, we performed X-rays and blood biochemical tests. The goats were sacrificed at two time points, 8 weeks, and 16 weeks after operation. Calcein Green and Xylenol Orange were injected with a certain interval before sacrificed. The samples were further proceeded to HR-pQCT scanning, biomechanical test, histological, and immunohistochemical staining.

Results:
Ca, Mg, P, Cr, Urea, ALT, and AST concentrations showed no significant difference between control group and Mg group at pre-op, 4 weeks, 8 weeks and 16 weeks after surgery. The serum Mg2+ level was found within the normal range at all time points in both groups. Various organs including liver, kidney and heart were harvested for histological analysis at 8 and 16 weeks after implantation and no histological abnormalities were observed in these organs of goats implanted with Mg-containing screw compared to those received Ti screw fixation.

X-ray showed that both the control group and Mg group showed no signs of deformity or dislocation. For macroscopic evaluation, Mg group showed a relatively better outcome with more tissue connection and cartilage matrix at the tunnel aperture, which showed a higher Oswestry Arthroscopy Score than the control group at week 8 and week 16.

HR-pQCT results showed greater BV/TV in the Mg group, as compared to control group, at both week 8 and week 16. The trabecular number (Tb.N) and trabecular thickness (Tb.Th) were higher in Mg group than that in control group at week 16. At weeks 8 and 16, the tibial tunnel diameter in the Mg group was smaller than the control group (Fig.1B, C)

Discussion and conclusion:
The innovative Mg-containing hybrid interference screw was developed and tested for its feasibility for tendon-bone healing in ACL reconstruction in goats, which shows superior outcomes in terms of new bone formation around the bone tunnel, highlighting the great translational merit.

Acknowledgement: This project is fully supported by Areas of Excellence (AoE/M-402/20).

References:
A semi-synthetic mesenchymal stem cells (MSC)-derived extracellular matrix (ECM)-based biomaterial synthesized under defined hypoxic culture conditions accelerates diabetic skin wound healing

Mr. Kwok Keung Lit, Mr. Cheuk Kwan Li, Miss Sui Ki Chiu, Prof. Anna Blocki

1School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong Special Administrative Region of China. , 2Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong Special Administrative Region of China. , 3Center for Neuromusculoskeletal Restorative Medicine, Hong Kong Science and Technology Park, Shatin, New Territories, Hong Kong Special Administrative Region of China. , 4Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong Special Administrative Region of China.

Background: We have recently reported the development of a MSCs-derived ECM-based biomaterial with augmented pro-regenerative potentials in a skin wound model. [1] An advantage of the approach is that the bioactivity of this biologic can be modified and/or further enhanced through modification of culturing conditions.

Methods: Here we seek to optimise the synthesis of the biomaterial by synthesizing it in xeno- and serum-free (XF/SF) culture medium, as well as under hypoxic conditions to upregulate secretion of pro-angiogenic factors by the MSCs into the ECM. A choice of XF/SF-derived material was first determined by in vitro angiogenic assays. Wound healing in a diabetic mice wound model was then investigated to determine the therapeutic efficacy of the chosen material.

Results: Results showed that the materials synthesized under the different XF/SF conditions exhibited enhanced pro-angiogenic bioactivity in vitro, as compared to with serum supplemented culture. When coupled with hypoxic culture conditions, the resulting biomaterial was observed to accelerate wound healing in diabetic mice wound healing model.

Discussion and Conclusion: Collectively, we have demonstrated that the pro-regenerative potential of our ECM-based material can be enhanced through the conjunctual use of XF/SF and hypoxic culture, paving the way for a novel treatment alternative for diabetic chronic wounds.

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Development of a Hypertrophic Cartilage for Bone Regeneration

Dr. Bach Le¹, Weng Wan CHAN¹, Shashaank Abhinav VENKATESH¹, Hariharan EZHILARASU¹, Priya MURUGAN¹, Zach LEE¹, Deepak CHOUDHURY¹

¹Biomanufacturing Technology (BMT), Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore

Title: Development of a Hypertrophic Cartilage for Bone Regeneration

Authors: Bach Quang Le*, Weng Wan Chan, Shashaank Abhinav Venkatesh, Hariharan Ezhilarasu, Priya Murugan, Zach Lee, Deepak Choudhury*

Affiliations: Biomanufacturing Technology (BMT), Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore
Correspondence: *Bach_Quang_Le@bti.a-star.edu.sg, *Deepak_Choudhury@bti.a-star.edu.sg

Background: Bone regeneration is a complex process that requires the interaction of multiple cell types, signaling molecules, and extracellular matrix components. Hypertrophic cartilage, which naturally precedes bone formation during development, has been shown to be an effective template for bone regeneration. The natural source of hypertrophic cartilage is very limited, and thus tissue engineering is the only viable option to manufacture this tissue. In this study, we present a novel biomanufacturing strategy for the production of a hypertrophic cartilage template for bone regeneration.

Methods: Our approach involves the use of donor mesenchymal stem cells (hMSCs) seeded onto a proprietary scaffold made of biocompatible material. Our unique scaffold design supports the cell assembly and maturation into hypertrophic cartilage tissue. Our approach is optimized for high throughput and large-scale manufacturing of cartilage tissue, with consistent differentiation results.

Results: The resulting hypertrophic cartilage constructs were then evaluated in vitro for their ability to support bone regeneration. Our results showed that the cartilage templates were successfully made, as evidenced by increased expression of cartilage-specific markers and histological stains. The biomanufactured hypertrophic cartilage constructs also displayed enhanced mechanical properties compared to traditional tissue engineering scaffolds.

Discussion and Conclusion: Our approach has potential applications in the field of cartilage and bone tissue engineering, where there is a critical need for effective and safe regenerative therapies. Our study demonstrates the biomanufacturing capability of cartilage tissue as a template for bone regeneration. Further optimization of the production process and future animal studies will be required to fully translate this technology to clinical applications.

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Towards Biomanufacturing of a Biomimetic Hydrogel-based Dermal Template for Skin Regeneration

**Dr. Bach Le**, Weng Wan CHAN, Gibson SOO, Chun Ting GOH, Kerk Wai TAT, Lok Boon KENG, May Win NAING, Deepak CHOUHDURY

1Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore; 2Singapore Institute of Manufacturing Technology (SIMTech), Agency for Science, Technology and Research (A*STAR), Singapore

**Title:** Towards Biomanufacturing of a Biomimetic Hydrogel-based Dermal Template for Skin Regeneration

**Authors:** Bach Quang Le#1, Weng Wan Chan#1, Gibson Soo2, Chun Ting Goh1, Kerk Wai Tat2, Lok Boon Keng2, May Win Naing2, Deepak Choudhury*1, # Contributed equally to this work.

**Affiliations:** 1Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore. 2Singapore Institute of Manufacturing Technology (SIMTech), Agency for Science, Technology and Research (A*STAR), Singapore.

**Correspondence:** *Deepak_Choudhury@btia-star.edu.sg*

**Background:** Dermal injuries are a significant healthcare concern, and current therapies have limitations. There is a growing interest in developing effective dermal templates for regenerative medicine, and collagen-based hydrogels have emerged as a promising candidate.

**Methods:** In this study, we present a novel collagen hydrogel dermal template formulated from a UV-crosslinkable methacrylated bovine collagen type I material (Col-MA). Our hydrogel is formulated from animal collagen type I, which is widely used in skin regeneration applications. We modified the collagen by methacrylation to make it photo-crosslinkable. The UV-crosslinking process was fast and efficient, allowing for scale-up manufacturing capability. We then performed another secondary chemical crosslink (CC) to enhance the stiffness and handleability of the hydrogel template.

**Results:** The UV/CC crosslinking method provided excellent structural support for hydrogel. Furthermore, it allowed us to finetune the mechanical stiffness and degradation profile of the hydrogel. We then optimized the hydrogel formulation and crosslinking conditions to achieve optimal properties for skin regeneration. Physical and chemical characterization of the hydrogel confirmed its uniformity and reproducibility. In vitro studies showed that the hydrogel supported the adhesion and proliferation of human dermal fibroblasts, demonstrating its biocompatibility and regenerative potential.

**Discussion and Conclusion:** Overall, our Col-MA hydrogel offers a promising dermal template for skin regeneration. Future studies will be required to evaluate the efficacy and safety of this approach in animal models and clinical trials. This research is part of the programmatic grant Additive Manufacturing for Biological Materials (AMBM) in Singapore, which aims to develop the next generation wound dressing comprising of regenerative dermal template, printable cells, and sensor in a scaled-up fabrication process.

**Acknowledgement:** This research is supported by A*STAR Project Additive Manufacturing for Biological Materials (AMBM) – Grant no A18A8b0059.
Temperature-controlled radiofrequency ablation with self-expandable electrode to ablate evenly malignant biliary obstruction

Ms. Yubeen Park¹, Mr. Dong-Sung Won¹, Ms. Jinmi Park¹, Mr. Sang Soo Lee², Mr. Jung-Hoon Park¹
¹Biomedical Engineering Research Center, Asan Institute for Life Sciences, Asan Medical Center, Seoul, Republic of Korea, ²Department of Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

Background: Radiofrequency ablation (RFA) was increasingly being presented to palliate unresectable malignant biliary obstruction (MBO) and prolong stent patency. However, conventional methods such as catheter-type electrode have limitations in that the ablation range is incomplete due to insufficient contact between the tissue and the electrode.

Methods: In this work, we developed bipolar self-expandable electrode (SE) and a customized RF generator. In addition, the efficacy and safety of the bipolar SE with a customized RF generator were investigated and the ablation ranges of the SE according to different ablation protocols was validated ex vivo and in vivo.

Results: Ablation ranges were proportionally and significantly increased with RF power and time in the porcine liver (p < 0.001) and common bile duct (CBD) (p < 0.001). RF power was more influenced the ablation length (β = 0.736 vs. 0.644) and depth (β = 0.727 vs. 0.574) compared with time in porcine CBD. Degrees of tissue damage, luminal narrowing, and histological findings including TUNEL- and HSP 70-positive areas were significantly increased in RF parameter-dependent manners (all p < 0.05).

Discussion and Conclusion: We demonstrated that the technical feasibility and safety of the RFA with bipolar SE and customized RF generator. Endobiliary RFA with the bipolar SE and customized RF generator generated even ablation areas and ablation ranges can be easily controlled by adjusting RF power and ablation time.

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Exploring Genes Associated With Hair Graying Using Hair Follicle Organoids

Ms. Shan Tu1, Dr. Tatsuto KAGEYAMA1,2, Prof. Junji Fukuda1,2

1Graduate School of Engineering Science, Yokohama National University, , Japan, 2Kanagawa Institute of Industrial Science and Technology, , Japan

Authors: Shan Tu1, Tatsuto Kageyama1,2, Junji Fukuda1,2*

Affiliations: 1Graduate School of Engineering Science, Yokohama National University, 79-5 Tokiwadai, Hodogaya-ku, Yokohama, Kanagawa, Japan. 2Kanagawa Institute of Industrial Science and Technology, 3-2-1 Sakado Takatsu-ku, Kawasaki, Kanagawa, Japan. (* Correspondence)

Category: Tissue Engineering and Regeneration

Background: Hair graying is caused by a variety of factors, including aging, heredity, and health decline. However, the molecular and cellular mechanisms underlying hair graying remain poorly understood. Most studies in this field have utilized hair follicle units, which have provided valuable insights. However, the collection of human hair follicles and maintaining them in culture for extended periods of time presents significant challenges, making it difficult to conduct repeated drug testing and genetic analysis. To address this issue, this study aims to establish in vitro models of hair graying that enable scalable and reproducible experiments and allow for observation of morphological changes throughout hair growth.

Methods: To prepare hair follicle organoids (HFOs), fetal mouse skin-derived epithelial and mesenchymal cells (1:1 ratio) were cultured with a 2% v/v Matrigel-containing medium (T. Kageyama et al., Science Advances, 8(42) eadd4603). HFOs were used for evaluation of various factors associated with hair pigmentation and gene knockdown experiments using pigmentation-related siRNAs.

Results: The epithelial and mesenchymal cells formed core-shell shaped HFOs through spontaneous organization on day 2, and hair follicle organogenesis was initiated from the core-shell interface on day 4 of culture. The pigmented hair shafts were sprouted with approximately 100% efficiencies on 6 days. Incorporating hair pigmentation promoting factors (including melanocyte-stimulating hormone) into the culture medium significantly improved the darkness of the hair shafts. The siRNA knockdown of genes involved in melanin synthesis (Bcl2, MITF) and transport (MyoX, PAR2, Rab11b) resulted in increased formation of gray hair follicles compared to the control group.

Discussion and Conclusions: We have successfully prepared HFOs and could observe the hair pigmentation of HFOs. This culture model has the potential to serve as a valuable tool for drug testing aimed at developing therapies for gray hair. By monitoring changes in hair color in HFOs, researchers can evaluate the efficacy of candidate compounds. Moreover, siRNA-mediated knockdown of key genes in this in vitro hair follicle model provides a means of investigating the mechanisms underlying hair pigmentation, from melanosome production to transport. This approach holds promise for elucidating the roles of specific genes in hair pigmentation and for identifying novel targets for therapeutic intervention.

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Absorbable Magnesium Stent for Treating Eustachian Tube Dysfunction in a Porcine Model: A Preliminary Study

Mr. Jeon Min Kang¹, Ms. Song Hee Kim¹, Dr. Jung-Hoon Park¹

¹Biomedical Engineering Research Center, Asan Institute for Life Sciences, Asan Medical Center, Seoul, Republics of Korea

Background: Absorbable magnesium (Mg) stents have fascinating biocompatibility and biodegradability, but their degradable behavior and efficacy in the Eustachian tube (ET) have not yet been investigated. Herein, we were to evaluate the efficacy and safety of Mg stent in the porcine ET by investigating their degradable behavior and luminal patency.

Methods: In this study, the degradable behavior of the Mg stent in artificial nasal mucus was evaluated. The Mg stent placement into the porcine ET model were also investigated to evaluate their luminal patency and histological changes.

Results: The mass loss rate of the Mg stents gradually decreased over time in the artificial nasal mucus. The Mg stent structure was not maintained, the bridges between the struts were disconnected, and the strut was sequentially separated after two weeks. The Mg stent placement was technically successful in all the porcine ETs without any stent-related complications. Based on histological findings, the thickness of submucosal tissue hyperplasia and the degree of inflammatory cell infiltration significantly decreased at four weeks compared with two weeks. Biodegradation of the Mg stent occurred before tissue proliferative reactions, and the ET patency was successfully maintained without stent-induced tissue hyperplasia at four weeks.

Discussion and Conclusion: We demonstrated that the temporary Mg stent placement can suppress stent-induced tissue hyperplasia formation while maintaining the ET patency within four weeks. The Mg stent that biodegrades rapidly seems to be effective and safe in porcine ET. Even though further preclinical studies are required to verify the optimal stent shape and indwell period in the ET, the application of the Mg stent in the ET has therapeutic potential for ET dysfunction.
A novel rapidly mineralized biphasic calcium phosphate with high acid-resistance stability for long-term treatment of dentin hypersensitivity

Dr. Sheng Long Tan¹
¹School Of Stomatology, China

Treating dental hypersensitivity (DH) rapidly and maintaining long-term effectiveness remains challenging. We aimed to address this problem by fabricating a novel rapidly mineralized biphasic calcium phosphate (RMBCP), which could rapidly elicit mineralization to form hydroxyapatite (HA) and perform excellent acid-resistant stability, thus effectively blocking the exposed dental tubules and protecting them from acid attack.
In Vivo Stable Allogenic Cartilage Regeneration in a Goat Model Based on Immunoisolation Strategy Using Electrospun Semipermeable Membranes

Ms. Yuyan Sun1, Mr Yujie Hua2,3, Ms. Yingying Huo2,3

1Research Institute of Plastic Surgery, Weifang Medical University, Weifang, China., 2Department of Plastic and Reconstructive Surgery, Shanghai 9th People’s Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, 3Shanghai Key Laboratory of Tissue Engineering, Shanghai, China

Authors: Yuyan Sun1, Yujie Hua2 3,Yingying Huo2 3

Affiliations:
1Research Institute of Plastic Surgery, Weifang Medical University, Weifang, Shandong, China.
2Department of Plastic and Reconstructive Surgery, Shanghai 9th People’s Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
3Shanghai Key Laboratory of Tissue Engineering, Shanghai, China

Category: SYIS (Student and Young Investigator Section) plus Tissue Engineering and Regeneration

Background: Tissue engineering is a promising strategy for cartilage defect repair. However, autologous cartilage regeneration is limited by additional trauma to the donor site and a long in vitro culture period. Alternatively, allogenic cartilage regeneration has attracted attention because of the unique advantages of an abundant donor source and immediate supply, but it will cause immune rejection responses (IRRs), especially in immunocompetent large animals. Therefore, a universal technique needs to be established to overcome IRRs for allogenic cartilage regeneration in large animals.

Methods: In the current study, we developed a hybrid synthetic-natural electrospun thermoplastic polyurethane/gelatin (TPU/GT) semipermeable membrane (SPM), tested its biocompatibility, semipermeability and immunoisolation function in vitro, and further explored the feasibility of stable allogenic cartilage regeneration in vivo by an immunoisolation strategy.

Results: In vitro results demonstrated that the rationally designed electrospun TPU/GT membranes has ideal biocompatibility, semipermeability, resistance to degradation and immunoisolation function. In vivo results further showed that the SPM efficiently blocked immune cell attack, decreased immune factor production, and cell apoptosis of the regenerated allogenic cartilage. Importantly, TPU/GT-encapsulated cartilage-sheet constructs achieved stable allogeneic cartilage regeneration in a goat model.

Scheme. The immunoisolation mechanism in allogenic cartilage regeneration: SPM prevents immune cell attack through physical barrier and blocks the negative regulatory effect mediated by destructive inflammatory factors.

Discussion and Conclusion: We demonstrated that our rationally designed SPM can inhibits immune cells attack and support cartilage metabolism thereby resulting in enhanced allogenic cartilage regeneration in vivo. The current work demonstrated a promising strategy to design biomimetic biomaterial facilitating allogenic engineered cartilage regeneration and supplies a new cartilage donor source to repair various cartilage defects.

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Engineering embryonic stem cell-induced retinal pigment epithelium monolayer with electrohydrodynamic jet-printed scaffolds

Dr. Hang Liu¹, Dr Zengping Liu¹, Dr Bhav Harshad PARIKH¹, Professor Dejian HUANG², Dr Xinyi SU¹
¹Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, ²Department of Food Science and Technology, Faculty of Science, National University of Singapore, Singapore
Authors: Hang Liu¹, Zengping Liu¹, Bhav Harshad Parikh¹, Dejian Huang²*, Xinyi Su¹*,

Affiliations: ¹Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore. ²Department of Food Science and Technology, Faculty of Science, National University of Singapore. (* Correspondence)

Category: Tissue Engineering and Regeneration, SYIS (Student and Young Investigator Section)

Background: Age-related macular degeneration (AMD) is a major cause of visual loss and has been estimated to bring a substantial global burden [1]. Currently, the most promising methods for late-stage AMD is cell therapy, which aims to generate allogeneic retinal pigment epithelium (RPE) in vitro and transfer cells to the degenerate retina. Electrohydrodynamic jet (E-jet) printing technique emerges as a possible solution for the maturation and delivery of stem cell-derived RPE monolayer.

Methods: Scaffolds were designed and fabricated with fibre diameter and pore size close to human RPE cell sizes (~10 µm and ~20 µm) via a modified E-jet printed system with polycaprolactone (PCL) biomaterial inks [2]. Effects of E-jet printed scaffolds on RPE monolayer formation and maturation were compared with Transwell® polyethylene terephthalate (PET) membrane by seeding and culturing embryonic stem cells derived RPE (ESCs-RPE) for eight weeks.

Results: Compared with PET membranes, E-jet printed scaffolds have a more evenly distributed structure, higher porosity, and higher biodegradability. And these scaffolds’ overall thickness and biomechanical properties are close to human Bruch’s membrane. The in vitro studies showed that the scaffold with 10 µm pores facilitates ESCs-RPE to proliferate and form a confluent cell monolayer comparable to a PET membrane. Furthermore, scaffolds with 10 µm pores and PET membranes can support ESCs-RPE to present cell integrity and barrier function. The phagocytosis function evaluation has shown that all the engineered RPE monolayers can actively phagocytosis fluorescence-labelled porcine photoreceptor outer segments. Moreover, the RPE-specific morphology, including microvilli and intracellular organelle shape, has indicated that scaffolds with 10 µm pores can enhance the maturation of ESCs-RPE monolayers.

Discussion and Conclusion: E-jet printed scaffolds can provide biomimetic mechanical environments and their cell-sized fibres and pores support cell growth and maturation. Thereby, functional ESCs-RPE monolayers can be regenerated in vitro. The current work showed a promising strategy for generating and delivering RPE monolayers with biodegradable scaffolds.


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Enhancing Chondrogenic and Adipogenic Differentiation of Stem Cells in Spheroid Culture using Hyaluronic Acid Hydrogel

Ms. Hye-Eun SHIM¹,³, Mr. Kyoung Hwan PARK³, Prof. Kang Moo HUH³, Ph.D Sun Woong KANG²
¹Research Group for Biomimetic Advanced Technology, Korea Institute of Toxicology, Daejeon, Korea,
²National Toxicity Policy Center, Korea Institute of Toxicology, Daejeon, Korea, ³Department of Polymer Science and Engineering, Chungnam National University, Daejeon, Korea

Background: Stem cells have garnered significant attention across diverse fields due to their ability to differentiate into various cell types. However, spheroid culture, which is frequently utilized for chondrogenic and adipogenic differentiation, has limitations in that necrotic cores form when nutrients and oxygen fail to reach the center of the spheroid. To address these limitations, hydrogels, particularly those based on hyaluronic acid (HA), are gaining attention for their excellent biocompatibility, biodegradability, and ability to facilitate the transport of oxygen and nutrients.

Methods: This study employed an HA hydrogel to promote chondrogenic and adipogenic differentiation of stem cells in spheroid culture. HA particles were produced using the W/O emulsion method and embedded between cells to form spheroids.

Discussion and Conclusion: Compared to conventional spheroid culture methods, spheroids with HA particles exhibited significantly enhanced chondrogenic and adipogenic differentiation efficacy. This culture technique has the potential for application in various fields.

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Development of Multifunctional Cationic Polysaccharide Hydrogels for Inhibition of Postoperative Tumor Recurrence and Metastasis

Ms. Hee Seung Seo1, Mr Jaesung Lim1, Mrs. Se-Na Kim2, Mr Wooram Park3, Mr ChunGwon Park1
1Department of biomedical engineering, Sungkyunkwan university, Suwon, Republic of Korea, 2Research and development center, MediArk Inc., Cheongju, Republic of Korea, 3Department of integrative biotechnology, Sungkyunkwan university, Suwon, Republic of Korea

Title: Development of Multifunctional Cationic Polysaccharide Hydrogels for Inhibition of Postoperative Tumor Recurrence and Metastasis

Authors: Hee Seung Seo1, Jaesung Lim1, Se-Na Kim2, Wooram Park3, Chun Gwon Park1*

Affiliations: 1 Department of Biomedical Engineering, SKKU Institute for Convergence, Sungkyunkwan University (SKKU), Suwon, Gyeonggi 16419, Republic of Korea. 2 Department Research and Development Center, MediArk Inc., Cheongju, Chungbuk, 28644, Republic of Korea. 3 Department of Integrative Biotechnology, College of Biotechnology and Bioengineering, Sungkyunkwan University, Suwon 16419, Republic of Korea. (*Correspondence)

Category: Design and Application of Biomaterials

Background: Cancer recurrence and metastasis after primary tumor resection surgery remains the primary cause of treatment failure. To address these limitations, we prepared a Multifunctional Cationic Polysaccharide (MCP) Hydrogels system. Since Cationic Polysaccharide (CP), the main material of MCP hydrogel, have several biological properties, they have been considered as promising biomaterials in drug delivery. In addition, CPs are natural adjuvants, thus can induce dendritic cell activation to promote T cell mediated immune responses, thereby controlling tumor growth. Therefore, in this study, MCP hydrogel was fabricated utilizing the inherent multifunctional properties of CPs, to confirm its potential as an effective drug delivery system for anticancer treatment.

Methods: To synthesize the MCP hydrogel, CPs was dissolved in 10 mL of water to form a 2 w/v% solution (pH 9.0). 2% (w/v) CP solution and methacrylate were mixed and stirred for 48 hours, followed by dialysis for 2 days and then were lyophilized. The morphology of the MCP hydrogel was observed with scanning electronic microscope (SEM). The release and degradation profile was analyzed using an in vivo imaging system (IVIS).

Results: The MCP hydrogel represented a cylindrical morphology with a diameter of ≈9 mm and a thickness of ≈7 mm. The photographic and SEM images (Fig. 1A and B) of the MCP hydrogel showed a macro porous structure. When we examined the release kinetics of biologics in vivo, we discovered that the MCP hydrogel can substantially extend the local release of immunomodulatory compounds relative to local delivery of the same biologics as a free solution (Fig. 1C and 1D). To confirm in vivo degradability of the hydrogel, MCP hydrogel conjugated with a fluorescent dye was placed into the tumor resection site of a female BALB/c mouse. Noteworthy degradation of the hydrogel was observed starting from 7 weeks, with complete resorption achieved by 14 weeks (Fig. 1E and F).

Discussion and Conclusion: The MCP hydrogel with macro porous structure were designed to release the loaded components in a localized and sustained manner. In addition, the sustained released behaviors and slow degradation rate of the MCP hydrogel offer the opportunity to prevent systemic and acute toxicity effects. To investigate the anti-tumor effect of MCP hydrogel, we will perform further experiments to ensure that the MCP hydrogel can improve the efficacy of the immune therapeutics with minimal side effects.
Acknowledgement: This work was supported by a Basic Science Research Program grant (2023-00208913 to C.G.P.) through the National Research Foundation of Korea (NRF) grants funded by the Ministry of Science and ICT (MSIT) of the Republic of Korea. This work was also supported by the Korean Fund for Regenerative Medicine (KFRM) grant funded by the Korean government (MSIT and the Ministry of Health & Welfare) (KFRM 21A0501L1 to C.G.P.).
A temporary “bloodstream”: oxygenating hydrogels increase functional integration of neural stem cell grafts

Dr. Yi Wang¹,²
¹Department of Biomedical Engineering, Faculty of Engineering and Information Technology, The University of Melbourne, Melbourne, Australia, ²The Graeme Clark Institute, The University of Melbourne, Melbourne, Australia

Background: Injectable biocompatible nanostructured hydrogels have great potential for the treatment of brain injury as cellular delivery vectors. However, there are a number of hurdles that must be overcome before cell transplantation with these materials have the necessary reliability and efficacy for clinical translation. Injectable hydrogels suffer from issues relating to hypoxia, poor cell survival and cell differentiation, and functional integration within new stem cell grafts owing to the lack of an established vascular network and limited oxygen supply.

Methods: In this work, we have engineered oxygenating hydrogels which can mimic “bloodstream” by protein-engineering techniques and solid-phase peptide synthesis. We have characterised those oxygenating hydrogels and explored oxygen release kinetics, followed by the assessment of the functional integration in vivo after 28 days.

Results: The oxygenating hydrogels can perform nanofibrous scaffolds with mechanical support, as well as being the functional state for oxygen binding and releasing. The oxygenating hydrogels have no effect on host inflammatory response while supporting cell survival and graft innervation. Most importantly, the oxygenating hydrogel can promote the differentiation of primary stem cells into neurons, which are considered essential for brain injury.

Discussions and Conclusions: We have successfully engineered oxygenating hydrogels that are capable of concomitantly delivering stem cells and oxygen within the brain to support engraftment until vascularisation can occur naturally. These oxygenating hydrogels can promote extensive innervation, ensuring functional synaptic connectivity while increasing of neuronal differentiation, demonstrating modulating cell fate specification. These oxygenating hydrogels are the promising approach for the treatment of neural injuries and diseases affecting the central and peripheral nervous systems.
Photo-crosslinked gelatin methacryloyl (GelMA)/hyaluronic acid methacryloyl (HAMA) composite scaffold using anthocyanidin as photo-initiator for bone tissue regeneration

Prof. Ren-Jei Chung¹, Dr. Yu-Chien Lin, Ms. Yu-Wen Huang
¹Department of Chemical Engineering and Biotechnology, National Taipei University of Technology (Taipei Tech), Taipei, Taiwan
Title: Photo-crosslinked gelatin methacryloyl (GelMA)/hyaluronic acid methacryloyl (HAMA) composite scaffold using anthocyanidin as photo-initiator for bone tissue regeneration

Authors: Ren-Jei Chung*, Yu-Wen Huang

Affiliations: Department of Chemical Engineering and Biotechnology, National Taipei University of Technology (Taipei Tech), Taipei, Taiwan (* Correspondence)

Category: Design and Application of Biomaterials

Background: Gelatin and hyaluronic acid are natural biopolymers that are important components of soft tissues in the human body, and both have been used in several biomedical applications. However, further functionalization is required to achieve certain properties, such as tunable mechanical properties and controllable degradation.

Methods: To increase the applicability of gelatin and hyaluronic acid, methacrylic acid and a photoinitiator have been used to modify photo-crosslinkable gelatin methacryloyl (GelMA) and hyaluronic acid methacryloyl (HAMA). GelMA and HAMA have been widely used in bone regeneration and tissue applications because of their controllable mechanical properties and ability to fabricate scaffolds. One of the most commonly used photoinitiators is Irgacure 2959 (I2959); however, free radicals released from I2959 are cytotoxic. Therefore, two different photo-initiators, I2959 and anthocyanidin, are used in this study for scaffold fabrication to compare the structural properties of the scaffolds.

Results, Discussion and Conclusion: In vitro and in vivo studies are performed to evaluate the biocompatibility of the scaffolds and their ability to promote the restoration of osteochondral defects. Additionally, other tests, such as fluorescence, degradability, bioactivity, and intracellular alkaline phosphatase (ALP) assays, are also performed.
The biophysical cues of cell-derived extracellular matrix (cECM) are associated with its osteogenesis-mediating bioactivity

**Dr. Lih Ying Shin**

1The Chinese University of Hong Kong, , Hong Kong SAR

**Introduction:**

Upon dextran sulfate (DxS) supplementation into osteogenically induced mesenchymal stem cell (MSC) cultures, enhanced ECM deposition and enrichment of osteogenic factors within the ECM material were observed. The resulting decellularized matrices exhibited the strongest osteo-inductive effects on re-seeded naïve MSCs. Besides the accumulated biochemical factors, ECM has the ability to signal via biophysical cues, such as topographical features. We thus hypothesized that changes in topographical features will result in changes in bioactivity of these ECM materials.

**Methods:**

To test this hypothesis, structurally-modified matrices were manufactured by two individual methods, each affecting the collagen I network within the ECM. Collagen I was chosen as it is one of the most abundant protein components in the bone ECM and a major component determining ECM architecture. Alteration of ECM topography was either achieved by interfering with lysyl oxidase (LOX)-mediated crosslinking using β-minopropionitrile (BAPN) or enzymatically digesting deposited collagen after decellularization by collagenase.

**Results:**

While both approaches successfully altered the native ECM architecture, they resulted in opposing changes in the ECM’s bioactivity. BAPN-disrupted ECM resulted in the loss of its original osteo-inductive properties in a dose-dependent manner, while collagenase-digested ECM exhibited enhanced osteogenic bioactivity for lower collagenase concentrations and a decrease in bioactivity with high collagenase concentrations.

**Discussion and Conclusion:**

We concluded that different treatments resulted in distinct degrees of matrix disruption and that low collagenase concentration treatments may have indeed increased the bioavailability of sequestered factors within the ECM. In contrast, a strong disruption of ECM architecture by either BAPN or high concentration-collagenase treatment would result in partial loss of bioactivity. As the alteration of collagen architecture by either of the two approaches induced changes in other biophysical properties of the ECM, including mechanical properties, bioavailability of bioactive factors, and even the overall composition of the ECM, these results raise the awareness that processing of ECM and the format in which it is delivered may play a role in its therapeutic efficacy.

**References:**

Repurposing Human Hair Keratins into Novel Fibers Based on Interfacial Polyelectrolyte Complexation

Ms. Laura Li-En Foo1,2, Prof Kee Woei Ng1,3
1School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore, 2Nanyang Environment and Water Research Institute, Interdisciplinary Graduate Programme, Nanyang Technological University, Singapore, 3Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One, 637141, Singapore

Title: Repurposing Human Hair Keratins into Novel Fibers Based on Interfacial Polyelectrolyte Complexation

Authors: Foo Li-En Laura1,2, Ng Kee Woei1,3*

Affiliations:
1 School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore
2 Nanyang Environment and Water Research Institute, Interdisciplinary Graduate Programme, Nanyang Technological University, Singapore
3 Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One, 637141, Singapore

*Correspondence

Category: SYIS (Student and Young Investigator Section), Design and Application of Biomaterials

Background: Millions of tonnes of human hair accumulate in landfills or are incinerated annually, contributing to environmental pollution. However, human hair contains keratins which is a valuable biomaterial as they are biocompatible, biodegradable and bioactive. In addition, as the keratins are of human origin, there is a lower risk of interspecies disease transmission compared to animal-derived biomaterials. Interfacial Polyelectrolyte Complexation (IPC) is a straightforward method to produce micrometer-sized fibers. However, no research has focused on the use of human hair keratins to produce IPC fibers.

Methods: In this work, novel human hair keratin IPC fibers were produced and their physico-chemical and mechanical properties were evaluated. Some characterization techniques that were used are FESEM, FTIR and mechanical tester.

Results: The novel keratin fibers can be produced under neutral conditions and the fibers are formed through electrostatic interaction, hydrogen bonding and disulfide bonding. The predominant secondary structure found in the fibers are β-sheets. Through tensile testing, the novel keratin fibers exhibited good mechanical properties and are comparable to existing suture materials.

Discussion and Conclusion: We have shown that these novel human hair keratin IPC fibers have the potential to be used for biomedical applications such as sutures. In addition, we have shown a new method to produce micrometer-sized keratin fibers that have not been reported before. Furthermore, repurposing human hair by extracting keratins for functional applications will help to reduce negative environmental impacts.

Acknowledgement: This work was supported by the Ministry of Education, Singapore (AcRF Tier 1: RG7/22).
Scaffold-free Tracheal Engineering via a Modular Strategy Based on Cartilage and Epithelium Sheets

**Dr. Hai Tang**\(^1\,^2\), Weiyan Sun\(^1\,^2\), Yi Chen\(^1\,^2\), Chang Chen\(^1\,^2\)

\(^1\)Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University School Of Medicine, China, \(^2\)Shanghai Engineering Research Center of Lung Transplantation, China.

**Title:** Scaffold-free Tracheal Engineering via a Modular Strategy Based on Cartilage and Epithelium Sheets

**Authors:** Tang Hai\(^1\,^2\), Sun Weiyan\(^1\,^2\), Chen Yi\(^1\,^2\), Chen Chang\(^1\,^2\)*

**Affiliation:** \(^1\)Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University School Of Medicine, China. \(^2\)Shanghai Engineering Research Center of Lung Transplantation, China. (*Correspondence)

**Category:** Tissue Engineering and Regeneration

**Background:** In clinical practice, tracheal resection with primary end-to-end anastomosis is the only curative treatment for tracheal stenosis. Unfortunately, extended tracheal resection often leads to tracheal defects, and practical clinical substitutes with complex functional structures and can avoid the adverse influences from exogenous bioscaffolds are still lacking.

**Methods:** A cartilage sheet (Cart-S) prepared by high-density culture was laminated and reshaped to construct a cartilage tube as the main load-bearing structure. A temperature-sensitive culture technique was used to construct the monolayer epithelium sheet (Epi-S). Epi-S could be integrally transferred to the inner wall of the cartilage tube, forming a scaffold-free complex tracheal substitute (SC-trachea), prevascularized, and transplanted in situ.

**Results:** After being cultured for two weeks in vitro, chondrocytes self-assembled into a tough monolayer cell sheet (Figure a) containing trap structures and cartilage matrix (Figure b). Cart-S positively affect the growth and differentiation of the epithelium in the Epi-S, and the genes CK14 and Pan-CK were upregulated (Figure c). In vivo, after prevascularization, the SC-trachea lumen was patent and peripherally encapsulated by connective tissue rich in blood vessels of recipient origin (Figure d), indicating good integration and vascularization. After two weeks in vivo, the stress-strain curve of the cartilage tube was similar to that of the native trachea (Figure e). After in situ transplantation (Figure f), Five rabbits survived longer than two months with stable breathing while in good condition. One rabbit died of anastomotic fistula. After gross examination, the lumen of the trachea was unobstructed, and the inner wall was smooth (Figure g), with no apparent inflammatory exudation or granulation tissue proliferation. Immunofluorescence showed high expression of epithelial-specific CK14 (Figure h), and clear ciliated structures were observed at the tops of some cells under SEM (Figure i).

**Discussion and Conclusion:** The SC-trachea improved survival rates and perfectly replicated normal tracheal function following extensive tracheal reconstruction, providing a promising future for the permanent functional reconstruction of tracheal defects.
Development of a Liver-on-chip Model to Simulate Liver Inflammatory Response

Ms. Dhvanii Chawla1,2, Mr. Chun Keung PANG2, Professor Hon Fai CHAN1,2, Professor Rocky TUAN1,2

1Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, Hong Kong Special Administrative Region of China, 2School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, Hong Kong Special Administrative Region of China

Introduction: Inflammation is a common response observed in various liver diseases and is usually managed by immunosuppressive therapies. Uncontrolled inflammation can lead to irreversible tissue damage and liver failure that may require liver transplantation. Since human cell-based in vitro models can potentially mimic our biology more accurately than animal models, organ-on-a-chip platforms developed using cell and organoid culture have recently been employed to investigate disease mechanisms and perform drug testing.

Subjects and Methods: In this project, HepG2 cells and human embryonic stem cells (hESC) that were differentiated into hepatocyte-like cells (HLCs) were cultured in 2D cell culture and as 3D spheroids using various techniques for modelling liver inflammatory conditions and screening potential therapeutic agents such as drugs and stem cell-derived products. To simulate inflammation, cultures were exposed to conditioned medium derived from human monocyte cells (ThP-1 cell line) polarized into pro-inflammatory macrophages using phorbol 12-myristate 13-acetate (PMA) and treated with lipopolysaccharide (LPS) to obtain conditioned medium.

Results: We showed that hESCs successfully differentiated into HLCs and exhibited hepatic markers in 2D, similar to cultured HepG2 cells, and formed hepatic spheroids in 3D culture systems. Treatment with a macrophage conditioned medium, shown to contain cytokines such as IL-6, IL-1β, TNF-α (Fig. 1A), was used to simulate inflammatory response. After treatment, HepG2 cells and the HLCs cultured in both 2D and 3D showed a significant drop in cell number and albumin production, as measured by CCK8 assay and ELISA, respectively (Fig. 1B), indicating detrimental impact on the cultured cells. These results demonstrated preliminarily the feasibility of using a biological, human cell-based system to simulate the response of liver cells to inflammatory cytokines.

Discussion and Conclusion: This work has laid the foundation for the development of more advanced hepatocyte cultures such as 3D organoid and organ-on-chip models (Fig. 1C) to simulate inflammation in the liver, investigate disease mechanisms, and screen potential therapeutic agents. It would be of interest to investigate possible co-culturing with other liver cell types and integration of multiple organ-on-chips with biosensing monitoring and perfusion systems to model multi-organ diseases in the future.

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The PCK2-glycolysis axis assists three-dimensional-stiffness maintaining stem cell osteogenesis

Dr. Zheng Li¹, Yongsheng Zhou
¹Peking University School of Stomatology, , China
Title: The PCK2-glycolysis axis assists three-dimensional-stiffness maintaining stem cell osteogenesis

Authors: Zheng Li, Yongsheng Zhou*

Introduction: Understanding mechanisms underlying the heterogeneity of multipotent stem cells offers invaluable insights into biogenesis and tissue development. Extracellular matrix (ECM) stiffness has been acknowledged as a crucial factor regulating stem cell fate. However, how cells sense stiffness cues and adapt their metabolism activity is still unknown.

Methods: In this work, we experimentally mimicked the physical characteristics of 3D trabeculae network of normal and osteoporotic bone with different microstructure and stiffness using the GelMA with tunable stiffness. In addition, we explored the novel role of mitochondrial phosphoenolpyruvate carboxykinase (PCK2) in enhancing osteogenesis in 3D ECM via glycolysis using this hydrogel system.

Results: We reported the different microstructure and stiffness of trabeculae network of normal and osteoporotic bone, and the novel role of the key rate-limiting enzyme of gluconeogenesis, mitochondrial phosphoenolpyruvate carboxykinase (PCK2) in manipulating osteogenesis in physiological-mimetic normal bone (stiff) and pathology-mimetic osteoporotic bone (soft) in vitro and in vivo. We observed that PCK2 promotes osteogenesis in 3D ECM with tunable stiffness in vitro and in vivo. Mechanistically, PCK2 enhances the rate-limiting metabolic enzyme pallet isoform phosphofructokinase (PFKP) in 3D ECM, and further activates AKT/extracellular signal-regulated kinase 1/2 (ERK1/2) cascades, which directly regulates osteogenic differentiation of MSCs.

Discussion and Conclusion: Collectively, our study elucidates the unraveling role of PCK2 in promoting osteogenesis in 3D ECM with tunable stiffness. The underlying mechanism is through regulating PFKP-mediated glycolysis. These findings highlight the critical importance of structural mechanisms of aging and metabolic disease, and implicate an intricate crosstalk between cell mechanics and metabolism, and provide new perspectives for strategies of osteoporosis.

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Exudate Absorbing and Antimicrobial Hydrogel Integrated with Multifunctional Curcumin-loaded Magnesium Polyphenol Network for Facilitating Burn Wound Healing

Ms. Yan Gong1, Ms. Pei Wang1, Dr. Xiansong Wang1

1Department of Thoracic surgery, Shanghai Key Laboratory of Tissue Engineering, Shanghai Ninth People’s Hospital, Department of Plastic and Reconstructive Surgery, Shanghai Key Laboratory of Tissue Engineering, Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 200011, China, Shanghai, China

Abstract
Title: Exudate absorbing and antimicrobial hydrogel integrated with multifunctional curcumin-loaded magnesium polyphenol network for facilitating burn wound healing

Authors: Yan Gong1, Pei Wang1, Xiansong Wang1

Affiliations: 1Department of Plastic and Reconstructive Surgery, Shanghai Key Laboratory of Tissue Engineering, Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 200011, China (* Correspondence).

Category: Design and Application of Biomaterials

Background: Burns are one of the most common traumas in the world, but reducing the healing time of deep burn wound is still a challenging clinical issue. Traditional dressings not only have a lengthy medical procedure, but also cause unbearable pain and secondary damage to patients. At the same time, infection and pain from burn wounds are also leading causes of death from shock. Reduced healing time of deep burn wounds is associated with decreased mortality and scar formation.

Curcumin (Cur) is an active ingredient in turmeric, and has demonstrated efficacy in anti-inflammatory therapy, antioxidant therapy, inhibition of hypertrophic scar formation, attenuation of pain, and promotion of wound healing. Therefore, Cur has great potential for use in burn wound healing. However, the current clinical applications of Cur are limited by its low stability, low water solubility, rapid decomposition, and poor bioavailability. Because of the extensive wound exudates of burns, traditional hydrogels seem to be inefficient for long-term wound closure and drug delivery. Therefore, there is an urgent need to develop novel hydrogel dressings with high morphological plasticity and wet adhesion to suit the special wound conditions of burns while slowly releasing well-protected bioactive drugs to promote wound healing from different aspects.

Methods: In this work, we developed an exudate absorbing and antimicrobial hydrogel with curcumin-loaded magnesium polyphenol network (Cur-Mg@PP) to promote burn wound healing. That hydrogel was composed of an epsilon-poly-L-lysine (ε-PLL)/polymer poly (γ-glutamic acid) (γ-PGA) hydrogel (PP) and curcumin-loaded magnesium polyphenol network (Cur-Mg). Meanwhile, we also tested its efficacy on treating burn wounds in vitro and in vivo.

Results: We succeeded in fabricating Cur-Mg@PP with highly enhanced stability and bioavailability of Cur via the application of magnesium-organic microspheres. The structural stability of the Cur-Mg@PP hydrogel allowed it to maintain wound protection during treatment. Meanwhile, the Cur-Mg@PP demonstrated excellent therapeutic effects against gram-negative bacilli and gram-positive cocci, indicating its broad-spectrum antimicrobial effects. The biodegradable nature of Cur-Mg@PP prevented pain and secondary damage caused by repeated dressing changes. The synergies induced by Cur-Mg@PP promote angiogenesis, enhance cell proliferation, and exert antioxidant, anti-inflammatory, and antibacterial effects. Moreover, successful wound closure by the adhesive Cur-Mg@PP hydrogel and the therapeutic effects of the released Cur and Mg2+ on accelerating the healing of burn wounds were achieved both in vivo and in vitro.

Discussion and Conclusion: Cur-Mg@PP combines the therapeutic effects of Cur, Mg2+, and PP hydrogels, facilitating the healing process of burns. The therapeutic effects of Cur-Mg@PP were optimized by i) enhancing the stability and bioavailability of Cur; ii) antioxidant, anti-inflammatory, antibacterial, analgesic, and cell proliferation promoting effects enabled by Cur; iii) cell migration and angiogenesis improved by Mg2+; iv) repolarization of M2 macrophages facilitated by Mg2++; v) formation of a physical barrier and prevention of secondary damage by the application of the PP
hydrogel; and vi) exudate absorbing and anti-bacteria realized by PP hydrogel. Various functions of Cur-Mg@PP were verified both in vivo and in vitro. This multifunctional Cur-Mg@PP preserves the biological activity of Cur, providing a novel approach for protecting wounds and accelerating burn healing.

Acknowledgement: This work was financially supported by the National Natural Science Foundation of China (31971271).
Elastin domain-derived proteins, new types of elastin-like protein that bring the characteristics of human elastin

**Mr. Seung Kyeum Cho**

1Pohang University Of Science And Technology, , South Korea

Category: Design and Application of Biomaterials

Background: Elastin is an attractive biomaterial owing to its unique properties including high resilience, long half-life, and remarkable interaction with cells. Elastin provides structural integrity, biological cues, and persistent elasticity to a range of important tissues including vasculature and lungs. Elastin is also an important load-bearing tissue in the bodies of vertebrates and used in places where mechanical energy is required to be stored. Human elastin consists of 36 domains and the structure consists of repeats of hydrophobic and crosslinking domains.

Methods: In this research, we newly developed human elastin domain-derived proteins (EDDPs) which can mimic the properties of human elastin and overcome the limits of elastin-like proteins (ELPs).

Results: EDDPs were designed with the domains from human elastin. It differs from previous ELPs which shows great mechanical properties but has limitation form bringing out the diverse properties of human elastin. Here in this study we confirmed that EDDP showed similar features shown in human elastin such as LCST, mechanical, cell viability and migration properties.

Discussion and Conclusion: Here in this study we showed the newly developed EDDP which we mimicked from the design of human elastin. We demonstrated that EDDP shared the diverse properties of human elastin which in the future may be effectively applied into various applications for human tissue defects.
Photo-crosslinkable bio-adhesive for reinforced bone graft fixation

**Ms. Jinyoung Yun**¹, Hyung Joon Cha¹

¹Department of Chemical Engineering, Pohang University of Science and Technology (POSTECH), Pohang, South Korea

**Title:** Photo-crosslinkable bio-adhesive for reinforced bone graft fixation

**Authors:** Jinyoung Yun¹, Hyung Joon Cha¹*

**Affiliations:** ¹Department of Chemical Engineering, Pohang University of Science and Technology (POSTECH), Pohang, South Korea (* Correspondence)

**Category:** Tissue Engineering and Regeneration

**Background:** Bone grafting is a common procedure that involves the use of synthetic or natural materials to replace missing bone or stimulate bone growth. One of key aspects of this treatment is the use of a binder to hold the graft material in place. However, the binding materials currently available have limitations, such as poor biocompatibility, inadequate mechanical properties, and weak adhesive strength. Additionally, mixing the bone graft material with the binder in advance may result in difficulty filling irregular bone defects.

**Methods:** In this study, we present an in situ photo-crosslinkable bioadhesive which is composed of a bioengineered mussel adhesive protein (MAP) fused with the cell-adhesive peptide, arginine-glycine-aspartate (RGD).

**Results:** The tyrosine residue of MAP allows for photo-crosslinking, enabling hydrogel to be quickly and easily polymerized under harmless visible light. In addition to its ability to fill in irregular bone defects, the adhesive hydrogel also possesses other desirable properties including controllable biodegradation rate, high compressive strength, underwater adhesiveness, and biocompatibility. The incorporation of RGD in the MAP enhances in vitro cell adhesion and proliferation, which are essential for promoting bone formation and osseointegration. In vivo experiments demonstrated that the adhesive hydrogel improved bone regeneration in rat calvarial bone defects, with better results compared to commercial product.

**Discussion and Conclusion:** The bioadhesive provides a promising alternative to traditional fixation methods, with the potential to improve implant stability and promote bone regeneration. Further studies are required to investigate the long-term performance and clinical efficacy of this bioadhesive in biomedical applications.
Human adipose-derived and synovial-derived mesenchymal stem cells synergistically improves the therapy for osteoarthritis

Dr. Jianqun Wu, Yangyi Yu, Wenfeng Dai, Gui-ang Liu, Guangheng Li

1Division of adult joint reconstruction and sports medicine, Department of Orthopedic Surgery, Shenzhen People’s Hospital (the First Affiliated Hospital, Southern University of Science and Technology; the Second Clinical Medical College, Jinan University), , China

Title: Human adipose-derived and synovial-derived mesenchymal stem cells improve osteoarthritis treatment in a synergistic manner

Authors: Jianqun Wu  Yangyi Yu  Wenfeng Dai  Gui-ang Liu  Guangheng Li*

1Division of adult joint reconstruction and sports medicine, Department of Orthopedic Surgery, Shenzhen People’s Hospital (the First Affiliated Hospital, Southern University of Science and Technology; the Second Clinical Medical College, Jinan University)

*Corresponding author

Li Guangheng, MD, PhD (Email: liguangheng@hotmail.com)

Category: Stem Cells and Cell-Based Therapies

Background: Osteoarthritis (OA) is the most chronic pain disease that can be treated with stem cell therapy, but stem cells from different tissues have specific characteristics. The adipose-derived mesenchymal stem cells (ADSCs) demonstrated a potent anti-inflammatory effect, whereas cartilage regeneration is limited. While Synovium-Derived Stem Cells (SDSCs) demonstrate superior chondrogenic differentiation potential. Consequently, the mixed ADSCs and SDSCs may exert the dual properties of inhibiting OA inflammation and enhancing cartilage regeneration, and may maximize the synergistic effect of the two cell types, which can treat or even reverse the progression of OA.

Methods: ADSCs, SDSCs, and chondrocytes were isolated and cultured from OA patients undergoing total knee arthroplasty. By co-culturing chondrocytes with Trans-well or 3D Pellet, the effect of different ratios of ADSCs and SDSCs (4×10^5) was examined. ADSC to SDSC ratios were 1:0 (4×10^5 ADSC alone), 0:1 (4×10^5SDSC alone), 8:2 (3.2×10^5ADSC, 0.8×10^5SDSC, 8A2S), 5:5 (2×10^5ADSC,2×10^5SDSC, 5A5S), and 2: 8(0.8×10^5ADSC, 3.2×10^5SDSC, 2A8S). After co-culturing chondrocytes for 14 and 21 days, the Col I, II, X, and SOX-9 genes were analyzed using qPCR, while proteoglycan and Collagen II were analyzed using Safranin O and immunohistochemistry. OA induced by partial meniscectomy (pMMx) in immunodeficient rats for 4 or 12 weeks.  The therapeutic effects were evaluated 12 or 20 weeks after intra-articular injection of different ratios of ADSCs and SDSCs (10^6 cells), including 1) the retention and localization of stem cells labeled with fluorescent nanoparticles (fNPs); 2) Knee MRI and CT examination; 3) gait analysis and Von Frey test; 4) pathological staining and immunohistochemical analysis of possible mechanisms; and 5) RNA-seq analysis of potential mechanisms.

Results: We successfully isolated and identified ADSCs and SDSCs of high purity. It was discovered that 8A2S and 5A5S significantly promoted the gene expression of Col II and SOX-9 and proteoglycan, and inhibited the gene expression of Col I and X, when co-cultured with Trans-well or 3D Pellet; fNPs tracking demonstrated that stem cells were diminished to 20% at 4 weeks and localized at damaged cartilage or synovium at 12 weeks. The 5A5S treatment significantly reduced OA degeneration and pain, which was superior to the ADSC treatment alone. 1) MRI and CT revealed that the 5A5S group had a lower incidence of bone cysts and bone remodeling in subchondral bone, as well as a lower BV/TV and BMD. Gait analysis and Von Frey test demonstrated that the 5A5S group had higher average footprint pressure intensity and mechanical pain threshold, and lower expression of the CGRP and NPY positive nerve fibers in subchondral bone than the OA group; 2) HE and Safranin O staining demonstrated that the thickness of hyaline cartilage was increased and with a lower OARSI score in the 5A5S group; 3) Immunohistochemistry revealed that 5A5S group inhibited the loss of Col II and ACAN, decreased synovial inflammation scores and the proportion of TNF- and IL-1 positive cells; 4) RNA-seq analysis revealed that 5A5S group improved the therapy for OA by promoting chondrocyte autophagy by activating the FoxO1 signaling pathway.
Discussion and Conclusion: Mixed ADSCs and SDSCs in a 5:5 ratio could effectively reduce degeneration and pain in mild-moderate OA, outperforming ADSCs or SDSCs alone. Hybrid stem cells could be a new alternative method for treating OA.

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Key words: Adipose-derived mesenchymal stem cells, synovial stem cells, Osteoarthritis
Axial compressive loading attenuates early osteoarthritis by reducing subchondral bone remodeling

Dr. Jianqun Wu1, Yonghao Pan2, Chao Liu2, Guangheng Li1

1Division of adult joint reconstruction and sports medicine, Department of Orthopedic Surgery, Shenzhen People’s Hospital (the First Affiliated Hospital, Southern University of Science and Technology; the Second Clinical Medical College, Jinan University), , China, 2Department of Biomedical Engineering, College of Engineering, Southern University of Science and Technology, , China

Title: Axial compressive loading attenuates early osteoarthritis by reducing subchondral bone remodeling

Authors: Jianqun Wu1 Yonghao Pan2 Chao Liu PhD 2* Guangheng Li1*

1Division of adult joint reconstruction and sports medicine, Department of Orthopedic Surgery, Shenzhen People’s Hospital (the First Affiliated Hospital, Southern University of Science and Technology; the Second Clinical Medical College, Jinan University)

2 Department of Biomedical Engineering, College of Engineering, Southern University of Science and Technology

*Corresponding author

Guangheng Li, MD, PhD (Email: liguangheng@hotmail.com); Chao Liu, PhD (Email: liuc33@sustech.edu.cn)

Category: Tissue Engineering and Regeneration

Background: Subchondral bone remodeling is one of the earliest features of OA development. Mechanical loading and alendronate (ALN) can be used as a non-invasive physical therapy method for OA. However, the timing and efficacy for treatments are unknown. This study aims to determine whether the timing of mechanical loading and ALN influences the pathobiological changes of OA.

Methods: ACLT-induced OA mice were subjected to either early (1-3 weeks) or late (5-7 weeks) axial compressive dynamic load or intraperitoneal injection of ALN. Changes in gait, subchondral bone, cartilage, osteophyte, and synovitis were assessed using micro-CT, tartrate-resistant acid phosphatase staining, pathological section staining, and immunohistochemistry at 1, 2, 4, and 8 weeks.

Results: At 1, 2, and 4 weeks, the OA limb had lower average footprint pressure intensity and lower BV/TV in the subchondral bone, and more osteoclasts. At 4 weeks, early loading or ALN or Load+ALN treatments induced less cartilage destruction, with corresponding reduction in OARSIs and increased hyaline cartilage thickness. They also had fewer number of osteoclasts, higher BV/TV and BMD of subchondral bone, suppressed inflammation and IL-1β- and TNFα-positive cells in synovium. Early loading was superior to ALN in improving knee function and gait. At 8 weeks, early loading or Load+ALN improved the average footprint pressure intensity and knee flexion. At 8 weeks, early Load+ALN had a synergistic effect on protecting hyaline cartilage and proteoglycans. Footprint pressure intensity and cartilage destruction were worse in late loading limbs, and no difference in BV/TV, BMD, osteophyte formation and synovium inflammation were found between late-Load, late-ALN, and late Load+ALN groups and ACLT groups.

Discussion and Conclusion: Dynamic axial mechanical loading or ALN in the early stages of knee trauma protected against OA by suppressing subchondral bone remodeling. However, late loading promoted cartilage degeneration in advanced OA, indicating that reduced loading should be performed in the late stages of OA to avoid the acceleration OA. For patients with mild to severe OA, loading reduction by brace protection or maintaining joint stability by early ligament reconstruction surgery may ameliorate OA exacerbation.

Keywords: Axial compressive loading; Alendronate; Posttraumatic osteoarthritis; Subchondral bone remodeling

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Steroid-associated osteonecrosis in cortical bone of rabbits and PDGF-BB therapy

Dr. Huijuan Cao

Authors: Huijuan Cao¹, Keda Shi¹, Jing Long¹, Yanzhi Liu¹, Lingli Li¹, Yiqing Zhao¹, Tianluo Ye¹, Cuishan Huang¹, Yuxiao Lai¹, Ling Qin² *, Xinluan Wang¹ *

Affiliation: ¹ Centre for Translational Medicine Research & Development, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, PR China; ² Musculoskeletal Research Laboratory, Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, PR China. (* Correspondence)

Category: Stem cells and cells-Based Therapies

Introduction: The pathophysiologic features of steroid-associated osteonecrosis (SAON) in cortical bone are unclear. Platelet-derived growth factor-BB (PDGF-BB) has also been commercialized for promoting bone regeneration. This study was designed to investigate the pathologic changes of cortical bone, and further evaluate the effect of PDGF-BB on preventing cortical bone necrosis and improving cortical bone structure after SAON induction.

Subjects and Methods: In this study, SAON was induced by repeated lipopolysaccharide (LPS) and methylprednisolone (MPS) injections in rabbits. At 2, 4, and 6 weeks after SAON induction, PDGF-BB was intramedullary injected into the proximal femora. Xylenol orange and calcein green were injected subcutaneously into rabbits on days 14 and 4 before euthanasia. At 3 days after last drug treatment, micro-fil perfusion was performed for angiography. Then the femoral diaphysis was dissected for micro-computed tomography (μCT)-based angiography, μCT-based cortical bone geometry, and histological analysis.

Results: In present study, results shown that SAON induction protocol could mediate the incidence of osteonecrosis (ON) lesion of the cortical bone in femur reach to 100%, the ON lesion area took approximately 43.3% of cortical bone, and the neighboring bone marrow appeared necrosis and lesions in various degree. Meanwhile, the periosteum was degenerating, and the intraosseous vessels and nerves disappeared in the central area of the necrotic cortical bone, as well as the necrotic cortical bone was rarely remodeled by newly formed bone in SAON samples. Finally, the cortical bone 3D geometry and histological microarchitecture in femur were impaired post-SAON induction. In the other hand, we found that PDGF-BB treatment could improve blood flow and reduce the occurrence of SAON in the femoral diaphysis. Data showed that the incidence of ON lesion on the cortical bone reduced to 25-37.5% after PDGF-BB treatment, and the ON lesion areas narrowed to 9.1-16.5% of cortical bone, the normal bone marrow areas enlarged to 86.3-88.8%. Importantly, PDGF-BB treatment could recover the cortical bone 3D geometry and histological microarchitecture through increasing cortical mineralization ability both on periosteal and endosteal surfaces after SAON induction.

Discussion and Conclusion: SAON induction led to the cortical bone structure-function destruction. PDGF-BB might be a potential candidate stimulus for preventing ON lesion and protecting the cortical bone structure and function from SAON induction.

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Meniscus regeneration promotion using “mechanical pre-trained” mesenchymal stem cells through YAP-mediated mechanosensing

Dr. Liya AI¹, Dr. Mingze DU¹, Prof. Lisha ZHENG², Prof. Dong JIANG¹
¹Department of Sports Medicine, Peking University Third Hospital. Institute of Sports Medicine of Peking University. Beijing Key Laboratory of Sports Injuries. Engineering Research Center of Sports Trauma Treatment Technology and Devices, Ministry of Education, Beijing, China, ²Key Laboratory of Biomechanics and Mechanobiology (Beihang University), Ministry of Education, Beijing Advanced Innovation Center for Biomedical Engineering, School of Biological Science and Medical Engineering, Beihang University, Beijing, China

Background: Mesenchymal stem cells (MSCs) emerge as a perspective for regenerative medicine applications. In bone and joint regeneration, the therapeutic efficiency of MSCs primarily influenced by their targeted differentiation capacity and adaptability to their microenvironment after transplantation in vivo. Identifying appropriate cues continuously supporting MSCs lineage-specific differentiation without adverse side effects is critical for tissue healing and cellular therapy.

Methods: In this work, we employed meniscus-specific cyclic tensile strain (CTS) to pre-train synovial mesenchymal stem cells (SMSCs) in the presence of meniscus fibro-chondrocytes (MFCs) co-culture, and tested their role in guiding meniscus targeted differentiation and meniscus tissue regeneration in vitro and in vivo. The maintenance effect and mechanism of CTS in meniscus tissue healing, as well as SMSCs mechanical sensitivity and memory effect, have also been investigated.

Results: CTS combined with MFCs co-culture effectively promotes SMSCs differentiation, and SMSCs could memorize the effect of CTS. The in vitro studies showed that the CTS pre-trained SMSCs have higher mechanical sensitivity and meniscus-specific differentiation capacity than un-trained SMSCs. CTS-induced YAP nuclear translocation and downstream activation involve differentiation and mechanical memory of SMSCs. Meanwhile, cell nuclear shape and cytoskeleton were remodeled after CTS, which also accelerated the activation of YAP. Furthermore, after transplanted in vivo, the CTS pre-trained SMSCs are better adapted to the mechanical environment and promote meniscus tissue healing by regenerating more meniscus-specific cells and ECM.

Discussion and Conclusion: We demonstrate that meniscus-specific CTS can promote SMSCs targeted differentiation and upregulate its mechanical sensitivity, thereby improving the coordination of the cell-tissue mechanical environment and enhancing meniscus regeneration in vivo. The current work demonstrated a promising strategy for stem cell-based therapy, which can enhance the therapeutic function of stem cells and promote further clinical applications of stem cells.

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Animal Horn Polypeptide-coupled Thermosensitive Hydrogels in Bone Tissue Repair

Ms. Nannan Xue1,2, Ms. Wenjian Zhao1, Ms. Ziyi Zhou1, Mr. Rizhong Huang1, Dr. Jun Chen1, Dr. Jin-ao Duan1, Dr. Pei Liu2, Dr. Yiwei Wang1,2,3

1Jiangsu Provincial Engineering Research Center of Traditional Chinese Medicine External Medication Development and Application, Nanjing University of Chinese Medicine, Nanjing 210023, China, Nanjing, China, 2Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, National and Local Collaborative Engineering Center of Chinese Medicinal Resources Industrialization and Formulae Innovative Medicine, Jiangsu Key Laboratory for High Technology Research of TCM Formulae, Nanjing University of Chinese Medicine, Nanjing 210023, China, Nanjing, China, 3Burns Injury and Reconstructive Surgery Research, ANZAC Research Institute, University of Sydney, Concord Repatriation General Hospital, Concord 2137, Australia, Sydney, Australia

Background: Bone tissue has the ability to self-repair and regenerate. However, when the bone defect exceeds the critical size threshold, it will lead to bone nonunion, deformity union, requiring transplantation and surgical treatment. Traditional bone transplantation materials undertake external molding after implantation, which in risk of material migration and postoperative immune rejection. As a suitable bone defect filling material, hydrogel can be utilized to refill the defects within easy operation, non-toxic and better biocompatibility. The present study aims to introduce and characterize a new injectable temperature-sensitive hydrogel system (PNPHO) by coupling it an active animal horn polypeptides (AHPs) to promote bone tissue repair.

Methods: In this work, the selected animal horn polypeptide (AHPs) with capability in bone regeneration was prepared with a thermosensitive composite hydrogel (AHPs-PNPHO hydrogel) and examined for pharmacodynamics by in vivo and in vitro experiments.

Results: In results, AHPs was found to promote the cell proliferation of MC3T3-E1 cells and increase the expression of Runx-related transcription factor 2 (RUNX2), osteocalcin (OCN) and osteopontin (OPN) which are markers for cell transformation into osteoblasts. AHPs incorporated PNPHO thermosensitive hydrogel can reduce its Gelling temperature within a shorten gel time. AHPs-PNPHO was also proved to have better biocompatibility and low immunogenicity in vitro. In a mouse femoral bone defect model, results showed that AHPs-PNPHO hydrogel can significantly promote bone regeneration in situ and accelerate the repair of bone defects.

Discussion and Conclusion: In the present study, we found that AHPs can accelerate cell differentiation of anterior osteocytes to osteoblasts, and AHPs-PNPHO has better biocompatibility and low immunogenicity, which are suitable for use in minimally invasive procedures, and accelerates the regeneration of bone tissue while filling bone defects.

Reference:


Combination of irreversible electroporation and gold-doped mesoporous silica nanoparticles for cancer therapy

Dr. Ratchapol Jenjob

Inha University, South Korea

Title: Combination of irreversible electroporation and gold-doped mesoporous silica nanoparticles for cancer therapy

Authors: Ratchapol Jenjob, Yixin Jiang, Phuong Hoa Tran, Hosun Jung, Nattha Suwanprakorn, and Su-Geun Yang

Affiliations: Department of Biomedical Science, BK21 FOUR program in Biomedical Science and Engineering, Inha University College of Medicine, Incheon 22212, South Korea

Category: Design and Application of Biomaterials

Background: Irreversible electroporation (IRE) is an ablation technique that is currently being developed for cancer adjuvant therapy. IRE transmits microsecond electrical pulses across the target tissue, creating permanent nanoscale pores on the cell membrane. In this way, IRE disrupts cell membrane integrity and eventually causes a complete collapse of cellular homeostasis and cell death. The most prominent cellular response after IRE treatment is the explosive generation of reactive oxygen species (ROS, peroxides, superoxide, hydroxyl radical, and singlet oxygen) that can cause oxidative damage to proteins, DNA, fatty acids, and other biological molecules. ROS is related to various types of cell death, including apoptosis, necrosis, and ferroptosis.

Methods: In this work, we developed the Au-doped mesoporous silica nanoparticles (Au-MSNs) to enhance the efficiency of IRE and investigated its potential as a new therapeutic strategy for breast cancer. We evaluated the in vitro cytotoxicity of Au-MSNs in combination with IRE on breast cancer cells. The cellular ROS generation and lipid peroxidation were measured under IRE treatment with different concentrations of Au-MSNs. We also assessed the cell membrane permeability of cancer cells in the presence of Au-MSNs under IRE treatment, along with therapeutic cytotoxicity.

Results: The synthesized Au-MSNs had around 80-100 nm of particle size and were successfully end-doped with Au nanoparticles. Cytotoxic cell death and cell membrane permeability increased by 28% and by 25-fold, respectively, under a combination treatment of IRE (800 V/cm) and Au-MSNs (100 μg/ml) over single IRE. Moreover, ROS and lipid peroxidation of cancer cells were significantly increased by 14- and 265-fold, respectively, under combination treatment of IRE (800 V/cm) and Au-MSNs (100 μg/ml).

Discussion and Conclusion: IRE, when combined with Au-MSNs, precipitated the generation of ROS for killing breast cancer cells. Combined treatment of IRE with Au-MSNs over the 50 μg/ml concentration more abundantly produced cellular ROS than IRE alone treatment, effectively induced the oxidation of phospholipid of the cell membrane and led to cell death. These combined reactions eventually enhanced the therapeutic functions of IRE treatment. Our findings strongly suggested that Au-MSNs have the potential to improve IRE-mediated tumor ablation.

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PhaseX (Patient-derived Hydrogel-ASsisted EXplants); A novel approach for evaluating patient response to immune checkpoint inhibitors

**Mr. Kanishka Fernando**

National University of Singapore, Singapore

Title - PhaseX (Patient-derived Hydrogel-ASsisted EXplants); A novel approach for evaluating patient response to immune checkpoint inhibitors

Authors – 1 Kanishka Fernando, 2 Christabella Adine, 1, Nicholas Ching Wei Ho, 3,5 Hong Sheng Quah, 4 Samantha Shu Wen Ho, 4 Karen Wei Weng Teng, 3,5 Camille Arcinas, 4 Ling Li, 1 Kelly Ha, 1 Joey Wei Ling Chew, 1 Chenhui Wang, 5 Nathaniel Sheng Hua Too, 3 Hariraman Bhuvaneswari, 6,7,8 Rahul Nagadia, 9 Joe Poh Sheng Yeong, 3,5, N Gopalakrishna Iyer, 1,2,10, * Eliza Li Shan Fong

1 Department of Biomedical Engineering, National University of Singapore, Singapore, 2 The N.1 Institute for Health, National University, of Singapore, Singapore, 3 National Cancer Centre Singapore, Singapore, 4 Translational Medicine Research Centre, MSD, Singapore, 5 Duke-NUS Medical School, National University of Singapore, Singapore 6 Department of Head and Neck Surgery, National Cancer Centre Singapore, Singapore, 7 Department of Oral and Maxillofacial Surgery, National Dental Centre Singapore, Singapore 8 Department of Dental Medicine, Karolinska Institute, Stockholm, Sweden, 9 Institute for Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore, 10 Cancer Science Institute, National University of Singapore, Singapore, * Correspondence.

Category - Enabling Technologies & SYIS (Student and Young Investigator Section)

Introduction

The emergence of immune checkpoint inhibitors (ICIs) has revolutionised cancer treatment over the last decade, with more than 50% of diagnosed cancer patients being deemed eligible for ICI treatments. However, less than 20% of these patients actually respond to the treatment. Limitations in the accurate prediction of the patient’s response to ICIs, stem from the lack of available tumor models that can provide a complete understanding of the tumor microenvironment (TME), which plays a significant role in the patient’s response to ICIs.

Methods

In this work, we developed PhaseX, an engineered hyaluronan (HA) hydrogel-based tumor explant model. The critical hydrogel parameters, which influence the long-term culture of patient-derived tumor slices (PDTS), were optimised for head and neck squamous cell carcinoma (HNSCC) patients. In-depth characterisation of the model was evaluated after seven days against the original tumor. The effect of PD-1 blockade was determined by analysing the expression of cytokines, chemokines, and T-cell markers on the supernatant.

Results

We observed that the stiffer HA hydrogel (330Pa) with integrin binding has a higher number of total live cells and better maintains the tissue morphology, in comparison to conventional PTFE cell culture inserts. PhaseX model showed that major cell subpopulations, cancer pathways, immune compartment and cell-cell interactions of the original tumor were well-preserved. Supernatant analysis clustered two different patient groups as potential responders and non-responders. The predicted patient response successfully matched with the available clinical response in two cases with prolonged exposure. In one case, where the patient was clustered as a non-responder on Day 2, was clustered as a responder on Day 4.

Discussion and conclusion
We demonstrate, for the first time, that modulating the matrix properties of HA hydrogels can maintain the viability and architecture of PDTS for at least 7 days, which is superior to the conventional PTFE cell culture inserts. The ability of PhaseX to preserve the original tumor composition and gene expression for this period enables the detection of temporal readouts to better predict the efficacy of immune checkpoint inhibitors.

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Dense-layered culture of human dermal papilla cells promotes proliferation while maintaining trichogenic activity

Dr Lei YAN¹, Dr Tatsuto KAGAYAMA, Dr Junji FUKUDA
¹Kanagawa Institute of Industrial Science and Technology, Kawasaki, Japan, ²Yokohama National University, Yokohama, Japan

Background: Dermal papilla cells (DPCs) are a specific type of mesenchymal cells found in the hair follicles and possess the critical function of maintaining and regulating hair growth, shape, and cycle. DPCs are considered to play important roles in hair regeneration for the treatment of androgenic alopecia. However, when DPCs are isolated from the scalp, trichogenic functions are rapidly lost in in vitro passage culture. Various approaches have been studied to maintain their functions, including co-culturing with other types of cells, the use of specific growth factors, and spheroid culture. In this study, we propose dense-layered culture for a large scale preparation of human DPCs with restored functions.

Methods: Human DPCs were cultured for 30 days without trypsinization. Cell proliferation, multilayer formation, trichogenic gene expression were investigated during 30 days of culture. Hair regeneration ability of DPCs were examined by transplanting them into the back skin of mice. As a comparison, DPCs were cultured as spheroids for 30 days. The experiments were performed using purchased and frozen DPCs and primary DPCs from the human scalp.

Results: During 30 days of culture, DPCs grew and stacked on themselves and formed multilayered structures. The number of cells increased about *-fold compared to the seeding number in dense-layered culture, while there was almost no increase in the number in spheroid culture. In addition, trichogenic gene expression was significantly recovered in dense-layered culture. Furthermore, patch assays with mice showed that DPCs in dense-layered culture generated approximately *-fold more hair follicles than those in spheroid culture.

Discussion and Conclusion: The results demonstrated that dense-layered culture promotes the growth of DPCs while maintaining their functionality. This approach may have significant potential for use in hair regeneration therapy for hair loss treatment.
Serglycin in skin microenvironment promotes skin self-renewal in a CD44-dependent manner based on single-cell RNA sequencing data

Mr. Fan Bie1, Dr Xiaogang Liu1, Dr Lijuan Liu1, Dr Fan Yang1, Dr Yahui Xiong1, Prof Lei Chen1, Prof Shaohai Qi1
1The First Affiliated Hospital Of Sun Yat-sen University, , China

Background: Skin is the outermost protective organism and composed of two main layers: epidermis, dermis. The epidermis functions as a protective barrier and consists predominantly of keratinocytes. The dermis mainly comprises the extracellular matrix (ECM) and dermal fibroblasts and also includes immune cells to provide the skin with the ability to against foreign pathogens. The skin functional decline is influenced by natural aging and photoaging which induce early-onset morphological changes. However, the difference between two types of aging has not been analyzed systematically yet. The molecular and functional properties of various skin cell types in skin aging remained poorly understood.

Methods: The natural aging and photoaging single-cell RNA sequencing datasets were obtained from the Gene Expression Omnibus (GEO) database (GSE130973) and Genome Sequence Archive (GSA) database (HRA000395). Seurat R package (version 4.1.0) was used to further analyze the single-cell RNA-seq data. Cell cluster analysis was conducted. Cell types were identified. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analyses were performed on hub genes. Key genes were predicted by protein-protein interaction (PPI) and molecular docking. CellCall was used to identify potential interactions between and within fibroblasts and other dermal cell populations.

Results: The single-cell transcriptome profiling identified eight cell types, as well as four subpopulations of fibroblasts. Further analysis revealed progressive decline of mesenchymal-like fibroblast in two types of aging. GO analysis revealed the upregulation of the expression of the ECM genes in the mesenchymal-like fibroblast subpopulation, which is downregulated in the other subpopulations. CellCall revealed a dense communication network among fibroblasts and other cells in both natural aging and photoaging. PPI found that chondroitin sulfate proteoglycan serglycin (SRGN), a CD44-interacting factor, underwent decline in photoaging but remained in natural aging, inhibition of SRGN in fibroblast decreased the proportion of CD44+ mesenchymal fibroblast and promoted cellular senescence in photoaging. Lastly, we found that supplement of SRGN alleviated cellular senescence of dermal fibroblasts. In summary, these findings have important implications for understanding the molecular framework of SRGN-CD44 interaction in human skin aging and provide potential targets for skin aging related disease therapies.

Discussion and Conclusion: Based on scRNA sequencing of photoaging and natural aging dataset, we described an overall cellular composition of skin and cell-type-specific DEGs. Specifically, the upregulation of pathways related to the generation of ECM was observed in both aging related datasets. However, in the photoage dataset, the expression of CD44-related genes decreased so that the dermis fibroblast can not migrate to photodamaged sites to supplement collagen, which was high expressed in natural aging dataset. Meanwhile, the expression of the ligand of CD44 such as SRGN and HAS2 had a high correlation in both datasets. The above results indicated photoage demaged the intrinsic anti-aging pathway and promoted the atrophy of elastic networks in dermis skin. These findings were useful to identify novel therapeutic targets or compounds against human skin aging and related diseases.

Acknowledgement: Sun Yat-Sen University-Yat Sin Group (YSG) Skin Health Precision Research Joint Laboratory
Annexin A5 Accelerate Diabetic Wounds Healing by Regulating Skin Inflammation

Ms. Bijun Kang

1Shanghai 9th People's Hospital, , China

Introduction: Cell-free fat extract (CEFFE), a kind of liquid mixture from adipose tissue, is enriched more than 1700 proteins. And we confirmed that CEFFE could promote diabetic wound healing and modulate inflammation about M1 and M2 polarization of macrophages. Then we found that annexin A5 (AnxA5) is of high concentration in CEFFE by mass spectrum analysis. It is reported that AnxA5 plays significant role in tissue regeneration and inflammation1,2. Therefore we suppose that the anti-inflammatory effect of CEFFE is mainly functional through AnxA5.

Subjects and Methods: Full-thickness skin wound with a diameter of 6mm was produced in db/db mice in vivo and treated with AnxA5@GelMA. Wounds were observed on day 0, 3, 7, 10, 14 and harvested on day 14. Wound healing was evaluated by tissue section-staining and relative gene expressions. We also detected effect of AnxA5 in vitro on macrophages, human vascular endothelial cells (HUVEC), human fibroblast (HFB) and human immortal keratinocyte cells (HaCaT) for inflammatory regulation, tube formation, cell proliferation and migration.

Results: AnxA5 could accelerate wound healing in db/db mice and immune-histological staining showed that AnxA5 treatment could decrease expression of CD68. Pro-inflammatory factors, such as IL-1β and TNF-α, were reduced after AnxA5 treatment in db/db mice. AnxA5 could regulate macrophages by inhibiting the M0 to M1 polarization of macrophages and reducing expressions of pro-inflammatory factors in vitro. However, AnxA5 played modest effects in HUVEC, HFB and HaCaT in vitro.

Discussion and Conclusion: In inflammatory environment, phosphatidylserine (PS) is exposed on cell surface. AnxA5 is a member of annexin family and binding to PS-exposing membrane. Our results suggested that AnxA5 mainly effect on inflammatory cells in skin but normal HFB and HaCaT. Diabetic wound is healing under inflammatory environment. In the early stage of wound healing, AnxA5 regulate skin inflammation by reducing release of pro-inflammatory factors, and result in quickening wound healing.

References:
Expansion of human salivary acinar cell spheroids/organoids in 3D hydrogel platforms

Dr Yu Li ZHANG¹, Dr. Simon TRAN¹

¹Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Canada

Background: There are two groups of patients living with severe dry mouth (xerostomia, salivary hypofunction): a) over 600,000 new cases of head and neck cancer annually worldwide treated with radiotherapy and b) nearly four million North Americans with Sjögren’s syndrome (SS; the second most prevalent rheumatic diseases). Because of this severely reduced (or lack) of saliva secretion, both groups of patients experience considerable morbidity and discomfort such as difficulty in swallowing, tooth decay, oral ulcers and infections.

Prior to developing treatments for these patients, a valid treatment-testing salivary gland (SG) model is needed to investigate the physiological and pathological processes of damaged salivary glands. However, a significant challenge toward this model development is the lack of a proper ex vivo substitute material for the extracellular matrix (ECM). This material must have physicochemical, functional, and biological properties that mimic the native SG ECM.

A protein-based ECM extracted from Englebreth-Holm-Swarm tumors in mice, called Matrigel®, is currently considered the gold standard scaffold for 3D culture. However, the raw material of the Matrigel® can only be obtained by inducing cancer into mice and then euthanizing them. In addition to ethical issues, the widespread use of Matrigel has been limited by high costs, promoting researchers to develop more accessible and cheaper alternatives for 3D culture scaffolds.

Subjects and Methods: Hence, we propose Alginate-Egg White (AEW) novel hydrogels, which could be a promising alternative scaffold to Matrigel® for salivary tissue engineering. The egg white material provides ECM-like proteins that mimic the ECM microenvironment and replicate human cells’ developmental conditions. At the same time, the ionic crosslinking property of alginate allows for modification of the porosity, strength, and rigidity of the scaffold, which are important for 3D spheroid formation. Furthermore, compared to Matrigel®, egg white material does not require euthanizing any animals, making the EWA a great, cheaper, more environmentally friendly, and ethical alternative material for 3D culture scaffolds.

Results: Thus far, we developed and tested four hydrogels’ composition with different concentrations of alginate (A), gelatin (G), and egg white (EW): 1) A1EW7, 2) A1G7EW7, 3) A1EW5, and 4) A1G7EW5, where the number stands for the concentration (% w/v) of each polymer. All these scaffolds showed the capability of supporting salivary cell growth, proliferation, and spheroid formation. Among these 4 hydrogel groups, A1EW7 showed the highest cell viability and A1G7EW7 was the best for promoting the formation of large SG spheroids within 14-21 days of culture. Besides, A1G7EW7 has been tested with culturing human primary salivary functional units (SFU) and proved its ability of maintaining human SG primary cell proliferation and viability for 28 days of 3D culture.

Discussion and Conclusion: It is well known that matrix composition plays an important role in spheroid/organoid formation and cell expansion. Here, we proved that the increase of EW concentration in hydrogel promoted SG cell proliferation and spheroid/organoid formation. The AEW hydrogels have great potential to be an alternative candidate of Matrigel for in vitro 3D culture, due to their low-cost production, biocompatibility, and accessibility. And further, it is promising to be used as a potential source for cell transplantation (i.e., using this platform as an ex vivo cell expansion source) and a 3D organoid platform.
Skin substitutes reshaped the commensal wound microbiota is associated with diabetic wound healing

Mr. Zeyu Xu¹, Ms. Qinghan Tang¹, Ms. Xiaotong Ding¹, Ms. Ruihan Jiang¹, Mr. Jun Chen¹,², Mrs. Yiwei Wang¹,²

¹Jiangsu Provincial Engineering Research Center of TCM External Medication Development and Application, Nanjing University of Chinese Medicine, Nanjing, China, ²Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Nanjing University of Chinese Medicine, Nanjing, China

Category: Design and Application of Biomaterials

Background: In the clinical practice, IntegraTM and MatriDerm® dermal substitute is reported to have higher wound infection rate compared to the synthetic dermal templates. This study aims to investigate how the commensal microbiota are altered by different skin substitutes which can affect wound healing.

Methods: Two synthetic nanofibrous structure scaffolds, polycaprolactone (PCL) and polylactic acid-co-glycolic acid copolymer (PLGA) were produced using electrospinning, while commercial dermal templates including IntegraTM and MatriDerm® were also examined characteristically. Db/db mice with a 1cm² wound on the dorsal area were utilized as the diabetic wound healing model. Wound healing rates were measured at day 0, 3, 7, 14, 21 and compared between four skin substitute groups. Wound tissues were collected for histological and molecule analysis. The wound microbiota was harvested and collected at each time point prior to 16s rRNA gene sequencing. Serum was also harvested to detect the expression of inflammatory markers at day 3 and 7. To explore the effect of internal structure on wound microbiota, 3D printed PCL scaffolds was prepared while electrospun collagen scaffolds were also fabricated for further examination.

Results: Two synthetic electrospun PCL and PLGA scaffolds were successfully fabricated within nanofibrous structure. In the animal study, these two scaffolds were found to significantly accelerate wound healing compared to commercial dermal templates IntegraTM and MatriDerm®. The results of 16s rRNA showed that on day 7, the PCL group and PLGA group has significantly higher alpha diversity estimators than that of the MatriDerm® group. On day 14, only PCL group alpha diversity estimators were significantly higher than the MatriDerm® group. Additionally, two synthetic electrospun scaffolds can alter the genus level of wound microbiotas, significantly increasing Escherichia-Shigella and reduced Staphylococcus. The metagenomic analysis indicated that the main strain in the genus-level of Escherichia-Shigella is E. coli, and the main strain in Staphylococcus are S. xylosus and S. aureus. However, the inflammation was not influenced by altered wound microbiota or scaffolds over healing process. We then examined whether the internal structure of the skin substitute is the secondary factor affecting wound microbiota. The internal structural were characterized between the best and the worst healing rate skin substitutes, PCL vs. MatriDerm®. By using the alcohol immersion method to calculate porosity, it was found that the porosity of the PCL group was significantly higher than that of the MatriDerm® group. ImageJ analysis showed that the pore size of the MatriDerm® group was significantly larger than that of the PCL group. The water vapor transmission of the MatriDerm® group was found to penetrate more compared to the PCL group.

Discussion and Conclusion: In this study, we examined both natural and synthetic skin substitutes on wound microbiota and wound healing. The result showed that nanofibrous synthetic skin substitutes can increase the diversity of wound microbiotas, resulting in significantly enhanced wound repair in diabetic mice.
Red blood cell-mimicking liposomes loading curcumin promote diabetic wound healing by absorbing toxins and mediating inflammatory response

Ms. Qinghan Tang¹, Dr Yiwei Wang¹², Dr Jun Chen¹²

¹Jiangsu Provincial Engineering Research Center of TCM External Medication Development and Application, Nanjing University of Chinese Medicine, Nanjing, China, ²Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Nanjing University of Chinese Medicine, Nanjing, China

Background: The excessive inflammatory response in diabetic skin ulcer wounds and the toxins produced by bacteria at the wound area further aggravating inflammation, which greatly inhibit the healing process. Therefore, design of a novel biomaterial-based system that can improve the inflammatory microenvironment and absorb the bacterial toxins in wound area is urgently needed.

Methods: In this study, a red blood cell-mimicking liposomes loading curcumin (RC-Lips) was fabricated to act as a "sponge", aiming to adsorb bacterial toxins. Moreover, curcumin was loaded in the liposome as it is known to prevent the excessive inflammation. The “sponge” liposomes were investigated for the cell polarization of macrophages, wound healing rate, and inflammatory markers both in vitro and in vivo.

Results: The red blood cell-mimicking liposomes fused with the red blood cell membrane were prepared successfully with desirable particle size, stability, a good encapsulation rate, and proved in vitro adsorption of bacterial toxins. Cell culture study showed that red blood cell-mimicking liposomes can inhibit the transformation of M0 macrophages to pro-inflammatory M1 type, while promoting the cell polarization of M2 with anti-inflammatory effect. In addition, red blood cell-mimicking liposomes can alleviate the damage and pro-apoptotic effects of bacterial toxins on keratinocytes, thereby promoting the cell proliferation and migration. In the animal studies, it was found that red blood cell-mimicking liposomes significantly reduced the wound area and promoted re-epithelialization. Molecular analysis further revealed that red blood cell-mimicking liposomes significantly down-regulated inflammatory response and increased the expression of anti-inflammatory markers.

Discussion and Conclusion: We demonstrated that the red blood cell-mimicking liposomes have dual effects of regulating the balance of inflammatory response in diabetic wounds as well as adsorbing and alleviating the bacterial toxins, thereby significantly accelerating wound healing in diabetic mice.
A Tunable Crosslinked Collagen-Based Dermal Template for Third Degree Burn Wound

Weng Wan CHAN1, Keya Rani ROY2, Dr. Bach Le1, Hariharan EZHILARASU1, Xiaqian ZHANG2, Ryan Yi Da LIM2, Avinanda BANERJEE2, Mariya KURIAKOSE2, Krystle Joy NG2, Priya MURUGAN1, Chun Ting GOH1, May Win NAING3, SriKala RAGHAVAN2, Deepak CHOUDHURY1


Authors: Weng Wan Chan#1, Keya Rani Roy#2, Bach Quang Le1, Hariharan Ezhilarasu1, Xiaqian Zhang, Ryan Yi Da Lim, Avinanda Banerjee, Mariya Kuriakose, Krystle Joy Ng, Priya Murugan1, Chun Ting Goh1, May Win Naing3, SriKala Raghavan*2, Deepak Choudhury*1. # Contributed equally to this work.


Correspondence: Deepak_Choudhury@bti.a-star.edu.sg

Background: Third-degree burns are severe injuries that result in the complete destruction of the skin's epidermal and dermal layers. Current treatment options for these types of burns are limited, and there is a critical need for effective and safe regenerative therapies. Many skin grafts have been developed using xenogeneic collagen from bovine, porcine, or ovine sources as the base biomaterial. These collagens have been shown to be well-integrated in the wound site and serve as a scaffold for neodermis formation. However, existing products such as the Integra dermal template utilize dehydrothermal (DHT) crosslinking, which is time-consuming and often results in inconsistent crosslinking, particularly for large scaffolds.

Methods: In this study, we present a novel fabrication process for a collagen dermal template that consists of a tunable freeze-drying and crosslinking process. This process allows us to fine-tune the crosslink density and degradation profile of the template. We performed in vitro characterization as well as in vivo validation in a full-thickness skin wound mouse model. For control, we used the IntegraTM dermal template collagen sponge.

Results: We demonstrated the fabrication reproducibility using physical and chemical characterization methods to measure the template's pore structure, crosslinking density, and in vitro degradation profile. These parameters are known to affect both take rate and regenerative capacity of the template in vivo. Furthermore, our collagen-based dermal template is cell-free, reducing the risk of rejection and simplifying the manufacturing process. In vitro studies demonstrated that our collagen dermal template had similar pore size range and equivalent degradation profile to the Integra sponge. However, our collagen template supported the proliferation of human fibroblast cell line WS-1 better than the Integra template after 2 weeks in culture. In vivo data suggested a similar level of skin regeneration/repair between our sponge template compared with IntegraTM. We did not see any abnormal angiogenic responses or a heightened immune response with our scaffolds.

Discussion and Conclusion: Our approach offers a promising alternative to existing collagen-based skin grafts. For future work, we will incorporate various bio-additives to enhance angiogenesis and test the template in a burn wound animal model. Following proof-of-concept fabrication, we will utilize a biomanufacturing process to demonstrate industrial-scale production. This research is part
of the programmatic grant Additive Manufacturing for Biological Materials (AMBM) in Singapore, which aims to develop the next generation wound dressing comprising of regenerative dermal template, printable cells, and sensor in a scaled-up fabrication process.

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Improving Cell Adhesion on Plant-based Scaffolds for Tissue Manufacturing

Dr Ratima Suntornnond, Ms Wee Swan Yap, Ms Priyatharshini Murugan, Mr Hariharan Ezhilarasu, Ms Yiting Liu, Dr Deepak Choudhury

1Biomanufacturing Technology, Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore

Title: Improving Cell Adhesion on Plant-based Scaffolds for Tissue Manufacturing

Authors: Ratima Suntornnond, Yap Wee Swan, Priyatharshini Murugan, Hariharan Ezhilarasu, Yiting Liu, Deepak Choudhury

Affiliations: 1Biomanufacturing Technology, Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore (Correspondence: *Deepak_Choudhury@bti.a-star.edu.sg)

Category: Design and Application of Biomaterials

Background: Plant-derived scaffolds are increasingly used in various applications due to their abundant natural sources and sustainability advantages over animal-derived scaffolds. However, while plant-based scaffolds can replicate the shape, mechanical properties, and macrostructure required for cellular penetration, they are limited by the absence of the extracellular matrix (ECM) that exists in native tissue. This usually leads to inadequate and inconsistent cell adhesion. Therefore, it is crucial to develop strategies to improve the surface adhesion of these scaffolds to ensure their long-term viability and efficacy. Here, we introduce our in-house developed coating reagents to modify the surface properties of the scaffolds, which can enhance their ability to support cell adhesion and proliferation.

Methods: The proprietary coatings were formulated using non-animal origin ingredients to ensure their safety and sustainability. The plant-derived scaffolds used in this study were fabricated via a decellularisation process. Water contact angle (WCA) measurement and Bicinchoninic acid assay (BCA) were used for characterising the coated scaffolds. Preliminary cell culture has been carried out using C2C12 mouse myoblasts.

Results: The water contact angle (WCA) method was used to assess the degree of hydrophobicity of the developed scaffolds and that indicated that the functionalised scaffolds exhibited a lower degree of hydrophobicity compared to non-modified scaffolds. The Bicinchoninic acid assay (BCA) is used to measure protein adsorption. The BCA assay showed that the functionalised scaffolds exhibited significantly higher protein adsorption than non-modified scaffolds. The cell viability and cell proliferation test using C2C12 mouse myoblasts demonstrate that functionalised scaffolds perform better than non-functionalised scaffolds.

Discussion and Conclusion: Coating reagents have been shown to enhance cell adhesion on plant-derived scaffolds. Cell studies on C2C12 emphasise that the modification process significantly improved cell proliferation compared to no coating condition. These findings highlight the potential of these functionalised scaffolds for use in various tissue engineering applications – ranging from regenerative medicine to cell therapy to cellular agriculture.

References
Acknowledgement: This work is supported by the National Research Foundation, Singapore, and the Agency for Science, Technology and Research (A*STAR), under the Singapore Food Story R&D Programme (Grant Number H20H8a0003 awarded to Deepak Choudhury).
Sprayable CIP-loaded Ti3C2 MXene/SA Hydrogel for Wound Healing and Drug Release System

Mr. Hyeongtaek PARK¹, Ph.D Hwan KIM¹,²,³
¹Department of IT Convergence (Brain Korea Plus 21), Korea National University of Transportation, Chungju, Republic of Korea, ²Department of Polymer Science and Engineering, Chungju, Republic of Korea, ³Department of Biomedical Engineering, Chungju, Republic of Korea

Background: Wound healing is essential for maintaining tissue and organ function and protecting the body from infections and harmful stimuli. Despite our body's excellent wound regeneration ability, if the wound healing time is delayed due to aging, diabetes, or eating habits, bacterial infection can cause secondary problems. Therefore, studying how to inhibit bacterial infection and effectively heal wounds is necessary.

Methods: To achieve effective wound healing, ciprofloxacin (CIP) antibiotic was loaded onto MXene, and the CIP-loaded MXene was mixed with sodium alginate (SA) at a 1:1 ratio, then transferred to a spray bottle and sprayed with a 4wt% CaCl₂ solution to form a CIP-loaded MXene/SA hydrogel with excellent antibacterial activity. The wound-healing ability of the hydrogel was tested in vitro and in vivo.

Results: CIP-loaded MXene/SA hydrogel was prepared by mixing ciprofloxacin-loaded MXene with sodium alginate and spraying CaCl₂ and showed excellent photothermal conversion effect and biocompatibility under NIR irradiation. In addition, drug release by NIR control showed enhanced antibacterial activity against E. coli and S. aureus. MXene/SA hydrogel characteristics accelerated wound healing in vitro and in vivo.

Discussion and Conclusion: We designed a CIP-loaded MXene/SA hydrogel to provide rapid drug release via NIR, promote wound healing, and prevent bacterial infection. This drug delivery system demonstrated its effectiveness in achieving efficient wound healing based on its excellent photothermal conversion ability and antibacterial activity. This promising approach has shown potential in future tissue engineering applications for wound healing.

Acknowledgment: This work was supported by the Korean Fund for Regenerative Medicine (Code: KFRM 22A0105L1-11) and the Ministry of Science and ICT of Korea (NRF-2021R1C1C2004576).
Decellularized Plant-based Scaffolds for Cultured Meat Production

Dr. Deepak Choudhury

BTI A*STAR, Singapore

Introduction: Cultured meat/Cultivated Meat is gaining popularity as a substitute for animal agriculture. It involves isolating and expanding animal muscle cells in a lab1,2. However, the creation of animal-free scaffolds is crucial to fabricate a structured meat product that mimics conventional meat which should be edible, dense, nutritious, and possesses suitable mechanical properties for cell growth3.

Subjects and Methods: We have created highly macro-porous and tuneable plant-based edible scaffolds that provide an advantage in producing textured meat. These scaffolds have unique properties that promote cell alignment and orientation, essential for muscle cell differentiation. We evaluated the attachment, proliferation, and differentiation of various mammalian cells on these scaffolds.

Results: We used our proprietary scaffolds with C2C12 myoblasts to develop a model system for a cultured meat prototype. In vitro studies demonstrated that the scaffold's microarchitecture and stiffness support cell attachment, proliferation, and differentiation into muscle tissue. To demonstrate the feasibility, we cultured porcine adipose-derived mesenchymal stem cells (pADMSCs) and differentiated them into muscle tissue in our scaffolds, co-cultured with differentiated fat cells.

Discussion and Conclusion: PrestoBlue and Live/Dead staining of C2C12 and pADMSCs in our plant-based scaffold revealed enhanced cell attachment and proliferation. Upon serum starvation, the proliferated cells differentiated into myotubes, as confirmed by gene expression analysis and immunofluorescence staining of muscle markers such as myosin heavy chain and Desmin. Co-culturing porcine-derived adipocytes with muscle cells brings the prototype closer to actual meat.

Figure1: Realization of a Cultured Meat prototype using a plant-based scaffold

References:


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Efficient biocompatible magnetoelectric nanoparticles for biomedical applications

Dr. Roman Chernozem
Tomsk Polytechnic University, , Russian Federation

Title: Efficient biocompatible magnetoelectric nanoparticles for biomedical applications

Authors: Roman Chernozem1,*, Maria Surmeneva1, Andrei Kholkin1,*, Roman Surmenev1,*

Affiliations: 1Piezo- and magnetoelectric materials center, Tomsk Polytechnic University, Tomsk, Russia. (*Correspondence)

Category: Design and Application of Biomaterials

Background: Nanoscale structures offer an excellent opportunity as minimally invasive surgical tools because of their strong towing force and functionality. Recently, wirelessly powered nanorobots (NRs) based on the magnetoelectric (ME) materials have attracted great research interest in the diverse biomedical fields from targeted drug delivery to environmental remediation. Due to the presence of both piezoelectric and magnetic phases, ME NRs can be precisely delivered and polarized under external magnetic field, allowing to wireless locomotion and on-site triggered therapeutic release.[1] Despite the appealing features of ME NRs, there are several aspects that require considerable research efforts as follows: biocompatibility, reproducibility, biodistribution, etc. Thus, the development of technological approach for the fabrication of novel ME NRs using biocompatible materials is essential.

Methods: For the first time, we designed core-shell ME nano-heterostructures based on the biocompatible magnetostrictive MnFe2O4 (MFO) and ferroelectric Ba0.85Ca0.15Zr0.1Ti0.9O3 (BCZT) relaxors using a cost-effective microwave-assisted hydrothermal synthesis. The extensive characterization has been performed to study the morphology, composition, structure, phase interface, magnetic properties and catalytic behavior of the developed ME NRs.

Results: It was demonstrated that a variety of the duration and temperature of the hydrothermal synthesis leads to in the morphology changes of MFO cores from quasi-spherical to rod-like with a subsequent increase in the magnetization values and reduction in microstress values. The analysis of the composition and structure revealed the hetero-epitaxial growth of the thin BCZT-shell (2-5 nm) on the surface of PVP- and OA-functionalized MFO cores as shown in images (Figure 1A) of High-resolution transmission electron microscopy (HRTEM) and Energy-dispersive X-ray spectroscopy mapping (EDX). Whereas, no shell was revealed in the case of pristine MFO cores. Raman spectroscopy confirmed the presence of ferroelectric non-centrosymmetric BCZT phases, such as tetragonal, pseudo-cubic, orthorhombic. The formation of the thin (2-5 nm) BCZT shell reduced the magnetization of PVP- and OA-functionalized MFO cores from 63.99 ± 0.31 to 18.68 ± 0.13 emu/g and from 67.43 ± 1.30 to 20.74 ± 0.22 emu/g, respectively. Nevertheless, developed core-shell ME NRs promoted the degrading of the Rhodamine B (RhB) pollutant with more than 95% efficiency within 2.5 h under AC magnetic field (150 mT, 100 Hz) treatment, indicating the ROS-groups generation due to the direct ME effect (Figure 1B).

Figure 1. (A) HRTEM and EDX mapping images for core-shell ME MFO@BCZT NRs based on the OA-functionalized MFO cores: red lines and white arrows indicate the BCZT shell. (B) Dependence of the concentration of a model organic dye, RhB, from different concentrations of ME MFO_PVP@BCZT (star symbol) and MFO_OA@BCZT (triangle symbol) NRs subjected under the magnetic field (150 mT, 100 Hz) for different times: orange – 1 mg/ml; green – 4 mg/ml; red – 8 mg/ml.
Discussion and Conclusion: We demonstrated that the developed biocompatible ME MFO@BCZT NRs have high magnetic properties and an efficient ME conversation, leading to catalytic degradation of some dyes/pollinations, such as RhB. Thus, the designed ME NRs based on the biocompatible MFO and BCZT can be considered as a promising tool for non-invasive biomedical applications, such as regenerative medicine.

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SFRP4+stromal cell subpopulation with IGF1 signaling in human endometrial regeneration

Ms. Yu Li1,2,3, Bingbing Wu1,2,3,4, XiaoHui Zou1,2,3*

1Clinical Research Center, the First Affiliated Hospital, School of Medicine, Zhejiang University, Zhejiang, China, 2Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cell and Regeneration Medicine, Zhejiang University, Zhejiang, China, 3Zhejiang Provincial Key Laboratory of Tissue Engineering and Regenerative Medicine, Zhejiang, China, 4International Institutes of Medicine, The 4th Affiliated Hospital of Zhejiang University School of Medicine, Zhejiang, China

Title: SFRP4+stromal cell subpopulation with IGF1 signaling in human endometrial regeneration

Authors: Yu Li1,2,3, Bingbing Wu1,2,3,4, XiaoHui Zou1,2,3*

Affiliations: 1Clinical Research Center, the First Affiliated Hospital, School of Medicine, Zhejiang University, Zhejiang University, Hangzhou, Zhejiang 310003, PR China. 2Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cell and Regeneration Medicine, Zhejiang University, Zhejiang, China. 3Zhejiang Provincial Key Laboratory of Tissue Engineering and Regenerative Medicine, Hangzhou, Zhejiang 310058, PR China. 4International Institutes of Medicine, The 4th Affiliated Hospital of Zhejiang University School of Medicine

Category: Tissue Engineering and Regeneration

Background: Full-thickness injury or dysfunction of the human endometria causes intrauterine adhesion, miscarriage, and uterine factor infertility. However, little is known about the underlying mechanism of human endometrium maintaining its highly dynamic properties of repeated injury and scar-less repair during the menstrual cycle.

Methods: First, single-cell RNA-seq was used to profile single-cell atlas from seven full-thickness normal human uterine tissues from two menstrual phases (3 from the proliferative-NP and 4 from secretory phase-NS). Next, in vitro cell coculture of endometrial stromal cells with endometrial epithelial organoids was performed to confirmed cell-cell interaction. Last, an in vivo rat model of full-thickness endometrial injury was constructed to verify the effect of the key IGF1 signal molecule secreted from SFRP4+ stromal cells.

Results: We dissected cell heterogeneity of main cell types (epithelial, stromal, endothelial, and immune cells) of the full thickness uterine tissues, cell population architectures of human uterus cells across the menstrual cycle. We identified an SFRP4+ stromal cell subpopulation highly enriched in the regenerative stage of the human endometria during the menstrual cycle, and the SFRP4+ stromal cells could significantly enhance the proliferation of human endometrial epithelial glands and full-thickness endometrial injury through IGF1 signaling pathway in vivo.

Conclusion: The cell atlas of full-thickness uterine tissues revealed the cellular and molecular mechanisms regulating the monthly regeneration of human endometria, which provides insights into the biology of human endometrial regeneration and the development of regenerative medicine treatments against endometrial damage and intrauterine adhesion.

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LINC00346 suppresses PI3K/AKT/NF-κB signaling pathway through miR-25-3p/PTEN axis in inflammatory conditions

Prof. Won-Ha Lee¹, Min-Ji Kim¹, Su-Geun Lim¹, Dong-Hyung Cho¹, Jun-Yeong Lee¹, Kyoungho Suk²
¹School of Life Sciences, BK21 FOUR KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea, ²Department of Pharmacology, Brain Science & Engineering Institute, BK21 FOUR KNU Biomedical Convergence Program, Kyungpook National University School of Medicine, Daegu, South Korea
Title: LINC00346 suppresses PI3K/AKT/NF-κB signaling pathway through miR-25-3p/PTEN axis in inflammatory conditions

Authors: Min-Ji Kim¹, Su-Geun Lim¹, Dong-Hyung Cho¹, Jun-Yeong Lee¹, Kyoungho Suk², and Won-Ha Lee¹,*

Affiliations: ¹ School of Life Sciences, BK21 FOUR KNU Creative BioResearch Group, Kyungpook National University, Daegu 41566, Republic of Korea ² Department of Pharmacology, Brain Science & Engineering Institute, BK21 FOUR KNU Biomedical Convergence Program, Kyungpook National University School of Medicine, Daegu 41944, Republic of Korea (* Correspondence)

Category: Design and Application of Biomaterials

Background: Long intergenic non-coding RNA 346 (LINC00346) has been reported to be involved in developing atherosclerosis and specific cancers by affecting signaling pathways. However, its function in inflammation has not been thoroughly studied. Therefore, its expression pattern and function were determined in the human macrophage-like cell line THP-1.

Methods: LncRNA levels were detected using qRT-PCR, Protein-level expression was detected using Western blot, RNA-RNA interaction was blocked using decoy RNA, Cytokine secretion was detected using ELISA, and NF-κB activation was detected using a dual-luciferase reporter assay.

Results: Lipopolysaccharide (LPS) treatment induced the expression of LINC00346. LPS-induced NF-κB activation and proinflammatory cytokine expression were suppressed or enhanced by the overexpression or knockdown of LINC00346, respectively. Analyses using dual luciferase assay and decoy RNAs that could block RNA–RNA interactions indicated that LINC00346 improves phosphatase and tensin homolog (PTEN) expression by sponging miR-25-3p. Subsequently, PTEN suppresses phosphoinositide-3 kinase (PI3K)-mediated conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3) as well as consequent activation of protein kinase B (AKT) and NF-κB. Interestingly, database analysis revealed that the expression levels of LINC00346 and PTEN were simultaneously decreased in breast cancer tissues. Further analyses conducted using a breast cancer cell line, MDA-MB-231, confirmed the functional relationship among LINC00346, miR-25-3p, and PTEN in LPS-induced activation of NF-κB.

Discussion and conclusion: These results indicate that miR-25-3p-sponging activity of LINC00346 affects the balance between PTEN and PI3K as well as the downstream activation of AKT/NF-κB pathway in inflammatory conditions.

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Biomimetic composite gelatin methacryloyl hydrogels for improving survival and osteogenesis of human adipose derived stem cells in 3D microenvironment

Ms. Eunhyung Kim¹, Ph.D Heungsoo Shin¹
¹Department of Bioengineering, Hanyang University, Seoul, South Korea

Title: Biomimetic composite gelatin methacryloyl hydrogels for improving survival and osteogenesis of human adipose derived stem cells in 3D microenvironment

Authors: Eunhyung Kim¹, Heungsoo Shin¹, *

Affiliations: ¹Department of Bioengineering, Hanyang University, Seoul, Korea (* Correspondence)

Category: Tissue Engineering and Regeneration

Background: Cell-laden gelatin methacryloyl hydrogel (GelMA) has been widely used in bone tissue engineering because of its similarity to natural extracellular matrix (ECM), biocompatibility, and ease of modulating mechanical properties. However, reactive oxygen species (ROS) generated by photocrosslinked hydrogels can induce limited osteoinductive properties of the hydrogels, negatively affecting viability and differentiation of encapsulated cells. Consequently, GelMA hydrogels must be engineered to remove ROS and induce osteogenesis in stem cells in order to engineer bone tissue.

Methods: We synthesized novel nanoparticles by self-assembling the tannic acid (TA) and simulated body fluid biominerals through metal-phenolic network. Then, composite GelMA hydrogels were fabricated with tannic acid mineral nanoparticles (TMN) and human adipose-derived stem cells (hADSCs) (7.5 w/v% GelMA, 8 mm diameter, 2 mm height, cylindrical shape). Then, we analysed the delivery of instructive signals to cells by particles encapsulated in the hydrogel and osteogenic differentiation of stem cells both in vitro and in vivo.

Results: As the amount of TMN in the hydrogel was increased, the radical scavenging effect was enhanced, leading to improved cell viability. Live and dead staining showed a reduced number of dead cells in the hydrogel in the presence of TMN compared to pristine GelMA hydrogel after 7 days of culture. Moreover, encapsulated hADSCs in hydrogels containing TMN resulted in higher levels of deposited calcium ions and osteogenic gene expression compared to pristine hydrogels. In vivo experiments also showed greater osteogenic differentiation in encapsulated cells in hydrogels containing TMN.

Discussion and Conclusion: In this study, we developed a composite GelMA hydrogel containing TMN. The particles were made from TA and biominerals to address the limitations of cell encapsulated GelMA hydrogels, such as oxidative stress and limited cell induction. The results showed that TMN in the hydrogel successfully enhanced cell viability and proliferation by alleviating oxidative stress-induced cell death. In addition, TMN in the hydrogel induced the encapsulated cells to differentiate into osteogenic lineages. In vivo tests demonstrated osteogenesis of the transplanted cells, suggesting that TMN solves the problems of stem cell encapsulated photocrosslinkable hydrogels and can be used to treat bone defects.

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Ultrashort Echo Time Magnetization Transfer Imaging of Knee Cartilage After Long-Distance Running

Ms. Dantian Zhu¹
¹Fifth Affiliated Hospital, Sun Yat-sen University, China

Introduction: Long-distance running is related to injury of the knee cartilage, which can eventually develop into osteoarthritis. Non-invasive MR monitoring of early changes in the cartilage allows for timely intervention of osteoarthritis progression and can prolong the life of exercise. This study aims to assess the detection of changes in the knee cartilage of amateur marathon runners before and after long-distance running using 3D ultrashort echo time magnetization transfer (UTE-MT).

Subjects and Methods: We recruited 23 amateur marathon runners (46 knees) in this prospective cohort study. MRI scans using ultrashort echo time magnetization transfer (UTE-MT) sequences were performed 3 times (pre-race, 2 days post-race, and 4 weeks post-race). UTE-MT ratio (UTE-MTR) were measured for knee cartilage (eight subregions) before and after running.

Results: For most subregions of cartilage, the UTE-MTR values decreased 2 days post-race and increased after 4 weeks of rest. The UTE-MTR values in lateral tibial plateau, central medial femoral condyle, and medial tibial plateau showed a significant decrease at 2 days post-race compared to the other two time points (P < 0.05).

Discussion and Conclusion: The UTE-MT technique characterizes the tissue system with a two-pool model which includes the water and macromolecular proton pools [1]. The magnetizations in these two pools are constantly being exchanged. Even though the UTE sequence is unable to detect the signal from macromolecular protons [2], the UTE-MT technology provides indirect information about the content and integrity of macromolecules such as collagen and proteoglycans by using exchange effects. Our study found that UTE-MT technology can monitor the injury and repair of macromolecular components in knee cartilage after long-distance running. UTE-MTR is a promising method for the detection of dynamic changes in knee cartilage after long-distance running.

References
Effects of the peptide bond cis-trans isomerization of peptoid residues on the collagen triple-helix structure

Mr. Rong Mao QIU¹, Ms. Xiao Jing Li¹, Doc. Yang Li¹
¹The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, China

Title: Effects of the peptide bond cis-trans isomerization of peptoid residues on the collagen triple-helix structure

Authors: Rongmao Qiu1, Xiaojing Li1, Yang Li1*

Affiliations: 1 Guangdong Provincial Key Laboratory of Biomedical Imaging and Guangdong Provincial Engineering Research Center of Molecular Imaging, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, China (* Correspondence)

Category: Design and Application of Biomaterials

Background: Collagen is the most abundant protein in the human body and a major component of the extracellular matrix. All of the peptide bonds in collagen must adopt the trans conformation to form its hallmark structural motif, the triple helix. Previous studies using synthetic collagen mimetic peptides (CMPs) found that proline derivatives with a lower trans: cis ratio tend to destabilize the collagen triple helix. However, these studies were mainly based on proline analogs, and the direct effect of cis-favoring residues on the triple helix is unclear.

Methods: In this work, we used collagen mimetic peptides (CMPs) with GlyProHyp repeat sequence as the host peptide X-CMP to investigate the effect of the peptoid residue guest X on the collagen triple-helix structure including stability and folding. Based on the specific properties of peptoid residues, we also designed and synthesized a novel CMP that can maintain a single-chain state but have strong triple-helix hybridization ability and verified their ability to target denatured collagen in myocardial infarction.

Results: The circular dichroism (CD) spectrum showed that strongly cis-favoring peptoid residues can form a fairly stable collagen triple-helix but slow down its folding rate significantly. Based on the properties of strongly cis-favoring peptoid residues, we designed and synthesized N2pic3-CMP, which cannot fold triple-helix at pH 3.0, but can rapidly fold into a hyperstable triple-helix at pH 7.4. The in vivo evaluation showed that Cy5 labeled N2pic3-CMP can target denatured collagen in the fibrotic lesion of myocardial infarction.

The scheme shows that cis-favoring peptoid residues can form stable collagen triple-helix but slow down the folding rate.

Discussion and Conclusion: The cis-trans propensity of peptoid residues had little effect on the stability of the collagen triple-helix, but affected the folding rate of the collagen triple-helix. Our work expanded the fundamental understanding of the relationship between cis-trans isomerization and protein folding rates, and showed that cis-peptide bond favoring peptoid residues could be used for the design and construction of new peptidomimetics with specific folding and biological activities.

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Morphology-based prediction of extracellular vesicle production potency of mesenchymal stem cells

Mr. Taiki FURUTANI1, Dr. Yuto TAKEMOTO1, Mr. Kenjiro TANAKA1, Dr. Ryuji KATO1,2
1Graduate School of Pharmaceutical Sciences, Nagoya University, Pharmaceutical Build. 302, Furocho, Chikusaku, Nagoya, Japan, 2Institute of Nano-Life-Systems, Nagoya University, NIC, Furocho, Chikusaku, Nagoya, Japan

Introduction: Despite the growing expectations on cell-based products by the advancement of regenerative medicine, manufacturing of live and delicate cells with medical-grade quality has been a great challenge. Since conventional cell assessment techniques in molecular biology has been invasive, such as staining or expression profiling, it is not compatible with cell-based product manufacturing, which requires to manufacture intact cells as final product. Microscopic observation of cell morphology is still the most practically used technique to check the cellular status in cell manufacturing, however it is not-quantitative and heavily relying on expert’s decisions. Therefore, there are great demand for the development of novel cell assessment technology to enable effective cell quality control.

Subjects and Methods: Our group has been reporting “Morphology-based cell quality prediction technology” by combining the recent image processing and AI-related machine learning technologies to enable non-invasive real-time cell quality control technique during their manufacturing process [1, 2]. In this study, we challenged to apply this technology to predict “extracellular vesicle (EV) production potency” in Mesenchymal stem cells (MSCs) only from their time-course microscopic images. Several lots of MSCs were cultured w/wo EV stimulation, and more than 3000 images were obtained by automatic imaging system. By image processing, we extracted multi-parametric morphological profiles from the images, and constructed machine learning model to discriminate the “high EV production status” of MSCs only from their morphological information. EV production rates were evaluated experimentally as their ground truth, and validated.

Results: Our morphology-based AI model was found to show high performance of discriminating “bad EV production status” only from their images and was evaluated to be effective for early quality monitoring system for cell manufacturing.

Discussion and Conclusion: Our results indicated that the morphological profile of MSCs can tell their quality of “EV production performance”, therefore it can be used not only for the cellular in-process monitoring, but also for donor screening to select the elite donor for the most efficient product manufacturing. Such image-based automatic quality assessment technology will accelerate the automation of cell manufacturing to achieve more cost efficient and higher safety cell-based products.

Reference:

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Clinical Study Of Arthroscopic Repair Of Rotator Cuff Tears

Prof. Jun Chen, Yuting Zhong, Luyi Sun, Han Guo, Shiyi Chan, Chengxuan Yu

Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai, China

Authors: Yuting Zhong, Luyi Sun, Han Gao, Chengxuan Yu, Jun Chen, Shiyi Chen
Affiliations: Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China.

Category: Tissue Engineering and Regeneration

Introduction: Arthroscopic surgery is one of the most common treatment options for repairing rotator cuff tears in clinical practice. However, a certain proportion of tendon retears still occur after rotator cuff repair. It is necessary to explore the risk factors affecting the clinical efficacy after the surgery.

Subjects and Methods: The study is a retrospective cohort study. Patients who were diagnosed with medium-large rotator cuff tears and underwent arthroscopic rotator cuff repair in our department between January 2017 to January 2019 were included and undertaken a minimum 2-year follow-up. Postoperative pain (VAS score), shoulder active range of motion (ROM), shoulder function (CMS, ASES, FUSS score), and magnetic resonance imaging (MRI) outcomes were measured and analyzed to evaluate postoperative clinical efficacy. Meanwhile, according to the tendon healing results under MRI, patients were divided into two groups: the intact group (Sugaya 1-3) and the retear group (Sugaya 4, 5). The factors affecting tendon healing were analyzed by univariate and multivariate analyses. Furthermore, subgroup analysis was conducted between the better healing group (Sugaya 1, 2) and the poor healing group (Sugaya 3-5).

Results: A total of 72 patients (33 patients with medium-sized tears and 39 patients with large-sized tears) were included in this study. The mean age of all patients at surgery was 61.5 years old, and the mean follow-up time was 33.7 months. The overall retear rate was 13.9%. Postoperative flexion (160.1 ± 15.5° vs. 97.6 ± 28.6°; p<0.001), abduction (151.9 ± 26.0° vs. 81.2 ± 28.0°; p<0.001), external rotation (63.8 ± 15.9° vs. 26.5 ± 15.7°; p<0.001), internal rotation (T10 - T11 vs. L4 - L5; p<0.001), VAS (1.4 ±1.5 vs. 6.7 ± 1.5; p<0.001), and ASES score (86.9 ± 11.3 vs. 46.7 ± 12.3; p<0.001) were significantly improved. Compared with the intact group (n=62), patients in the retear group (n=10) was significantly older (66.8 ± 5.3 vs. 60.7 ± 6.4, p=0.005), with a greater postoperative external rotation angle (73.5 ± 10.6° vs. 62.3 ± 16.1°, p=0.037), and a lower postoperative contralateral shoulder abductor muscle strength (7.5 ± 1.4 lbs vs. 9.2 ± 3.6 lbs, p=0.041). Multivariate Logistic regression analysis showed that age was risk factors for retear (OR=1.156, 95%CI [1.009, 1.325]), while tear size, preoperative VAS score, and preoperative joint stiffness did not have significant effects on retear. In subgroup analysis, compared with the better healing group (n=50), patients in the poor healing group (n=22) was significantly older (65.4 ± 5.2 vs. 59.8 ± 6.4, p=0.001), with a larger tear size (3.1 ± 1.0 cm vs. 2.3 ± 0.9 cm, p=0.004) and a lower postoperative ASES score (82.8 ± 15.6 vs. 88.7 ± 8.4, p=0.041). Multivariate Logistic regression analysis showed that risk factors for retear included age (OR=1.166, 95%CI [1.041, 1.306]), tear size (OR=2.495, 95%CI [1.179, 5.278]) and preoperative joint stiffness (OR=7.057, 95%CI [1.493, 33.358]).

Discussion and Conclusion: The overall mid-term clinical efficacy of arthroscopic rotator cuff repair in the treatment of medium-large rotator cuff tears is satisfactory. Patients’ pain was relieved, shoulder function were significantly improved and no obvious limitation of ROM was found. However, retear still occurred in part of patients. Age, tear size, and joint stiffness were factors related to postoperative poor healing.
Finite Element Analysis Of Patch Bridging Reconstruction Of Rotator Cuff Tears

Prof. Jun Chen¹, Yuting Zhong¹, Luyi Sun¹, Han Guo¹, Chengxuan Yu¹, Shiyi Chen¹
¹Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai, China

Authors: Yuting Zhong, Luyi Sun, Han Gao, Chengxuan Yu, Jun Chen, Shiyi Chen
Affiliations: Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China.
Category: Tissue Engineering and Regeneration

Introduction: Patch bridging reconstruction can reduce suture tension and rebuild the mechanical integrity of the rotator cuff. However, problems such as failure of the tendon-patch suture site still exist and may result in retear. Therefore, it is important to explore the biomechanical changes of rotator cuff patch after bridging reconstruction of rotator cuff tears.

Subjects and Methods: This study is a biomechanical finite element analysis. Firstly, geometric reconstruction of the CT and MRI data of a healthy human shoulder joint was conducted by the three-dimensional reconstruction software Mimics, smoothing processing and solid construction were completed by Geomagic software, and the structures of the normal shoulder joint were further reassembled and established in Hypermesh software. After that, a full-thickness defect and retraction model of the supraspinatus tendon was constructed. Meanwhile, the patch, suture and anchor models were constructed as well, and the Marlow method was used to assign material properties. Subsequently, three different suturing models were further establish: single simple suture, single mattress suture and double mattress sutures. Ultimate failure load, failure mode, and stress distribution of each structure and other biomechanical results under different models were calculated and compared.

Results: The ultimate failure load of single mattress suture (71.3 N) was 5.6% greater than that of single simple suture (67.5 N). As for mattress suture, the ultimate failure load (81.5 N) of double mattress sutures was 14.3% greater than that of single mattress suture. The stress distribution on the patch and supraspinatus tendon was concentrated in the perforation of suture. In this study, the failure of bridging reconstruction mainly occurred at the suture perforation of the patch, and the specific damage forms included cutting through and button-holing through.

Discussion and Conclusion: A finite element model for the patch bridging reconstruction of full-thickness rotator cuff tear was successfully established. Among the conditions involved in this study, double mattress sutures were optimal for patch bridging reconstruction of full-thickness rotator cuff tear.
Tyrosinase-mediated Redox System for Tissue Engineering

Prof. Su-Hwan Kim

Dong-A University, South Korea

Hydrogel system based on enzyme-mediated mild crosslinking reaction has been a promising approach in tissue engineering. Inspired by skin melanin synthesis and marine mussel adhesion, tyrosinase-mediated hydrogel crosslinking has been exploited as cell-friendly reactions and explicit reaction mechanisms. The tyrosinase-mediated reaction is based on oxidation in the active site of the enzyme. Without any cofactor, tyrosinase oxidizes phenolic moieties using only oxygen molecules. As phenolic moieties (e.g., tyrosine, catechol, and polyphenols) are universally expressed in human protein and can be easily conjugated to hydrogel, tyrosinase has been devoted to enzyme-mediated protein and hydrogel crosslinking for tissue engineering and regenerative medicine. Hydrogel prepared by tyrosinase exhibits appealing properties as a dynamic scaffold for cell delivery and as a bioink for 3D bioprinting. Recapitulating the structure of the native extracellular matrix (ECM), innovative tyrosinase-mediated hydrogel crosslinking has now shifted to the field of translational medicine. Biomimetic hydrogel with in situ tyrosinase crosslinking can be efficiently and easily applicable to the disease model for therapeutic purposes. We demonstrate that the novel enzyme-based crosslinking hydrogel has a robust potential in tissue engineering and regenerative medicine.
Addressing the critical quality attribute in Mesenchymal stem cell therapies via CIRSPR/Cas9 mediated gene knockout.

Mr. Ryota IISAKA¹,², Mr. Evan FUNG¹, Mr. Jeffery HO¹, Mr. Jason CHOI¹, Mrs. Hikari KUROGI¹,², Dr. Eiji KOBAYASHI¹,²
¹Rohto Advanced Research Hong Kong Ltd, Hong Kong, China, ²Regenerative Medicine Research and Planning Division, ROHTO Pharmaceutical Co., Ltd., Kyoto, Japan

Introduction:
Mesenchymal stem cells (MSCs) are multipotent stem cells which are widely known for its immunomodulatory properties and paracrine effects, thereby presenting a great value in various therapeutic applications. It has been reported that several proteins expressed and/or secreted by MSCs play a role in anti-apoptosis as well as anti-fibrosis, and immunosuppressive effect etc. Various genes are commonly regarded as key regulators in the MSC mode of action (MoA), and however, the specific quality attribute (CQA) to address the MSC-MoA has not been elucidated yet to date. This study aimed to address this question by selecting three genes and creating immortalized MSC cell lines that either one, two or all three genes have been knocked out from the genome. Various characterization tests have been conducted to identify which gene expressions play a crucial role in MSC mediated therapeutic effects.

Subject and Method:
Human tissue derived-primary and immortalized MSCs were used to knock out gene X, Y, Z*, using CRISPR-Cas9 system. These established KO-MSCs were then tested under various in vitro assays to test its efficacy including anti-fibrotic, anti-inflammatory and immunomodulatory effect.

Results:
Obtained KO-MSCs presented partial decline in the effect in some of the tested assays, indicating the gene that has been deleted from the genome has an important role in presenting the specific efficacy. Further testings are ongoing to contribute to identifying the CQA of MSC based therapies.

Discussion:
This study utilizes the immortalized MSCs to conduct multiple gene knockout within the same batch, and highlighted the complexity of MSC-MoA in the tested assays. However, these three tested genes have been replenished their important role in the MSC based therapy.

*The three genes selected and the details of experiments could be disclosed on the day of the presentation (currently under discussion related to patent filing).
Effect of different storage conditions of dermal fibroblast conditioned medium (DFCM) on biological properties of fibroblast cells

Ms. VINOOTHINI KARUNNANITHY², Associate professor Mh Busra FAUZI¹, Associate Professor Yogeswaran LOKANATHAN¹, Associate Professor Ng MIN HWEI¹, Dr Manira MAAROF¹
¹Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Cheras, Malaysia

Title: Effect of different storage conditions of dermal fibroblast conditioned medium (DFCM) on biological properties of fibroblast cells

Author: Vinoothini Karunnanithy¹, Mh Busra Fauzi¹, Yogeswaran Lokanathan¹, Min Hwei Ng¹, Manira Maarof¹*

Affiliations: ¹Centre of Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras Kuala Lumpur Malaysia (*Corresponding author’s email: manira@ppukm.ukm.edu.my)

Category: Tissue Engineering and Regeneration

Introduction: Dermal fibroblast conditioned medium plays a significant role in skin regeneration in vitro and vivo studies. However, the stability of the protein secreted remains undiscovered. This study aimed to investigate the effect of the DFCM stored in different storage conditions on the biological properties of fibroblast cells.

Methods: The confluence human dermal fibroblast at passage 3 were washed and incubated at 37 °C in a 5% CO2 incubator for 72 h with fresh serum-free fibroblast-specific medium ((F-12: Dulbecco’s Modified Eagle medium without serum; Sigma, USA) (referred to as FM), and the spent medium was collected as DFCM-FM. The DFCM-FM was stored in different storage temperatures (room temperature, 4 °C, -20 °C, and -80 °C) and storage times (1, 3, and 6 months). The protein profile was studied by conducting a BCA assay and SDS-PAGE. The cell attachment, proliferation, and migration analysis was done to study the effect of DFCM. The immunocytochemical staining (ICC) was conducted by staining the DFCM-treated fibroblast cells with Ki67 and Collagen type I antibody to study the proliferation efficacy and expression of collagen type I respectively.

Results: Protein concentration of DFCM-FM stored at -80°C was maintained for up to 6 months. The SDS-PAGE result showed the same protein bands in DFCM-FM at all different temperatures for 6 months. The DFCM-FM stored for 1, 3, and 6 months have a higher attachment and proliferation rate compared to the control group regardless of the different temperatures. Fibroblast treated with DFCM-FM stored at different temperatures for up to 6 months shows a comparable migration rate compared to control groups. Immunocytochemical staining results showed that the fibroblasts treated with DFCM-FM stored at every condition expressed Collagen type I protein. Meanwhile, rabbit monoclonal Ki67 stained fibroblast cells stored at -20°C and -80°C, exhibited higher proliferative cells compared to other groups.

Conclusion: This study concludes that the secreted proteins in DFCM-FM stored at -20 °C and -80 °C up to 6 months maintained exhibited low protein degradation with the molecular weight of proteins in a range of 35 -180 kDa. The findings showed that DFCM can be stored up to 6 months in -20 °C and -80 °C and maintains the effect on fibroblast biological properties which includes cell attachment,
proliferation, migration and protein expressions. However, further studies are needed to specify the proteins maintained in the different storage conditions.

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Ambient intelligence for cell manufacturing digital transformation

Mr. Kengo Momose¹, Mr. Takeru Shiina¹, Dr. Yuto Takemoto¹, Mr. Kenjiro Tanaka¹, Dr. Kei Kanie¹, Dr. Ryuji Kato¹,²

¹Graduate School of Pharmaceutical Sciences, Nagoya University, Pharmaceutical Build. 302, Furocho, Chikusaku, Nagoya, Japan, ²Institute of Nano-Life-systems, Institutes of Innovation for Future Society, NICT, Furocho, Chikusaku, Nagoya, Japan

Title: Ambient intelligence for cell manufacturing digital transformation
Authors: Kengo Momose¹, Shiina Takeru¹, Yuto Takemoto¹, Kenjiro Tanaka¹, Kei Kanie¹, Ryuji Kato¹,²*

Affiliation: ¹Graduate School of Pharmaceutical Sciences, Nagoya University, ²Institute of Nano-Life-systems, Institutes of Innovation for Future Society, Nagoya University

Category: Enabling Technologies

Introduction: Cell-based therapies represent a promising next-generation medical modality for traditionally incurable diseases and defects. As a result, there is a rapidly increasing demand for large-scale, reliable, and stable cell manufacturing. However, the current cell culture process is heavily reliant on manual operations, and the technological development of the cell manufacturing industry lags behind other manufacturing industries.

The rapid advancement of image processing technology has greatly facilitated the quantification of human activities, which were previously difficult to comprehend with quantitative data. In the medical field, the application of image-based quantitation for expert operations and patient activities is known as "ambient intelligence" [1]. This approach is considered an important data transformation (DX) challenge for establishing data-driven intelligent facility management. These studies suggest that DX represents an efficient approach for enhancing manual-dependent processes.

Subjects and Methods: In this work, we challenged to apply image-based quantification “ambient intelligence” approach on cell culture operation to quantitatively evaluate the operator’s culture skill and make the operation education more efficient. Using the video data in clean bench operations from several operators, we applied the skeleton estimation algorithm to extract the track data of operator’s cell-passage operation. We also developed original data summarization algorithm to convert the track data into operation actions and compared their operation characteristics and their educational effects.

Results: The results of our study demonstrate that data-driven operation profiling using video analysis is an effective method for both operators and educators to standardize operational skills and facilitate the effective training of operators to perform consistent operations.

Discussion and Conclusion: Our findings indicate that ambient intelligence represents an effective technique for DX of unstable cell culture processes and for enhancing the performance of novice operators through the use of quantitative, objective data. These results suggest that ambient intelligence-assisted cell manufacturing represents the next-generation approach for standardizing complex cell culture operations and improving the efficiency and safety of cell manufacturing.


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Enabling Cultivated Meat with Tissue Engineering Technologies

Dr. Yeshi Liang 1
1GFI Consultancy, Shanghai, China

Introduction: Facing the emerging food security and climate challenges, alternative proteins appear as one of the most promising solutions to produce food more sustainably, efficiently, and safely. Among the three pillars of alternative proteins, cultivated meat aims to create meat via tissue engineering technologies rather than growing meat via animals which consumes high energies and produces large quantities of greenhouse gases. This report outlines the latest innovations and trends in the cultivated meat field and the corresponding whitespaces and opportunities.

Methods: Literature review and analysis

Results and Discussion:
- Cultivated muscle and fats
- Restructuring meat: cell spheroid and organoids, cell sheets, 3D printing
- Cell isolation, immortalization, and differentiation
- Low-cost serum-free culture media: plant-based hydrolysates, fermentation products, flavonoids, media recycling technologies
- Scaffolding: industrial scale fabrication techniques and materials variety
- Cultivated meat company and academy landscape

References:
Functional Trachea Reconstruction Using 3D-Bioprinted Native-Like Tissue Architecture Based on Designable Tissue-Specific Bioinks

**Dr. Yingying Huo**, Yujie Hua¹,², Guangdong Zhou¹,²,³

¹Department of Plastic and Reconstructive Surgery, Shanghai 9th People’s Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China; ²Shanghai Key Laboratory of Tissue Engineering, Shanghai, China

Title: Functional Trachea Reconstruction Using 3D-Bioprinted Native-Like Tissue Architecture Based on Designable Tissue-Specific Bioinks

Authors: Yingying Huo¹, Yujie Hua¹ ², Guangdong Zhou¹ ²*

Affiliations: 1Department of Plastic and Reconstructive Surgery, Shanghai 9th People’s Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, PR China. 2Shanghai Key Laboratory of Tissue Engineering, Shanghai, PR China. (*Correspondence)

Category: SYIS (Student and Young Investigator Section) plus Tissue Engineering and Regeneration

Background: Functional segmental trachea reconstruction remains a remarkable challenge in the clinic. To date, functional trachea regeneration with alternant cartilage-fibrous tissue-mimetic structure similar to that of the native trachea relying on the three-dimensional (3D) bioprinting technology has seen very limited breakthrough. This fact is mostly due to the lack of tissue-specific bioinks suitable for both cartilage and vascularized fibrous tissue regeneration, as well as the need for firm interfacial integration between stiff and soft tissues.

Methods: In the current study, we developed a novel strategy for 3D bioprinting of cartilage-vascularized fibrous tissue-integrated trachea (CVFIT), utilizing photocrosslinkable tissue-specific bioinks.

Results: Both cartilage- and fibrous tissue-specific bioinks created by this study provide suitable printability, favorable biocompatibility, and biomimetic microenvironments for chondrogenesis and vascularized fibrogenesis based on the multicomponent synergistic effect through the hybrid photoinitiated polymerization reaction. As such, the tubular analogs are successfully bioprinted and the ring-to-ring alternant structure is tightly integrated by the enhancement of interfacial bonding through the amidation reaction. The results from both the trachea regeneration and the in situ trachea reconstruction demonstrate the satisfactory tissue-specific regeneration along with realization of mechanical and physiological functions.

Scheme. The construction of 3D-bioprinted CVFIT for trachea regeneration in nude mice and in situ trachea reconstruction of rabbits.

Discussion and Conclusion: The current study developed a novel strategy for functional trachea reconstruction using a 3D-bioprinted biomimetic cartilage-vascularized fibrous tissue-integrated trachea. The tubular analogues featuring alternant cartilage and vascularized fibrous tissue rings were successfully bioprinted and the ring-to-ring architecture was tightly integrated through chemically enhanced interfacial bonding. The functional trachea reconstruction in both mechanical and physiological characteristics was successfully achieved due to the alternant stiff-to-soft tissue structure, which were very close to that of the native trachea. This study thus suggests the 3D-bioprinted native tissue-like trachea as a promising alternative for future clinical trachea reconstruction.

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3D Spheroid-microvasculature-on-a-chip For Tumor-endothelium Mechanobiological Crosstalk

Ms. Yingqi Zhang

The University Of Sydney, , Australia

Introduction
Tumor metastasis is tightly regulated and coordinated by hydrodynamic forces. The understanding of cancer metastasis in most of the studies involves individual circulating tumor cells (CTCs) that undergo epithelial-to-mesenchymal (EMT) transition and escape from the primary tumor and intravasate into the blood stream.1 While the importance of CTCs has been emphasized, it is also proposed that CTC clusters, referred to as tumor-derived microemboli or circulating tumor aggregates, may break off from primary tumors and lodge into distal capillaries to initiate metastatic growth. The hematogenous cancer metastases could originate from CTC clusters at the site of primary tumor cell attachment to the vascular wall.2 Therefore, the underlying mechanobiology interplay between CTC clusters and endothelial cells is a fascinating open question to study. In this study, we investigate the effect of Piezo1 (a mechanosensitive ion channel) in regulating 3D tumor spheroid-endothelium crosstalk in an endothelialized microfluidic device cultured with tumor spheroids. Our model enabled a thorough investigation of 1) the EMT transition of, 2) the spreading of, and 3) the effect of shear stress on the MCF-7 wildtype and piezo1 knockout with and without the presence of endothelium.

Methods
Using CRISPR-Cas9 genome editing technology, we knocked out the Piezo1 gene of MCF-7 cells, confirmed by western blotting. Using standard soft lithography microfabrication process, we created parallel microfluidic channels to model venous capillary anatomies on a PDMS chip. Upon microfluidic fabrication, the biofunctionalization of the microfluidic device consists of four major steps: 1) Generate MCF-7 wildtype and piezo1 knockout tumor spheroids using the liquid dome arrays; 2) Seed and culture endothelial cells in the microfluidic channel; 3) Add Piezo1 modulator to the formed tumor spheroids; 4) Seed 3D spheroids into the bare or endothelialized chip; and 4) Apply fluid shear stress.

Results and Discussion
We found that both MCF-7 wildtype and piezo1 knockout tumor spheroids degraded the endothelial junctions upon its invasion and the extravasating tumor impose compressive force on adjacent endothelial cells. VE-cadherin degradation is important in the context of tumor metastasis. The biomechanical compression on endothelium could reflect the higher solid stress and stiffness developed in tumor spheroids. Moreover, MCF-7 wildtype spheroids exhibited significantly slower expansion rates compared to the piezo1 knockout MCF-7 spheroids. This indicates that the existence of piezo1 can help slower the MCF-7 spheroid spreading in the metastasis process. Also, venous shear exposure downregulated the MCF-7 wildtype tumor migration and spreading but not the piezo1 knockout spheroids, which may implicate that the shear activated piezo1 in MCF-7 wildtype, leading to upregulated calcium influx to the spheroids.

References
Biomimetic mechanically strong one-dimensional hydroxyapatite poly(D,L lactide) composite inducing formation of anisotropic collagen matrix

Dr. Yonggang Zhang¹, Prof. Jiaping Li², Prof. Pamela Habibovic²

¹Dalian University of Technology, Dalian, China, ²Maastricht University, Maastricht, Netherlands

Title: Biomimetic mechanically strong one-dimensional hydroxyapatite/poly(D,L lactide) composite inducing formation of anisotropic collagen matrix

Authors: Yonggang Zhang¹*, Jiaping Li² and Pamela Habibovic²

Affiliations: 1School of Bioengineering, Dalian University of Technology, Linggong Road, Dalian 116024, China (* Correspondence); 2MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Instructive Biomaterials Engineering, Maastricht University, Universiteitssingel 40, 6229 ER Maastricht

Category: Design and Application of Biomaterials

Background: Natural bone is a complex composite, consisting predominantly of collagen and hydroxyapatite (HA), which form a highly organized, hierarchical structure from nano- to macroscale. Because of its biphasic, anisotropic, ultra-fine structural design, bone tissue possesses excellent mechanical properties. Herein, inspired by the composition and microstructure of natural bone, a biphasic composite consisting of highly aligned one-dimensional hydroxyapatite (1D HA) and poly(D,L lactide) (PDLA) was developed.

Methods: Biomimetic 1D HA/ Poly(D,L lactide) (PDLA) composite scaffold: First, one dimensional (1D) Sr-doped HA and Cu-doped HA were synthesized through hydrothermal reaction. Desired amount of Sr/Cu-doped 1D HA was mixed with PDLA in acetone and then dried. The biomimetic 1D HA/PDLA composite scaffold with desired dimensions was made by extruding the solutions through a nozzle (24G) by using a 3D plotting system (Bioplotter, Japan).

Results: In biomimetic 1D HA/PDLA composite, the Sr/Cu-doped 1D HA were uniformly distributed within the PDLA matrix and highly aligned along their crystallographic c-axes, similar to HA nanocrystals in natural bone. The presence and alignment of Sr/Cu-doped 1D HA crystals resulted in mechanical reinforcement of the polymer matrix, including compressive and tensile strength, modulus, fracture toughness, swelling resistance and long-term structural stability. Biologically, the biomimetic composite facilitated mineral deposition from simulated body fluid and supported attachment, proliferation and alkaline phosphatase activity of hMSCs. Moreover, the highly aligned Sr/Cu-doped 1D HA crystals in the 3D porous scaffolds induced the alignment of hMSCs and secretion of anisotropic collagen fiber matrix in 3D.

Discussion and Conclusion: The biomimetic 1D HA/PDLA composite presented here contributes to the current efforts aiming at design and development of load-bearing bioactive synthetic bone graft substitutes. Moreover, the biomimetic composite may serve as a 3D platform for studying cell-ECM interactions in bone tissue. The method and design principles applied here may provide inspiration for development of advanced composites for bone regeneration.

Acknowledgement: This work was supported by the Materials Driven Regeneration (MDR) program.
Co-axial electrospinning of hyaluronan-based nanofibrils with spontaneous assembly to cells-nanofibrils matrix for the recovery of osteochondral defects

Ms. Hoai-Thuong Duc Bui1, Ms. Oanh-Vu Pham-Nguyen1, Ms. Heewon Kim2, Professor Byung-Jae Kang2, Professor Hyuk Sang YOO1

1Department of Medical Biomaterial Engineering, Kangwon National University, Chuncheon-si, Republic of Korea, 2Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

Introduction: Hyaluronic acid (HA) is a natural glycosaminoglycan in the extracellular matrix (ECM) of various soft tissues, especially in the synovial fluid of joints. Fabricating HA nanofibers as drug delivery systems or tissue bio-scaffolds has attracted much attention owing to the biodegradability and biocompatibility of HA. Nanofibrous structure has high porosity, large surface area, and interconnected pores, which is highly potential as a bio-mimicking scaffold for promoting cell growth and maintaining cell functions. Electrospinning of pure and/or unmodified HA is difficult to handle due to its high viscosity, high hydrophilicity, and high surface tension. In this study, HA-based nanofibers were fabricated via co-axial electrospinning with poly(ɛ-caprolactone) (PCL) and polyethylene glycol (PEG) as sheath and HA as core (PCL@HA NFs) for self-assembly into a matrix with adipose-derived stem cells (ADSCs), which further facilitates chondrogenic differentiation.

Methods: Co-axial electrospinning was conducted employing PCL/PEG dissolved in chloroform as the outer layer and HA/ polyvinyl alcohol dissolved in 50% ethanol as the inner core. The HA core was subsequently crosslinked by 1,4-butanediol diglycidyl ether (BDDE). PEG at the outer layer was removed by excessive wash with MeOH, and confirmed by SEM and FT-IR. The presence of HA in the NFs after PEG removal was determined by TGA, alcian blue staining, swelling, and mass erosion test, and FITC conjugated HA used for fabrication of PCL@FITC-HA NFs was also visualized by CLSM. The electrospun nanofibrous meshes were further fragmented into nanofibers (NFs) before mixing with ADSCs to form a cells-NFs matrix. To study the effects of PCL@HA NFs on chondrogenesis, after 24-h formation, cells-NFs matrixes were cultured in a reduced chondrogenic medium. The expression of chondrogenic genes (COL1, COL2, ACAN, SOX9) was evaluated by qRT-PCR, and the whole transcriptome RNA-sequencing of ADSCs after 10 days of chondrogenesis was analyzed. In vivo study was conducted on 10-week-old male rats, and the effects of ADSCs-PCL@HA NFs complex on the recovery of osteochondral defects were analyzed by micro-CT and histological staining.

Results: The surface of NFs became rougher and the diameter was also decreased when PEG was eroded. CH2–O–CH2 peak at 1100 cm⁻¹ on the FT-IR spectrum of PCL@HA NFs disappeared after PEG washing steps. The CLSM images of nanofiber meshes showed that the FITC-HA was co-localized within the nanofibers, and the fluorescence intensity of FITC-HA was significantly increased after PEG removal. PCL and PCL/PEG@HA nanofiber exhibited similar swelling profiles and the swelling ratio remained stable, whereas the swelling ratio of PCL@HA nanofiber rapidly increased after 120 min. After 10 days of cultivation of the ADSCs-NFs complex, cell viability was significantly reduced in TCPS groups and PCL@HA NF-contained groups showed higher viability. Moreover, the expression of chondrogenic genes was elevated in all groups after 7 days of cultivation, in which the expression of SOX9 and ACAN was highest when ADSCs were cultured with mixture of PCL/PCL@HA NFs. In addition, the expression of COL2 was highest in PCL@HA NFs groups. The whole transcriptome RNA-sequencing of ADSCs showed that ADSCs in PCL@HA NFs exhibited remarkable up-regulation of chondrogenesis-related genes (ACAN, IL11RA, COMP, MMP13, COL1A2, COL3A1…) and down-regulation of MSCs markers related genes (CD59, CD44, ENG, BMP…). GO enrichment analysis also revealed that ADSCs cultured on PCL@HA NFs contained matrix showed higher relation with cartilage development, collagen fibrils organization, and skeletal morphogenesis. In vivo results evinced that bone volume/total volume of the rats treated with PCL@HA NFs contained matrix was higher than those treated
with PCL NFs matrix only. Histological staining demonstrated that the group treated with PCL@HA NFs contained matrix exhibited a higher cartilage regeneration rate compared to others.

Discussion and Conclusion: Those results suggested that HA was successfully co-electrospun with PCL, and the successful removal of PEG enabled the exposure of HA on PCL@HA NFs. In vitro and in vivo results confirmed the chondrogenesis-inducing role of HA on ADSCs which supports the improvement of cartilage regeneration. Our study shows a promising strategy for the recovery of osteochondral defects.
Cost-effective and cell-instructive porous PCL scaffolds as mesenchymal stem cell expansion and carrier platforms

Mr. Johnny Kuan Un Wong

The University Of Sydney, Australia

Background:
Mesenchymal stem cells (MSC) hold great promise for research and clinical applications, but the high manufacturing cost of cells, compounded by high therapeutic dosages and inconsistent cell quality, hinders the accessibility of stem cell therapies. Three-dimensional (3D) cost-effective, cell-instructive, functionally tailorable, and high surface area-to-volume porous polymeric scaffolds can address the limitations of conventional planar cell expansion systems.

Method:
Biodegradable macroporous polycaprolactone (PCL) scaffolds were 3D printed with extrusion-based 3D printers. Scaffolds were surface modified using a novel customized high voltage plasma system, called packed-bed plasma immersion ion implantation (PBPI3), which allows uniform treatment within the porous structures. A mitogenic factor, fibroblast growth factor 2 (FGF2), was immobilized onto the internal scaffold surfaces to functionally recapitulate the native microenvironment of MSCs. A holder-assisted cell seeding method was employed to promote efficient and uniform cell seeding. Cell yield was determined by DNA quantification, and cell distribution within scaffolds was imaged by confocal microscopy. Cell phenotype was validated by flow cytometry.

Results:
Homogenous plasma surface modification of 3D PCL scaffolds was demonstrated by the covalent retention and uniform distribution of Cy5-conjugated proteins on treated PCL scaffolds after stringent detergent washing conditions. Long-term covalent binding capacity was demonstrated by the covalent retention of FGF2 on treated PCL scaffolds stored for up to 6 months. Custom-built holders improved the efficiency of cell seeding and the uniformity of cell distribution on treated PCL scaffolds, which maximised the available surface area for MSC proliferation, resulting in significantly improved cell yield. Plasma-treated scaffolds coated with FGF2 further enhanced the growth kinetics of MSCs compared to that on conventional tissue culture vessels or bare scaffolds, independent of surface area. The phenotype of cells harvested from the functionalized scaffolds was robust, as demonstrated by characteristic MSC surface marker expression.

Discussion and conclusion:
Our plasma-treated and biofunctionalized biodegradable scaffolds reduce labour costs associated with repeated passaging over 2 weeks, decrease growth factor usage by 26 fold, and fulfill dual roles for MSC expansion and in vivo MSC delivery. This work demonstrates the potential of our technology to manufacture cost-effective and cell-instructive polymer scaffolds, with versatility for different biomolecules, multiple cell types, and for both ex vivo and in vivo applications.

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Morphology-based super-early quality prediction for efficient development of elite cell bank

**Prof. Ryuji Kato**¹,²

¹Graduate School of Pharmaceutical Sciences, Nagoya University, , Japan, ²Institute of Nano-Life-Systems, Institutes of Innovation for Future Society, Nagoya University, , Japan

Author: Ryuji Kato¹,²*

Affiliation: ¹Graduate School of Pharmaceutical Sciences, Nagoya University, ²Institute of Nano-Life-Systems, Institutes of Innovation for Future Society, Nagoya University

Introduction: To ensure consistent distribution of cell-based products, the establishment of a high-quality cell bank is of paramount importance. However, the establishment of allogeneic cell-based products requires significantly larger cell banks than autologous products, resulting in substantial establishment costs. Commonly, the establishment of a cell bank is a challenging task, as there is a significant trade-off between maximizing the final cell yield and preserving cellular activity, which commonly decreases with their expansion process. Another technical challenge is the small number of "starting cells", which makes it difficult to evaluate using conventional cell assessment methods. Furthermore, since cell bank starting cells cannot be destructed, non-invasive cell assessment technologies are required not only to evaluate the precious starting cells but also to facilitate better cell bank quality.

Cell morphology represents a critical and reliable indicator for non-invasive monitoring of cellular status. Advances in hardware technology, which enable automated and comprehensive imaging, coupled with the rapid growth of artificial intelligence (AI)-related technologies, have facilitated the use of image-derived morphological information as a new biomarker for quantitative evaluation of cells. Our research group has reported various applications of an AI-guided morphological cell evaluation method for cellular quality assessment. This technique is now regarded as one of the most effective tools for monitoring cell quality during industrial cell manufacturing for cell-based products.

Subjects and Methods: In this study, we applied our AI-guided morphology-based cell quality evaluation technology to predict the quality of culturing cells non-invasively, which is typically not possible without biological experiments. We experimentally determined several critical cell quality attributes, such as growth potential, passage limitation, and differentiation potency in mesenchymal stem cells (MSCs) and constructed an AI model for predicting super early potency.

Results: Our findings reveal the feasibility of predicting diverse "future potencies" of MSCs only based on the morphological profiles reflecting their population heterogeneity during the initial 1-2 days of the culture period [1, 2].

Discussion and Conclusion: We established multiple artificial intelligence (AI) models to forecast the future potencies of MSCs in order to improve the efficiency of cell bank establishment. Our cell quality evaluation technology has the potential not only to identify elite donors but also to minimize the cell bank establishment effort by selecting elite starting cells in their process.


Acknowledgement

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Effect of Silk Fibroin/ Nano-Hydroxyapatite Composite on Immune Responses

Ms. Kallista Wong1, Professor James Goh1
1National University of Singapore, Department of Biomedical Engineering, Singapore

Title: Effect of Silk Fibroin/ Nano-Hydroxyapatite Composite on Immune Responses

Authors: Kallista Wong, Prof James Cho Hong Goh
Affiliations: National University of Singapore, Department of Biomedical Engineering
Category: Design and Applications of Biomaterials, SYIS (Student and Young Investigator Section)

Background: In recent years, a hybrid knitted silk scaffold with porous silk sponge consisting of silk fibroin (SF) and low crystallinity nano-hydroxyapatite (nHA) has been developed to improve the integration of tendon graft to the bone for anterior cruciate ligament reconstruction. However, unpublished data has shown that the SF/nHA scaffold was a moderate irritant to the surrounding bone tissues in a 12-week implantation study in rabbits. It was unclear whether the immune response was pro-inflammatory or anti-inflammatory. Since the scaffold is made of degradable materials, the local irritancy effects caused by the degradation process cannot be ruled out.

Macrophages play an important role in dictating the long-term clinical outcome of biomaterials. They have high plasticity and can be polarized to pro-inflammatory M1 or anti-inflammatory M2 phenotypes in response to the local environment. M1 macrophages secrete pro-inflammatory cytokines (TNF-α, IL-6) to initiate the healing process and accelerate the degradation of biomaterial. Thereafter, macrophages switch to M2 phenotype and secrete anti-inflammatory cytokines (IL-10, TGF-β1) to facilitate tissue repair and regeneration. However, persistent activation of either macrophage phenotype can be detrimental and may lead to chronic inflammation or fibrous encapsulation. Persistent activation of macrophages can cause macrophages membrane to be fused into foreign body giant cells (FBGCs) and adhere to the surface of the implant. Furthermore, activated fibroblasts are differentiated into myofibroblasts, resulting in collagen deposition, forming a barrier between the tissue and the implant. This eventually results in fibrous encapsulation, leading to the failure of the implant. Therefore, it can be hypothesized that during the degradation process, as the SF/nHA composite is degraded, the degradation products can contribute to the polarization of macrophage phenotypes from pro-inflammatory M1 to anti-inflammatory M2 to facilitate tissue repair and regeneration (Figure 1).

Methods: Firstly, the SF and SF/nHA composites were fabricated and subsequently degraded in vitro using protease XIV. Thereafter, the immune response of the THP-1 derived macrophages towards the degradation products of the composites was characterised using quantitative PCR and ELISA assays.

Results & Discussion: After 24 days of degradation, the SF/nHA scaffold had a lesser extent of degradation compared to the SF scaffold due to higher β-sheet (Silk II) content and tightly packed crystalline structures. The cell viability assay showed that the degradation products from SF/nHA and SF scaffolds were non-cytotoxic and that the addition of nHA did not affect the cell viability. Additionally, from the PCR and ELISA results, THP-1 derived macrophages initially expressed higher levels of pro-inflammatory cytokine (TNF-α, IL-6) when cultured with the degradation products of SF/nHA than the degradation products of SF. By 24 days of degradation of SF/nHA in protease, the pro-inflammatory expression has significantly decreased. Additionally, the anti-inflammatory (IL-10, TGF-β1) expression of the macrophages when cultured with Day 24 degradation products of SF/nHA and SF have significantly increased. However, the degradation product of SF in protease has better anti-inflammatory effect than that of SF/nHA due to significantly higher expression of IL-10. These observations can be attributed to the amino acids found in the degradation products.

Conclusion: As both the SF and SF/nHA composite were degraded in protease, the initial (Day 1) degradation products elicited a pro-inflammatory response while the latter (Day 24) degradation products elicited an anti-inflammatory response, suggesting that the degradation products can contribute to the polarisation of the THP-1 derived macrophages from pro-inflammatory M1 to anti-inflammatory M2 phenotype. The degradation products from SF in protease have higher glycine,
alanine, and serine amino acids concentration which could exert an anti-inflammatory effect on the macrophages.
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Topological self-induced functional reconstruction of biological and biomechanical heterogeneous meniscus

Dr. Mingze Du¹, Dr. Liya Ai¹, Prof. Jun Yin², Prof. Dong Jiang¹

¹Department of Sports Medicine, Peking University Third Hospital. Institute of Sports Medicine of Peking University. Beijing Key Laboratory of Sports Injuries. Engineering Research Center of Sports Trauma Treatment Technology and Devices, Ministry of Education, Beijing, China, ²The State Key Laboratory of Fluid Power Transmission and Control Systems, Key Laboratory of 3D Printing Process and Equipment of Zhejiang Province, School of Mechanical Engineering, Zhejiang University, Hangzhou, China

Title: Topological self-induced functional reconstruction of biological and biomechanical heterogeneous meniscus

Authors: Mingze Du1, Liya Ai1, Jun Yin2, Dong Jiang1*

Affiliations: 1Department of Sports Medicine, Peking University Third Hospital. Institute of Sports Medicine of Peking University. Beijing Key Laboratory of Sports Injuries. Engineering Research Center of Sports Trauma Treatment Technology and Devices, Ministry of Education. Beijing, China. 2The State Key Laboratory of Fluid Power Transmission and Control Systems, Key Laboratory of 3D Printing Process and Equipment of Zhejiang Province, School of Mechanical Engineering, Zhejiang University. Zhejiang, China. (* Correspondence)

Category: Tissue Engineering and Regeneration

Background: Osteoarthritis (OA) is the most common chronic joint disease, and one of the most important pathogenic factors of OA is meniscus injury, which unfortunately still cannot be functionally restored. The state-of-art tissue-engineered meniscus (TEM) is far from able to restore meniscal biofunctions due to the current inability to biologically and biomechanically reconstruct the gradient heterostructure of the natural meniscus.

Methods: In this work, we presented an innovative 3D-printed TEM with a unique gradient-sized diamond-pored microstructure (GSDP-TEM) through two-stage temperature control system, and tested its effects on biological and biomechanical heterogeneities reconstruction in vitro. Functional meniscus restoration and chondroprotection ability induced by GSDP-TEM were also evaluated in vivo.

Results: Under the synergistic effect of transforming growth factor (TGF)-β3 and connective tissue growth factor (CTGF), the unique gradient microtopology of the GSDP-TEM allows the spatially heterogeneous differentiation of seeded mesenchymal stem cells into their index cell phenotypes, triggering the gradient transition of the extracellular matrix (ECM) from the inside out. The GSDP-TEM also presents excellent biomechanical heterogeneity with a circumferential tensile modulus and energy dissipation similar to those of the natural meniscus. After implantation in a rabbit knee model, the GSDP-TEM induces heterogeneous biomimetic neomeniscus regeneration and efficiently alleviates joint degeneration.

Discussion and Conclusion: We presented a novel design of GSDP-TEM to mimic the heterogeneous architecture of the natural meniscus, introducing the effect of micro-topology to dually rebuild the heterogeneities and biofunctions of meniscus. The present study also provided a new perspective of the topology-induced biomechanical and biochemical heterogeneities reconstruction, which could be promoted to the construction of multiple heterogeneous load-bearing tissues in human body.

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Recellularized dLhCG (rLhCG) for Bone Reparation and Regeneration

Mr. Cheng MA, Dr. Dong-an WANG

1Department of Biomedical Engineering, College of Engineering, City University of Hong Kong, Hong Kong SAR, 2Karolinska Institutet Ming Wai Lau Centre for Reparative Medicine, HKSTP, Hong Kong SAR

Population ageing has become the focus of attention these years. Aged people can have higher chances of suffering from osteoarthritis since the loss of synovial fluid may lead to the abnormal friction of cartilage tissue and induce damage, resulting in the loss of protection for bone tissue. This will further bring about the abrasion of the subchondral bone and cause pain. Meanwhile, accidental injury can also cause bone defects and influence the patient’s movement patterns. For terminal-stage bone loss, joint arthroplasty is widely used in clinical to improve patients’ living standards. Metal, ceramic and polymers are materials that are generally used for transplantation. Researchers are now trying to design and fabricate novel materials that are available for not only bone reparation but help regeneration, realizing the restoration of defects to normal bone tissue to the greatest extent. Some research teams used stem cells, for example, mesenchymal stem cells, to realize bone regeneration. Hydrogels or decellularized bone grafts can also be used as materials for the therapy of bone defects. Previously, our research team developed an artificial cartilage graft called decellularized living hyaline cartilage graft (dLhCG). dLhCG is a 3D-sponge-like scaffold that contains only endogenous cartilaginous extracellular matrix. The procedure of fabricating dLhCG is to encapsulate the chondrocyte with hydrogel, waiting for the formation of an interpenetrating network with hydrogel and extracellular matrix, followed by gel-removal and decellularization. In this research, the dLhCG is used as a cartilage graft, and bone marrow-derived mesenchymal stem cells (bMSCs) are seeded in dLhCGs to mimic endochondral ossification in vitro. bMSCs-recellularized dLhCG (rLhCG) are divided into several groups. Samples of the chondrogenic group are treated with HS medium to induce bMSCs chondrogenic and hypertrophic differentiation. Hypertrophic chondrocytes have the capability to trans-differentiate into osteoblasts. Samples of the osteogenic differentiation group are treated with HS+ medium to induce direct differentiation from bMSCs to osteoblasts. After 5-week cell culturing, rLhCGs are no longer pure cartilage grafts but artificial bone grafts. rLhCGs undergo characterization processes, including qPCR, RNA-seq, ELISA, immunohistochemical staining and composition determination. rLhCGs are also transplanted into nude mice’s great omentum for 14 days to evaluate the capability of vascularization. Additionally, rLhCGs are transplanted to defects of rats’ femurs after decellularization, and treated femurs are taken out for micro-CT scanning and immunohistochemical staining to determine bone reparation capability. The results show that two groups can realize the transition from cartilage graft to bone graft. Several genes expression is related to chondrocyte hypertrophic, for example, Col10A1, indicating that the chondrogenic differentiation group realize the transition mentioned above through in vitro endochondral ossification. The results of animal experiments suggest that as a bone graft, rLhCG shows high biocompatibility and can realize bone reparation and vascularization. To sum up, the artificial bone graft, rLhCG, we fabricated with the tissue engineering method, has the capability of bone reparation and regeneration and can be used as a novel bone graft for transplantation.
In vivo reprogramming by nano-hypoxia of spleen to instruct vasculogenic cell homing

Dr. Seyong Chung¹, Dr. Seung Eung Yu, Prof. Hak-Joon Sung¹
¹Yonsei University College Of Medicine, South Korea

Introduction: Several clinical trials have investigated the use of bone marrow (BM) mononuclear cells as potential vasculogenic cells for treating ischemic sites. However, these trials have only resulted in marginal effects, with a major limitation being the small number of cells obtained through extraction and the lengthy invasive process. The spleen contains abundant mononuclear cells, which inherently target ischemic sites. If these cells can be reprogrammed in vivo, plentiful vasculogenic cells can be efficiently deployed to ischemic sites.

Subjects and Methods: Splenic mononuclear cells were reprogrammed based on hypoxic conditions, both in vitro and in vivo. After reprogramming, these cells were evaluated for their inherent targeting ability and therapeutic effects on ischemic sites, using hindlimb ischemia and partial hepatectomy models in mice.

Results: Hypoxia induced vasculogenic differentiation in splenic mononuclear cells, as evidenced by changes in cell morphology and increased expression of vasculogenic cell markers. A hypoxia-mimetic agent (CoCl₂) also exerted similar effects in vitro, and hypoxia-mimetic agent-loaded liposomes were developed to deliver "nano-hypoxia" to the spleen. Both nano-hypoxia and splenic artery ligation increased the expression of vasculogenic cell markers in the spleen, resulting in a ten-fold increase in the number of vasculogenic cells compared to BM. These reprogrammed cells retained their targeting ability to ischemic sites, while also exhibiting therapeutic effects. When hindlimb ischemia was induced after reprogramming, nano-hypoxia showed increased expression of vasculogenic cell markers and facilitated blood recovery compared to conventional strategies using BM cells. Enhanced angiogenesis with nano-hypoxia was also effective in a partial hepatectomy model, as it facilitated hepatic regeneration and improved hepatic function compared to the conventional strategy group.

Discussion and Conclusion: In vivo reprogramming is a simple and non-invasive strategy for vasculogenic cell treatment of ischemia, which can overcome previous limitations of insufficient cell numbers.
Maintenance of proliferative capacity of human mesenchymal stem cells by methylglyoxal

Ms. Kai Torng ANG¹, Dr. Mee-Hae KIM¹, Prof. Masahiro KINO-OKA¹,²
¹Graduate School of Engineering, Osaka University, Suita, Japan, ²Research Base for Cell Manufacturability, Graduate School of Engineering, Osaka University, Suita, Japan

Title: Maintenance of proliferative capacity of human mesenchymal stem cells by methylglyoxal

Authors: Kai Torng Ang¹, Mee-Hae Kim¹, Masahiro Kino-oka¹ ²

Affiliations: ¹Department of Biotechnology, Graduate School of Engineering, Osaka University, Suita, Osaka, Japan. ²Research Base for Cell Manufacturability, Graduate School of Engineering, Osaka University, Suita, Osaka, Japan

Category: Stem Cells and Cell-Based Therapies, SYIS

Background: In order for human mesenchymal stem cells (hMSCs) to be clinically effective in regenerative medicine, culture strategies for producing a sufficient quantity of cells with good quality are required. During the expansion culture of hMSCs, local cell density increases exponentially in the culture vessel along with changes in cell behaviors, causing cell contact inhibition and reduced cell proliferation. Previous studies on stem cell proliferation have reported that secretion and structural changes of the extracellular matrix (ECM) are important for maintaining cell proliferative potential. In this study, we investigated the proliferative effect of hMSCs by using methylglyoxal (MG), a precursor of advanced glycation end products which is capable of modifying ECM structure.

Materials and Methods: hMSCs were seeded at a seeding density of 3.0 × 10^3 cells/cm² and cultured for 144 h. At t = 48 h, hMSCs were exposed to MG at 0.125 nM for 24 hours, and MG was removed from the culture at t = 72 h. Assessment of cell density (cells/cm²) was performed by trypan blue exclusion method using automated cell counter and apparent specific growth rate μ^app (h^-1) was calculated. The assembly and structural changes of the ECM main components, fibronectin and collagen type I after MG exposure (t = 72 h and 144 h) were examined by immunofluorescence staining.

Results and Discussion: In cells without MG exposure, the apparent specific growth rate μ^app (h^-1) increased to (2.05 ± 0.3) × 10^-2 h^-1 in the early phase of culture (t = 48-72 h), while the apparent specific growth rate decreased to (0.89 ± 0.04) × 10^-2 h^-1 in the late phase of culture (t = 72-144 h). On the other hand, the apparent specific growth rate of cells with MG exposure in the late phase of culture was (1.50 ± 0.1) × 10^-2 h^-1, confirming the maintenance of apparent specific growth rate of cells throughout the culture. Furthermore, we confirmed that MG disrupted fibronectin and collagen fibril formation as well as changes in their structure, however it did not influence fibronectin and collagen type I secretion. These results suggested that MG could reorganize ECM and the reconstruction of the scaffold environment leads to the maintenance of cell proliferative capacity. This current work demonstrated a promising strategy for establishing an expansion culture system.

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3D bio-printing tubular model with vascularization for mimicking the human intestine

Mr. Seunghun Son¹, Mr. Dongjin Lee¹, Mr. Jimin Seok¹, Ms. Dahong Kim¹, Ms. Sua Park¹, Mr. Junhee Lee¹
¹Korea Institute of Machinery and Materials, Daejeon, South Korea

The human intestine is a dynamic and complicated organ that is essential for immunological protection, waste removal, and nutrient absorption. Realistic and functioning in vitro models that closely resemble the anatomical and functional properties of the human intestine are required to better understand intestinal activities and create effective treatments for intestinal illnesses. In this paper, we introduce a method for fabricating a vascularized tubular intestine model using 3D bio-printing technology.

The bio-printing process involved using GelMA bioink composed of CCD-18Co (Myo-fibroblasts), Caco-2 (Colorectal adenocarcinoma), and HUVEC (Human Umbilical Vein Endothelial) cells. The tubular model was fabricated using a 4-axis (X,Y,Z,Rotation) controlled bio-printer, and vascularization was achieved by embedding endothelial cells encapsulated in the bioink. In order to structurally maintain the tubular shape, the PCL scaffold was printed first, and Caco-2, HUVECs, and CCD-18Co cells were printed on the inner and outer surface.

The inner diameter of bio-printed tubular intestine model is 2mm, the length is 36mm and composed of 4 layers including polymer and cell laden bio-inks. We characterized the cell viability (Live&Dead, CCK-8 Assay), Caco-2 cell differentiation (ALP&Protein Assay, Teer measurement), HUVECs differentiation (CD-31) and morphology of the model using SEM (Scanning Electron Microscope).

In conclusion, our study shows the potential of 3D bio-printing technology for creating functional models of the human intestine that can be used for drug screening, disease modeling, intestine implant model and personalized medicine. Vascularized tubular intestine model fabricated by 3D bio-printing shows significant advancement in developing realistic and physiological intestine models.
Construction of 3D vasculature using stem cell-derived extracellular matrix scaffold

Ms. Byoungha An¹,², Mr. Jae Won Kwon¹,², Mr. Kwideok Park¹,²
¹Korea Institute of Science and technology, Seoul, South Korea, ²University of Science & Technology, Daejeon, South Korea

Title: Construction of 3D vasculature using stem cell-derived extracellular matrix scaffold
Authors: Byoungha An¹,², Jae Won Kwon¹,², Kwideok Park¹,² *
Affiliations: ¹ Center for Biomaterials, Korea Institute of Science and Technology (KIST), Seoul 02792, Korea.
² Division of Bio-Medical Science and Technology, KIST School, University of Science and Technology (UST), Seoul 02792, Korea. (* Correspondence)

Category: Tissue Engineering and Regeneration

Background: The mature vasculature in three-dimensional tissue engineered construct is crucial not only for tissue regeneration but also for basic research and drug development toward cardiovascular diseases and cancers.

While this goal is still challenging, the coordinated assembly of vascular cells and extracellular matrix (ECM) may provide a chance to recapitulate mature vasculature in vitro. Here, we propose the construction of 3D vasculature using cell-derived ECM scaffold and vascular cells.

Methods: We obtained the stem cell-derived extracellular matrix (scECM) by decellularization of umbilical cord stem cells. The human umbilical vein endothelial cells (HUVECs), mesenchymal stem cells (MSCs) and pericytes were seeded separately or together in the scECM scaffold to induce vascularization and maturation as well. The vascularized scECM scaffold was evaluated for tubular structure using confocal laser scanning microscopy. Those cells were recovered from the scECM scaffold and subjected to RT-qPCR for the examination of the mRNA expression level of marker genes.

Results: The scECM scaffold was quite stable while maintaining the structure during long-term culture of HUVEC, MSCs and pericytes. The cells seeded in the scaffold could attach and spread throughout the scECM scaffold and the cell population was consistent both in the surface and in the middle of the scaffold. After 7 day of cell culture, the capillary formation was observed not only in the HUVEC, MSC and pericytes co-cultured scECM scaffold but also in the scECM scaffold where HUVEC were cultured alone. But stabilization and maturity of the engineered vasculature was significantly more pronounced when MSCs or pericytes were present.

Furthermore, the vascularized scECM scaffold was loaded in the transwell system and evaluated for their capacity as an in vitro blood vessel model.

Discussion and Conclusion: Our results demonstrated that scECM scaffold successfully provided structural
support for cell to build the vascular structure. The analysis of mature vasculature formation suggested that the cells may remodel the scECM scaffold, which seemed to be essential for capillary maturity. Taken together, scECM scaffold was effective in creating mature vascular capillaries.

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Structural and biofunctional evaluation of decellularized jellyfish matrices in wound healing

**Ms. Wenjun Yu**^1,2^, Mr. Jie Zhao^1^, Mr. Yang Li^1,2^, Mr. Hong Shan^1,3^  
^1^Guangdong Provincial Engineering Research Center of Molecular Imaging, Zhuhai, China, ^2^Department of Radiology, Zhuhai, China, ^3^Department of Interventional Medicine, Zhuhai, China  

**Title:** Structural and biofunctional evaluation of decellularized jellyfish matrices in wound healing  

**Authors:** Wenjun Yu^1,2^, Jie Zhao^1^, Yang Li^1,2^, Hong Shan^1,3^  

**Affiliations:**  
^1^Guangdong Provincial Engineering Research Center of Molecular Imaging, 2Department of Radiology, and 3Department of Interventional Medicine, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, China. (*Correspondence)  

**Category:** Tissue Engineering and Regeneration; SYIS (Student and Young Investigator Section)  

**Background:** Extracellular matrices decellularized from marine animal tissues are emerging scaffolds in tissue engineering. Jellyfish tissues are suitable for making functional and safe decellularized matrices in part due to their simple structure, high water content, and low risk of pathogen transmission to humans. Jellyfish are some of the most prevalent marine animals, but their decellularized matrices have remained largely undeveloped.  

**Methods:** In this work, we evaluated the structures and functions of the jellyfish (Rhopilema esculentum) matrices decellularized with seven commonly reported decellularization reagents individually, including 2% 3-[3-cholamidopropyl] dimethylammonio]-1propanesulfonate (CHAPS), 5% sodium deoxycholate (SD), 5% IGEPAL CA-630 (IGEPAL), 5% Tween-20 (Tween), 5% Triton X-100 (Triton), 1% sodium N-dodecanoylsalcosinate (SNL), and 1% sodium dodecyl sulfate (SDS). We tested its biofunction in vitro and in vivo.  

**Results:** The seven decellularization reagents showed effectiveness in removing the cellular components. Scanning electron microscopy and mechanical testing revealed that the decellularized matrices mostly retained the native microstructures, whereas only SDS and SNL distorted the matrices’ multilayered and fibrous architecture. The collagen hybridizing peptide fluorescence staining showed that SDS, SNL, Triton X-100, IGEPAL, and Tween-20 denatured the jellyfish collagen molecules to varying degrees while CHAPS and SD protected the collagen’s triple-helix conformation. Furthermore, the decellularized jellyfish matrices showed similarity to different types of mammalian collagen and supported the adhesion and proliferation of human dermal and cornea fibroblasts and mouse chondrocytes in 3D culture. The decellularized jellyfish matrix also facilitated wound healing in vivo by reducing inflammation while promoting angiogenesis and tissue remodeling.  

**Discussion and Conclusion:** Our study demonstrated that the decellularized jellyfish matrices are an easy-to-prepare, biocompatible, and potentially widely applicable scaffold for regenerative medicine.  

**Acknowledgment:** This work has just been published (J. Mater. Chem. B, 2023, 11, 3740-3751).
A Review of the Research Status of Recombinant Human Collagen in China and Worldwide

Mr. Haihang Li

1Jiangsu Trautec Medical Technology Co. Ltd, China

Abstract:
Recombinant human collagen forms a new type of biomaterial, based on the concept and technical route of synthetic biology. The collagen protein obtained through large-scale fermentation in heterologous hosts such as Pichia pastoris has the same amino acid sequence as human collagen, which brings numerous benefits, including high bioactivity, low immunogenicity, and high biocompatibility. After more than 30 years of technological developments, recombinant collagen materials have been widely accepted by the market. Studies have shown that treating wounds with hydrogel materials composed of recombinant type III collagen significantly increases the total content of type III collagen and the ratio of type III/type I collagen in the healed dermis, ultimately leading to the significant reduction of scar formation after wound healing. A multi-center clinical study of artificial cornea prepared based on recombinant type III collagen as fault replacement in treating corneal diseases has been carried out in mainland China. Recombinant collagen materials have also been extensively used in the regeneration of other tissues such as bone and cartilage. Taken together, in this report, I will discuss the recent trend of academic research and industrial development of recombinant collagen in China and across the world. I will also elaborate on the latest achievements of our research team in skin regeneration and repair.

References:
Importance of data design for developing robust image processing with deep learning

Mr. Takeru SHIINA\textsuperscript{1}, Ms. Kazue KIMURA\textsuperscript{1}, Mr. Yuto TAKE\textsuperscript{1}, Mr. Kenjiro TAKANA\textsuperscript{1}, Prof. Ryuji KATO\textsuperscript{1,2}

\textsuperscript{1}Graduate School of Pharmaceutical Sciences, Nagoya University, Pharmaceutical Build. 302, Furocho, Chikusaku, Nagoya, Japan, \textsuperscript{2}Institute of Nano-Life-Systems, Institutes of Innovation for Future Society, Nagoya University, NIC, Furocho, Chikusaku, Nagoya, Japan

Introduction: Label-free image analysis offers a balanced combination of high throughput and low cost, therefore considered as one of the most powerful modalities to make non-invasive assessment of cells used for cell therapies. Although morphological monitoring had long been a practical technique to maintain cellular quality in daily culture, such manual technique heavily relying on human sense is no longer compatible for assuring high efficiency and safety of cell-based products. Our group has been proposing effective usage of image processing technology combined with machine learning for label-free morphology-based cell assessment, to enable “automatic cell quality check” only from their microscopic images as cell quality control technology. However, there are still several limitations in such label-free image analysis. The difficulty is the precision and robustness of cell recognition in the label-free images. Compared to the high content imaging in pharmaceutical analysis using stained images, label-free images obtained in regenerative medicine studies lacks ground-truth. Moreover, since label-free image analysis for cell quality assessment must handle various primary cells from patients, which are much more heterogenous than cell lines used in drug assays. Therefore, commonly, it is difficult to obtain robust cell segmentation with label-free images in regenerative medicine. To tackle such difficulty, deep learning (DL) models offer a new possibility. There are growing numbers of research trying to introduce DL for cell assessment, however there are still few reports that discuss “how should we collect sufficient data for DL.”

Subjects and Methods: Our group has been trying to establish the concept for obtaining robust image processing model to achieve flexibility for different types of cell morphologies in the real-world data\textsuperscript{[1]}. The subject of this study was to investigate the effective data construction concept for robust cell segmentation performance using DL. Using four types of morphologically characteristic cells, we collected 2592 images for training combined with the ground-truth using live cell staining. We optimized the training dataset sizes and combinations and evaluated the effect of “morphological types included in training data” on the U-Net cell segmentation performance.

Results: Our results clearly indicated that the DL model segmentation performance was greatly influenced by the “morphological types” in the training datasets. We also found to obtain robust DL model with images consisting of specific cell types.

Discussion and Conclusion: We investigated the characteristics of effective data quality for constructing robust DL model for stable cell segmentation of label-free images in cell manufacturing processes. Our results indicate that DL models trained with appropriate dataset variation can provide universal performance to support image-based cell quality assessment.


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Hydrogel as An Adhesive Coating Layer for Preventing Stent Migration in Rat Esophagus

Ms. Chu Hui Zeng, Ms. Song Hee Kim, Mr. Jeon Min Kang, Dr. Ji Hoon Shin, Dr. Jung-Hoon Park

1Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

Introduction: Stent migration is a common complication of stent implantation that lowers the overall therapeutic effects. When it occurs, patients may have to undergo repeat procedures for stent reinsertion or removal despite technical difficulties. Although numerous approaches have been developed to lower the migration rate, the efficacy of stent migration prevention is anticipated to be further improved. By far, not many studies investigated hydrogel-based stents and their usefulness to prevent migration. In addition, the three-dimensional (3D) nano-networked silica film (NSF) is believed to enhance the adhesion of the hydrogel to the stent surface. Therefore, this study aimed to develop a hydrogel-based anti-migration stent, with or without the 3D NSF, and investigate its safety and efficacy for preventing stent migration in rat esophagus.

Subjects and Methods: A total of 18 male Sprague–Dawley rats (300–350 g; 9 weeks old) were randomly allocated to three groups of six rats: Group A (control) received bare self-expandable metallic stent (SEMS); Group B received hydrogel-coated SEMS; and Group C received NSF-hydrogel–coated SEMS. Follow-up examinations, including body weight measurement and stent position/shape check under fluoroscopy, were completed at 3 days and 1, 2, and 4 weeks after stent placement. Hematoxylin and eosin staining was performed to determine the degree of submucosal inflammatory cell infiltration, thickness of the epithelium, and thickness of submucosal fibrosis. Masson’s trichrome staining was used for assessing collagen deposition. The degrees of α-SMA– and Ki-67–positive deposition were subjectively determined by immunohistochemistry.

Results: Stent placement was successful in all rats, reaching a technical success rate of 100%. Two (11.1%) of the 18 rats experienced death (n = 1; Group C) or early stent migration (n = 1; Group A) and were therefore excluded from further study. The remaining 16 (88.9%) rats survived until the end of the study without stent-related complications. As the stent migrated in four (4/6; 66.67%) rats in Group A but none in Group B or C, Group A generated a significantly higher stent migration rate than the other two groups (P > 0.05). Body weight steadily increased in all living rats (P > 0.05). Compared with Group A, the mean thickness of the epithelium, mean thickness of submucosal fibrosis, and mean degrees of collagen, α-SMA–, and Ki-67–positive deposition were all significantly lower in Group C (all P < 0.05). However, when comparing between Groups B and C, statistically significant difference was not identified in any of the findings (all P > 0.05), though the numbers were, in general, lower in Group C than in Group B.

Discussion and Conclusion: The newly developed NSF-hydrogel–coated stent not only safely and effectively prevented stent migration but also suppressed stent-induced tissue hyperplasia in rat esophagus. It may represent a solution to stent migration in the esophagus and other luminal organs in the clinic. The potential of this NSF-hydrogel coating layer as a drug delivery platform for treating tissue hyperplasia is worth further investigation.
Targeting collagen damage for sustained in situ antimicrobial activities

Ms. Xiaoyun Mo1, Ms. Suwen Zhao1, Professor Yang Li1

1Guangdong Provincial Key Laboratory of Biomedical Imaging and Guangdong Provincial Engineering Research Center of Molecular Imaging, the Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, 519000, China, Zhuhai, China

Title: Targeting collagen damage for sustained in situ antimicrobial activities

Authors: Xiaoyun Mo1, Suwen Zhao1, Jie Zhao1, Yang Li1,*, and Hong Shan1,2,*

Affiliations: 1Guangdong Provincial Engineering Research Center of Molecular Imaging and 2Department of Interventional Medicine, the Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, 519000, China.

Category: Design and Application of Biomaterials

SYIS (Student and Young Investigator Section)

Background: Antimicrobial peptides (AMPs) are promising agents for treating infections, but their applications are limited by the short-term retention at the infection site, as well as the non-targeted uptake and adverse effects on normal tissues. Since infection often follows an injury (e.g., in a wound bed), directly immobilizing AMPs to the collagenous matrix of the injured tissues may help overcome these limitations by transforming the extracellular matrix microenvironment of the infection site into a natural reservoir of AMPs for sustained in situ release.

Methods: We developed a peptide conjugate consisting of a dimeric AMP construct of Feleucin-K3 and a collagen hybridizing peptide, which enables the conjugate to specifically anchor to the damaged and denatured collagen molecules in the injured and infected tissues.

Results: We demonstrated that the dimeric Flc design exhibited potent and broad-spectrum antimicrobial activities, and the conjugate could bind to the damaged but not the normal skin tissues for days ex vivo, while considerably enhancing the retention and antimicrobial efficacy as well as facilitating tissue repairing in vivo in a rat wound healing model.

Discussion and Conclusion: Because collagen damage is ubiquitous in almost all injuries and infections, our strategy of collagen-damage targeting may open up new avenues for antimicrobial treatments in a range of infected tissues.

Acknowledgment: This work is currently in revision for publication in the Journal of Controlled Release.
The long non-coding RNA IGFBP7-AS1 accelerates stem cells from human exfoliated deciduous teeth odontogenic differentiation by regulating IGFBP7 expression

Dr. Dan Wang\(^1\), Dr. Ningxin Zhu\(^1\), Miss Fei Xie\(^1\), Dr. Man Qin\(^1\), Dr. Yuanyuan Wang\(^2\)

\(^1\)Department of Paediatric Dentistry, Peking University School and Hospital of Stomatology, Beijing, China

Title: The long non-coding RNA IGFBP7-AS1 accelerates stem cells from human exfoliated deciduous teeth odontogenic differentiation by regulating IGFBP7 expression

Authors: Dan Wang, Ningxin Zhu, Fei Xie, Man Qin, Yuanyuan Wang*

Affiliations: School and Hospital of Stomatology, Peking University, BEIJING, CHINA. #22 Zhongguancun Nandajie, Haidian District, Beijing 100081, China(* Correspondence)

Category: Stem Cells and Cell-Based Therapies

Background: Stem cells from human exfoliated deciduous teeth (SHED) are considered to be one of the important seed cells in tissue regeneration engineering of dental pulp because of their excellent properties. Inducing the directional differentiation of SHED into odontoblast is a crucial point of the regeneration of dental pulp. Long non-coding RNA (IncRNA) refers to non-coding RNA with a length greater than 200 bp. It has been proved that IncRNA played a key role in regulating the directional differentiation of stem cells. This study aims to analyze the RNA expression profile during the odontogenic differentiation of SHED by high-throughput sequencing, verify the effect of IncRNA insulin-like growth factor binding protein 7 antisense RNA 1 (IGFBP7-AS1) in vivo and in vitro, explore the mechanism of IGFBP7-AS1 in the odontogenic differentiation of SHED, and provide new targets for the directional odontogenic differentiation of SHED.

Methods: In this work, RNA sequencing (RNA-seq) was used to obtain the expression profile of IncRNAs and mRNAs during the odontogenic differentiation of SHED. The effect of IGFBP7-AS1 on odontogenic differentiation of SHED was assessed in vitro and in vivo. The underlying mechanism of IGFBP7-AS1 during SHED odontogenic differentiation was analyzed through bioinformatics prediction. The effect of IGFBP7 and the relationship between IGFBP7-AS1 and IGFBP7 were confirmed by correlation analysis, qRT-PCR and western blotting.

Results: RNA sequencing results showed that IncRNA and mRNA were differentially expressed during the odontogenic differentiation of SHED. IGFBP7-AS1 is a key IncRNA in the process of odontogenic differentiation of SHED. Its expression is gradually up-regulated and is significantly positively correlated with the expression of odontogenic differentiation related markers. The in vitro studies showed that knockdown of IGFBP7-AS1 inhibited alkaline phosphatase staining, alizarin red staining and the expression of odontogenic differentiation related markers, whereas overexpression of IGFBP7-AS1 enhanced them. The in vivo studies showed that overexpression of IGFBP7-AS1 could promote the formation of odontoblast-like cells and the formation of new dentin. The expression of odontogenic differentiation related markers was also increased. IGFBP7, as the downstream target gene of IGFBP7-AS1, could promote the odontogenic differentiation of SHED in vitro. The regulation of IGFBP7 by IGFBP7-AS1 may be achieved by enhancing the stability of IGFBP7 mRNA through forming RNA duplex.

Discussion and Conclusion: IGFBP7-AS1 plays an important role in the odontogenic differentiation process of SHED. IGFBP7-AS1 could promote the odontogenic differentiation of SHED in vivo and in vitro. The molecular mechanism of IGFBP7-AS1 to promote the odontogenic differentiation of SHED is increasing the stability of downstream mRNA IGFBP7.
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RGD-based Targeted Photothermal Tumor Therapy of Surface-Modified MXene

Ms. Soojin Kim¹, Mr. Hwan Kim¹
¹Korea National University Of Transportation, South Korea

Introduction: Cancer is a significant cause of death among non-infectious diseases, and treatment methods such as surgery, chemotherapy, and radiation therapy are used, but it is known to have side effects on drugs. Recently, studies have been conducted, such as photothermal therapy, a method of killing tumor cells with a certain amount of thermal energy to tumors. However, because it is a non-specific characteristic, it is likely to affect tumor cells and surrounding cells. As a method of targeting tumor cells, there is a method of using integrin αVβ3, which is known to be overexpressed in tumor cells.

Methods: In this study, we identify characteristics such as the photothermal conversion of a material called MXene according to particle size control. In addition, MXene nanoparticles were surface-modified: 1) Combine poly(ethylene glycol) (PEG), which acts as a polymer spacer, and 2) Conjugate RGD peptides that can target tumor cells. The photothermal treatment ability of MXene according to modification is confirmed, and tumor targeting ability is evaluated compared to normal cells.

Results: First, as a result of experimenting by dividing particle sizes into nano-size and micro-size to confirm the properties of MXene’s material, the nano-size MXene particles showed a higher photothermal conversion effect. Accordingly, after NIR irradiation, it showed excellent tumor-killing ability. As a next step, MXene nanoparticles were surface-modified with RGD peptides, and then successful synthesis was demonstrated through analysis. Finally, the photothermal therapy ability of MXene according to modification was confirmed, and the tumor targeting ability was evaluated by comparing it with normal cells.

Discussion and Conclusion: The properties of MXene, such as hydrophilicity and biocompatibility, were used for biological and medical applications and tumor treatment. The particle size was measured by controlling the size of the MXene particles. In particular, the improved photothermal properties and tumor-killing ability were confirmed in nano-sized particles. In conclusion, it showed the ability of MXene nanoparticles to target and kill tumors.
Evaluation of the cartilage regeneration of polydactyly derived chondrocyte sheets-mini using a xenogeneic transplantation model.

Dr. Makoto Ogawa¹,², Mrs. Eriko Toyoda¹,², Ms. Shiho Wasai¹,², Mr. Tatsumi Tanaka¹,², Ms. Ryoka Uchiyama¹,², Mrs. Miki Maehara¹,², Mr. Tadashi Akamatsu³, Mr. Masahiko Watanabe¹,², Mr. Masato Sato¹,²

¹Department of Orthopaedic Surgery, Surgical Science, Tokai University School of Medicine, Isehara, Japan, ²Center for Musculoskeletal Innovative Research and Advancement (C-MiRA), Graduate School of Medicine, Tokai University, Isehara, Japan, ³Department of Plastic Surgery, Surgical Science, Tokai University School of Medicine, Isehara, Japan

Introduction:
We are currently conducting a clinical study for articular cartilage repair using allogeneic polydactyly-derived chondrocyte sheets (PD sheets). However, the transplantation of PD sheets requires open-knee surgery and is currently performed in conjunction with open-wedge high tibial osteotomy (OWHTO), which is highly invasive. In order to establish a less invasive treatment, we evaluated PD sheets-mini, fabricated on a special culture dish, which may be injected into the knee-joint using a needle. Here, we report the characteristics of PD sheets-mini and the regenerative effects of PD sheets-mini using a xenogeneic transplantation model.

Methods:
Chondrocytes isolated from cartilage tissue of polydactyly patients were seeded at 0.75×10⁴ cells/cm² on RepCell™, which is a temperature-responsive culture dish with 3 mm × 3 mm grid walls etched on the culture surface. Chondrocytes were cultured for 2 weeks and characterized through ELISA (TGF-β1, melanoma inhibitory activity (MIA), DKK-1, ESM-1, MMP-3 and MMP13), qPCR (COL1A1, ACAN, SOX9, RUNX2, and MMP3), flowcytometry (CD26, CD29, CD31, CD44, CD45, CD49a, CD73, CD81, CD90, CD146 and CD166).

Twenty-four 12-week-old rats (F334/NJcl-rnu/rnu; Clea Japan, Tokyo, Japan), were used in the experiments. PD sheets, PD sheets-mini and PD cells were allocated equally to each transplantation group. An osteochondral defect (diameter 2 mm; depth 1 mm) was created on the patellar groove of the femur using a hand drill. And, PD sheets, PD sheets-mini and PD cells were transplanted as:
Group A, a mixture of hyaluronic acid and saline (without transplantation); Group B, PD cells and a mixture; Group C, PD sheets-mini and a mixture, and Group D: PD sheets. For Group A, B and C, transplant products were injected after the quadriceps femoris muscle and tendon were sutured. For Group D, PD sheet was transplanted and then the quadriceps femoris muscle, tendon, and skin were sutured. The animals were euthanized by administration of high-dose anesthesia at 4 weeks after transplantation. The operated knee was opened, and the distal portion of the femur was excised and 8 μm sections were cut near the center of the defect area. Standard protocols were used for histological staining.

Results:
The expression of mRNA for matrix metalloproteinase 3 (MMP3) was significantly higher in PD sheets-mini than in PD cells, the expression of mRNA for collagen, type I, alpha 1 (COL1A1) and Runt-related transcription factor 2 (RUNX2) was significantly lower in PD sheets-mini than in PD cells. PD sheets-mini produced higher amounts of MIA (PD cells 23.5 ng/1.0 × 10⁶ cells; PD sheets-mini 139.6 ng/1.0 × 10⁶ cells) and DKK-1 (PD cells 84.6 ng/1.0 × 10⁶ cells; PD sheets-mini 122.6 ng/1.0 × 10⁶ cells) but lower amounts of TGF-β1 (PD cells 19.6 ng/1.0 × 10⁶ cells; PD sheets-mini 13.7 ng/1.0 × 10⁶ cells), MCP-1 (PD cells 62 ng/1.0 × 10⁶ cells; PD sheets-mini 18 ng/1.0 × 10⁶ cells) and MMP3 (PD cells 23.2 ng/1.0 × 10⁶ cells; PD sheets-mini 5.8 ng/1.0 × 10⁶ cells) than PD cells. The surface markers related to differentiation and cartilage formation capacity differed between PD cells and PD sheets-mini. No significant differences in surface markers between PD sheets and PD sheets-mini were detected.
Four weeks after transplantation, the defects in Group D were filled with repaired tissue. In Groups A, B and C, the defects were not filled or filled with fibrous tissue that did not stain for Safranin O. Immunostaining showed more expression of COL I staining than COL II staining in the tissue. In Groups B and C, the repaired tissue showed partly Safranin O although the most did not stain for Safranin O. Immunostaining showed more expression of COL I staining than COL II staining in most of the tissue. In Group D, the repaired tissue showed good staining for Safranin O, and immunostaining showed more expression of COL II staining than COL I staining in the tissue.

Discussion and Conclusion:
PD sheets-mini showed properties similar to PD sheets, but the cartilage repair effect when administered intra-articularly was not as good as that of PD sheet. In order to improve cartilage repair effect of PD sheets-mini, it was considered necessary to investigate a method of administering and retaining it locally to the cartilage defect.
Technology Transfer for GMP-Compliant Manufacturing Process in Accordance with the Revised PIC/S Annex 1

Dr. Nicodemus Wong\textsuperscript{1,2,3}, Dr. Belle Yu-Hsuan Wang\textsuperscript{1,2,3}, Prof. Wayne Yuk-Wai Lee\textsuperscript{1,2,3,4}

\textsuperscript{1}Center for Neuromusculoskeletal Restorative Medicine, CUHK InnoHK Centres, Hong Kong Science Park, Hong Kong, \textsuperscript{2}Department of Orthopaedics and Traumatology, Faculty of Medicine, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, \textsuperscript{3}Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, \textsuperscript{4}SH Ho Scoliosis Research Laboratory, Joint Scoliosis Research Center of the Chinese University of Hong Kong and Nanjing University, The Chinese University of Hong Kong, Shatin, Hong Kong,

The revised Annex 1 of the PIC/S GMP Guide on the manufacture of sterile products was published in September 2022 and entered into force in August 2023. Driven by PIC/S and the EMA inspectors’ Working Group, this first full revision aimed to restructure the Annex 1 and add clarity to the requirements for manufacturing sterile advanced therapy medicinal products (ATMP) including active substance, excipient, primary packaging material and finished dosage form to minimize risks of microbial, particulate and endotoxin/pyrogen contamination. In this revised version, guidance is provided for ATMP pack sizes, processes and technologies. Whether the ATMP is packaged in single unit or multiple units, manufactured with highly automated systems or manual processes, using state-of-the-art technologies or classical manufacturing systems, the implementation of contamination control strategies is essential for the manufacturing of ATMP and requires detailed technical and process knowledge. Elements to be considered are outlined in the revised Annex 1.

For the adaptation to the revised Annex 1, a collaborative process of technology transfer is critical for bridging the gap between research and practice. By supporting the flow of scientific findings, knowledge and intellectual property among different innovation stakeholders, difficulties confronted during this process will be resolved with perspective from each party and elevate the chance of successful commercialization. For instance, critical process parameters for the technical manufacturing processes, critical quality attributes for quality controls and the release of the finished products for the purpose of technology transfer from the perspectives of upstream R&D setting. Specifically, validation of reputable sources for ATMP starting materials and raw materials are critical for the manufacturing process because direct contact is involved with the final products. These materials must be obtained from a traceable and reputable origin and tested for relevant and defined product quality specifications such as viral testing, mycoplasma, endotoxin and performance testing. Moreover, full composition and potency of the final products shall be well-defined at the beginning of the development process as measurable parameters to determine safety and therapeutical efficacy of the products. Furthermore, current technologies for ATMP rely on computerized operation for both manufacturing processes and analysis, where electronic records and signatures play a significant role for traceability. While developing these processes and analytical methodology, systems compatible with FDA guidelines 21 CFR Part 11 shall be chosen at the beginning of the developmental process to avoid unnecessary delay due to incompetent systems.

In this report, we will discuss potential pitfalls during technology transfer to encourage early development of alternative approaches and contingency plans for effective and efficient translation. With active communication among different stakeholders, the chance of a successful commercialization will vastly increase and be completed in a timely manner.

Acknowledgements:
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References:
Annexin-V alleviates osteoarthritis via macrophage mediated immunomodulation and chondrocyte protection

Mrs. Zhuoxuan Jia, Dr. Wenjie Zhang
1 shanghai ninth peopole hospital, shanghai, China, 2 shanghai ninth peopole hospital, shanghai, China

• Introduction
Osteoarthritis (OA) is a degenerative joint disease characterized by the gradual destruction of joint cartilage and synovial inflammation. In our previous study, we proposed the use of cell-free fat extract (CEFFE) derived from discarded adipose tissue, which is rich in various protein components, and demonstrated through in vivo and in vitro experiments that CEFFE has therapeutic effects on early-stage OA. To further investigate the specific components in CEFFE that contribute to its therapeutic effects, we fractionated CEFFE by salt extraction and used a macrophage polarization model for screening. We discovered that Annexin-V protein (A5), present in CEFFE, has the ability to inhibit the transition of M0 macrophages to M1 macrophages. Therefore, we hypothesize that Annexin-V may have a therapeutic effect on early-stage OA.

• Subjects and Methods
In the in vivo experiment, we divided SD rats into four groups, with 6 rats in each group, namely Control, Model, low-dose A5 group, and high-dose A5 group. An intra-articular injection of 50 µL MIA solution was used to construct an in vivo OA model. Control group was not treated, while the other groups were given either physiological saline (Model group) or the corresponding dose of A5 (low-dose or high-dose) once every 2 weeks for a total of 4 times. The evaluation indicators included behavioral experiments: paw withdrawal threshold and gait analysis; pathological studies: HE staining, Safranin O-Fast Green staining, Toluidine blue staining, CD68 and CD206 immunohistochemistry. In the in vitro experiment, we used RAW264.7 and bone marrow-derived macrophages, induced by LPS and IFNγ to transform from M0 to pro-inflammatory M1 type, and explored the anti-inflammatory effect of A5 using flow cytometry and qRT-PCR. We also investigated the protective effect of A5 on chondrocytes with or without IL-1β and TNF-α using primary mouse cells.

• Results
The in vivo experiment results showed that A5 can alleviate MIA-induced OA pain symptoms, improve articular cartilage fibrosis, and reduce the occurrence and/or degree of chondrocyte proliferation, degeneration, necrosis, and erosion. At the same time, A5 can also promote the increase of anti-inflammatory macrophages and improve the inflammatory state of the joint cavity. The in vitro experiment showed that A5 can reduce the proportion of M1 macrophages, and the inhibitory effect of high-concentration A5 was most significant. A5 has the ability to reduce chondrocyte apoptosis, and related matrix metalloproteinases such as ADAMT5, MMP3, MMP9, and MMP13 decreased after A5 treatment, and these changes were concentration-dependent.

• Discussion and Conclusion
In summary, we confirmed the effectiveness of A5 treatment in an in vivo OA model, demonstrated the ability of A5 to inhibit macrophage transformation from M1 to pro-M1 in an in vitro macrophage model, and confirmed the ability of A5 to reduce chondrocyte apoptosis and decrease the production of degradation enzymes in an in vitro primary chondrocyte model.
Digital Light Processing (DLP) of Biomimicking Radially Graded Scaffolds for Bone Tissue Engineering

Ms. Yue WANG1,2, Dr. Jiaming BAI2, Dr. Min WANG1
1The University of Hong Kong, Hong Kong, Hong Kong, 2Southern University of Science and Technology, Shen zhen, China

Introduction: Bone tissue engineering (BTE) has advanced greatly in recent decades. Geometrical features, e.g., pore size, pore morphology and porosity of BTE scaffolds are of great significance for bone tissue regeneration. It is believed that scaffolds with geometrical characteristics similar to the host tissue can have better functionality and efficiency. With rapid progresses in computer aided design and additive manufacturing, it is natural to develop functionally graded scaffolds to mimic the structures and functions of natural bone.

Materials and Methods: Four types of biomimicking scaffold designs based on triply periodic minimal surfaces (TPMS)-gyroid (G) or -primitive (P) units with graded pore sizes from the periphery to the center were attempted. Scaffolds of these designs at two porosity levels (65 and 75 vol.%) were fabricated via digital light processing (DLP) 3D printing using biphasic calcium phosphate (BCP) bioceramic. Properties of BCP slurries for DLP and optimal DLP processing parameters were systematically investigated. The dimensional accuracy and compressive properties of BCP scaffolds were analyzed. Other aspects of sintered BCP scaffolds were also studied.

Results: Four types of biomimicking scaffolds were designed and fabricated. The slurry of nano-sized BCP with 70 wt.% solid loading had a relatively low viscosity and good curing accuracy, enabling successful fabrication of graded scaffolds at the two porosity levels. Scaffolds of the G-G design displayed better dimensional accuracy and compressive strength than G-P, P-G, and P-P scaffolds. The compressive strength of G-G scaffolds with 65 and 75 vol.% porosity could reach 12.23±0.82 and 5.55±0.65 MPa, respectively. Sintered BCP fabricated via current process exhibited excellent biocompatibility and bioactivity, indicating their high potential for BTE.

Discussion and Conclusions: Four types of biomimicking radially graded scaffold designs based on TPMS units were achieved using a computer-assisted method. The porosity and pore size could be simultaneously controlled in a specific range by adjusting unit cell size and thickness. The G-G scaffolds showed good dimensional accuracy and compressive properties because of the inherent geometrical features of G structure. The compressive strength of BCP scaffolds in this study was competitive owing to the high densification of the ceramic grains and the refined ceramic grain size of the sintered bioceramic. The methodology established in this study advances the design of biomimicking BTE scaffolds and fabrication of complex bioceramic scaffolds.

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Regenerative Role of Type II Collagen Solution Derived from Supercritical Carbon Dioxide Decellularized Cartilage in Medial Meniscectomy-Induced Osteoarthritis Model

Dr Srinivasan Perisamy, Dr Yun-Ju Chen, Dr Dur-Zong Hsu, Dr Dar-Jen Hsieh

1R&D Center, ACRO Biomedical Co., Ltd. 2nd. Floor, No.57, Luke 2nd. Rd., Luzhu District, Kaohsiung City 82151, Taiwan, Kaohsiung, Taiwan, 2R&D Center, ACRO Biomedical Co., Ltd. 2nd. Floor, No.57, Luke 2nd. Rd., Luzhu District, Kaohsiung City 82151, Taiwan, Kaohsiung, Taiwan, 3Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, 138 Sheng-Li Rd., Tainan 70428, Taiwan., Tainan, 4R&D Center, ACRO Biomedical Co., Ltd. 2nd. Floor, No.57, Luke 2nd. Rd., Luzhu District, Kaohsiung City 82151, Taiwan, Kaohsiung, Taiwan

Background: Osteoarthritis (OA) is a deteriorating synovial joint disease affecting the articular cartilage and subchondral bone. About 9.6% of men and 18.0% of women over the age of 60 are affected by OA, estimated by the Worldwide Health Organization (WHO). Nonoperative OA therapy has the potential to manage symptoms, improve function, and delay the need for joint replacement. Viscosupplementation with hyaluronan is a well-established therapy choice in OA.

Methods: In the present study, we extracted type II collagen solution from supercritical carbon dioxide decellularized porcine cartilage and used it to treat the surgical medial meniscectomy (MNX) induced post-traumatic osteoarthritis (PTOA) model in rats.

Results: OA progression was attenuated dose-dependently in the intra-articular administration of type II collagen 1, 5, 10 mg/mL which significantly decreased the pain in MNX-induced OA. The pain was evaluated by a capacitance meter, type II collagen attenuated dose-dependently the pain. Type II collagen dose-dependently attenuated articular cartilage damage as evidenced by Micro-CT. The bone volume significantly increased with increasing dose of type II collagen in OA-treated animals. The therapeutic efficacy of type II collagen on articular cartilage damage is due to its role in the expression of type II collagen, aggrecan and SOX-9.

Conclusion: We demonstrated that the intra-articular administration of type II collagen solution reestablished the injured cartilage and decreased osteoarthritis in the experimental PTOA model.
Hemostatic Efficacy of Supercritical Carbon Dioxide Decellularized Acellular Dermal Matrix (ADM) Scaffold Powder and Paste in Rat Spleen Rupture Model

Dr Yun-Ju Chen, Dr Srinivasan Perisamy, Dr Dar-Jen Hsieh

1R&D Center, ACRO Biomedical Co. Ltd, Kaoshiung, Taiwan, Kaoshiung, Taiwan, 2R&D Center, ACRO Biomedical Co. Ltd, Kaoshiung, Taiwan, Kaoshiung, Taiwan, 3R&D Center, ACRO Biomedical Co. Ltd, Kaoshiung, Taiwan, Kaoshiung, Taiwan

Background: Hemostasis is important to decrease blood loss and alleviate the danger of death from severe bleeding. During surgery, the common problem for physicians is stopping bleeding. Apart from the advances in endoscopic surgeries and related bleeding remains hard to manage with increased failure and rebleeding. In the present study, the hemostatic efficacy of supercritical carbon dioxide decellularized acellular dermal scaffold and paste in rat spleen rupture model was evaluated.

Methods: In the present study, we decellularized the porcine acellular dermal matrix (ADM) using supercritical carbon dioxide (SCCO2) and subsequently freeze-milled into powder and mixed with saline to make a paste. The spleen is an organ related to immunity and hematopoiesis. It is hard to stop bleeding compared to other organs in clinical practice. Therefore, the rat spleen rupture model is used to evaluate the hemostatic function.

Results: Hemostasis time and blood loss are important parameters for the evaluation. In comparison to the sham group, the predicate device and ADM scaffold powder and paste group provided an instantaneous hemostasis effect. The blood loss in the sham group was significantly higher than those in the predicate device and ADM scaffold powder and paste groups. Histopathological evaluations showed the inflammation score in the sham group was similar to that in the predicate device group and ADM scaffold powder and paste, indicating no inflammatory response. Hemorrhage results showed that the postoperative splenic local hemorrhage was high in the sham group compared to the ADM scaffold powder and paste groups.

Conclusion: In the present study, we produced the SCCO2 decellularized ADM scaffold powder and paste. ADM scaffold powder and paste group provided an instant hemostasis effect. In addition, ADM scaffold powder and paste decreased blood loss and inflammation.
In Vivo Reconstruction of Supercritical Carbon Dioxide Decellularized Kidney in the Rabbit Model.

Dr. Dar-jen Hsieh, Dr Yin-Chih Fu, Dr Srinivasan Perisamy, Dr Wen-Chih Liu, Dr Jia-Jung Lee, Dr Peir-In Liang

1Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan, Kaohsiung, Taiwan, 2R&D Center, ACRO Biomedical Co. Ltd, Kaohsiung, Taiwan, Kaohsiung, Taiwan, 3Department of Orthopadics, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan, Kaohsiung, Taiwan, 4Division of Nephrology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan, Kaohsiung, Taiwan, 5Department of Pathology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan, Kaohsiung, Taiwan, 6R&D Center, ACRO Biomedical Co. Ltd, Kaohsiung, Taiwan, Kaohsiung, Taiwan

Background: Chronic kidney disease is an important cause of mortality and morbidity worldwide, affecting largely the adult population. Renal regeneration approaches offer great potential for the end-stage treatment of chronic kidney disease, but their accessibility remains inadequate to the clinical challenges due to the intricacy of this organ.

Methods: In the present study, we decellularized a rabbit kidney using supercritical carbon dioxide (SCCO2) and subsequently a direct orthotropic transplantation approach to another rabbit was done, after 4 weeks the kidney was harvested and markers of regeneration were studied.

Results: Decellularized kidneys were stained by hematoxylin and eosin and 4,6-diamidino-2-phenylindole (DAPI) staining and DNA depicted complete removal of cells by SCCO2. In vivo recellularization of the kidney was evaluated by computerized tomography, ultrasonography and nuclear scan. The expression of metanephric mesenchyme, PAX2 and WT1 had a modulating expression in the transplanted kidney, indicating regeneration. The stem cell marker CD34 was found to be expressed in the transplanted kidney, indicating the recruitment of stem cells in the transplanted kidney leading to regeneration. Pre-tubular aggregate and renal vesicle marker PAX8 were found to express in the transplanted kidney, indicating the formation of the functional unit of the kidney leading to regeneration. The expression of CK and CK7 in the transplanted kidneys indicates cell proliferation and regeneration. Nuclear renal scan depicted urine formation around the reconstructed kidney, indicating a partial renal function in vivo.

Conclusion: In the present study, we established that the SCCO2 decellularized kidney did not have immune related-rejection. Furthermore, the expression of metanephric mesenchyme and renal vesicle markers indicate the formation of the functional unit of the kidney. In addition, the recruitment of stem cell markers and cell proliferation markers occurs in the direct orthotopic transplanted SCCO2 decellularized kidney. We have demonstrated that decellularized kidneys can be reconstructed in vivo with likely partial biological function. This brings hope for those patients waiting in line for a donated kidney.
In vivo, direct xenotransplantation of supercritical carbon dioxide decellularized artery enabled complete recellularization and depicted physiologic-like artery

Dr. Srinivasan Periasamy, Dr Shih-Ying Sung, Dr Yi-Wen Lin, Dr Chin-Chen Wu, Dr Chih-Yuan Lin, Dr Po-Shun Hsu, Dr Balaji Nagarajan, Dr Yi-Ting Tsai, Dr Chien-Sung Tsai, Dr Feng-Yen Lin, Dr Dar-Jen Hsieh

1ACRO Biomedical Co. Ltd, Kaoshiung, Taiwan, 2Graduate Institute of Medical Sciences, National Defense Medical Center, Taipei, Taiwan, Taipei, Taiwan, 3Division of Cardiovascular Surgery, Department of Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, 4Institute of Oral Biology, National Yang-Ming Chiao-Tung University, Taipei, Taiwan, Taipei, Taiwan, 5Department and Graduate Institute of Pharmacology, National Defense Medical Center, Taipei, Taiwan, Taipei, Taiwan, 6Institute for Structural Biology, Drug Discovery and Development, Virginia Commonwealth University, Virginia, USA, Virginia, USA, 7Taipei Heart Institute, Taipei Medical University, Taipei, Taiwan, Taipei, Taiwan, 8Division of Cardiology and Cardiovascular Research Center, Taipei Medical University Hospital, Taipei, Taiwan, Taipei, Taiwan, 9Department of Internal Medicine, College of Medicine, School of Medicine, Taipei Medical University, Taipei, Taiwan, Taipei, Taiwan

Background: In humans synthetic vascular graft usage is associated with a lesser percentage of patency and an elevated level of infection of arteriovenous fistulas. Higher rates of thrombosis and infection are observed after the use of common synthetic vascular grafts such as Dacron and expanded polytetrafluoroethylene.

Methods: In the present study, we used the direct xenotransplantation (XTP) approach of supercritical carbon dioxide (SCCO2) decellularized rabbit artery and implanted in the ACI/NKyo rat to assess the main concept of tissue engineering, the decellularization-recellularization approach and compared with sodium dodecyl sulfate (SDS) decellularized artery.

Results: In vivo, the recellularization of the artery was complete, with endothelial cell distribution in the recellularized blood vessel. The SCCO2 decellularized artery depicted good biocompatibility, improved chemotactic migration of endothelial progenitor cells, decreased risk of vasculopathy, decreased inflammatory and splenic immune responses, and good physiological-like tension responses after xenotransplantation in ACI/NKyo rats compared with detergent SDS decellularized artery. The SCCO2 decellularized artery depicted physiological conditions for artery regeneration with contractions and dilations similar to the native artery. However, SDS decellularized artery was not equivalent to the native artery. The aneurysmal alterations and vasculopathy in the SCCO2 decellularized artery were negligible compared to SDS decellularized artery in XTP rats.

Conclusion: We demonstrated that the SCCO2 decellularized artery decreased inflammatory and immune-rejection responses, displayed chemotaxis of vascular progenitor cells, and competently established into a functional artery in XTP rats.

3D Printing of Reinforced Blend Hydrogel Tissue Engineering Scaffolds Loaded with an Anticancer Drug

Ms. Xiaodie Chen¹, Ms. Hanqi ZHU¹, Prof. Min WANG¹

¹Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China

Title: 3D Printing of Reinforced Blend Hydrogel Tissue Engineering Scaffolds Loaded with an Anticancer Drug

Authors: Xiaodie Chen, Hanqi Zhu, Min Wang*

Affiliation: Department of Mechanical Engineering, The University of Hong Kong, Hong Kong. (*Corresp. auth.)

Category: Design and Application of Biomaterials

Introduction: Tissue engineering scaffolds assist human body tissue regeneration. Even after surgical removal of a tumor, additional therapies may still be necessary for eliminating any residual cancer cells. An effective solution is the implantation of a tissue engineering scaffold that contains an anticancer drug at the resection site. In recent years, 3D printing has been increasingly used for fabricating advanced tissue engineering scaffolds. Also, hydrogels are popular materials for new scaffolds and matrices for tissue regeneration. Combining 3D printing, hydrogels and anticancer drugs can lead to new multifunctional tissue engineering scaffolds.

Materials and Methods: In this study, to produce reinforced hydrogel scaffolds via 3D printing, methacrylate-modified chitosan (CSMA) and methylcellulose (MC) were mixed as new printing inks. Rheological properties of hydrogel blend inks, printing resolution and scaffold properties such as surface morphology, water absorption, biodegradability, compression modulus, etc. were systematically investigated. Furthermore, doxorubicin (DOX)-containing hydrogel blend inks were 3D printed into scaffolds. The release and effects of DOX were studied.

Results: CSMA/MC hydrogel blend scaffolds were successfully fabricated via 3D printing. The addition of MC to CSMA hydrogel led to sufficient viscoelastic behavior of inks and enabled their good printability, which allowed printing of multilayer lattice structure without collapsing and ensured structural integrity of printed structures. An increase in MC concentration resulted in a decrease in the degradation rate of scaffolds. DOX was released from 3D printed CSMA/MC hydrogel blend scaffolds in a sustained manner. DOX-loaded CSMA-MC scaffolds could serve as support structures and a drug-delivery system for cancer patients.

Discussion and Conclusions: CSMA/MC hydrogel blend scaffolds loaded with DOX could be 3D printed. MC acted effectively as the reinforcement in the scaffolds. The scaffolds supported the proliferation of rBMSCs, indicating their good biocompatibility. These scaffolds provide dual functions for cancer patients: ablation of residual cancer cells, and promoting tissue regeneration.

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4D Printing of Hydro-thermal Responsive Tissue Engineering Scaffolds and Their Properties

Ms. Xiaodie Chen¹, Dr. Jiahui LAI¹, Prof. Liwu ZHENG², Prof. Min WANG¹
¹The University of Hong Kong, Hong Kong, China, ²Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR

Title: 4D Printing of Hydro-thermal Responsive Tissue Engineering Scaffolds and Their Properties

Authors: Xiaodie Chen 1, Jiahui Lai 1, Liwu Zheng 2, Min Wang 1, *

Affiliation: 1 Department of Mechanical Engineering, The University of Hong Kong, Hong Kong. 2 Faculty of Dentistry, The University of Hong Kong, Hong Kong (*Corresponding author)

Category: Tissue Engineering and Regeneration

Introduction: 3D printed tissue engineering scaffolds are static in shape and properties during their application and cannot respond dynamically during tissue regeneration. Shape-morphing tissue engineering scaffolds may satisfy the rigorous demands in certain clinical applications and adjust to the environments of specific regions of the body. 4D printing, which uses shape memory polymers (SMPs) and 3D printing, can fabricate dynamic structures which can change their shape, properties or functions under appropriate stimuli. But most SMPs react to only one stimulus whereas human bodies can provide multiple stimuli. By combining an SMP with a biocompatible hydrogel when both can respond to different stimuli, unique scaffolds can be made and utilized.

Materials and Methods: To fabricate scaffolds with tailored properties that could respond to dual stimuli, thermal-responsive poly(D,L-lactide-co-trimethylene carbonate) (PDLLA-co-TMC, “PTMC” in short) and gelatin methacryloyl (GelMA) which responds to water were mixed as an ink for 4D printing. The surface morphology, chemical composition, structure and tensile properties of 4D printed blend scaffolds were investigated. Shape morphing of blend scaffolds at 37 °C in water was studied. Human umbilical vein endothelial cells (HUVECs) were seeded onto scaffolds for assessing biological properties of scaffolds.

Results: PTMC/GelMA blend scaffolds were made by 4D printing using extrusion 3D printing. Compared to pure PTMC scaffolds, PTMC/GelMA blend scaffolds had a higher Young's modulus and stretchability. PTMC/GelMA scaffolds could utilize depth-changing UV irradiation and temperature stimuli to cope with the complex environment in vivo. In addition, the degradation rate of PTMC/GelMA blend scaffolds were significantly increased after the addition of GelMA. Furthermore, PTMC/GelMA blend scaffolds exhibited good biocompatibility and facilitated cell attachment and proliferation.

Conclusions: 4D printed PTMC/GelMA blend scaffolds possessed desired properties/functions, including good biocompatibility and designed shape morphing ability owing to response to dual stimuli. They were hydro-thermal responsive and may find applications for tubular tissues such as the vasculature and the gastrointestinal tract.

Acknowledgement: This work was financially supported by Hong Kong’s Research Grants Council (RGC) through grants 17200519, 17202921, 17201622 and N_HKU749/22.
Development and pathogenesis of patellar tendinopathy in a rat model

Ms. Ying Rao1,2, Prof. Rocky, S. Tuan1,2,3, Prof. Dan, Michelle Wang1,2,3

1School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China, 2Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China, 3Center for Neuromusculoskeletal Restorative Medicine, Hong Kong Science Park, Hong Kong SAR, China

Title: Development and pathogenesis of patellar tendinopathy in a rat model

Authors: Y. Rao1,2, R.S. Tuan1,2,3*, D.M. Wang1,2,3*

Affiliations: 1School of Biomedical Sciences, Faculty of Medicine, 2Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China; 3Center for Neuromusculoskeletal Restorative Medicine, InnoHK Center, Hong Kong SAR, China (*Correspondence)

Category: Tissue Engineering and Regeneration

Background: Calcific tendinopathy (CT) is a debilitating condition characterized by the presence of apatite deposits within the tendon, resulting in chronic pain, functional decline, and reduced exercise tolerance among patients. [1] Despite various clinical treatments, outcomes have been inconsistent, highlighting the need for a better understanding of the underlying pathogenesis. [2] The pathogenesis of CT is multifactorial and complex, with one potential mechanism being the erroneous differentiation of tendon stem/progenitor cells (TSPCs) into chondrocytes or osteoblasts. [1, 2] Additionally, immune-stem cell crosstalk may also play a role in this process, necessitating further investigation. [3] Hence, the objective of this study is to establish a dynamic landscape of tendinopathy development and explore the mechanisms underlying TSPC differentiation and immune-cell crosstalk in CT.

Method: To establish a rat model of patellar tendinopathy, 10-12 week-old, Sprague Dawley (SD) rats were used in this study. These rats were randomly divided into two groups: (1) phosphate buffer solution (PBS) injection control group; and (2) collagenase (250 U)-treated group (COL), with the contralateral tendon serving as an intact control group. The tendinopathy model was characterized via gross morphology, histopathology, and gene expression analysis. Additionally, transcriptomics investigations were performed on 2-, 4- and 8-week post-surgery to assess the mechanisms involved in cell lineage switch (Figure 1A).

Result: Our findings showed that: (1) The COL groups exhibited swelling, yellowish tendons, as well as an abundant secretion of mucus compared to the PBS groups (Figure 1B); (2) Histologically, the COL groups showed a complete loss of architecture, irregular fibers, and abnormal tenocytes at week 2. Chondrocyte-like cells, shown histologically as round cells within lacunar space, were first seen at week 4. At week 8, a large area of calcification and more chondrocyte-like cells were observed (Figure 1B); and (3) The COL tendinopathy group displayed distinct gene expression patterns compared to the control groups. Specifically, significant enrichment in gene ontology (GO) patterns and pathways associated with chondrogenesis, osteogenesis, and inflammation within the tendinopathy group were observed. Moreover, an increase in the proportion of certain immune cells was noted, indicating the presence of a cartilaginous transcriptional program and chronic inflammation in rat patellar tendinopathy (Figure 1C).

Discussion and conclusion: The rat tendinopathy model we established exhibited a chondrogenic phenotype, heterotopic ossification, tendon matrix degradation, and chronic inflammation processes similar to models reported in the literature. The preliminary transcriptomic data also confirmed a cartilaginous transcriptional program and chronic inflammation in the rat patellar tendinopathy model. In future work, we will apply a multi-omics approach, integrating transcriptomics landscape and proteomics profiles to investigate the underlying mechanism of rat patellar tendinopathy.

Figure 1: Characterization and transcriptomic analysis of rat tendinopathy model. (A) Schematic of experimental design. (B) Gross morphology and histology analysis. (C) RNA-seq analysis of rat patellar tendinopathy model. (N=2-3)
Acknowledgments: Research Grants Council of Hong Kong SAR (GRF 14118620 and 14121121, DW); The Hong Kong Innovation and Technology Commission (Health@InnoHK, DW, RST).

Soft-photolithographically Defined Acoustic Template for Arbitrarily Patterned Acoustic Bioassembly

Pu Chen¹,², Dr. Sihan Chen¹
¹Tissue Engineering and Organ Manufacturing (TEOM) Lab, Department of Biomedical Engineering, Wuhan University TaiKang Medical School (School of Basic Medical Sciences), Wuhan, China, ²TaiKang Center for Life and Medical Sciences, Wuhan University, Wuhan, China

Title: Soft-photolithographically defined acoustic template for arbitrarily patterned acoustic bioassembly

Authors: Chen Sihan¹ Chen Pu¹,²*

Affiliations: ¹Tissue Engineering and Organ Manufacturing (TEOM) Lab, Department of Biomedical Engineering, Wuhan University TaiKang Medical School (School of Basic Medical Sciences), Wuhan, China. ²TaiKang Center for Life and Medical Sciences, Wuhan University, Wuhan, China. (*Corresponding author: puchen@whu.edu.cn)

Category: Enabling Technologies

Background: Acoustic bioassembly is recently regarded as a highly efficient biofabrication technique to generate functional tissue construct. However, most acoustic bioassembly techniques are currently limited to assembling cells into some specific simple types of periodic and symmetric patterns, which presents a significant challenge to constructing tissues with complex cytoarchitectures.

Methods: To address this issue, we herein demonstrate a novel acoustic template based bioassembly technique that enables to assemble live cells into predefined arbitrary multicellular structures. To realize this technique, we employed soft lithography to fabricate PDMS-based microchannel that works as an acoustic template and modulates near-field acoustic waves for the formation of predefined force potential distribution. Cells in the acoustic field self-assemble into closely-packed cytoarchitecture just above the microchannels where force potential is minimized. Using this technique, we assemble HUVEC spheroids and HepG2 cells into liver lobule-like microtissues.

Results: We investigate the geometric dimensions of the acoustic template on bioassembly. The function signal generator provided a 1.9 MHz AC sine electrical signal to the power amplifier, and the piezoelectric transducer was connected to the power amplifier. We found that the geometries of the matching layer of the micro-machinated gas have a better acoustic modulation effect at the sub-wavelength scale. The optimal conditions of the matching layer of the micro-machinated gas (the film thickness is 20 μm; the cavity height is 60 μm, the cavity spacing is 400 μm), which can form the minimum sound pressure above the gas matching layer, and realize the arbitrary pattern assembly of the particles/cells above it. To further quantitatively evaluate the liver metabolism and synthesis function of the hepatic lobule-like liver model, we detected albumin secretion and urea production. Albumin and urea levels in the assembled hepatic lobule-like liver model were higher than those in the unassembled hepatic lobule-like liver model. The albumin secretion and urea production of the hepatic lobule-like liver model reached the highest level on the sixth day. The albumin concentration in the supernatant was 17.3 ng/mL, and the urea concentration was 1.6 mg/dL, indicating that the hepatic lobule-like liver model can reconstruct some key liver-specific functions.

Discussion and Conclusion: Advanced biofabrication technique is essential for tissue engineering and regenerative medicine. Bioassembly is recently regarded as a critical technical route for biofabrication due to its advantages in direct cell manipulation, improved tissue functions, and highly efficient fabrication process [1]. However, it’s limited to generate tissue construct with some simple
types of specific patterns. Here, we overcome this big challenge and extend bioassembly applications to form tissue constructs with arbitrary complex structures. We employed near-field acoustic waves and modulated acoustic pressure fields using a soft-photolithographically defined microchannels. Numerical simulations and microparticle assembly experiments indicate that low pressure regions formed above the microchannels and aggregated microparticle into a pattern exactly like the structure of microchannels. Furthermore, we explore this technique to assemble HUVEC spheroids and HepG2 cells into liver-lobule like structures where HUVECs were radially arranged. On day 7 post bioassembly, the formed liver microtissues displayed liver-specific functions including albumin secretion, urea synthesis, glucose metabolism and lipid storage. We expect this technique will be broadly used to construct complex functional tissues for tissue engineering and regenerative medicine.

References

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Injectable cryogel microspheres act as modularized cell delivery carriers and sustainable Simvastatin release nano-in-micro multistage system for tissue regeneration

Dr. Xiaojing Yuan¹, postdoctor Zuoying Yuan², Professor Yuanyuan Wang¹, Professor Chunyang Xiong², Professor Yuming Zhao¹, Professor Lihong Ge¹

¹Department of Pediatrics, Peking University School & Hospital of Stomatology, Beijing, China, ²Department of Mechanics and Engineering Science, College of Engineering, Peking University, Beijing, China

Title: An injectable shape memory co-delivery micro platform for tissue regeneration

Authors: Yuan Xiaojing¹, Yuan Zuoying², Wang Yuanyuan¹, Xiong Chunyang²*, Zhao Yuming¹*, Ge Lihong¹*

Affiliations: 1. Department of Pediatrics, Peking University School & Hospital of Stomatology, #22 Zhongguancun South Avenue, Beijing, 100081, China.
2. Department of Mechanics and Engineering Science, College of Engineering, Peking University, Beijing, 100871, China.

Category: Design and Application of Biomaterials

Background: Cell therapeutics hold tremendous regenerative potential and the therapeutic effect depends on the effective delivery of cells. In this study, the injectable shape memory co-delivery platform was constructed with improved cell vitality and enhanced cell function to prompt functionalized tissue regeneration.

Subjects and Methods: By combining the emulsion technique with gradient-cooling cryogelation, the pore size of methacrylated gelatin microspheres (CMS) was precisely controlled. The cell protective ability of CMS was evaluated by live/dead assay, and the stemness and differentiation potential of SHED and BMSC were detected in vitro. The drug-cell co-delivery and modularized cell delivery platforms were constructed respectively, and the regeneration effect of the micro platform was verified both in vitro and in vivo.

Results: CMS-30 could quickly recover to the original morphology after external force without losing integrity, therefore, SHED and BMSC loaded on CMS-30 showed higher cell vitality. Cells loaded on CMS showed tight adhesion. Nanog and Sox-2 gene expression were higher in cells loaded on CMS-30 group. Through the construction of a nano-in-micro system based on Simvastatin nanoparticles encapsulated in CMS, the controllable dual delivery platform of simvastatin and SHED was realized, which significantly promoted the proliferation and odontogenic differentiation of SHED, and achieved pulp-like tissue regeneration in the root canal in vivo. The pro-angiogenic CMS module loaded with HUVEC and the osteogenic CMS module loaded with BMSC were prepared respectively, and the functional tissue with high expression of OCN and CD31 were successfully regenerated after subcutaneous injecting both modules into nude mice.

Discussion and Conclusion: CMS-30 with optimized porous structure and shape memory characteristics could effectively protect stem cells vitality, improve their stemness and retain differentiation potential. The co-delivery micro platform can realize tissue functional regeneration.
In vitro intestinal model with bacterial beads and dynamic stimuli

Mr. Mioto Nishino¹, Mr Naoya Ito, Mr Toshihiro Usui, Mr Junji Fukuda
¹Yokohama National University, Japan

Introduction: Intestine is the main organ where most of the orally administered drugs are absorbed into the body. In vitro intestinal model would therefore provide important insights for drug development. Although enterobacteria play critical roles in some drug metabolisms and absorption, it is still challenging to co-culture intestinal epithelial cells and enterobacteria due to the quite large difference in growth rates. Moreover in vivo intestinal epithelial cells are constantly exposed to dynamic stimulation through the transport of the contents. The purpose of this study is to recapitulate these environments in vitro to develop an intestinal model.

Subjects and Methods: The intestinal model is composed of bacterium-encapsulated beads, an epithelium cell layer, and a seesaw stage. Bacterium-encapsulated beads were prepared by falling droplets of sodium alginate solution containing E. coli into a calcium chloride solution by centrifugal force. Human Caco-2 intestinal epithelial cells were seeded on the 6 well inserts and cultured for 1 day for adhesion. Then, bacterial beads were placed on the epithelium cell layer and cultured for 6 days with shaking on the seesaw stage.

Results: X-Gal staining revealed that E. coli were survived after encapsulation into the beads. The barrier function of epithelium cell layer was maintained for at least 6 days in co-culture with the bacterial beads. In addition, villi-like structures of ~80 µm in height were formed only when cultured on the seesaw stage with bacterial beads.

Discussion and Conclusion: Further studies including details analysis of tissue structures and functions and the interaction between epithelial cells and bacteria will be needed. This model could be an important tool for better understanding intestinal absorption and drug development.
Seeking and Identifying Time Window of Antibiotic Treatment Under In vivo Guidance of PbS QDs Clustered Microspheres Based NIR-II Fluorescence Imaging

Dr. Sijia Feng, Shiyi Chen, Yunxia Li, Jun Chen
1Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai, China
Title: Seeking and Identifying Time Window of Antibiotic Treatment Under In vivo Guidance of PbS QDs Clustered Microspheres Based NIR-II Fluorescence Imaging
Authors: Sijia Feng, Shiyi Chen, Yunxia Li, Jun Chen
Affiliations: 1Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China. (Correspondence)
Category: Design and Application of Biomaterials

Background: To date, little is known about the time window of antibiotic treatment, including both the initiation and termination. During this time window, the efficacy of antibiotics could be maximized, along with timely administration executed and prolonged use avoided, thus urgently calling for more advanced imaging technology for in vivo visualization and monitoring of bacterial infection in assistance of seeking the time window of antibiotic treatment.

Methods: This study presented a NIR-II fluorescence imaging strategy based on PbS QDs clustered microspheres (PbS QDs@microspheres), which not only demonstrated excellent NIR-II fluorescence properties, but also could facilitate seeking and identifying the time window of antibiotic treatment by monitoring bacterial infection in vivo in a real-time manner.

Results: PbS QDs@microspheres with an average size of 510 nm were prepared through cross-linking process, demonstrating nine times higher PL intensity than the original PbS QDs, along with deep optical tissue penetration in muscle and 50% fat+50% muscle. Monitoring of bacterial infection in a mouse model of joint infection was achieved by utilizing PbS QDs@microspheres to label S. aureus in vivo. Instructed by the NIR-II fluorescence signals from the intra-articularly injected S. aureus, 1 h to 3 d post-infection was sought to be a period of bacteria burst, along with relatively low immune cells counts. Four therapeutic regimens (1 h, 2 h, 1 d and 3 d post-infection) of antibiotic treatment were implemented, among which the 3 d group resulted in a survival rate of 50%, significantly lower than the other three groups (P = 0.028). The results of blood culture and histopathological analysis denoted that antibiotic administration at 3 d post-infection induced higher inflammatory reaction during the recovery period.

Discussion and Conclusion: Under the guidance of PbS QDs@microspheres based NIR-II fluorescence imaging, 1 h to 3 d post-infection was sought as the time window of antibiotic treatment for mouse models with joint infection. Antibiotic administration at 1 h post-infection prevented bacteremia, while at 3 d post-infection induced higher inflammatory reaction.
Spatio-temporally Deciphering Peripheral Nerve Regeneration in vivo after Extracellular Vesicles Therapy under NIR-II Fluorescence Imaging

Dr. Sijia Feng1, Jun Chen2, Sijia Feng2, Yan Wo1
1Department of Anatomy and Physiology, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, 2Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai, China

Title: Spatio-temporally Deciphering Peripheral Nerve Regeneration in vivo after Extracellular Vesicles Therapy under NIR-II Fluorescence Imaging

Authors: Yueming Wang1, Jun Chen2*, Sijia Feng2*, Yan Wo1*

Affiliations: 1Department of Anatomy and Physiology, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China. 2Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China. (* Correspondence)

Category: Tissue Engineering and Regeneration

Background: As the EVs therapy is expected to be a novel potential for optimizing peripheral nerve regeneration in the treatment of PNI, it is crucial to understand when, where and how the EVs exert their therapeutic effect. However, the migration time, spatial distribution, and retention of EVs in vivo, which are closely related to the effectiveness and efficacy of EVs therapy during peripheral nerve regeneration remain unknown, due to a lack of real-time monitoring modalities. Moreover, non-invasive analysis and prediction of functional recovery in the course of treatment are anticipated for timely adjustment of EVs administration.

Methods: In the present work, an innovative approach for EVs labeling that preserves their morphology and physiological characteristics as well as longitudinal monitoring of EVs in vivo was performed, providing a spatio-temporal map of EVs and allowing for in-depth interpretation of nerve regeneration after EVs therapy.

Results: Our results demonstrate that the injected EVs migrated from the uninjured site to the injured site of the nerve, with an increase in fluorescence signals detected from 4 to 7 days post-injection, indicating the release of contents from the EVs with therapeutic effects. Immunofluorescence and behavioral tests revealed that EVs therapy promoted nerve regeneration and functional recovery at 28 days post-injection. We also found a relationship between functional recovery and NIR-II fluorescence intensity change pattern, providing novel evidence for the therapeutic effects of EVs therapy using real-time NIR-II imaging at the live animal level.

Discussion and Conclusion: Our findings indicated that sciatic nerve injury treated with SKP-SC-EVs therapy resulted in nerve regeneration and neurofunctional recovery, the process of which could be precisely deciphered by NIR-II fluorescence imaging in vivo, displaying considerable potential for future PNI treatment under real-time monitoring.
Functional and Structural Outcomes After Arthroscopic Rotator Cuff Repair With or Without Preoperative Corticosteroid Injections

Dr. Sijia Feng¹, Huizhu Li¹, Yuting Zhong¹, Jun Chen¹, Yuzhou Chen², Shiyi Chen¹
¹Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai, China, ²Department of Orthopedic Surgery, Xin Hua Hospital affiliated to School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Title: Functional and Structural Outcomes After Arthroscopic Rotator Cuff Repair With or Without Preoperative Corticosteroid Injections

Authors: Sijia Feng¹, Huizhu Li¹, Yuting Zhong¹, Jun Chen¹, Yuzhou Chen²*, Shiyi Chen¹*

Affiliations: ¹Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China. ²Department of Orthopedic Surgery, Xin Hua Hospital affiliated to School of Medicine, Shanghai Jiao Tong University, Shanghai 200092, China. (*Correspondence)

Category: Tissue Engineering and Regeneration

Background: Corticosteroid injections (CSIs) are effective in alleviating pain in patients with rotator cuff tears, but controversy still exists regarding their potential adverse effects on clinical outcomes after rotator cuff repair. The purpose of the current study was to compare both structural changes and functional outcomes in patients who underwent arthroscopic RCR, either with or without preoperative CSIs.

Methods: A retrospective cohort study was carried out among patients who underwent arthroscopic rotator cuff repair for partial- and full-thickness tears between 2015 and 2019. The patients who received preoperative CSIs were included in the CSI group and compared with a group without preoperative CSIs (non-CSI group), matched at a ratio of 1:2 based on tear size, age, and follow-up time. Both functional evaluation and structural assessments using magnetic resonance imaging (MRI) were performed at the final follow-up. Clinical outcomes—including retear rate as the primary outcome; pain; shoulder functional scores including the Constant-Murley score, American Shoulder and Elbow Surgeons score, and Fudan University Shoulder Score; range of motion (ROM); tendon integrity; tendon healing type; and cartilage thickness—were compared between the 2 groups with a statistical significance of P < 0.05 and power of 0.9.

Results: Thirty-one patients were included in the CSI group, and 62 were included in the non-CSI group. After a mean 3-year follow-up, the 2 groups demonstrated no significant differences in retear rate; visual analog scale for pain; shoulder functional scores; and active ROM including forward flexion, abduction, external rotation, and internal rotation. No significant differences were observed on postoperative MRI scans of the rotator cuff tendon (tendon integrity, healing type, residual tendon attachment area, etc), cartilage thickness, and muscle atrophy.

Discussion and Conclusion: No significant differences were found at a mean 3-year follow-up in the retear rates, pain, ROM, and glenohumeral structure on postoperative MRI scans after arthroscopic rotator cuff repair with or without preoperative CSIs.
Bankart Repair With Remplissage Restores Better Shoulder Stability Than Bankart Repair Alone, and Medial or Two Remplissage Anchors Increase Stability but Decrease Range of Motion: A Finite Element Analysis

Dr. Sijia Feng¹, Huizhu Li¹, Yuzhou Chen¹, Jun Chen¹, Xiaoxi Ji¹, Shiyi Yi¹
¹Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai, China

Title: Bankart Repair With Remplissage Restores Better Shoulder Stability Than Bankart Repair Alone, and Medial or Two Remplissage Anchors Increase Stability but Decrease Range of Motion: A Finite Element Analysis

Authors: Sijia Feng¹, Huizhu Li¹, Yuzhou Chen¹, Jun Chen¹*, Xiaoxi Ji¹*, Shiyi Chen¹*

Affiliations: ¹Sports Medicine Institute of Fudan University, Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China. (* Correspondence)

Category: Tissue Engineering and Regeneration

Background: Although a standard arthroscopic Bankart repair with remplissage (BR) was described when the procedure was first invented, the specific surgical process varies among surgeons. The current study aimed to investigate the effects of the number and location of anchors for remplissage on postoperative glenohumeral biomechanics. Medially placed anchors and 2 anchors rather than 1 for remplissage were hypothesized to achieve better shoulder stability at the cost of a loss of range of motion (ROM).

Methods: A biomechanical study was conducted involving finite element model constructed based on data from the intact glenohumeral joint. Seven models were established, including a normal model, a model of Bankart lesion combined with “off-track” Hill-Sachs lesion, a model of Bankart repair alone, and 4 models of Bankart repair with remplissage based on different remplissage anchor numbers and locations. The effects of the number and location of the remplissage anchors on glenohumeral stability were studied through calculation and comparison of (1) the stress and its distribution on the joint capsule, cartilage, labrum and anchors as well as (2) the displacement of the humeral head.

Results: Finite element analysis demonstrated that contact stress on the glenohumeral cartilage decreased when medial or 2 anchors were used and was minimized in the combined repair model with 2 medial anchors. The stress on remplissage anchors was greater when the anchors were placed medially. The humeral head displacement was maximized in the combined lesion model. The combined repair models with 2 medially placed anchors showed the largest slope on the force displacement curve, indicating the largest strain on the humeral head.

Discussion and Conclusion: Based on a finite element analysis, Bankart repair with remplissage restored better shoulder stability compared with Bankart repair alone in the treatment of anterior shoulder instability involving Bankart lesion combined with “off-track” Hill-Sachs lesion. When the anchor for remplissage was medially placed or 2 anchors were used, the stability of the glenohumeral joint increased but with a loss of range of motion. The results of this study will assist in choosing the number and location of anchors for remplissage during shoulder stabilization surgery although with some limitations.
Investigation of shear flow effect on vascular endothelium under a dynamic flow system.

Mr. Vadym Kopych¹ ², Mr. Avelino DOS SANTOS DA KOSTA¹, Kwideok Park¹ ²

¹Center for Biomaterials, Korea Institute of Science and Technology, Seoul, Republic of Korea, ²Division of Bio-Medical Science and Technology, KIST School, University of Science and Technology (UST), Seoul, Republic of Korea

Title: Investigation of shear flow effect on vascular endothelium under a dynamic flow system.

Authors: Vadym Kopych¹ ², Avelino Dos Santos Da Kosta¹, Kwideok Park¹ ²

Affiliations: ¹Center for Biomaterials, Korea Institute of Science and Technology, Seoul, Korea ²Division of Bio-Medical Science and Technology, KIST School, University of Science and Technology (UST), Seoul, Korea

Category: SYIS Design and Application of Biomaterials

Background: Endothelial cells (ECs) are constantly exposed to the blood flow and resultant shear stress. Understanding how ECs can sense blood flow has been a critical subject for many years to elucidate the underlying mechanisms of vascular pathophysiology. To investigate such mechanisms, many in vitro endothelium models have been developed over the past few decades. In this work, we developed a dynamic flow system using PDMS and examined the shear flow effect on endothelial cells.

Methods: All the tests were done using human umbilical vein endothelial cells (HUVEC) cultured on gelatin-covered glass. We cultivated HUVECs in antibiotic-free media for 5 days to form a monolayer and they were then subjected to wall shear stress (WSS) of 2 dynes/cm² and 7 dynes/cm² for 12 hours. We used confocal microscopy to compare cell alignment and nucleus aspect ratio between atheroprone and atheroprotective flow conditions. RT-qPCR, Western Blot were performed to explore changes in gene expression and protein levels. For statistical analysis, GraphPad Prism 9 software was used.

Results: Our newly developed dynamic fluid system recreated the physiologically relevant response of endothelial cells. Atheroprotective flow condition caused the rearrangement of F-actin filaments of the cytoskeleton as well as a change in the morphological structure of the cell nucleus. Endothelial cells have been shown to alter the expression of main mechanosensors under atheroprone and atheroprotective conditions. Moreover, our result showed that Hb-α expression, which was previously thought to be only present between endothelial cells and pericyte, increased in response to the atheroprotective shear stress.

Discussion and Conclusion: We demonstrated that our system is capable of imitating the physiologically relevant wall shear stress and its effect on gene expression patterns and protein levels. Further study, with the co-culture model, is needed to simulate exact physiological conditions.

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Engineering Electrospun Membranes as Functional Biomimetic Tendon Sheath for Enhanced Anti-Adhesion and Tendon Repair

**Mrs. Qiao Yang**, Yaobin Wu, Ling Wang

1Biomaterials Research Center, School of Biomedical Engineering, Southern Medical University, Guangzhou, China, 2Guangdong Provincial Key Laboratory of Medical Biomechanics, National Key Discipline of Human Anatomy, School of Basic Medical Sciences, Southern Medical University, Guangzhou, China

Title: Engineering Electrospun Membranes as Functional Biomimetic Tendon Sheath for Enhanced Anti-Adhesion and Tendon Repair

Authors: Qiao Yang1, Yaobin Wu2*, Ling Wang1*

Affiliations: 1Biomaterials Research Center, School of Biomedical Engineering, Southern Medical University, Guangzhou, China. 2Guangdong Provincial Key Laboratory of Medical Biomechanics, National Key Discipline of Human Anatomy, School of Basic Medical Sciences, Southern Medical University, Guangzhou, China (* Correspondence)

Category: Tissue Engineering and Regeneration

Background: Tendons are composed of hierarchical collagen fibers and various sheath structures. When a tendon injury occurs, there is a risk of tendon sheath infection, which can cause adhesion and weaken the integrity of the tendon-sheath structure [1]. Although some physical membranes have been developed to prevent adhesion by inhibiting cell attachment and proliferation, they may also impede tendon healing[2]. An alternative strategy is developing dense fibrous films containing polymers related to the extracellular matrix, which can promote tendon healing and prevent adhesion. However, fibrous films are needed to create effective methods to address inflammation and promote healing in tendons[3].

Methods: We fabricated an aligned coaxial polycaprolactone/gelatin methacryloyl nanofibers (P/GM nanofibers) loaded with celecoxib (an NSAIDs drug could anti-inflammatory) via coaxial electrospinning and tested its effects on tenogenic differentiation of tendon stem/progenitor cells (TSPCs) on the unloaded membranes, and then investigated the anti-inflammatory of celecoxib both in vitro and in vivo.

Results: The SEM results indicated that both the coaxial P/GM nanofibers, whether loaded with celecoxib or not, have uniform diameters which were about 800 nm and exhibited low water angles around 20°. Moreover, while TSPCs cultured on these membranes, these aligned coaxial P/GM membranes could improve the expression of tendon-related genes, including TNMD and SCX which were ninefold and fivefold respectively compared to the PCL groups through the RT-qPCR results. Furthermore, the P/GM films loaded with celecoxib displayed a long-term drug release of about 72% at day 47. Compared with the unloaded group, these celecoxib-loaded membranes showed an excellent anti-inflammatory ability with the RT-qPCR results. In addition, the P/GM nanofibers with 6% drug concentration exhibited more than fourfold the expression of TNMD as much as the unloaded group in an inflammatory environment. The in vivo evaluation was evaluated in a rat patellar tendon defect model. The representative sections were stained with special markers such as type I collagen (COL 1) and type III collagen (COL 3) to illustrate the period of tendon regeneration. These fluorescence images showed that there was an insignificant difference between P/GM group and P/GM-6% celecoxib group on the expression of COL 1 after two weeks of regeneration, but the expression of COL 3 of P/GM-6% celecoxib membranes was about 1.5 times than P/GM membranes. Based on the fluorescence images showed that the expression level of IL-6 decreased consistently.
from 2-week to 4-week regeneration. Additionally, the P/GM-6% celecoxib groups performed about 2 times lower in the expression of IL-6 as much as the P/GM group owing to the release of celecoxib.

Discussion and conclusion: We prepared the coaxial P/GM membrane with a GelMA shell that would enhance cell viability due to the incorporation of RGD motifs. Therefore, GelMA served as a shell that provided an ECM-like local environment for cell attachment and spread, which enabled the promotion of cell differentiation. celecoxib-loaded nanofibers performed uniform diameters and smooth surfaces, with lower water angle contact, because of covering the GelMA shell. Furthermore, we demonstrated that TPSCs’ attachment and proliferation well on these celecoxib-loaded membranes owing to GelMA coated. Interestingly, these celecoxib-loaded membranes could inhibit collagen secretion, this phenomenon indicated that the release of celecoxib could decrease the adhesion formation. Besides, RT-qPCR results also showed that these membranes with celecoxib release could suppress pro-inflammatory cytokines expression. These results indicated that the celecoxib-loaded P/GM membranes could promote TSPCs maturity and anti-adhesion due to limiting the collagen secretion in vitro research. As for in vivo experiments, aligned coaxial membranes could induce collagen and cell orientation in the rat patellar defect model compared to the injury-only group. Celecoxib-loaded coaxial P/GM nanofibrous films could reduce peritendinous formation owing to the lower expression of pro-inflammatory cytokines, compared with unloaded P/GM scaffolds. Importantly, after 4 weeks of regeneration, there was observed that decrease in IL-6 expression in all groups, which meant that the period of tendon healing from the inflammation stage transmitted to the proliferation stage. And the celecoxib-loaded films performed a swift speed on tendon repair with a higher ratio of the expression of COL 1 in the injured sites compared to others. Therefore, the current work demonstrated a promising strategy to design a biomimetic tendon sheath capable of anti-adhesion and formation to promote tendon healing.

Acknowledgment: This work was supported by the National Natural Science Foundation of China (32000955)

Reference:
4D Bioprinted Trilayer Biomimetic Scaffolds with Hierarchical Structure for Uterine Tissue Regeneration

Mr. Shangsi Chen¹, Liwu Zheng², Min Wang¹

¹Department of Mechanical Engineering, The University of Hong Kong, , Hong Kong SAR, ²Faculty of Dentistry, The University of Hong Kong, , Hong Kong SAR

Title: 4D Bioprinted Trilayer Biomimetic Scaffolds with Hierarchical Structure for Uterine Tissue Regeneration

Authors: Shangsi Chen 1, Liwu Zheng 2, Min Wang 1 *

Affiliation: 1Department of Mechanical Engineering, The University of Hong Kong, Hong Kong. 2Faculty of Dentistry, The University of Hong Kong, Hong Kong. (*Corresponding author)

Category: Tissue Engineering and Regeneration


Materials and Methods: New scaffolds consisted of three layers: poly(L-lactide-co-trimethylene carbonate) (PLLA-TMC)/thermoplastic polyurethane (TPU) (PLLA-TMC/TPU) polymer blend scaffold layer, estradiol (E2)-containing poly(lactide-co-glycolide) (PLGA)/gelatin fiber layer, and bone-marrow derived mesenchymal stem cells (BMSCs)-containing gelatin methacryloyl (GelMA)/gelatin hydrogel layer. They were constructed via, sequentially, 4D printing, electrospinning and bioprinting. These biomimicking and hierarchical trilayer scaffolds were cultured at 37°C. Cell viability and behavior were studied. Other properties were also investigated.

Results and Discussion: When the PLLA-TMC:TPU ratio was above 0.25:1, 4D printed PLLA-TMC/TPU scaffolds exhibited programmed shape morphing when cultured at 37°C. With PLLA-TMC:TPU at 0.25:1, PLLA-TMC/TPU scaffolds had excellent mechanical properties: plastic strain over 400% and tensile strength around 0.5MPa, which are comparable to human uterus. After deposition of E2-PLGA/gelatin fibers, tensile tests showed that the bilayer scaffolds were still very stretchable. E2 release was sensitive to pH of the environment. The bilayer scaffolds displayed good biocompatibility. Moreover, BMSCs were homogeneously distributed in 3D bioprinted hydrogel layer and exhibited very high cell viability and cell survival. At 37°C, trilayer scaffolds could change from planar into tubular structure automatically within a short time.

Conclusions: Trilayer biomimetic scaffolds with hierarchical structure were successfully made for uterine tissue regeneration. They possessed high elasticity, good biocompatibility, controlled biomolecule delivery and shape morphing ability. These novel scaffolds are highly promising for uterine tissue regeneration.

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Tailoring the multiscale mechanics of tunable elastomeric decellularized extracellular matrix (dECM) for wound healing through immunomodulation

**Authors:** Pu Luo1, Shoucheng Chen2, Zhuofan Chen2, Wei Qiao3, Kelvin W. K. Yeung1

**Affiliations:** 1Department of Orthopaedics and Traumatology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China; 2Hospital of Stomatology, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Stomatology, Guangdong Research Center for Dental and Cranial Rehabilitation and Material Engineering, Guangzhou, China; 3Applied Oral Sciences and Community Dental Care, Faculty of Dentistry, the University of Hong Kong, Hong Kong SAR, China

**Category:** Tissue Engineering and Regeneration

**Background:** With the recognition of the essential role of macrophages in tissue regeneration, different immunomodulatory techniques have been proposed to modify traditional biomaterials for wound healing. Decellularized extracellular matrix (dECM) is a widely used material in clinical tissue injury treatment due to its favorable biocompatibility and similarity to the natural tissue environment. However, most decellularization protocols can damage the native structure of dECM, which undermines its inherent advantages and potential clinical applications. Given the potential to modulate macrophage responses by tailoring the mechanical properties of biomaterials, we introduce a tunable elastomeric dECM and a mechanics-immunomodulation-based strategy to enhance wound healing in this study.

**Methods:** In this work, we fabricated a tunable elastomeric dECM by optimizing the freeze-thaw method. We systematically investigated the multiscale mechanical properties, tested the biocompatibility, biodegradability, immunomodulatory ability in vitro and in vivo, and evaluated the efficacy of the tunable elastomeric dECM for promoting full-thickness wound healing in a rat model.

**Results:** The microscopic stiffness of dECM was significantly enhanced after three freeze-thaw cycles. We demonstrated that the mechanical properties of dECM, altered by the cyclic freeze-thaw process, contributed to distinct macrophage-mediated immune responses to the material. Our sequencing data further revealed that the immunomodulatory effect of dECM was induced via the mechanotransduction pathways in macrophages. Enhanced micromechanical properties of three freeze-thaw cycles treated dECM significantly promoted M2 macrophage polarization, leading to superior wound healing.

**Discussion and Conclusion:** Our findings indicate that the immunomodulatory property of dECM can be efficiently modulated by tailoring its inherent micromechanical properties during the decellularization process. This mechanics-immunomodulation-based strategy holds a great promise for clinical translation into the field of soft tissue regeneration.
Cell membrane vesicles derived from hBMSCs and hUVECs enhance bone regeneration

**Dr. Dandan Wang**¹, Yaru Guo², Bin Xia¹, Xuliang Deng²

¹Department of Paediatric Dentistry, Peking University School and Hospital of Stomatology, Beijing, China, ²Beijing Laboratory of Biomedical Materials, Department of Geriatric Dentistry, Peking University School and Hospital of Stomatology, Beijing, China

**Title**: Cell membrane vesicles derived from hBMSCs and hUVECs enhance bone regeneration

**Authors**: Dandan Wang ¹, Yaru Guo ², Bin Xia¹,** and Xuliang Deng ²,*

**Affiliations**: ¹ Department of Pediatric Dentistry, School and Hospital of Stomatology, Peking University, Beijing 100081, P. R. China ² Beijing Laboratory of Biomedical Materials, Department of Geriatric Dentistry, Peking University School and Hospital of Stomatology, Beijing 100081, P. R. China

* Corresponding author. Beijing Laboratory of Biomedical Materials, Department of Geriatric Dentistry, Peking University School and Hospital of Stomatology, Beijing 100081, P. R. China, 010-62173403

** Corresponding author. Department of Pediatric Dentistry, School and Hospital of Stomatology, Peking University, Beijing 100081, P. R. China, 010-82395361

**Introduction**: Bone tissue renewal can be enhanced through co-transplantation of bone mesenchymal stem cells (BMSCs) and vascular endothelial cells (ECs). However, there are apparent limitations in stem cell-based therapy which hinder its clinic translation. Therefore, the exploration of stem cell substitutes is a highly promising research direction in tissue regeneration. Here, we focused on cell membrane vesicles (CMVs) induced by cytochalasin B (CB), which display favorable bioactivity and are amenable to large-scale manufacturing.

**Methods**: In this study, we successfully prepared human bone marrow mesenchymal stem cells derived CMVs (BMSC-CMVs) and human umbilical vein endothelial cells derived CMVs (EC-CMVs) induced by cytochalasin B (CB) through differential centrifugation, and tested their effects on angiogenesis and bone formation in vitro and in vivo.

**Results**: The results showed that BMSC-derived cell membrane vesicles (BMSC-CMVs) possessed membrane receptors involved in juxtacrine signaling and growth factors derived from their parental cells. EC-derived cell membrane vesicles (EC-CMVs) also contained BMP2 and VEGF derived from their parental cells. BMSC-CMVs enhanced tube formation and migration ability of hUVECs, while EC-CMVs promoted the osteogenic differentiation of hBMSCs in vitro. Besides, BMSC-CMVs and EC-CMVs could promote osteogenesis and lead to bony tissue formation in vivo.

**Discussion and Conclusion**: Using a rat skull defect model, we found that co-transplantation of BMSC-CMVs and EC-CMVs could stimulate angiogenesis and bone formation in vivo. CMVs can play a key role in mediating the interaction between hBMSCs and hUVECs, which in turn synergistically enhanced bone defect healing in vivo. Hence, CMVs derived from hBMSCs and hUVECs might be applied to tissue regeneration. Therefore, our research might provide an innovative and feasible approach for cell-free therapy in bone tissue regeneration.

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Alginate hydrogel patch combined with extracellular matrix significantly enhances the delivery efficiency of mesenchymal stem cell-derived secretomes for advanced skin wound healing

**Mr. Jae Won Kwon**¹ ², Dr. Cininta Savitri¹, Mr. Seung Won Yang¹ ², Dr. Kwideok Park¹ ²

¹Center for Biomaterials, Korea Institute of Science and Technology (KIST), Seoul, Republic of Korea,
²Division of Bio-Medical Science and Technology, University of Science and Technology (UST), Daejeon, Republic of Korea

Authors: Jae Won Kwon¹ ², Cininta Savitri¹, Seung Won Yang¹ ², Kwideok Park¹ ² *

Affiliations: ¹ Center for Biomaterials, Korea Institute of Science and Technology (KIST), Seoul 02792, Republic of Korea
² Division of Bio-Medical Science and Technology, University of Science and Technology (UST), Daejeon, 34113, Republic of Korea (* Correspondence)

Category: Tissue Engineering and Regeneration

Background: Human mesenchymal stem cell (hMSC) secretomes contain various therapeutic factors, such as growth factors (GFs), cytokines and other extracellular molecules. Extracellular matrix (ECM) is a complex of diverse macromolecules, and it has many functions, including GFs control through their numerous binding site. We hypothesize that ECM in alginate patch can more effectively hold and deliver hMSC secretomes and thereby enables significantly better therapeutic effects. We investigated an alginate patch that contains hMSC secretomes and decellularized human fibroblast-derived matrix (hFDM) together for advanced wound healing.

Methods: ECM was obtained from decellularization of in vitro cultured human lung fibroblasts. To obtain hMSC-derived secretomes, hMSCs were cultivated in serum-free media (SFM) and the collected SFM was process further to obtain concentration concentrated conditioned medium (CCM) using Centrifugal Filter Unit. We had four different test groups to investigate the role of ECM in the patch and the therapeutic effect of secretomes as well: alginate/SFM (AS), alginate/ECM/SFM (AES), alginate/CCM (AC), and alginate/ECM/CCM (AEC). Each patch was loaded in the transwell inserts, where test cells were seeded on the bottom and cultivated in SFM condition. Moreover, murine full-thickness wounds were then created by using a biopsy punch (8 mm) under sterile surgical condition. Those patches were transplanted and replaced every 2-3 days. The wound tissues were assessed via H&E, Herovici staining and immunohistochemistry.

Results: we have successfully developed an alginate-based hydrogel patch (AEC) that contains hMSCs-derived secretomes and human fibroblast-derived, decellularized matrix (FDM). We found that the secretomes contained diverse therapeutic factors, such as VEGF, HGF, IGFBPs, IL-6, and IL-8, as assessed via growth factors antibody array and cytokine proteome microarray. BCA assay and ELISA confirmed that AEC patch could hold significantly larger amounts of secretomes and release them longer over AC. AEC patch was also very effective in stimulating not only cell migration and cell proliferation but also the collagen synthesis of hSFB, and assembly of HUVECs. The AEC patch-treated wounds disclosed significantly better wound healing indications, such as faster wound closing, accelerated cell-recruitment, increased neovascularization, fast keratinocyte migration at 7 day and normalized epidermis thickness, improved mature collagen deposition at day 14. In particular interest, AEC patch enabled a smooth phenotype shift of myofibroblast into fibroblast over time and advanced maturation of neovessels at 14 day.

Discussion and Conclusion: We believe that our AEC could provide a favorable environment for secretomes enrichment, due mainly to the presence of ECM that has multiple bind sites for GFs and cytokines. Taken together, our AEC patch proved significantly advanced wound healing capability.
Acknowledgement: This research was supported by the Korean Fund for Regenerative Medicine (KFRM). The Ministry of Science and ICT, Republic of Korea (21A0102L1-12).
Long-term umbilical cord blood-derived platelet-rich plasma treatment delays ovarian aging in middle-aged female mice

Ms. Ying Gu1, Bingbing Wu1, Jian Xu1,2
1Fourth Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang, China, 2Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China

Title: Long-term umbilical cord blood-derived platelet-rich plasma treatment delays ovarian aging in middle-aged female mice

Authors: Gu Ying1, Hu Qianyu1, Wu Bingbing1, Xu Jian1 2*
Affiliations: 1Fourth Affiliated Hospital, Zhejiang University School of Medicine, No. 1 Shang Cheng Avenue, Yiwu, 322000, Zhejiang, China. 2Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China (* Correspondence)

Category: SYIS/ Tissue Engineering and Regeneration

Background: Ovarian aging clinically mainly includes in following stages: diminished ovarian reserve (DOR), premature ovarian insufficiency (POI), early menopause and menopause. Age related decrease in follicle numbers and oocyte quality dictates the onset of cycle irregularity and the final cessation of menses, which contributes to the gradual decline in fertility and the final occurrence of natural sterility. The relative non-response to assisted reproductive technology (ART) is also adverse. Restoring the fertility from aging is critical in nowadays of late marriage and late childbirth.

Methods: In this work, we manually extracted platelet-rich plasma (PRP) from umbilical cord blood of healthy neonates. Female ICR mice (10 months old) were given PRP or PBS intraperitoneally until 16 months of age to tests the effects on ovarian reserve and female fertility by follicle counting and serum hormone measurement.

Results: PRP extracted from umbilical cord blood demonstrated the safety of use in vivo by no sign of lesions. The total number of ovarian follicles decreased with age; however, it was greater in PRP-treated mice than that from control animals, as were the numbers of primordial, primary, and antral follicles. Also, the number of atretic follicles in PRP group were less than the control group. Serum sexual hormone measurement, including AMH (anti-Müllerian hormone), FSH (follicle-stimulating hormone) and estradiol, showed parallel results with follicle counting.

Discussion and Conclusion: Our work demonstrated that long-term umbilical cord blood-derived platelet-rich plasma treatment can restore ovarian function from aging. The current work showed a promising strategy to store ovarian function in middle-age by using a kind of medical waste.
Large-scale preparation of hair microgels using a bioprinter for hair regenerative medicine

Ms Ayaka Nanmo¹, Dr. Tatsuto Kageyama¹,², Prof. Junji Fukuda¹,²
¹Graduate School of Engineering Science, Yokohama National University, Yokohama, Japan,
²Kanagawa Institute of Industrial Science and Technology (KISTEC), Ebina, Japan

Title: Large-scale preparation of hair microgels using a bioprinter for hair regenerative medicine
Authors: Ayaka Nanmo¹, Tatsuto Kageyama¹,², Junji Fukuda¹,²*
Affiliations: 1 Graduate School of Engineering Science, Yokohama National University, Yokohama, Kanagawa, Japan. 2 Kanagawa Institute of Industrial Science and Technology (KISTEC), Ebina, Kanagawa, Japan (* Correspondence)

Category: Tissue Engineering and Regeneration

Introduction: Hair regenerative medicine has emerged as a promising therapy for the treatment of hair loss. During development of hair follicles in vivo, reciprocal interactions between epithelial and collagen-rich mesenchymal layers in the hair follicle germs (HFG) trigger follicular morphogenesis. Fabrication of HFG-like aggregates in vitro has been studied by replicating such microenvironments to prepare tissue grafts for hair regenerative medicine. Since thousands of tissue grafts are required for a patient suffering from alopecia, it is important to prepare a large number of HFGs for clinical application. In this study, we propose a scalable and automated approach using a bioprinter for the large-scale preparation of collagen-enriched HFGs, termed hair microgels (HMGs)¹.

Methods: Mouse epithelial and mesenchymal cells were isolated from the embryonic skin and suspended in 2.4 mg/mL of collagen gel solution, respectively. A drop (2 µL) of each collagen solution was placed adjacent to each other using an electromotive pipette or bioprinter to prepare HMGs (Fig. 1). Changes in the HMGs' diameter were observed during 3 days of culture. To investigate the relationship between spontaneous contraction of HMGs and trichogenous gene expression (ALP and Lef1), a myosin II ATPase inhibitor, blebbistatin, was added to the culture medium. HMGs after 3 days of culture were transplanted into shallow stab wounds prepared on the back of nude mice. The number of hairs generated per transplanted site was evaluated after 3 weeks of transplantation.

Results: The HMGs showed spontaneous contraction by cell traction forces during 3 days of culture (Fig. 1). The long-side diameter of collagen drops reduced from 3.2 mm to 0.7 mm after 3 days of culture in bioprinting, wherein the cell density and collagen were enriched >10 times. The contraction was significantly inhibited in the presence of blebbistatin, and it played a crucial role in upregulating ALP and Lef1 gene expression in the HMGs. The HMGs regenerated hair follicles and shafts post transplantation more efficiently compared to the HFGs without collagen gel². Human dermal papilla cells instead of mouse mesenchymal cells were used to prepare HMGs, which also regenerated hairs on the back of nude mice. Moreover, more than 1,000 HMGs were automatically prepared within 12 minutes using a bioprinter.

Discussion and Conclusion: We demonstrated that a large number of HMGs can be automatically prepared using a bioprinter through spontaneous contraction of cell-suspended collagen microgels in the culture. The cell- and collagen-dense microenvironments were suitable for the improvement of trichogenic functions. Although it will be necessary to replace both cell types with human-derived cells to realize hair regeneration therapy, this approach may provide a promising strategy for advancing hair regenerative medicine.

References:
Microdevices for Engineering Small Tissues Using Human iPS-derived Cardiomyocytes for Cardiotoxicity Assays

Mr. Keiichiro Oiso¹

¹Yokohama National University, Japan

Background: Cardiotoxicity assays using human iPS-derived cardiomyocytes are expected to be an alternative method to animal experiments in drug discovery. However, a lack of tissue engineering approaches sufficiently replicating microstructures and in vivo responses using human cardiomyocytes is one of the key issues. The purpose of this study is to find an approach to engineering 3D tissues using human iPS-derived cardiomyocytes for cardiotoxicity assays.

Methods: Spheroid is a typical 3D culture method for various cell types including cardiomyocytes. Using iPS-derived cardiomyocyte spheroids, we first investigated effects of fatty acid-based medium without glucose on cardiac differentiation. Considering that the oxidation of fatty acids requires a large amount of oxygen, the microdevice for spheroid array culture was fabricated with oxygen permeable silicone rubber. We further modified the design of a microdevice so that cardiac tissues can be arrayed by simply seeding cells and then be applied tension in one direction by self-contraction.

Results: The oxygen-permeable spheroid culture device induced formation of cardiomyocyte spheroid with a uniform diameter and without hypoxic necrosis. Expression of cardiac differentiation makers, such as MYL2 in the spheroids were improved in spheroids cultured on fatty acid-based culture medium compared to typical glucose medium. Further maturation with oriented sarcomere structures was also observed in the modified microdevice.

Discussion and Conclusion: The microdevice fabricated in this study may provide suitable approach for in vitro cardiotoxicity assays. Our next subject is to miniaturize and arrange microdevices in parallel, and examine tissues with cardiotoxic compounds.
4D Printed Multilayered Tissue Engineering Scaffolds for Postoperative Bone Tumor Patients

Mr. Jizhuo CHEN¹, Prof. Min WANG¹
¹Department of Mechanical Engineering, The University of Hong Kong, Hong Kong

Title: 4D Printed Multilayered Tissue Engineering Scaffolds for Postoperative Bone Tumor Patients

Authors: Jizhuo Chen, Min Wang*

Affiliation: Department of Mechanical Engineering, The University of Hong Kong, Hong Kong. (*Corresp. auth.)

Category: Tissue Engineering and Regeneration

Introduction: Bone defects resulting from surgical removal of bone tumor not only affect the structural integrity and function of bone but also pose a risk of tumor recurrence owing to potential residual tumor cells. Tissue engineering (TE) offers a promising way to tackle the difficulties of killing residual tumor cells and regenerating bone in situ through creating multifunctional TE scaffolds. Polymer-based TE scaffolds enable the encapsulation of bioactive component to promote bone regeneration. Natural polymers usually have weaker mechanical strength while synthetic polymers are usually less biocompatible. It is therefore useful to combine these two types of polymers to obtained TE scaffolds with good properties both mechanically and biologically.

Materials and Methods: Multilayered scaffolds were made via 3D/4D printing. Briefly, bioactive tricalcium phosphate (β-TCP) nanoparticles and anticancer drug cisplatin were dispersed in PDLLA-co-TMC (“PTMC” in short) solution to make inks. The inks were 4D printed into scaffolds as the core structure. Thick layers of scaffolds from collagen with Poloxamer 407 thickener were 3D printed on top and bottom surfaces of cisplatin-containing β-TCP/PTMC composite scaffolds. After crosslinking of collagen by genipin, Poloxamer 407 was rinsed away.

Results: Rheological study and 3D/4D printing revealed much improved printability of PTMC and collagen with the addition of β-TCP and Poloxamer 407, respectively. SEM and EDX analyses showed that β-TCP particles were uniformly distributed within 4D printed PTMC struts. Young’s modulus and tensile strength of β-TCP/PTMC composite scaffolds were twice of those of PTMC polymer scaffolds owing to β-TCP reinforcement, matching mechanical properties of human trabecular bone. Shape memory polymer (SMP) PTMC made scaffolds shape morphable with a temperature change from 20 to 37°C. Cell culture experiments using rBMSCs showed good biocompatibility of multilayered scaffolds. In vitro release study indicated steady and sustained cisplatin release.

Discussion and Conclusions: The 3D/4D printed multilayered scaffolds possessed good mechanical and biological properties. Shape-morphing ability of multilayered scaffolds was provided by PTMC SMP, and β-TCP particles in scaffolds acted as a reinforcement and could also promote bone formation. Biocompatibility of multilayered scaffolds was enhanced by 3D printed collagen. Controlled release of cisplatin would help to prevent tumor recurrence. The new scaffolds have the potential for treating postoperative bone tumor patients.

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3D printing of bone tissue engineering scaffold with customized structures and biofunctions

**Prof. Changchun Zhou**, Boqing Zhang, Lina Wu, Kefeng Wang, Yujiang Fan, Xingdong Zhang

1National Engineering Research Center for Biomaterials, College of Biomedical Engineering, Sichuan University, Chengdu, China

**Title:** 3D printing of bone tissue engineering scaffold with customized structures and biofunctions

**Authors:** Changchun Zhou1*, Boqing Zhang1, Lina Wu1, Kefeng Wang, Yujiang Fan1, Xingdong Zhang1

**Affiliations:** 1National Engineering Research Center for Biomaterials, College of Biomedical Engineering, Sichuan University, Chengdu, 610064, China (*Correspondence: changchunzhou@scu.edu.cn)

**Category:** Design and Application of Biomaterials

**Background:** As an essential part of the human motion system, bone plays the role of locomotion, supporting, and protecting the body. Bone repair materials not only require a customized shape to match the defect but also need a specific porous structure to modulate their biological activity. Therefore, three-dimensional (3D) printing technology was introduced for the fabrication of bone tissue engineering scaffold. This technology provides better possibilities for optimizing the implants’ specific biofunctions.

**Methods:** The high-precision Ca-P bioactive ceramics were prepared by 3D printing technology, and the biological function of Ca-P bioactive ceramics was established. The HAp (P100, Baiameng, China) powder with mass fraction of 50%, 60%, and 70% was ball milled with photosensitive resin, dispersant (BYK-2155; BYK Chemie, Germany), and photoinitiator (diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide; TPO) for 10 h to prepare the printing inks. The complex shaped green body with high resolution was fabricated using a digital light processing printer (Admaflex 130 plus, Admatec, The Netherlands) (Figure 1). After cleaning and drying, the prepared green bodies were placed in a muffle furnace for sintering and densification to obtain the ceramics.

**Results:** The large-scale shape of the scaffold can be designed according to customized requirements. The maximum error was less than 0.15 mm, compared with the design model, and 95% of the error was distributed within 0.1 mm. The compressive strengths of the 50, 60, and 70 wt % specimens reached 19.75, 34.34, and 48.16 MPa, respectively. These scaffolds show good bone regeneration ability; the speed of new bone growth was about 769 ± 42 nm per day in subcutaneous tissue. In histological images, the newly formed tissue was found inside the porous scaffold, approximately 15.93 ± 2.31% of the available macropore area in the ceramic implants was occupied by the newly formed bone.

**Discussion and Conclusion:** With the development of biomedical materials, artificial materials have been able to achieve regeneration of diseased tissues and organs, and these bioactive materials will be an important development direction. Calcium phosphate (Ca-P) ceramics are promising bone repair materials because of their excellent biological properties. The composites based on calcium phosphate materials, such as the composites of calcium phosphate powders and degradable polymers have exhibited good bone regeneration properties.

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An in-vitro evaluation of novel braided PET artificial ligament for ACL Reconstruction

Mr. Shenglin Li1,2, Dr. Shuhan Wang2, Mr. Wenliang Liu2, Dr. Chao Zhang1, Dr. Jian Song1,3

1School of Biomedical Engineering, Shenzhen Campus of Sun Yat-sen University, Shenzhen, China, 2Shenzhen Institute for Drug Control, Shenzhen Testing Center of Medical Devices, Shenzhen, China, 3State Key Laboratory of Tribology, Tsinghua University, Beijing, China

Introduction: Anterior cruciate ligament (ACL) reconstruction with PET artificial ligament has been widely adopted clinically, however, the absence of bioactivity has significantly limited its further application. Inspired by the histological characterization of cortical bones, mimic overlapping bone lamellas layers consisting of soft collagen fibrils and hard hydroxyapatite should be promising in establishing an osseous connection between the graft and host bone.

Subjects and Methods: In this work, we proposed a novel braided PET artificial ligament for ACL Reconstruction with a shell-and-core composition by two kinds of PET fibers: 1) density of 150 D (denoted as “S” for stiffness) and 2) diameter of 0.3 mm (denoted as “H” for hard). The amount ratio of H and S fibers was set by 1:3 (H1S3) and 1:1 (H1S1), while the control group was completely composed of S fibers.

Results: Increasing H fiber contents led to better mechanical strengths and tribological performances. SEM images of H1S1 samples after friction tests revealed the role of mechanical bearing played by H fibers, while S fibers offered optimal apertures for cell ingrowth. The in-vitro cytotoxicity test indicated no observable cytotoxicity of graft and debris after friction.

Discussion and Conclusion: We conclude that this novel artificial ligament design contributes to providing better mechanical and tribological performance with promising biocompatibility and spatial conditions for cell ingrowth, thus lowering the risk of bone tunnel enlargement and demonstrating a promising strategy for ACL reconstruction.

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Construction of Potential miRNA-mRNA Regulatory Network in Liuwei Dihuang Pills on kidney yin deficiency postmenopausal osteoporosis by Bioinformatics Analysis

Dr. Jianjun Wu¹, Prof. Shi LIN, Dr. Jingyi CHEN, Dr. Zhuoma DONGZHI, Dr. Zhijia TANG, Prof. Hongxing HUANG

¹Guangzhou University of Chinese Medicine, , China

Introduction: Osteoporosis is a systemic bone disease characterized by reduced bone mass, changes to the construction of bone tissue, an increase in bone fragility, and a higher risk of fracture. In recent years, Chinese medicine's treatment of osteoporosis has achieved satisfactory results. Chinese medicine theory holds that congenital deficiency or postnatal deficiency is the pathogenesis of postmenopausal osteoporosis (PMOP), its clinical syndrome for kidney-yin deficiency. Liuwei Dihuang pills can nourish kidney yin and have a therapeutic effect on PMOP. However, the study of the miRNA-messenger RNA (mRNA) regulatory network in combination with Liuwei Dihuang Pills on kidney yin deficiency PMOP is still lacking.

Methods: To find differentially expressed genes (DEGs), the microarray datasets were downloaded from the Gene Expression Omnibus (GEO) database and put through an analysis using the R program (LIMMA). Afterwards, the potential target genes of DEGs were identified. Following that, the hub genes were identified using the cytoHubba plugin of the cytoscape tool, and additional bioinformatics tasks like protein-protein interaction (PPI) network analysis, module analysis, and miRNA-hub gene network creation were taken out.

Results: 498 DEGs in total were found, of which 495 were up-regulated and 3 were down-regulated. The ten overlapped genes were then predicted using cytoHubba and designated as hub genes. Furthermore, a network of miRNA-hub genes was developed to investigate the relationship between the hub genes and the miRNAs they target. A total of ten genes, including GENPE, RACGAP1, ECT2, AURKA, CCNB1, CCNA2, SMC2, CDC25C, BRCA1, RB1, and one novel key miRNA, hsa-miR-106b-5p, were discovered as a result of network construction.

Discussion and Conclusion: Potential therapeutic targets of kidney yin deficiency PMOP include GENPE, RACGAP1, ECT2, AURKA, CCNB1, CCNA2, SMC2, CDC25C, BRCA1, RB1, and one key miRNA, hsa-miR-106b-5p. To confirm these genes, additional research is needed. determining the precise functions of the novel miRNAs is crucial in order to further illuminate the mechanisms underlying kidney yin deficiency PMOP. This requires both in vivo and in vitro investigations of miRNAs and pathway interactions.

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High-Efficiency Proteoglycan Analog-Engineered Biomimetic Collagen Scaffold for Corneal Regeneration

Mr. Le Ma¹, Mr. Hongliang Jiang¹, Prof. Qiongyu Guo¹
¹Department of Biomedical Engineering, The Southern University of Science and Technology, Nanshan District, Shenzhen, Guangdong, China, Shenzhen, China

Corneal transplantation has proven to be a highly effective clinical treatment for corneal blindness but is highly limited by insufficient donor corneas; therefore, the research and exploration of artificial corneal scaffolds (ACSs) hold significant clinical relevance and importance. The utilization of collagen for artificial corneas via bioengineering methods has addressed the issue of poor biocompatibility commonly observed with traditional ACSs but still limited by their mechanical toughness. The small leucine-rich proteoglycans (SLRPs) in the cornea are integral in regulating the collagen fibril self-assembly to a distinctive lamellar arrangement, which also offers the cornea superior mechanical toughness and transparency. Therefore, controlling collagen fibril assembly to produce homogeneous and biomimetic structures is critical for improving collagen-based ACSs' mechanical toughness and transparency, which is also a highly sought-after research topic among the international scientific community.

In this work, we utilized type I collagen as raw material, incorporated the concept of proteoglycan analogs (PGAs) to regulate collagen fibril self-assembly during the long-term vitrification process, and studied the microstructural formation mechanism. We also evaluated the ACSs' physical properties and their in vitro and in vivo biocompatibility and comprehensively assessed their potential as a transplantable material for corneal replacement in clinical settings.

Results: By utilizing the physical interaction between PGAs and collagen fibrils under a controlled formation process, we obtained a parallel arranged fibrils structure in same lamellar and orthogonal arranged fibrils structure in adjacent lamellar that is similar to natural corneal collagen fibrils, which is also crucial for the high transparency and mechanical toughness of prepared ACSs. The in vitro cell migration demonstrated that ACSs selectively promote the proliferation and differentiation of corneal epithelial and stromal cells and maintain their original topological structure. Ex vivo defective cornea culturing model demonstrated that ACSs promote the re-epithelialization process. Similarly, in vivo corneal stromal-defect-re-transplantation model demonstrated that ACSs significantly inhibit fibroblasts or myofibroblasts formation, promote the corneal stromal regeneration, and maintain the original thickness of the defected cornea.

Our research has demonstrated a new concept of introducing PGAs and simulating the human corneal fibril maturing process, allowing collagen fibril self-assembly to the natural-corneal-like structure and finally resulting in improved transparency and mechanical toughness at the macroscopic level. Additionally, ACSs selectively promote corneal cell proliferation and differentiation, facilitating corneal tissue in-situ regeneration while inhibiting the formation of fibroblast or smooth muscle, thereby maintaining corneal transparency. Our work has shown promising results with collagen-based corneal scaffolds as a potential treatment for corneal blindness and shows excellent potential for clinical translation.
Integrating the Endosteal and Perivascular Compartments of the Bone Marrow Niche in a Microfluidic Device

Ms. Ho Ying Wan1,2, Dr. Benjamin CAO4,5, Prof. Susan K. NILSSON4,5, Prof. Rocky S. TUAN1,2,3, prof. Anna Maria BLOCKI1,2,3

1 Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong, Hong Kong; 2 School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, Hong Kong; 3 Department of Orthopaedics & Traumatology, Faculty of Medicine, The Chinese University, Hong Kong, Hong Kong; 4 Biomedical Manufacturing Commonwealth Scientific and Industrial Research Organisation (CSIRO), Melbourne, Australia; 5 Australian Regenerative Medicine Institute, Monash University, Melbourne, Australia

Ho-Ying WAN 1,2, Benjamin CAO4,5 , Susan K. NILSSON4,5, Rocky S. TUAN1,2,3 Anna Maria BLOCKI1,2,3

1 Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong; 2 School of Biomedical Sciences; 3 Department of Orthopaedics & Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR; 4 Biomedical Manufacturing Commonwealth Scientific and Industrial Research Organisation (CSIRO), Melbourne, Australia; 5 Australian Regenerative Medicine Institute, Monash University, Melbourne, Australia

Introduction
The bone marrow (BM) niche maintains hematopoietic stem cells (HSCs) and ensures a continuous supply of hematopoietic lineage cells throughout life (1). Within the BM niche, HSCs are found in close proximity to the bone lining surface, while remaining close to blood vessels, termed endosteal and perivascular compartments, respectively (1). As the BM niche greatly influences HSC maintenance and proliferation, mimicking key features of the BM niche in vitro can provide better understanding of how HSCs interact with both cellular and extracellular components of the BM to maintain HSC function. Such insights may lead to improved HSC expansion ex vivo, enabling their successful utilization in HSC transplantation for the treatment of hematopoietic cancers and disorders.

Methods
Using a microfluidics system, we engineered a BM niche in vitro that comprised both the endosteal and perivascular compartments. Human ECM-rich bone-like tissue surface with mature osteoblasts resembling the endosteal compartment were produced (2) and a physiologically relevant microvascular network (MVN) stabilized by macromolecular crowding (MMC) enabled the modelling of the perivascular compartment (3). Next, both engineered BM compartments were integrated in a microfluidic device and murine HSC (LSKSLAM) were introduced. After 7 days, the progeny of the seeded HSC were isolated, analysed and transplanted into irradiated recipient mice, where their functional potential was measured by competitive repopulation and limiting dilution transplant assays.

Results
The endosteal and perivascular compartments were successfully integrated in a microfluidic device enabling recapitulation of the BM niche in vitro. Incorporated HSCs demonstrated good cell survival and proliferation, while retaining the expression of HSC markers. Future studies will focus on determining the functionality of the cultured HSC, their interaction with various components of the BM niche, as well as the modelling of physiological processes such as HSC homing and mobilization.

Reference

Keywords
Microfluidics, Bone Marrow Niche, Tissue-on-a-Chip
A pH-neutral Bioactive Glass Empowered Gelatin-Chitosan Composite Scaffold for Skull Defect Repairing

Dr. Yu Liu¹, Dr. Yanlei Zhao, Dr. Bin Zhu, Dr. Dong Qiu, Dr. Xiaoguang Liu
¹Peking University Third Hospital, China

Background: Skull defects are frequently encountered in many clinical practices, including severe cranial trauma, neoplasia, and neurosurgical interventions etc., having affected millions of people worldwide. Existing skull repair materials have some shortcomings. The ideal skull defect repairing material should have good biocompatibility, mechanical properties, and contribute to osteogenesis.

Methods: In this study, we designed and fabricated biodegradable, bioactive and mechanically robust porous scaffolds composed completely of biological materials. We used gelatin-chitosan blend as the matrix, sodium phytate instead of toxic glutaraldehyde for cross-linking, and the pH-neutral bioactive glass (PSC) to improve biological activity and mechanical properties.

Results: We have established that gelatin-chitosan scaffolds crosslinked by 20wt% sodium phytate and containing 30wt% PSC have good mechanical, biocompatibility, and osteogenic properties, making them suitable bone substitute materials.

Discussion and Conclusion: In this work, a composite scaffold of gelatin, chitosan and pH-neutral bioactive glass, crosslinked by biogenic sodium phytate, was designed and prepared. Sodium phytate as a crosslinker is more biocompatible. The composite scaffold C-G-30% PSC/P had the best combination of biocompatibility, bioactivity, osteogenesis, mechanical properties and porous characteristics. Animal studies revealed that the C-G-30wt% PSC/P group had almost fully finished ossification and achieved a healed condition. This new type of composite scaffold had well tackled the troubles brought about by the non-degradability and non-osteogenesis of traditional skull defect repairing materials, the high toxicity of conventional aldehyde cross-linking agents, and the poor mechanical properties of pure chitosan or gelatin scaffold, is thus a promising material for skull defect repair.
Direct deposition of serum-free bioink on wounds for epidermis formation: in vitro and in vivo validation

Ms. Wan Ling Wong¹, Dr. Ruth Jinfen Chai¹, Dr. Cyrus Beh¹,²
¹Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore, ²Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore

Title: Direct deposition of serum-free bioink on wounds for epidermis formation: in vitro and in vivo validation

Authors: Wan Ling Wong¹, Ruth Jinfen Chai¹, Cyrus Weijie Beh²

Affiliations: ¹Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR), 61 Biopolis Drive, Proteos, Singapore 138673, Republic of Singapore.
²Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), 20 Biopolis Way, Centros, Singapore 138668, Republic of Singapore

Category: Tissue Engineering and Regeneration

Introduction: Current treatments for burn patients who suffer significant skin damage generally involve wound reconstruction using skin autografts. However, the lack of donor sites and donor site morbidity limit the effectiveness of such autografts. Alternative treatments such as cultured epithelial autografts and ‘spray-on’ skin have their drawbacks in terms of fragility of the tissues and difficulty in controlling cell deposition. In this paper, we discuss our work in developing an in-house formulated PDβ bioink that allows a contiguous layer of keratinocytes to be deposited directly onto burn wounds. The use of collagen in the bioink created a bioprintable gel that allowed better control of the deposition process, since the gel sets quickly after deposition and will not flow away from the site of application.

Methods: 3D in vitro experiments were carried out by depositing human primary keratinocytes in PDβ bioink onto a collagen-based dermal template containing dermal fibroblasts and cultured at air-liquid interface for 7 days to form a collagen-Human Skin Equivalent (c-HSE). For in vivo mouse studies, the keratinocyte laden PDβ bioink was deposited onto a skin flap that was incised on the dorsal side of a nude mouse. A piece of thin silicone was placed over the thoracic wall, and the flap was sutured back in place, then harvested 10 days later. Histological studies and immunofluorescence staining were performed on both c-HSE and mouse skin graft to detect the presence of a stratified epidermis through the expression of various cell proliferation and differentiation markers.

Results: Histological studies of c-HSE and mouse skin graft sections showed that an epidermis with stratified layers was successfully generated using PDβ bioink. Formation of a mature epidermis was further supported by immunofluorescence staining of proliferative markers Ki67 and keratin 14, human specific differentiation markers K10 and filaggrin as well as basement membrane protein collagen IV.

Discussion and conclusion: We have developed a serum-free PDβ bioink that enabled keratinocytes to proliferate and differentiate into a mature epidermis through a one-step deposition process. The resulting epidermis recapitulates native skin characteristics, as determined by histology and immunofluorescence. While further tests are required on large animal models to demonstrate its effectiveness as a burn treatment alternative, the current protocol can already produce donor-specific in vitro models for testing purposes.
Acknowledgement: This research is supported by Agency for Science, Technology and Research (A*STAR) under its RIE2020 Advanced Manufacturing and Engineering (AME) Programmatic Grant no. A18A8B0059 titled Additive Manufacturing for Biological Materials (AMBM).
Hyaluronic acid–tyramine based hydrogels encapsulating Lumacaftor-ivacaftor for the treatment of osteoarthritis through CFTR-AnxA1 mediated regulatory chondrocytes.

Dr. Ziyi Chen, Prof. Jiankun Xu
1The Chinese University of Hong Kong, , Hong Kong SAR

Introduction: Osteoarthritis (OA) is a disabling degenerative joint disease that remarkably affects quality of life, especially in the aging population. However, current non-surgical treatments exert limited therapeutic effects on attenuating OA progression. Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations of CF transmembrane conductance regulator (CFTR) that is responsible for chloride transmembrane transport. Moreover, the CF-related arthritis and bone disorder have been widely reported in CF patients, especially with increasing survival age of CF patients.

Subjects and Methods: The present work employed C57-Bl6 mice with DF508 mutation to establish destabilization of medial meniscus (DMM) induced OA model. Gait disparity and subchondral bone microarchitecture of tibial plateau were assessed by Catwalk XT 9.0 system and micro-CT. For in vitro experiment, the primary cultured chondrocytes were exposed to low (5 dyne/cm²) or high shear force (20 dyne/cm²). The injectable tyramine modified hyaluronic acid (HA–Tyr) hydrogels were developed by radical crosslinking reaction using H2O2 and horse-radish peroxidase encapsulating Lumacaftor-ivacaftor for sustained release in the arthritic joints.

Results: The expression of CFTR in cartilage and supportive subchondral bone declined during the pathological progression of OA in both mice and human. The DMM mice model with CFTR mutation exhibited accelerated cartilage degeneration and subchondral bone resorption. Moreover, both in vivo and in vitro data suggested that CFTR maintained the hyaline cartilage correlated with mechanical sensation and regulation of anti-inflammatory factor AnxA1. CFTR expression was co-stained with AnxA1 in the superficial layer of cartilage. Loss of CFTR increased inflammatory response with excessive high fluid shear stress in primary chondrocytes. These data suggested that CFTR might be a potential target for OA therapy. The proof of concept experiment showed that sustained release of lumacaftor-ivacaftor ameliorated OA progression in DMM mice using HA-Tyr based hydrogel in respect of gaiting behavior, cartilage integrity, subchondral bone volume and inflammatory response.

Discussion and Conclusion: OA is a complex degenerative disorder in the whole joint with abnormal mechanical stress. The present work unraveled the involvement of CFTR in a newly defined regulatory cartilage (superficial layer) during OA progression. CFTR mediated the differentiation and maintenance of chondrocytes via inflammatory and mechanical signaling. The HA-Tyr hydrogel sustained release of CFTR-modulators ameliorated the OA progression which suggested that biomaterial design targeting CFTR might be a promising strategy for cartilage regeneration.
An injectable hydrogel microsphere-integrated training court to inspire tumor-infiltrating T lymphocyte potential

Prof. Qin Shi, Mr. Jiachen HE, Mr. Junjie NIU, Lin Wang¹, Wen Zhang, Xu He, Xiongjinfu Zhang, Wei Hu, Huilin Yang, Jie Sun

¹The First Affiliated Hospital of Soochow University, Suzhou, P.R.China
Title: An injectable hydrogel microsphere-integrated training court to inspire tumor-infiltrating T lymphocyte potential

Authors: Jiachen He, Junjie Niu, Lin Wang1, Wen Zhang, Xu He, Xiongjinfu Zhang, Wei Hu, Huilin Yang, Jie Sun, Qin Shi

Affiliations: Department of Orthopedics, The First Affiliated Hospital of Soochow University, Orthopedic Institute of Soochow University, Suzhou Medical College of Soochow University, 899 Pinghai Road, Suzhou, Jiangsu, 215031, P. R. China.

Category: Design and Application of Biomaterials

Background: Tumor-infiltrating T cells (TIL-Ts) are crucial for solid tumor progression and outcome. They can traffic into the tumor, initiate an immune response and eradicate the tumor cells efficiently since they are capable of recognizing tumor antigens specifically. Unfortunately, their clinical application has been limited because of the immunosuppressive microenvironment.

Methods: In this work, we developed an injectable hydrogel microsphere-integrated training court (MS-ITC) to inspire the function of TIL-Ts and amplify TIL-Ts, and testes its effects on anti-tumor in vitro and in vivo.

Results: The MS-ITC was injected locally into the osteosarcoma tumor tissue in mice. MS-ITC suppressed the growth of primary osteosarcoma by more than 95%, accompanied with primed and expanded TIL-Ts in the tumor tissues, compromising significantly increased CD8+ T and memory T cells, thereby enhancing the anti-tumor effect.

Discussion and Conclusion: We demonstrated that MS-ITC could provide a TIL-Ts-trained reservoir that continuously "evokes" and expands inactivated TIL-Ts. These findings demonstrate the tremendous potential of MS-ITC for inspiring TIL-T potential and offering new perspectives in solid tumor immunotherapy.
Virus-engineered microsol electrospun scaffold promotes the reprogramming of fibroblasts to neurons

Prof. Qin Shi, Mr. Changpeng Liu, Mr. Xinzhui Liu, Xiongjinfu Zhang, Junjie Niu, Jie Sun, Chichi Chen, Huilin Yang
1The First Affiliated Hospital of Soochow University, Suzhou, P.R.China
Title: Virus-engineered microsol electrospun scaffold promotes the reprogramming of fibroblasts to neurons

Authors: Changpeng Liu, Xiongjinfu Zhang, Junjie Niu, Jie Sun, Chichi Chen, Huilin Yang, Qin Shi

Affiliations: Department of Orthopaedics, The First Affiliated Hospital of Soochow University, Orthopaedic Institute of Soochow University, Medical College of Soochow University, 899 Pinghai Road, Suzhou, Jiangsu 215031, P. R. China

Category: Tissue Engineering and Regeneration

Background: Lentiviral vector-based therapies are widely applied in treating various diseases but face certain limitations, including release burst, rapid clearance, and immune activation. Notably, a significant increase in polypyrimidine tract binding protein1 (PTB) has been observed in spinal cord injury (SCI) rats, presenting a potential target for intervention.

Methods: We propose a novel lentiviral vector delivery platform utilizing a virus-engineered microsol electrospun scaffold. This scaffold is composed of a hyaluronic acid (HA) core encapsulating brain-derived neurotrophic factor (BDNF), and a polydopamine (PDA)-modified linear poly-L-lactic acid (PLLA) shell. We then grafted shPTB lentiviral vectors (LV-shPTB) onto this scaffold via PDA.

Results: In vitro studies demonstrated that the LV-shPTB achieved an infection efficiency of 70%. These oriented scaffolds significantly reduced inflammatory factor expression, induced the reprogramming of fibroblasts into neurons, and sustained BDNF release for over two weeks. In vivo studies showed that the scaffolds provided physical support and neural guidance while releasing BDNF and LV-shPTB. LV-shPTB delivery led to the reprogramming of fibroblasts into neurons, and the sustained BDNF delivery supported neuron proliferation and growth.

Discussion and Conclusion: The use of LV-shPTB and sustained BDNF delivery promoted the recovery of neurological function in SCI rats, demonstrating the potential application of this virus-engineered delivery platform in SCI treatment and other medical fields. This work provides a promising pathway to overcome the limitations of conventional lentiviral vector-based therapies. Still, the construction and application of this delivery platform still needs to be studied and solved.
The Effect of Piper Sarmentosum Aqueous Extract on Human Osteoarthritic Articular Chondrocyte

Ms. Yi Ting Lee, Associate Professor Dr. Mohd Heikal MOHD YUNUS, Associate Professor Dr. Azizah UGUSMAN, Dr. Muhammad Dain YAZID

1Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Malaysia, 2Centre of Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Malaysia

Introduction: Osteoarthritis is a prevalent degenerative joint condition that primarily affects older people and may result in physical impairment. Anti-inflammatory and pain relief medication-based nonsurgical therapies only relieve symptoms temporarily and raise the possibility of cardiovascular disease while other surgical and cell-based techniques can lead to the creation of fibrous tissue devoid of the typical characteristics of articular cartilage. As a result, alternative therapies like phytomedicine may be worthwhile to investigate. Piper sarmentosum (PS), is a traditional medicinal plant that has long been used to alleviate joint pain in Southeast Asia. It has been proven to show anti-inflammatory and antioxidant effects. Hence, the purpose of this study is to determine the potential of PS aqueous extract in promoting regeneration reducing inflammation, and function as antioxidants in human OA articular chondrocytes.

Subjects and Methods: The human OA articular chondrocytes (HOC) isolated from knee joint cartilage were cultured with PS for 3 days. The cytotoxicity effect of different PS concentrations on HOC was evaluated by measuring the cell viability and proliferation rate using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The effect of PS on anabolic and catabolic differential gene expression was verified by real-time polymerase chain reaction (PCR). The nitric oxide (NO), prostaglandin E2 (PGE2) and sulfated glycosaminoglycans (sGAG) production by monolayer chondrocytes in medium was analyzed by colorimetric method.

Results: The chondrocytes grown in different PS concentrations have polygonal morphologies while retaining their chondrocyte characteristics. The anti-inflammatory and antioxidant effects of PS aqueous extract were discovered, as shown it promotes the induction of the cartilage-specific markers and anabolic genes (collagen type II, aggrecan core protein and SOX9) while decreasing the catabolic gene (COX2 and iNOS) expression. Additionally, the PCR results showed no significant difference in gene expression of interleukin 6, matrix metalloproteinase 1 and 13 which are the inflammatory cytokines involved in OA progression. However, an increase in gene expression of these inflammatory cytokines was observed. Moreover, the PS aqueous extract is also able to reduce NO and PGE2 production while increasing the sGAG production.

Discussion and Conclusion: These findings suggested that PS aqueous extract exhibited chondroprotective ability by effectively promoting chondrocyte growth while suppressing catabolic activities and oxidative damage. This study's findings indicated that phytomedicine can be a useful alternative for OA treatment. However, more research is required to better understand the specific mechanisms involved.

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Natural Polymer-based Composite Hydrogels and 3D Printed Scaffolds for Liver Tissue Engineering

Ms. Xinyang Zhang¹, Ms. Xirui Zeng, Prof. Min Wang
¹The University of Hong Kong, Hong Kong, China
Title: Natural Polymer-based Composite Hydrogels and 3D Printed Scaffolds for Liver Tissue Engineering

Authors: Xinyang Zhang, Xirui Zeng, Min Wang*
Affiliation: Department of Mechanical Engineering, The University of Hong Kong, Hong Kong. (*Corresp. auth.)

Category: Tissue Engineering and Regeneration

Introduction: The liver is a vital organ for balancing biochemical environments in the human body. Liver transplantation is the gold standard for patients with end-stage liver diseases. However, liver transplantation is severely constrained by the demand and availability of donor organs. Liver tissue engineering provides an effective alternative treatment for the patients. Hydrogels can play an important role in liver regeneration because their scaffolds offer a suitable microenvironment for hepatic cells. Hydrogels of natural polymers such as polysaccharides and gelatin are attractive but they have poor mechanical properties. Using composite hydrogels can be an effective way to take advantages of constituent polymers with an improved mechanical performance. 3D printing can produce hydrogel tissue engineering scaffolds with desirable structures and properties.

Materials and Methods: In this study, carboxymethyl chitosan and aldehyde-based hyaluronic acid were prepared first. They were then mixed with another natural polymer to form composite hydrogel inks for 3D printing. The gel-forming behavior and effects of component ratios of the composite hydrogel were investigated. Printability of the composite hydrogels was studied and optimized. The structure and mechanical properties of 3D printed hydrogel scaffolds were investigated and the biological performance was assessed.

Results: Rheological analysis revealed that hydrogel inks had good shear thinning behavior, which ensured successful 3D printing. Composite hydrogels contained abundant pores, which is conducive for cell adhesion and proliferation. Compression tests showed that the modulus of composite hydrogels was close to that of healthy liver tissue. Hydrogel inks could be 3D printed into grid scaffolds with high shape fidelity. Healthy hepatocytes seeded on 3D printed scaffolds proliferated very well, indicating scaffold suitability for liver tissue regeneration.

Discussion and Conclusions: The progress in 3D cell printing-based liver tissue engineering has been hindered by the lack of bioinks that have adjustable properties and mimic the in vivo environment of liver. We have developed and evaluated nature polymer-based hydrogel inks for 3D printing. The composite hydrogel inks showed good printability. Furthermore, 3D printed composite hydrogel scaffolds exhibited excellent biocompatibility for liver cells. The composite hydrogel appears to be a suitable material for 3D cell printing, providing appropriate biomechanical and biochemical microenvironments in liver tissue regeneration.

Acknowledgement: Support by HK RGC through grants (17200519, 17202921, 17201622 and N_HKU749/22).
3D Printed Polysaccharide-based Tissue Engineering Scaffolds Providing Local Chemotherapeutic Effect

Ms. Xinyang Zhang, Prof. Min Wang

1The University of Hong Kong, Hong Kong, China

Title: 3D Printed Polysaccharide-based Tissue Engineering Scaffolds Providing Local Chemotherapeutic Effect

Authors: Xinyang Zhang, Min Wang*

Affiliation: Department of Mechanical Engineering, The University of Hong Kong, Hong Kong.

(*Corresp. auth.)

Category: Design and Application of Biomaterials

Introduction: Surgery is a common method for treating hepatocellular carcinoma but physical removal of carcinoma tissue cannot provide 100% clearance. Patients still face a high risk of tumor recurrence after surgery. Residual cancerous cells are deeply and irregularly embedded in normal cells which form barriers for other treatments. Systemic administration of chemotherapy agents is also often accompanied by severe side-effects and drug resistance. Surviving tumor cells have stronger invasiveness and proliferation potential. It is thus necessary to eliminate cancerous cells in the initial treatment after surgery. Furthermore, the large space at the original tumor site and tumor-associated inflammatory microenvironment caused by tumor resection can potentially incur tumor recurrence. Also, normal liver tissue should be regenerated at the original tumor site for functional repair of tissue defect. Therefore, there is an urgent need to develop a postoperative adjuvant therapy platform that can eliminate residual tumor cells and assist liver tissue regeneration. Anticancer drug-carrying tissue engineering scaffolds are potentially such platforms, and 3D printing can efficiently produce these types of scaffolds.

Materials and Methods: In this study, natural polysaccharide-based scaffolds that carried an anticancer drug were fabricated by extrusion-type 3D printing for tissue repair after surgery and for preventing postoperative recurrence of hepatocellular carcinoma. In detail, sorafenib-containing PLGA microspheres were mixed with a modified carboxymethyl chitosan to form a printing ink. The ink was used to print designed hydrogel scaffolds. When cancer cells recur and proliferate, the pH-sensitive hydrogel is stimulated, which promotes the release of sorafenib from PLGA microspheres to kill tumor cells.

Results and Discussion: Rheological properties of hydrogels were investigated and their printability was assessed. The 3D printed scaffolds were studied using various techniques. The results of cell experiments showed that normal hepatocytes had a high proliferation rate on the scaffolds, indicating the potential of scaffolds for liver tissue regeneration. Sorafenib-containing PLGA microspheres rendered the scaffolds to have a good chemotherapeutic effect, killing cancer cells and thus preventing the local recurrence of hepatocellular carcinoma.

Conclusions: Using suitably formulated inks, natural polysaccharides-based scaffolds that contained an anticancer drug could be 3D printed with designed large pores. They could provide pH-responsive type release of the drug to achieve a chemotherapeutic effect locally. These types of 3D printed scaffolds have the potential for treating post-operative cancer patients, enabling prevention of tumor recurrence and assisting tissue regeneration.
Acknowledgement: Support by HK RGC through grants (17200519, 17202921, 17201622 and N_HKU749/22).
Scaffold Design and Mechanical Behavior Simulation for Bone Tissue Engineering Scaffolds

Ms. Yujie ZHANG¹, Mr. Jizhuo CHEN¹, Prof. Min WANG¹
¹Department of Mechanical Engineering, The University of Hong Kong, Hong Kong

Title: Scaffold Design and Mechanical Behavior Simulation for Bone Tissue Engineering Scaffolds

Authors: Yujie Zhang, Jizhuo Chen, Min Wang*

Affiliation: Department of Mechanical Engineering, The University of Hong Kong, Hong Kong.
(*Corresp. auth.)

Category: Tissue Engineering and Regeneration

Introduction: In scaffold-based tissue engineering (TE), porous scaffolds play important roles in not only providing a conductive environment for cell proliferation and growth and for new tissue formation but also giving mechanical support for the cell-scaffold construct and for the tissue being regenerated. Therefore, mechanical behavior and properties of TE scaffolds must be thoroughly investigated and assessed, which is particularly important for scaffolds for regenerating load-bearing hard tissues such as bone. The porous structure of scaffolds should also be properly designed for achieving best biological and mechanical performances. 3D printing can produce precise porous structures as designed. In this study, TE scaffolds were designed using computer softwares and fabricated via 3D printing. The mechanical behavior of scaffolds was simulated and compared with test results.

Materials and Methods: Lattice structure-type TE scaffolds were designed using the drawing function of COMSOL Multiphysics 5.6 software. Pores in scaffolds were square-shaped and the porosity for scaffolds was set at 50 and 75%, respectively. Simulation of the compression behavior of scaffolds was performed using the COMSOL simulation software. Manufacture of the designed scaffolds used selective laser sintering (SLS), a type of 3D printing technology, and nylon as scaffold material. Compression tests of scaffolds were conducted on a Model 810 MTS machine and deformation processes of scaffolds were recorded using a high-speed camera.

Results: 3D printed scaffolds via SLS had high structural fidelity for the designs at two porosity levels, and SLS could produce clean and well-defined TE scaffolds. Mechanical tests provided real data for 3D printed scaffolds and revealed real compressive deformation processes of scaffolds. The numerical results and experimental results showed that simulation experiments could model the scaffold compression behavior/processes fairly accurately and predict the mechanical performance of different scaffolds [compressive modulus of 53.51 MPa (simulated) vs. 53.55 (tested) for 75% porosity scaffolds], which is important for the design and development of TE scaffolds.

Discussion and Conclusions: For tissue engineering of bone and also other body tissues, a good control of scaffold characteristics is highly important. As shown in our investigations, simple scaffolds (in this study) and complex scaffolds (in our other reports) could be designed using commercial software and more importantly, could be realized via suitable 3D printing technology. SLS 3D printing proved to provide high fidelity for printed scaffolds from the designs. The COMSOL software provided simulation data that were verified by experimental results, suggesting the potential and importance of scaffold simulation in the development of new TE scaffolds.

Acknowledgement: Support by HK RGC through grants (17200519, 17202921, 17201622 and N_HKU749/22).
Comparative analysis of biological characteristics of meniscal progenitor cells across different species

Ms. Wan Ting Yan¹, Jing-Song Wang, Zheng-Zheng Zhang
¹Sun Yat-sen Memorial Hospital, China

Introduction:
Meniscal progenitor cells (MPCs) have emerged as promising candidates for meniscus regeneration, they are also critical for understanding the microenvironmental changes that occur in the meniscus during injury. To better investigate this complex process, it is essential to establish an appropriate animal model. In this study, we compared the biological properties of MPCs isolated by differential adhesion to fibronectin (DAF) from human, goat, rabbit, and rat, with the aim of providing guidance on the selection of a suitable animal model for investigating meniscal regeneration.

Subjects and Methods:
Meniscal progenitor cells (MPCs) were isolated from freshly digested meniscus cells of human, goat, rabbit, and rat by selective fibronectin adhesion. Their expression of mesenchymal stem cell (MSC) and fibronectin receptor markers were identified. Biological functions, including proliferation, colony-forming, multilineage differentiation, and migration abilities, were compared between MPCs and their corresponding mixed meniscus cell (MC) populations in each species.

Results:
MPCs were successfully isolated using DAF technique in all species. Human and goat MPCs exhibited a spindle-shaped morphology, while rabbit MPCs appeared more polygonal and rat MPCs had a cobblestone-like appearance. MPCs expressed fibronectin receptor CD49e, CD49c, and MSC markers CD166, CD90, CD44, and Stro-1. MPCs shown better multilineage differentiation ability than MCs, particularly in terms of chondrogenic differentiation. All MPCs exhibited strong colony-forming ability, while human MPCs tend not to form compact colonies during in vitro culture. Compared to MCs, MPCs exhibited greater multilineage differentiation ability in all species. Moreover, human, goat, rat MPCs exhibited greater proliferation capacity, while no significant differences were observed in rabbit models. No significant differences were found in the migration ability of MPCs and MCs in all species.

Discussion and Conclusion:
This study compared the biological characteristics of DAF-isolated MPCs in different animal models for the first time. Our results suggest that MPCs exhibit greater similarity between goats and humans, highlighting the importance of developing meniscus regeneration models in larger animal.
Electrosprayed Regeneration-Enhancer-Element Microspheres Power Osteogenesis And Angiogenesis Coupling

Mr. Tianpeng Xu¹, Dr Yuhe Yang², Dr Xin Zhao¹
¹Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China, ²Department of Biomedical Engineering, The Hong Kong Polytechnic University, Hong Kong, China

Background: Electrosprayed microspheres for bone regeneration are conventionally restricted by the lack of osteogenic modulation for both encapsulated stem cells and surrounding cells at the defect site. Unfortunately, most of the present electrosprayed microspheres lack appropriate stimulation to guide the regenerative fate of the encapsulated cells. More importantly, these studies only considered the modulation of encapsulated cells but overlooked the potential role of microspheres as a modulator for surrounding cells in the defect site through cell-cell/cell-material communication.

Methods: In this work, we electrospray sodium alginate microspheres encapsulating L-arginine doped hydroxyapatite nanoparticles (Arg/HA NPs) and rat bone mesenchymal stem cells (rBMSCs) as regeneration-enhancer-element reservoirs (Arg/HA-SA@BMSC) for bone healing. We evaluate the effects of such regeneration-enhancer-element reservoirs on promoting BMSC osteogenesis differentiation and endothelial cells (ECs) angiogenesis. We also evaluate the bone regeneration potential of our regeneration-enhancer-element reservoirs in vivo with rat critical-sized calvarial defect model.

Results: The Arg/HA NPs serve as a container of L-arginine and Ca²⁺ and the rBMSCs inside the microspheres could metabolize the released L-arginine into bioactive gas nitric oxide (NO) in the presence of Ca²⁺ to activate the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) signaling pathway. Meanwhile, the generated NO could diffuse out of the microspheres together with the Ca²⁺ and L-arginine as exterior enhancers to promote the osteogenesis-angiogenesis coupling of surrounding rBMSCs and ECs at the bone defect site, generating an internal/external modulation loop between the encapsulated cells and surrounding native cells.

Discussion and Conclusions: Our cell-laden and Arg/HA NP loaded microspheres could not only enhance the osteogenesis of encapsulated BMSCs but also realize the exterior cells' osteogenic and angiogenic potential. We demonstrate our Arg/HA-SA@BMSC microspheres' capability to accelerate new bone formation and neovascularization in a rat calvarial defect model. We envision that our microsphere system could streamline the vascularized bone regeneration therapy as a high throughput, minimal invasive yet highly effective strategy to accelerate bone healing.

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Engineered brain-like constructs for neural development model and transplantation treatment based on 3D-printed neural progenitor cells

Ms. Ke Gai¹, Wei Chen¹, Mengliu Yang², Wei Shi², Hefeng Zhang¹, Yu Song¹, Feng Lin¹

¹Biomanufactoring Center, Department of Mechanical Engineering, Tsinghua University, Beijing, China, ²Key Laboratory for Biomechanics and Mechanobiology of the Ministry of Education, Beijing Advanced Innovation Centre for Biomedical Engineering, School of Engineering Medicine, Beihang University, Beijing, China

Title: Engineered brain-like constructs for neural development model and transplantation treatment based on 3D-printed neural progenitor cells

Authors: Ke GAI¹, Wei CHEN¹, Mengliu YANG², Wei SHI², Caizhe XU¹, Hefeng ZHANG¹, Yu SONG¹, Feng LIN¹

Affiliations: 1 Biomanufactoring Center, Department of Mechanical Engineering, Tsinghua University, Beijing, China. 2Key Laboratory for Biomechanics and Mechanobiology of the Ministry of Education, Beijing Advanced Innovation Centre for Biomedical Engineering, School of Engineering Medicine, Beihang University, Beijing, China

Category: SYIS, Tissue Engineering and Regeneration

Background: The development process of nervous system is crucial for research of developmental regulation mechanism, treatment of relevant diseases and exploration of brain-like artificial intelligence. Recently prevalent models for such study include animal models, organoids, organ chips, biological 3D printing models and so on, among which the last one holds the advantages in controllability, engineering degree and repeatability.

Methods: In this work, we built brain-like constructs though biological 3D extrusion printing with human iPSC-derived neural progenitor cells and long-term stable hydrogel mixture as bioink. The constructs were cultured in vitro for from several weeks to several months and evaluated for cell viability, proliferation, differentiation and electrical functions. Moreover, the in vivo culture in rats brain lasted for a month to demonstrate biocompatibility and ability to integrate into brain tissue.

Results: The hydrogel mixture we developed for neural progenitor cells show printability, similar elastic modulus to brain tissue (5~17 kPa), and long-term stability in composition and mechanical properties. Human neural progenitor cells can live and proliferate inside the constructs for months. The stemness can be maintained with proper medium, while differentiation happened when the stemness maintenance environment was changed. Clear calcium ion signals were observed in DAY 14 structures which proved the basic function of in situ differentiated neurons. Local field potential signals were detected with a MED64 recording system and verified by TTX treatment, different types of signals (including some epilepsy-like signals) were recorded. For the in vivo test, constructs with neural progenitor cells were injected into the hippocampal cortex of rats and made into brain tissue section a month later to confirm the distribution and proliferation conditions.

Discussion and Conclusion: We built a hydrogel system of bioink that mimic the mechanical properties of brain and printed human neural progenitor cells with the bioink. In the brain-like constructs, the neural progenitor cells lived and proliferated for months, differentiated under certain conditions, and exhibited the functions of neurons such as calcium signals and local field potential signals. Moreover, in vivo culture experiments demonstrated good biocompatibility and integration capabilities. Compared with current neural developmental models, our model is highly controllable, engineered and reproducible, which endow it with great potential of application in complicated brain development model construction, stem cell treatment and epilepsy, Alzheimer and other neural diseases model construction.

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Biomaterial based implants caused remote liver fatty deposition through activated blood-derived Kupffer cells

Mr. Zhi Peng¹, MD Hongwei OUYANG²
¹The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, ²Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative Medicine, Hangzhou, China

Background: Understanding the biocompatibility of biomaterials is a prerequisite for the prediction of its clinical application, and the present assessments mainly rely on in vitro cell culture and in situ histopathology. However, remote organs responses after biomaterials implantation is unclear.

Methods: Here, by leveraging body-wide-transcriptomics data, we performed in-depth systems analysis of biomaterials - remote organs crosstalk after abdominal implantation of polypropylene and silk fibroin using a rodent model.

Results: Local implantation caused remote organs responses dominated by acute-phase responses, immune system responses and lipid metabolism disorders. Of note, liver function was specially disturbed, defined as hepatic lipid deposition. We also proved that blood derived monocyte-derived Kupffer cells in the liver underlying the mechanism of abnormal lipid deposition induced by local biomaterials implantation. Moreover, from the perspective of temporality, the remote organs responses and liver lipid deposition of silk fibroin group faded away with biomaterial degradation and restored to normal at end, which highlighted its superiority of degradability. These findings were further indirectly evidenced by human blood biochemical examination from 141 clinical cases of hernia repair using silk fibroin mesh and polypropylene mesh.

Discussion and Conclusion: This study provided knowledge of biomaterials-body interactions. It is of great important for future development of biomaterial devices for clinical application.
Epileptic Model of Engineered Brain-like Tissue Constructs via 3D Cell-printing Technology

Mr. Wei Chen

Tsinghua University, China

Introduction: Epileptic model based on patients lesion neural cells aims to investigate the development of epilepsy and provide reference for individual medicine. Compared to prevalent 2D cultures, 3D constructs are better at mimicking microenvironment. Neural behaviors within the constructs are alternative due to the microenvironments constructed based on biomimetic design. The fabricated sample of neural cells is exposed to potential drugs for the analysis of neuron responses during the development of epilepsy. 3D cell-printing as an emerging biofabrication technology has been widely used to mimic natural 3D models in in vitro tissue research, and its extension in epileptic models construction make it a powerful model method in neuroscience.

Subjects and Methods: In this study, in vitro layered brain-like tissue and human brain tissue constructs are first proposed and later developed by 3D cell-printing technology. After more than several weeks of in vitro culturing, the formation of neural circuits in structures equips them with the capability of sensitively responding to a stimulus.

Results: Live/dead and immunostaining imaging is used to verify the growth of neural cells in the printed structure. The survival rate of neural cells in 2D and 3D samples is compared, and the results demonstrate that the 3D-printed structures exhibit a better artificial culturing environment and a higher survival rate. Local field potentials are collected and validated by the Med64 recording system. Tetrodotoxin and Phenytoin are used to test the drug sensitivity of the constructs, and the results show that both tetrodotoxin and low concentrations of phenytoin could inhibit epileptic signals in the printed structures.

Discussion and Conclusion: Compared with 2D model, primary neural cells and human brain cells are less prone to apoptosis in 3D-printed structures, so higher survival rate is obtained in 3D constructs. Both cellular morphology and electrophysiological signals of cells within 3D structures are more similar to neural cells in vivo. This suggests that 3D models can be an ideal alternative with similar microenvironment and extracellular matrix. Furthermore, the excitatory postsynaptic potential signals of the physiological performance indicate that the 3D-printed structure has great potential as a drug testing model in the pharmaceutical study for epileptic treatments.

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Construction of choroid-like vasculature by iPS derived endothelial and retinal pigment epithelium cells with choroidal fibroblasts

Dr. Heonuk JEONG¹,², Dr. Kazuno NEGISHI¹, Dr. Toshihide KURIHARA¹,², Dr. Kazuo TSUBOTA¹,³
¹Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan, ²Laboratory of Photobiology, Keio University School of Medicine, Tokyo, Japan, ³Tsubota Laboratory Inc., Tokyo, Japan
Title: Construction of choroid-like vasculature by iPS derived endothelial and retinal pigment epithelium cells with choroidal fibroblasts
Authors: Heonuk Jeong¹,², Kazuno Negishi¹, Kazuo Tsubota¹,³, Toshihide Kurihara¹,²*
Affiliations: ¹Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan. ²Laboratory of Photobiology, Keio University School of Medicine, Tokyo, Japan. ³Tsubota Laboratory Inc., Tokyo, Japan. (* Correspondence)
Category: Tissue Engineering and Regeneration

Background: A highly vascularized choroid plays a crucial role in supplying oxygen and nutrients to the outer layer of the retina. It consists of several vascular layers, including choriocapillaris, medium, and large blood vessels. It is necessary to establish in vitro tissue model to apply to effective regenerative medicine and drug screening for choroidal abnormalities such as choroidal thinning in myopic eyes and choroidal atrophy. This study aims to establish a multi-layered vascular structure resembling the choroid by constructing the stroma using fibroblasts.

Methods: Choroidal fibroblasts were isolated from C57BL6J mouse eye and co-cultured with iPSC-derived endothelial cells (ECs) on the transwell insert. Additionally, iPSC-derived retinal pigment epithelium (RPE) cells were cultured on the bottom of the culture plate. After 1 week of culture, the self-constructed tissue by the cells was immunostained for endothelial cell markers or stromal markers and evaluated its vasculatures using AngioTool (National Cancer Institute) software.

Results: Self-constructed tissues were generated with the combinations of three cell types (fibroblasts, ECs, and RPE cells). Regardless of the presence or absence of RPE cells, constructing a multi-layered structure with only ECs was not possible, only a single layer was formed. Furthermore, the presence of RPE cells led to the formation of differentiated vascular structures. As co-cultured with fibroblasts, multilayered vascular structures were constructed and with the presence of RPE, differentiated vascular structures were observed. The tissue consisting of three cell types showed significantly higher vessel area and higher average vessel length than only ECs (0.099±0.004 mm² vs 0.106±0.001 mm², p<0.01 and 583±105 µm vs 1562±363 µm, p < 0.01).

Discussion and Conclusions: Our study demonstrates that co-culturing ECs with choroidal fibroblasts and RPE cells can successfully construct multi-layered vasculature structures resembling the choroid. However, further functional evaluations are required to fully understand the capabilities of our model. The choroid in vitro model in this study is expected to have applications in bioengineered tissue for regenerative medicine.

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Advanced Prediction of Printing Resolution using Rheology-Informed Hierarchical Machine Learning

Ms. Dageon OH¹, Mr. Masoud SHIRZAD¹, Mr. Min Chang KIM², Prof. Eun-Jae CHUNG³, Prof. Seung Yun NAM¹²

¹Industry 4.0 Convergence Bionics Engineering, Pukyong National University, Busan, Republic of Korea, ²Major of Biomedical Engineering, Pukyong National University, Busan, Republic of Korea, ³Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University College of Medicine, Seoul, Republic of Korea

Category: Enabling Technologies

Background: Extrusion-based bioprinting has been a widely used technique for research and commercial purposes. Recently, the printability prediction of extrusion-based bioprinting which leads to accurate, faster fabrication speed, and long-term table functionality attracted considerable attention. Initially, the physical model for printability prediction was adopted with the rheological modeling of significantly sensitive to the power law index that can be obtained by line fitting of the measured viscosity. Thus, small errors in rheological measurement and line fitting can have a significant effect on the accuracy of prediction. Recently, multiple studies of printability prediction employed machine learning methods to estimate printing resolution which focuses on the confined variation of bioink composition and printing parameters to overcome the limitations of conventional printability prediction methods.

Methods: The rheology-informed hierarchical machine learning (RIHML) model is designed with a multi-input neural network to calculate the printing resolution using three types of input data; the printing parameters (nozzle diameter, nozzle length, printing speed, and pressure) and two types of rheological properties that viscosity and storage modulus. Various bioink compositions of Pluronic F-127, gelatin, xanthan gum, alginate, calcium chloride, and cellulose nanocrystal were used to make datasets for machine learning.

Results: The rheology-informed hierarchical machine learning model was developed to improve the prediction accuracy of the printing resolution of constructs fabricated by extrusion-based bioprinting. The conventional machine learning models are compared with the RIHML. All the prediction results presented RIHML has higher accuracy than conventional models. Furthermore, the RIHML model can predict the resolution of the new compositions.

Discussion and Conclusion: We developed a rheology-informed machine learning model to improve the prediction accuracy of the printing resolution of constructs fabricated by extrusion-based bioprinting. The model was used to predict the printing resolution in three different cases including new printing parameters with trained bioink materials; new concentrations of the trained bioink constituents; and untrained bioink compositions with the new material. Interestingly, the results showed that the RIHML model exhibited the lowest error percentage in printability prediction for various conditions. Furthermore, it can easily generalize and expand compared to the conventional method. Overall, the results present that the RIHML model can be a versatile tool for predicting printability.

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Multifunctional Fibrous Membrane with Drug-loaded Core-shell Structure for Vascularized Bone Regeneration

Dr. Jin Shue1, Yubao Li2, Jidong Li2, Zongke Zhou1

1Department of Orthopedics Surgery, Orthopedic Research Institute, West China Hospital, Chengdu, China, 2Research Center for Nano-Biomaterials, Analytical and Testing Center, Chengdu, China

Multifunctional Fibrous Membrane with Drug-loaded Core-shell Structure for Vascularized Bone Regeneration

Shue Jin1, Yubao Li2, Jidong Li2* and Zongke Zhou1*

1Department of Orthopedics Surgery, Orthopedic Research Institute, West China Hospital, Sichuan University, Chengdu 610041, P. R. China
2Research Center for Nano-Biomaterials, Analytical and Testing Center, Sichuan University, Chengdu 610065, P. R. China

E-mail: nic1979@scu.edu.cn; zhouzongke@scu.edu.cn

Introduction

Bone tissue regeneration is a complex interdisciplinary course of biomedical science and materials science, in which the regulation of the immune microenvironment, the dynamic balance between bone formation and bone resorption, and the construction of neovascularization network are extremely important. Active regulation of the immune microenvironment in the early stage of implantation and subsequent vascular network formation is considered a prerequisite for successful functional bone regeneration because it was essential for cell recruitment, cytokine secretion and the transportation of oxygen, nutrients and metabolic waste. Despite the fact that various bone regeneration scaffolds have been developed, researchers have never stopped exploring the construction of a scaffold that can genuinely realize vascularized bone regeneration.

Subjects and Methods

In this work, we are committed to preparing a bone graft substitute with an extracellular matrix (ECM) like structure that can actively regulate the immune microenvironment and promote vascularized bone regeneration. Here, we propose a core-shell structure fiber strategy using PLGA and PCL in combination to maximize their advantages and overcome their shortcomings[1]. We investigated the anti-shrinkage performance of the coaxial fibrous scaffold in several common liquid media by shrinkage tests. We also experimentally tested the physico-chemical properties and BA release behavior. Finally, we evaluated the vascularized bone regeneration ability of PFC/PCL-BA through a critical-sized calvarial bone defect model.

Results

The core-shell structures fibrous scaffold maintained its size in PBS, normal saline, and medium (103.4 ± 4.4%, 103.1 ± 3.6% and 97.8 ± 9.5%, respectively), and shrank only mildly in 75% ethanol (88.8 ± 7.7%). TEM and CLSM images of PFC/PCL-BA fibers certified the core-shell structure. The release behavior of BA in vitro demonstrated that BA’s release mode in the PFC/PCL-BA fibrous scaffold was a combination of diffusion-based and degradation-based modes. The results of degradation experiments indicated that the presence of PCL could maintain the structural integrity and stability of the fibrous membranes, and the PFC of the shell layer could effectively accelerate the degradation rate of the whole fibrous scaffold. The in vitro experimental results demonstrated that BA in the scaffold could upregulate the expression of osteogenic related genes in BMSCs, which may be beneficial for accelerating bone regeneration. The subcutaneously implantation results show that the designed PFC/PCL-BA scaffold can create a pro-regenerative immune microenvironment by regulating the macrophage phenotype transition. The critical-sized calvarial bone defect model results demonstrated that the PFC/PCL-BA scaffolds could guide bone regeneration as desired, and BA in the scaffold may promote bone regeneration by enhancing the microarchitecture of the trabeculae. The anti-shrinkage test results revealed that introducing size-stable PCL as the core fiber significantly improved the PLGA-based fibrous scaffold's dimensional maintenance.

Discussion and Conclusion
We demonstrate a simple, effective and feasible method to address the shrinkage of Poly (lactic-co-glycolic acid) (PLGA) and the relatively weak cell responsiveness of poly-caprolactone (PCL) through a core-shell structure fiber strategy. Considering that the amorphous PLGA will shrink dramatically while the semi-crystalline PCL has a good stability of dimension, we utilized the characteristics of PCL and PLGA to combine them into core-shell structure fibers, thereby resisting the dimensional shrinkage through the axial and radial reverse interaction between them. We further utilized fish collagen to modify the PLGA shell layer (PFC) of coaxial fibers and loaded baicalin (BA) into the PCL core layer (PCL-BA) to endow fibrous scaffold with more functional biological cues. The initial release of BA may be beneficial for regulating the immune cells’ function and the expression of inflammatory cytokines to alleviate the acute inflammatory response after biomaterials implantation, providing a beneficial local immune microenvironment for bone tissue regeneration. The release of BA in the middle and later stages is conductive to regulating the formation and resorption of bone.

Foreign body reaction is a series of complex reaction of host tissue to resist excessive physiological response and stimulation caused by infection. The magnitude of the stress response of immune cells induced by implanted material plays a crucial role in material-mediated tissue repair. The coaxial structure fibrous scaffold performed well in the moderation of the local microenvironment. Of course, BA also played an anti-inflammatory role and inhibited the fibrosis role in the whole process of foreign body reaction. The PFC/PCL-BA scaffolds could guide bone regeneration as desired, and BA in the scaffold may promote bone regeneration by enhancing the microarchitecture of the trabeculae. BA provided a beneficial immune microenvironment for bone regeneration by regulating pro/anti-inflammatory mediators in the early implantation stage. Then, BA further regulated bone remodeling by the osteoblast-mediated bone formation and osteoclast-mediated related bone resorption.

Interestingly, we found that the TRAP-positive region in the PFC/PCL-BA group was precisely where the neovascularization occurred, indicating that osteoclasts provide channels for blood vessels’ formation. It was the coupling of angiogenesis and osteoclast differentiation that promoted functional bone regeneration in the defect area. This work tactfully combined PLGA and PCL to establish a drug release platform based on the core-shell fibrous scaffold for vascularized bone regeneration[2].

Reference
[1] The invention discloses a multifunctional fibrous membrane for bone tissue regeneration and a preparation method thereof, Patent number: 202010520744.1
Nondestructive Assessment of Viscoelasticity of Tissue-Engineered Constructs using Shear Wave Elastography

Ms. Garin KIM\textsuperscript{1}, Prof. Changhan YOON\textsuperscript{2}, Prof. Seung Yun NAM\textsuperscript{3}
\textsuperscript{1}Industry 4.0 Convergence Bionics Engineering, Pukyong National University, Busan, Republic of Korea, \textsuperscript{2}Department of Biomedical Engineering, Inje University, Gimhae, Republic of Korea, \textsuperscript{3}Major of Biomedical Engineering, Pukyong National University, Busan, Republic of Korea

Category: Enabling Technologies

Background: Mechanical properties of biomaterials, such as elasticity and viscosity, play an important role in tissue engineering as they significantly affect cell-material interactions. Conventional measurement and quantification of mechanical properties of tissue-engineered constructs have been based on destructive methods including rheometry and tensile tests, which require multiple samples and intensive labor. In this study, ultrasound shear wave imaging, which is nondestructive with the real-time data acquisition capability, was proposed to overcome these limitations. In conventional shear wave elasticity imaging, shear waves can be generated from acoustic radiation, and their speed was monitored using sequential detection of ultrasonic signals with high framerate. However, the conventional method is more focused on the measurement of elasticity, which can lead to large uncertainties in the assessment of viscoelastic biomaterials. Therefore, we analyzed shear wave dispersion to measure both viscosity and elasticity of the tissue-engineered constructs.

Methods: A programmable ultrasound system (Verasonics Inc., Redmond, WA) and a linear array transducer L7-4 (Philips Healthcare, Andover, MA) were used for the generation and detection of the ultrasound shear wave. To generate acoustic radiation force, 32 elements were excited to transmit a tunable push duration. For the detection of shear waves, frames were acquired with a high pulse repetition frequency (PRF) of 10kHz. For data processing, shear wave speed and dispersion were calculated to estimate the elasticity and viscosity of the tissue-engineered constructs. To validate nondestructively observed mechanical properties, the storage modulus ($G'$) and the loss modulus ($G''$) at each frequency were obtained using a rheometer (HR-2, TA instruments) with a parallel plate.

Results: Viscoelasticity of the tissue-engineered constructs was estimated using the proposed method. Validation of the estimated mechanical properties was carried out to evaluate the accuracy of the nondestructive measurement. Subsequently, the change in viscoelasticity of the tissue-engineered constructs was monitored at different time points.

Discussion and Conclusion: We demonstrated the nondestructive quantitative assessment of the viscoelasticity of the tissue-engineered constructs using ultrasound shear wave elasticity imaging and analysis of shear wave dispersion. In conventional imaging modalities, the estimation of mechanical properties was inaccurate as the loss modulus was neglected in the processing algorithm. The experimental results indicate that the suggested nondestructive method of viscoelasticity measurement can be a versatile tool to monitor the mechanical and rheological changes of various tissue-engineered constructs.

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The Potential of Overexpressing Stearoyl-CoA Desaturase1 (SCD1) in Mesenchymal Stem Cells for Therapy Targeting Osteoporosis Model

Mr. Young-Jin Seo¹, Hye-Seong Oh¹,², Jin-Ho Park¹, June-Ho Byun¹,²
¹Department of Oral and Maxillofacial Surgery, Institute of Health Sciences, School of Medicine, Gyeongsang National University, Gyeongsang National University Hospital, Jinju, South Korea, ²Department of Convergence Medical Science, Gyeongsang National University, Jinju, South Korea

Authors: Young-Jin Seo¹,², Hye-Seong Oh¹,², Jin-Ho Park¹, June-Ho Byun¹,²*

Affiliations: ¹Department of Oral and Maxillofacial Surgery, Institute of Health Sciences, School of Medicine, Gyeongsang National University, Gyeongsang National University Hospital, Jinju 52727, Korea, ²Department of Convergence Medical Science, Gyeongsang National University, Jinju 52828, Korea

Category: Stem Cells and Cell-Based Therapies

Background: The bone microenvironment of osteoporosis promotes the differentiation of MSCs into adipocytes due to low levels of Histone acetylation, leading to an increase in marrow adipose tissue (MAT). As a result, the trans-differentiation of MSCs into osteoblast adipocytes is promoted by adipocytes. In vitro, experiments have demonstrated that saturated fatty acids (FAs) inhibit osteogenic differentiation, unlike unsaturated FAs. Therefore, we aim to investigate the effects of SCD1, an enzyme that converts saturated FAs to monounsaturated fatty acids (MUFAs), on osteoporosis.

Methods: This study implemented a co-culture model using gas chromatography-mass spectrometry (GC-MS) to analyze the differences between the secretion patterns of fatty acids released by adipocytes in the bone microenvironment. In addition, the degree of adipocyte trans-differentiation was observed, and the degree of clinical bone regeneration effect was compared through the overexpression of SCD1 in rat OVX (Ovariectomy) model-derived BM-MSC.

Results: MSCs of the rat OVX group were differentiated into adipocytes, and fatty acids were analyzed through GC-MS. As a result, the OVX group showed a higher FAs saturation ratio than the Sham group. In addition, it was confirmed that SCD1 affects changes in MUFAs and the degree of differentiation of osteoblast, and overexpression of SCD1 restored the reduced osteoblast differentiation ability and showed a positive effects on bone regeneration in vivo. These results suggest that SCD1 could be a potential therapeutic target for the treatment of osteoporosis.

Discussion and conclusion: This study found that the increase in MAT in the OVX model was associated with an increase in fatty acids and that the differentiation of MSCs depended on the type, amount, and ratio of fatty acids. The findings suggest that SCD1 not only regulates the differentiation of MSCs in osteoporosis and promotes bone regeneration through enhancing osteoblast activity but also can be a potential therapeutic target for the treatment of osteoporosis. Further investigation on the effects of different fatty acids on MSC differentiation and bone regeneration could lead to the development of more effective treatments for osteoporosis.

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Advanced Approach to Ischemia Treatment through Enzyme-Mediated Oxygen-Releasing Polyphenol Particles

Mr. Jinwoo HONG¹, Prof. Su-Hwan Kim¹

¹Department of Chemical Engineering, Dong-A University, Busan, Republic of Korea

Introduction: Ischemia disease causes tissue necrosis and ulcers due to low blood flow and lack of insufficient oxygen and nutrition supply. It is important to provide sufficient oxygen for ischemic tissue to regenerate. Polyphenols are phenolic compounds that have proven beneficial functions such as ROS scavenging, antioxidant, and anti-inflammatory. The mechanism of oxygen release through polyphenol-enzyme particles by coating enzymes on polyphenol particles provides inspiration for ischemic disease treatment approaches to improve angiogenesis and tissue regeneration.

Method: EGCG, the main polyphenol of green tea used in this study, is oxidized to semiquinone radicals by O₂ or cell-generated ROS, and then forms superoxide anion radicals to produce hydrogen peroxide. Also, catalase (CAT) is an enzyme that decomposes hydrogen peroxide into water and oxygen. Co-coating EGCG and CAT on the particle surface to form EGCG@CAT particles. These particles lower the amount of over-expressed ROS in ischemic diseases to normal levels, build a sustainable oxygen release system that provides oxygen in tissues while maintaining the level of ROS, improve tissue regeneration, and reliably regenerate damaged tissues through anti-inflammatory reactions.

Result: Successful synthesis of polyphenol-enzyme particles has been visualized using fluorescent labeling proteins. We also demonstrated the efficacy of EGCG@CAT particles to exhibit oxygen release effects in ROS environments in the presence of hydrogen peroxide and to lower over-expressed ROS to normal levels through ROS scavenging activity. This shows that polyphenol and enzyme activity are maintained even after particle synthesis. The in vitro studies showed the viability of HUVEC and NIH-3T3 cultured in particle-containing groups and complete media, but there was no significant difference in cell viability between samples, and for HUVEC cells, angiogenesis stage was modeled through tube formation.

Discussion and Conclusion: We demonstrate the ability of EGCG@CAT particles to induce angiogenesis and tissue regeneration through oxygen release and ROS scavenging in ischemic disease environments, and tube formation in HUVEC cells through in vitro studies. In the future, in vivo studies verify particle efficacy in mouse ischemia models to establish verification as oxygen-releasing particles. This study provides new insights into the reliable treatment potential of ischemic diseases.

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Locally sustained delivery of hypoxia inducible factor-1 inhibitor and doxorubicin from iodinated oil based Pickering emulsion for tumor synergistic therapy

Dr. Zhihua Li, Dr. Xiaoya Liu, Mrs. Jingyu Xiao, Mr. Le Ma, Mr. Yucheng Luo, Ms. Meijuan Wang, Mr. Hongliang Jiang, Mr. Hanyang Yao, Dr. Qiongyu Guo

1Shenzhen Key Laboratory of Smart Healthcare Engineering, Department of Biomedical Engineering, Southern University of Science and Technology, Shenzhen, China

Background: Tumor hypoxic microenvironment activates the hypoxia inducible factor-1 (HIF-1), which is linked to angiogenesis, drug resistance, tumor metastasis and proliferation. In addition, the effectively tumor-targeted delivery and tumor hypoxia related angiogenesis as well as drug resistance are the major challenges for cancer chemotherapy.

Subjects and Methods: In this study, we employed hydrophobic silica nanoparticles (HBSNs) to stabilize the iodinated oil (IO) based emulsion for locally sustained codelivery of hypoxia inducible factor-1 (HIF-1) functional inhibitor acriflavine hydrochloride (ACF) and cytotoxic agent doxorubicin hydrochloride (DOX) to achieve tumor synergistic chemotherapy.

Results: The IO based Pickering emulsion exhibited excellent physical stability and sustained release of DOX and ACF, promoting the accumulation and retention of drugs in extravascular region. The combination of DOX and ACF delivered by Pickering emulsion demonstrated significant inhibition of tumor growth both in vitro and in vivo.

Discussion and Conclusion: The codelivery of anticancer drug and HIF-1 functional inhibitor improved the synergistic therapeutic efficacy through the directly cytotoxic effect to tumor cells and the function suppression of HIF-1 simultaneously. Consequently, the IO based Pickering emulsion stabilized by HBSNs provides a great drug sustained delivery platform for tumor locoregional chemotherapy via intratumoral administration or transarterial chemoembolization.
3D-Printing of Graphene-Based Scaffolds for Breast Cancer Treatment

Dr. Giordano Perini$^{1,2}$, Dr. Valentina Palmieri$^{2,3}$, Dr. Ginevra Friggeri$^2$, Dr. Alberto Augello$^2$, Dr. Daniela Fioretti$^2$, Dr. Sandra Iulescia$^4$, Dr. Monica Rinaldi$^4$, Professor Marco De Spirito$^{1,2}$, Professor Massimiliano Papi$^{1,2}$

$^1$Università Cattolica del Sacro Cuore, Roma, Lazio, $^2$Fondazione Policlinico Universitario “A. Gemelli” IRCCS, Roma, Lazio, $^3$Istituto dei Sistemi Complessi, CNR, Roma, Lazio, $^4$Istituto di Farmacologia Traslazionale, CNR, Roma, Lazio

Introduction: Tumor ablation through photothermal therapy is a promising alternative treatment for breast cancer patients, which involves destroying tumor cells using heat-based therapies. However, traditional photothermal therapies can cause damage to healthy tissues and cells, which can lead to complications. Biocompatible 3D printed graphene oxide scaffolds have been recently investigated as a potential alternative for photothermal therapy due to their high photothermal conversion efficiency and biocompatibility. In this study, we explore the efficacy of two different therapeutic approaches for tumor ablation using 3D printed graphene oxide scaffolds.

Methods: Two different therapeutic approaches were conducted in vitro and in vivo to investigate the efficacy of tumor ablation using 3D printed graphene oxide scaffolds. The first approach involved three near-infrared (NIR) laser radiations of 3 minutes each, while the second approach involved a single radiation of 9 minutes. In vitro experiments were conducted by analyzing the viability and free radicals of cells, along with the different types of cytokines produced and cell death. In vivo experiments were conducted on mice to evaluate the best approach.

Results: The results of in vitro experiments showed that the three NIR laser radiations of 3 minutes each resulted in higher cell death through apoptosis and cytokine release compared to the single radiation of 9 minutes. Additionally, the three NIR laser radiations of 3 minutes each resulted in higher levels of free radicals in tumor region, indicating less damage to healthy tissues. In vivo experiments on BALB/c mice also showed that the three NIR laser radiations of 3 minutes each resulted in better tumor ablation and reduced damage to healthy tissues, along with higher levels of infiltrating leukocytes.

Discussion and Conclusion: The results of this study suggest that three NIR laser radiations of 3 minutes each on biocompatible 3D printed graphene oxide scaffolds is a more effective and safer approach for tumor ablation compared to a single radiation of 9 minutes. The production of free radicals and higher apoptotic cell death observed in in vitro experiments indicate that this approach is less likely to cause damage to healthy tissues, while the in vivo experiments on mice support the strong efficacy of this approach for tumor ablation. Future studies can further investigate the potential of biocompatible 3D printed graphene oxide scaffolds for tumor ablation using different therapeutic approaches and in different animal models.

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Static and Dynamic Mechanical Properties of 3D-printed Auxetic Structures for Biomedical Applications

Mr. Masoud SHIRZAD¹, Prof. Seung Yun NAM¹,²
¹Industry 4.0 Convergence Bionics Engineering, Pukyong National University, Busan, Republic of Korea, ²Major of Biomedical Engineering, Pukyong National University, Busan, Republic of Korea

Category: Design and Application of Biomaterials

Background: Different structures have been utilized in biomedical engineering for various purposes. The mechanical and physical properties of the structures, besides the biological properties, characterize their appropriateness for biomedical applications. Metamaterials, specifically auxetic structures, with negative Poisson’s ratio can improve the mechanical properties of the scaffolds and mimic specific tissue, such as tendons, in the human body.

Methods: In the present work, an auxetic structure was developed to mimic the structure of a real tendon and bone load-bearing behavior. To fabricate the auxetic structure, fused deposition modeling (FDM) was deployed, and the static and dynamic mechanical properties of auxetic scaffolds were investigated by universal testing machine (UTM) and finite element method (FEM).

Results: The static mechanical test showed that the auxetic structure could increase energy absorption of the fabricated scaffolds and longitudinal strain with similar porosity. These phenomena were approved by both the tensile and compression mechanical tests. Additionally, the stress-strain curve of the tensile test of the auxetic structure exhibited a toe region followed by a linear area, which was analogous to the stress-strain curve of the real human tendon. Moreover, the auxeticity could improve the number of cycles to failure in both loading conditions.

Discussion and Conclusion: The present study demonstrated that auxetic structures could considerably improve the mechanical properties of scaffolds, such as energy absorption and longitudinal strain of the scaffolds under static tests. The improvement of the mechanical properties is not limited to the static tests; the auxetic structure increases the number of cycles to failure compared to the conventional rectangular structure.

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Fabrication of Multiscale Structure using Sequential Extrusion-Based Bioprinting for Vascularized Tissue Scaffolds

Ms. Jungeun Choi¹, Prof. Seung Yun Nam¹,²
¹Major of Biomedical Engineering, Pukyong National University, Busan, Republic of Korea, ²Industry 4.0 Convergence Bionics Engineering, Pukyong National University, Busan, Republic of Korea

Category: Tissue Engineering and Regeneration

Introduction: Biofabrication of large tissues, such as the liver, heart, muscle, and bone, requires microvascularization below the size of 10 μm to support the nutrient and gas exchanges. Current extrusion-based bioprinting techniques suffer from limited printing resolution, which is essential for the fabrication of vascularized tissue scaffolds. Therefore, in the present work, we fabricated multiscale structures to achieve high printing resolution and mimic vascularized networks using sequential extrusion-based bioprinting.

Subjects and Methods: The multiscale vascularized tissue scaffold was fabricated using sequential extrusion-based bioprinting which includes printed bioink in the barrel. In addition, cell viability and proliferation were assessed with the manufactured scaffolds.

Results: The vascular tissue scaffolds with heterogeneous filament structures were fabricated well using sequential extrusion-based bioprinting. It was demonstrated that sequential extrusion-based bioprinting can be a useful tool to increase the resolution of fabricated scaffolds. Cell viability and proliferation were also verified in the vascularized scaffolds.

Discussion and Conclusion: The present study demonstrated that a multiscale construct with sequential extension-based bioprinting can considerably improve the printing resolution to mimic vascularized network formation. Therefore, benefiting from high-resolution bioprinting of diverse structures, the extrusion-based bioprinting technique could be used in a wide range of 3D printing applications overcoming the limitations of conventional biofabrication technologies.

Acknowledgement: This research was supported by a National Research Foundation of Korea (NRF) grant (NRF-2021R111A3040459) funded by the Korean government (MOE). This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI22C1323).
Amniotic epithelial cells loaded GelMA-HAMA hydrogel improve large-area skin wounds healing

Dr. Yao Chen

1 Affiliated Hospital of Nantong University, , South Korea

Background: Skin wound, particularly large area skin damage, as well as scar formation is common clinical problem. Human amniotic epithelial cells (hAECs) have been shown to have repairing properties in a variety of tissues. Natural component hydrogels are ideal extracellular matrix (ECM) materials for wound healing because of their biocompatibility which can mimic the extracellular matrix. The goal of this study is to design the GelMA/HAMA hydrogel with human amniotic epithelial cells (hAECs). The mixed hydrogel loaded with cells was used to cover huge skin wound surface and crosslinked rapidly under 405nm blue light. The amniotic epithelial cells could release cytokines to promote wound skin regenerate by promoting granulation tissue and stimulating blood vessel formation.

Methods: In the experiment, methacrylic gelatin (GelMA) and methacrylic hyaluronic acid (HAMA) were dissolved in different proportion to form scaffold. Scanning electron microscope was used to examine the internal structure of the hydrogel stent, mechanical testing for the strength property, CCK-8 for biocompatibility to determine the optimum concentration ratio. Cover the mixed hydrogel-loaded hydrogel-loaded amniotic epithelium to rat skin wounds and measure the healing rate. HE staining, Masson staining, and CD31 immunohistochemistry were used to examine histological phenotype of wound area. RT-PCR was used to detect the expression of VEGF and IL-10.

Results: 15% GelMA-1% HAMA has a strong water retention performance and better biocompatibility with amniotic epithelial cells, making it excellent for skin wound healing. HE staining, Masson staining, and CD31 immunohistochemistry revealed that composite cell hydrogels improved wound healing, enhanced collagen generation and angiogenesis, and RT-PCR test revealed that VEGF was up-regulated while IL-10 was down-regulated.

Conclusions: GelMA/HAMA hydrogel with amniotic epithelial cells inhibits wound inflammation, promotes collagen generation and angiogenesis, is critical for huge skin wound healing.

Keywords: Gelatin methacrylate, hyaluronic acid methacrylate, human amniotic epithelial cells, skin wounds healing

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4D Printed Self-folding Scaffolds with Controlled Growth Factor Delivery for Vasculature Regeneration

Dr. Jiahui Lai, Prof. Liwu Zheng, Prof. Min Wang

1Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, 2Faculty of Dentistry, The University of Hong Kong, Hong Kong.

Affiliation: 1Department of Mechanical Engineering, The University of Hong Kong, Hong Kong. 2Faculty of Dentistry, The University of Hong Kong, Hong Kong. (*Corresponding author)

Category: Tissue Engineering and Regeneration

Introduction: 4D printing produces dynamic structures that can change their shape, properties, or functions over time under appropriate external stimulus such as temperature, water, or pH. In recent years, 4D printing has been increasingly used in tissue engineering for creating novel tissue engineering scaffolds with shape-morphing ability, customized architecture, or special functions. Self-folding scaffolds are able to reshape themselves to match the tubular shape of blood vessels after deployment in vivo. However, currently stimulus-responsive biomaterials for 4D printing in vascular tissue engineering are still limited and new materials need to be developed.

Materials and Methods: Shape memory polymer poly(D,L-lactide-co-trimethylene carbonate) (PDLLA-co-TMC, “PTMC” in short) was used to provide the shape memory property for scaffolds. Gelatin methacryloyl (GelMA) was used to load a growth factor, vascular endothelial growth factor (VEGF). As illustrated in Fig.1, the PTMC layer was generated through 3D printing, reshaping into tubular shape and re-flattening the tube into 2D shape, while the second layer of GelMA was fabricated via direct extrusion of GelMA hydrogels containing VEGF. These two layers were printed as porous structures and were combined to form PTMC/GelMA bilayer scaffolds. Various experiments were conducted to characterize and evaluate the bilayer scaffolds.

Results: Bilayer PTMC/GelMA scaffolds were successfully manufactured. SEM images showed that regular macropores were present on the surface of these scaffolds and the GelMA layer was tightly attached to the PTMC layer. By heating bilayer scaffolds to above 37 °C, scaffolds were able to change from 2D flat structure to 3D tubular shape according to our design. The self-folding process was quick and completed within 1 minute. VEGF showed a biphasic release behavior for bilayer scaffolds, with an initial rapid release and subsequent slower and sustained release. Mesenchymal stem cells seeded on scaffolds showed good attachment and viability during in vitro culture.

Discussion and Conclusions: 4D printing is powerful for creating dynamic scaffolds for tissue regeneration, and shape-morphing and biomolecule-delivering PTMC/GelMA bilayer scaffolds were successfully 4D printed in this study. The scaffolds possessed designed porous structures, had good shape-morphing ability (self-folding into tubular shape), provided controlled release of VEGF, and exhibited excellent cytocompatibility. These experimental results suggested good potential of 4D printed bilayer scaffolds for vasculature regeneration.

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Treatment of Atopic Dermatitis with ROS-controllable and Multifunctional Polyphenol Carbon Dots Hydrogel

미스터, Jeongmin Han

Dong-A University, , South Korea

Introduction: Atopic dermatitis (AD) is a chronic inflammatory disease associated with an unbalanced immune response in skin tissue. High oxidative stress derived from reactive oxygen species (ROS) promotes the release of inflammatory cytokines and T-cell differentiation, resulting in the onset and exacerbation of atopic dermatitis. EGCG-derived carbon dots nanoparticles have unique therapeutic effects due to their antioxidant, anti-inflammatory, and radical scavenger properties, as well as multifunctional physicochemical properties through strong fluorescence, and high optical and surface passivation. Here, we manufactured a hydrogel patch for AD treatment by incorporating EGCG-derived carbon dots nanoparticles into alginate hydrogels. Alginate hydrogel patches containing EGCG-derived carbon dots nanoparticles can be a possible strategy for treatment and management by inhibiting oxidative stress induced by ROS in atopic dermatitis lesions.

Methods: In this study, Ethylene diamine (EDA) was added to the Epigallocatechin gallate (EGCG), and carbon dots were synthesized using the hydrothermal method. Hydrogels with EGCG-derived carbon dots were manufactured by pH-regulated Ca2+ crosslinking of synthesized carbon dots and alginate polymers, and their effects on atopic dermatitis lesions were investigated through tests of ROS scavenger and SOD imitation activity in vitro and in vivo.

Results: The EGCG-derived carbon dots showed excellent concentration-dependent ROS scavenger ability and SOD mimic activity properties. In vitro, studies have shown that EGCG-CDs hydrogel cultured together in DNCB-treated HaCaT cells significantly reduces ROS levels occurring within the cells. In addition, the hydrogel containing EGCG-derived carbon dots exhibited strong fluorescence, indicating its high optical properties, as confirmed by cell imaging. An in vivo evaluation in a DNCB-sensitized atopic dermatitis-induced BALB/c mouse model showed that EGCG-CDs hydrogel could reduce the high oxidative stress produced in skin tissue at the lesion site and resulted in improved wound healing.

Discussion and Conclusion: We found that EGCG-derived carbon-based hydrogels have superior ROS scavenger ability and SOD mimetic activity, which significantly reduces intracellular ROS levels. In vivo, studies also demonstrate that these hydrogels can eliminate high oxidative stress generated in atopic dermatitis lesions and result in better rates of inflammatory skin wound healing. This study demonstrated a treatment strategy that can promote wound healing in inflammatory skin diseases with ROS oxidative stress-regulated nanoparticles.


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Development of a Granular Bioink for 3D Bioprinting of Porous Scaffolds

Ms. Theresa Seah\textsuperscript{1,2}, Ms. Jingyi Zhang\textsuperscript{1}, Dr. Cyrus Weijie Beh\textsuperscript{1}

\textsuperscript{1}Bioprocessing Technology Institute, Agency for Science, Technology and Research (A*STAR), Singapore, \textsuperscript{2}Department of Biomedical Engineering, National University of Singapore, Singapore

Background
The main goal of tissue engineering is to create artificial tissues to replace diseased or damaged organs. 3D bioprinting is often used to create complex 3D scaffolds that mimic the structure of native tissues, with high precision and accuracy. However, one major challenge in the development of bioengineered artificial organs is the lack of a network to supply embedded cells with sufficient oxygen and nutrients to preserve their viability and functionality. In this work, we developed a bioprintable granular hydrogel that can be used to print a highly porous scaffold structure, capable of improving the mass transport of oxygen and nutrients to embedded cells.

Method
We developed a granular hydrogel bioink capable of producing a highly porous 3D scaffold. The granular hydrogel bioink consists of gelatin methacryloyl (gelMA) particles suspended in culture medium, which can be pipetted or bioprinted using commercially available bioprinters, and subsequently photocrosslinked in the presence of a photoinitiator. The covalent crosslinks formed between the gelMA microparticles create interconnected pores within the 3D scaffold structure, resulting in high porosity that facilitates cellular growth and proliferation.

Results
The granular hydrogel bioink enables the bioprinting of a highly porous scaffold structure, prompting rapid flow of medium throughout the scaffold. This enhances the mass transport of oxygen and nutrients, which is critical for the viability of the embedded cells. This feature also enables the 3D scaffold to be seeded with cells effectively. When Human Umbilical Vein Endothelial cells (HUVEC) suspended in medium are pipetted on the surface of the scaffold, they permeate through the scaffold’s pores, coating the surfaces of the gelMA particles with the adherent cells (Fig. 1a). Additionally, the pores between the gelMA microparticles enable the embedding of larger structures like spheroids. We demonstrated that EndoC-BH1 spheroids can be mixed with the granular bioink and subsequently photocrosslinked, allowing them to be held in place by the crosslinked gelMA microparticles (Fig. 1b). Furthermore, the elastic modulus of the printed scaffold can be altered by varying parameters such as the degree of functionalization (DOF) of the hydrogel, hydrogel concentration, and concentration of photoinitiator. This tunability of the elastic modulus allows the precise control of the biomechanical properties of the scaffold. Our study showed that the formation of HUVEC endothelial sprouts can be influenced by altering the elastic modulus of the printed scaffolds.

Conclusion
We demonstrated the development of a granular hydrogel bioink which can be pipetted and bioprinted on a conventional 3D bioprinter. The resulting scaffold structure is highly porous, which increases the surface area to volume ratio for the diffusion of oxygen and nutrients into the structure, to improve the viability of the embedded cells. Furthermore, we demonstrated that the porous nature of the hydrogel scaffold facilitates the spreading of adherent cells on the hydrogel microparticle surfaces, and the embedding of spheroids between the hydrogel microparticles. We believe that the granular hydrogel bioink has the ability to improve the biomanufacturing of artificial tissues for regenerative medicine.
Development of an Injectable Hydrogel for Controlled Release of Menthol in the Treatment of Obesity and Metabolic Disorders

Ms. Nu Thu Uyen PHAM\(^1\), Ms Ting RUAN\(^2\), Mr Chih-Yu FU\(^1\), Mr Chih-Hung LIN\(^{1,2}\), Mr Kun-Chi CHOU\(^{1,2}\), Ms Yu-Jung LIN\(^1\)

\(^1\)Research Center for Applied Sciences, Academia Sinica, Taipei, Taiwan, \(^2\)School of Medicine, College of Medicine, Fu Jen Catholic University, New Taipei, Taiwan

Authors: Uyen Nu Thu Pham(a), Ting Ruan(b), Chih-Yu Fu(a), Chih-Hung Lin(a,b), Kun-Chi Chou(a,b), Yu-Jung Lin(a),*

Affiliations: (a)Research Center for Applied Sciences, Academia Sinica, Taipei, Taiwan. (b)School of Medicine, College of Medicine, Fu Jen Catholic University, New Taipei City, Taiwan. (*Correspondence)

Category: Design and Application of Biomaterials

Background: Obesity has become one of the most serious public health issues, causing significant social and economic losses due to its strong association with numerous metabolic diseases. Many research demonstrated that menthol can stimulate the browning of white adipocytes in vivo through the activation of the transient receptor potential melastatin subtype 8 (TRPM8) on white adipocytes, providing an ideal concept for the treatment of obesity and its associated metabolic disorders.

Methods: We developed an injectable hydrogel that comprises carboxymethyl chitosan and aldehyde-functionalized alginate that are crosslinked through dynamic Schiff-base linkages for delivering menthol-cyclodextrin inclusion complexes (IC) with sustain releasing effect. Moreover, to facilitate the dissolution of the as-developed hydrogel after releasing its payload, amino acid-loaded liposomes are designed and covalently grafted onto networks of the hydrogel. The effects of IC@Hydrogel on treating obesity were evaluated in vitro and in vivo.

Results: The IC@Hydrogel can sustainably release menthol and completely dissolve after releasing its payload. The released menthol induced the transformation of the white adipocytes in white adipose tissues into thermogenic beige adipocytes both in vitro and in vivo. Furthermore, in vivo data showed that the IC@Hydrogel triggered fat consumption and improved systemic metabolic functions, thus alleviating high-fat diet-induced obesity and its associated metabolic disorders.

Discussion and Conclusion: The developed IC@Hydrogel can induce adipocyte browning without causing any side effects. The concept of this study may serve as a promising therapeutic for treating obesity and its related metabolic disorders.
Development of Redox-Responsive Micelles for Modulating Tumor Microenvironment and Enhancing Cancer Immunotherapy

Ms. Thi-Lan-Huong NGO¹, Mr. Kuan-Lin WANG¹,², Ms. Ting RUAN², Ms. Yu-Jung LIN¹

¹Research Center for Applied Sciences, Academia Sinica, Taiwan, ²Graduate Institute of Biomedical and Pharmaceutical Science, School of Medicine, Fu Jen Catholic University, Taiwan

Authors: Thi-Lan-Huong Ngo, Kuan-Lin Wang, Ting Ruan, and Yu-Jung Lin

Affiliations: ¹Research Center for Applied Sciences, Academia Sinica, Taiwan. ²Graduate Institute of Biomedical and Pharmaceutical Science, School of Medicine, Fu Jen Catholic University, Taiwan (*Correspondence)

Category: Design and Application of Biomaterials

Background: The use of immune checkpoint inhibitors (ICIs) that block the interaction between programmed death ligand 1 (PD-L1) and programmed cell death protein 1 (PD-1) holds considerable promise for cancer treatment. However, the efficacy of PD-L1/PD-1 blockade is better in tumors with higher PD-L1 expression and tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment (TME). Nonetheless, a large proportion of tumors are unlikely to respond to the immunotherapeutic effects of PD-L1/PD-1 blockade due to their immunosuppressive TME, also known as cold tumors. Therefore, it is imperative to develop strategies to transform the TME from immunosuppressive to immunostimulatory (hot tumors). Capsaicin, a pungent ingredient in chili peppers, reportedly has numerous effects on the regulation of the TME. Nevertheless, the precise delivery of capsaicin to the TME remains challenging.

Methods: In this study, a redox-responsive micelle was proposed to carry capsaicin and a PD-L1/PD-1 inhibitor to modulate the TME. The effectiveness of the micelles in modulating TME and inhibiting tumor growth was assessed via both in vitro and in vivo studies.

Results: The as-proposed micelle was successfully synthesized through a process of self-assembly. The micelles showed good redox-responsiveness, as confirmed by the results of size distribution, cryo-TEM images, and drug release profiles before and after exposure to GSH. Moreover, flow cytometry analysis and immunofluorescence staining images displayed that the micelles can increase PD-L1 expression in tumor cells by release of capsaicin. Additionally, the micelles transformed the TME to immunostimulatory and inhibited the tumor growth in a tumor-bearing mouse model.

Discussion and Conclusion: By enabling the precise delivery of capsaicin and a PD-L1/PD-1 inhibitor to the tumor site, the newly developed redox-responsive micelle holds significant promise for modulating TME and enhancing cancer immunotherapy.

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ROS-responsive nanoceria composite carriers loaded with PTH1-34 for healing of osteoporotic bone defects

Ms. Baoyu TAN

1The University Of Hong Kong, , Hong Kong SAR

Background: With the aging of the population, the number of elderly patients with osteoporotic fracture is increasing. Elderly patients with osteoporosis have difficulties in fracture healing due to poor bone quality and low osteogenic ability. At present, osteoporotic bone defects still face great challenges. Reactive oxygen species (ROS) play a crucial role in tissue repair, including bone. The site of osteoporotic bone defects is associated with abnormal levels of reactive oxygen species (ROS). Therefore, regulation of ROS concentration is essential to promote bone regeneration.

Objective: We firstly prepared nanoceria that could decompose H2O2 to regulate the intracellular ROS concentration efficiently; Then, we used different polymers to make polymer coated nanoceria to improve its enhance their biomimetic enzyme activity by reducing interparticle agglomeration without affecting their ROS scavenging efficiency, and improving their biocompatibility; Furthermore, using polymer coated nanoceria for the delivery of PTH1-34 to realize controlled and sustainable release to facilitate bone density, and strengthening bone microarchitecture.

Research methods and contents:
1. Cerium oxide nanoparticles were prepared, their physicochemical properties were analyzed by TEM, XPS and DLS. The superoxide dismutase-like catalytic activity was investigated by a colorimetric assay using UV–vis spectroscopy and the peroxidase-like catalytic activity of bare and coated nanoceria was investigated using the particles as heterogeneous nanocatalysts in the oxidation of the 3,3′,5,5′-tetramethylbenzidine (TMB) by hydrogen peroxide.
2. Prepare dopamine coated nanoceria to improve the stability and dispersion ability of cerium oxide nanoparticles, and at the same time by optimizing the coating methods to maintain its ROS scavenging ability.
3. Dopamine-coated cerium oxide nanoparticles was used to adsorb PTH1-34, and its packing capacity and drug release manner were studied.
4. The ROS responsive nano-cerium oxide composite scaffold coated with PTH1-34 could promote MC3T3-E1 cell differentiate into osteoblast in vitro by ALP activity, alizarin red staining and RT-PCR analysis.
5. The osteoporotic bone defect model was constructed, and the ROS responsive nano-cerium oxide composite scaffold containing PTH1-34 system was filled with the defect, new bone and microstructure were observed by micro-CT; osteoblast activity and bone growth were observed by histological staining, and the mechanism of bone regeneration was observed by RT-PCR, including the expression of osteogenesis related genes.

Discussion and Conclusion:
Dopamine-coated cerium oxide nanoparticles have good decomposition ability of H2O2 and can keep more stable than pure cerium oxide nanoparticles. At the same time, in vitro experiments showed that nanoparticles containing PTH1-34 could gather around cells and release drugs to directly promote cell differentiation to osteogenesis. In vivo experiments further showed that ROS responsive nano-cerium oxide composite scaffold system can significantly improve bone density and enhance bone microstructure. This composite system suggests that by regulating ROS level and collaborating with bone regeneration related bio factors is an important strategy for the treatment of osteoporosis and bone defect related diseases.
AVIFJ For Printing Single Cell Containing Droplets And Examining The Effect Of Nozzle Size On Single Cell Printing Efficiency.

Mr. Austin Smith¹, Dr. Feng Lin¹, Dr. Song Yu¹
¹ Department of Mechanical Engineering Tsinghua University, Beijing, China

Background: Single cell printing has made significant advances with the improvement of cell printing technology. The importance of single cell printing to tissue engineering lies in its ability to precisely place and pattern cells as they might be in vivo. Therefore, for the development of more biomimetic tissues it is important to integrate the ability to precisely pattern single cells in large tissue constructs. Liu et al. demonstrated the potential of Alternating Viscous and Inertial Force Jetting (AVIFJ) to be integrated with extrusion printing to develop heterogeneous tissue models [1]. Liu et al. briefly examined the capacity of AVIFJ for single cell printing. This study seeks to further expand on the work done by Liu et al. by further examining the effects of nozzle size on the single cell printing efficiency.

Methods: 400 droplets in 5X5 or 5X10 matrixes were printed to provide a large sample size. The process was replicated 3 times for each nozzle size at each concentration. Printing was performed using culture media as the cell suspending material and nozzle sizes were selected at 40 µm, 50 µm, 70 µm, and 100 µm. Cell concentrations used were 50,000 cells/ml, 285,000 cells/ml, and 1X10⁶ cells/ml. Cells were imaged using brightfield microscopy and counted manually.

Results: AVIFJ was successfully able to print droplets at each cell concentration with each size nozzle. The single cell efficiency varied significantly depending on the nozzle size and cell densities. Cell densities around 3X10⁵ with smaller nozzle sizes showed a higher likelihood of a single cell in a single droplet the highest single cell efficiency achieved was around 33%. Figure 1 shows the cell matrix after printing and a bright field image of the cells in the droplets.

Discussion and Conclusions: AVIFJ was demonstrated to be able to print single cell containing droplets. It was found that the likelihood of single cell containing droplets increases at smaller nozzle sizes and cell densities around 285, 000. It was also found that at relatively low seeding densities around 285,000 cells/ml and a nozzle size of 40 µm cells it was possible to have 33% of the droplets contain single cells.

Assessing the Potential of Human Umbilical Cord Mesenchymal Stem Cell-Derived Small Extracellular Vesicles (UC-MSC-sEV) in Preventing Scarring

Ms. Li Ting Kee¹, Assoc. Prof. Dr. Min Hwei NG¹, Assoc. Prof. Dr. Jhi Biau FOO²,³, Dr. Chee Wun HOW⁴, Dr. Jia Xian LAW¹

¹Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000, Cheras, Malaysia, ²School of Pharmacy, Faculty of Health and Medical Sciences, Taylor’s University, 47500, Subang Jaya, Malaysia, ³Centre for Drug Discovery and Molecular Pharmacology (CDDMP), Faculty of Health and Medical Sciences, Taylor’s University, 47500, Subang Jaya, Malaysia, ⁴School of Pharmacy, Monash University Malaysia, Bandar Sunway 47500, Subang Jaya, Malaysia

Introduction: The cosmetic industry has witnessed a groundbreaking advancement through the integration of cosmetics with stem cell technology, resulting in the emergence of functional cosmetics. Recent research has demonstrated that umbilical cord-derived mesenchymal stem cell (UC-MSC) conditioned medium contains growth factors and exosomes that aid in wound healing and re-epithelialization. However, the application of UC-MSC-derived extracellular vesicles (UC-MSC-sEV) on skin for the purpose of scar prevention remains unexplored. This study aims to evaluate the effects of UC-MSC-sEV on the proliferation, migration, and extracellular matrix (ECM) synthesis in human dermal fibroblasts.

Methods: Small extracellular vesicles (sEV) were isolated from UC-MSCs and assessed for their impact on the proliferation and migration of human dermal fibroblasts (HDFs). Additionally, gene and protein expression analysis were conducted on HDFs co-cultured with varying concentrations of UC-MSC-sEV to evaluate their role in modulating ECM protein synthesis. The penetration of UC-MSC-sEV into human skin was examined using fluorescent microscopy.

Results: The results indicated that increasing concentrations of UC-MSC-sEV effectively enhanced HDF proliferation, while no significant variation was observed in the migration rate among different treatment groups. Furthermore, the presence of UC-MSC-sEV modulated ECM expression in HDFs, and the utilization of fluorescent labeled UC-MSC-sEV confirmed their penetration into the epidermis after 18 hours.

Discussion and Conclusion: The findings demonstrate that UC-MSC-sEV can promote HDF proliferation and modulate skin ECM synthesis, thereby playing a vital role in scar reduction. These promising results suggest the potential application of UC-MSC-sEV in cosmetic formulations within the cosmeceutical industry, potentially amplifying their effectiveness in preventing skin scarring.
High-Resolution Image Detection Using Bacteriorhodopsin and Micro-biomanufacturing Techniques

Mr. Mian Wu, Yu Song, Feng Lin
1Biomanufacturing and Rapid Forming Technology Key Laboratory of Beijing, Beijing, China, 2Department of Mechanical Engineering, Tsinghua University, Beijing, China, 3Key Laboratory of Advanced Materials Processing Technology of Ministry of Education, Beijing, China

Title: High-Resolution Image Detection Using Bacteriorhodopsin and Micro-biomanufacturing Techniques

Authors: Mian Wu, Yu Song, Feng Lin

Affiliations: Biomanufacturing Center, Department of Mechanical Engineering, Tsinghua University, Beijing 100084, China

Category: Design and Application of Biomaterials

Background: Traditional image sensors are usually based on toxic or non-biodegradable materials. Bacteriorhodopsin offers a biocompatible and environmentally friendly alternative. However, designing and fabricating bacteriorhodopsin-based sensors presents several challenges. In this study, we developed a micro-biomanufacturing approach for fabricating a high-resolution bacteriorhodopsin-based image sensor.

Methods: Bacteriorhodopsin was immobilized on the surface of anodic aluminum oxide (AAO) nanochannels. Micro-biomanufacturing techniques were then used to fabricate a supporting part containing individual units. The AAO nanochannel covered with bacteriorhodopsin was assembled with the supporting part to form a biomaterial-based image sensor. The photoelectric response of the image sensor was then measured.

Results: The bacteriorhodopsin-based image sensor exhibited photoelectric response under illumination. The light-detecting units with sensing area around 0.0025mm2 showed photocurrents up to 1nA, and no significant interfere was observed between adjacent light-detecting units. The image sensor containing 10,000 individual light-detecting units provide a resolution up to 100*100.

Discussion and Conclusion: This study demonstrates the potential of bacteriorhodopsin as a biocompatible and environmentally friendly alternative for fabricating image sensors. Using micro-biomanufacturing techniques and AAO nanochannels, high-resolution image sensors were assembled with promising photoelectric response and resolution. Overall, this work presents a novel approach for applications of biomaterial in image sensing and energy conversion fields.
ROS-responsive Mesalazine based mucoadhesive prodrug for IBD treatment

Mr. Tae Kyun KIM¹, Mr. Dong Yun LEE¹,²

¹Hanyang University, Seoul, ²Elixir Pharmatech, Seoul,

Background: Inflammatory bowel disease (IBD) is associated with excessive reactive oxygen species (ROS) leading to oxidative stress and inflammatory signaling pathway. Mesalazine is the most prescribed medication for IBD, and it functions as an inhibitor of COX-1 and COX-2. This inhibition leads to the activation of peroxisome proliferator-activated receptor gamma (PPARγ). However, without delivery system, the majority of Mesalazine is absorbed in the stomach and small intestine, resulting in only 20% of the administered dose reaching the colon.

Methods: In this work, we developed a mucoadhesive ROS responsive prodrug for the treatment of IBD. Mesalazine (M) was conjugated to Glycol chitosan (GC) by ROS responsive linker thioketal (TK), and we tested its anti-inflammatory effect in vitro and in vivo.

Results: The GC-TK-M prodrug is designed to release conjugated mesalazine in response to the presence of a ROS environment. We demonstrated that the GC-TK-Me prodrug can alleviate inflammation with scavenging both NO and ROS from LPS stimulated RAW 264.7 macrophages, which are known to produce excessive ROS. Mucoadhesive properties of glycol chitosan was explored by FITC labeling in vivo. The orally administered GC-TK-M prodrug recovered the body weight and colon length in 2.5% DSS-induced model. Moreover, GC-TK-M prodrug group diminished colonic-pro-inflammatory cytokines in mRNA and protein levels compared to DSS-induced group.

Discussion and Conclusion: The current work demonstrated a controlled and prolonged drug delivery system to inflammation site with mucoadhesive glycol chitosan and ROS sensitive thioketal linker.

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The Role of Nonsteroidal Anti-inflammatory Drugs as Inflammatory Cytokine Regulator to Cartilage Regeneration

Ms. Junghwa Choi¹, Mr Seul-Ki Kim¹, Ms Hyang Kim¹

¹Institute of New Horizon Regenerative medicine, Gyeonggido, Korea

• Background: Osteoarthritis (OA) is a common chronic inflammatory disease leading to pain and functional disability and classify development stage according to synovial tissue inflammation. An imbalance of inflammatory cytokines in the synovial membrane causes degeneration of adjacent tissues. An example such as the degeneration of the cartilage of the knee joint is representative. In these degenerative diseases, it is common to prescribe Nonsteroidal Anti-inflammatory Drug (NSAID) to control the inflammatory response and relieve pain caused by inflammation. In this study, we investigated the anti-inflammatory and anabolic effect of various NSAIDs on synoviocyte and chondrocyte derived from the same patient during inflammatory conditions induced by pro-inflammatory factors such as interleukin-1β (IL-1β) and tumor necrosis factor- α (TNF- α).

• Methods : We performed treatment of various NSAIDs(Acetaminophen, Desibuprofen, Talniflumate and Pelubiprofen) in inflamed synoviocytes and chondrocytes artificially induced by pro-inflammatory cytokines(1 ng/mL IL-1β and 5 ng/mL TGF- α). In this study, cell proliferation was evaluated using a water-soluble tetrazolium salt (WST) assay, and mRNA expression levels of pro- and anti-inflammatory cytokines, catabolic, anabolic, and intermediate factors were measured by semi-quantitative real-time PCR.

• Results : Cell proliferation results demonstrated that, NSAIDs except Pelubiprofen inhibited proliferation in human synoviocytes under IL-1β-induced inflammatory conditions than under TNF- α induced inflammatory conditions after 48h and 72h of treatment. We preformed qRT-PCR to confirm the expression levels of pro-inflammatory cytokines such as IL-1 β, TNF- α, and COX-2 in human inflamed synoviocytes treated with these 4 types of NSAIDs at 48h. As a result of confirming the mRNA expression levels of pro-inflammatory factors such IL-1β, TNF-α, and COX-2 in human synoviocytes under IL-1 β -induced inflammatory conditions, when treated Dexibuprofen in inflamed synoviocytes, IL-1β and COX-2 mRNA expression levels decreased by 29.135- and 121.812-fold, respectively. Otherwhiles TNF- α mRNA expression level increased by 0.845-fold. Also, when treated with Pelubiprofen, IL-1 β, TNF- α, and COX-2 mRNA expression levels decreased by 48.432-, 2.036-, and 96.371- fold, respectively.

• Discussion and Conclusion : : In this study, we investigated the anti-inflammatory effects of various NSAIDs on human synoviocytes under pro-inflammatory cytokine-induced inflammatory conditions. Through this study, it was confirmed that NSAIDs treatment increased cell proliferation and pro-inflammatory cytokines in inflamed synoviocytes, and the effect on tissue regeneration in chondrocytes is being tested. We predict that NSAIDs not only affect OA progression by regulating the balance of inflammatory cytokines, but also affect tissue regeneration by chondrocytes. The results of this study can be used in the joint-on-a-chip development study using musculoskeletal cells derived from the same patient.


• Acknowledgement : This study was approved by the Institutional Ethical Review Board of Myongji Hospital (MJH 2020-09-025, MJH 2020-11-012). This work was supported by the New Horizon grant of Myong Ji Hospital(2303-03-01) and was partly supported by the Korea Basic Science Institute(KBSI) grant funded by the Korean government(MSIT)(DC202304).
Dissecting the role of the tissue microenvironment in ovarian ageing and infertility

*Dr. Anna Jaeschke¹, Dr Matt S HEPBURN²,³, Dr Alireza MOWLA²,³, Prof Brendan F KENNEDY²,³,⁴, Prof Chii J CHAN¹,⁵*

¹Mechanobiology Institute, National University of Singapore, Singapore, Singapore, ²BRITElab, Harry Perkins Institute of Medical Research, QEII Medical Centre, Nedlands and Centre for Medical Research, The University of Western Australia, Perth, Australia, ³Department of Electrical, Electronic & Computer Engineering, School of Engineering, The University of Western Australia, Perth, Australia, ⁴Institute of Physics, Nicolaus Copernicus University, Torun, Poland, ⁵Department of Biological Sciences, National University of Singapore, Singapore, Singapore

Introduction: Recent studies have emphasised the role of the ovarian microenvironment for normal tissue homeostasis and follicle development, including ovulation. Upon ageing, an altered molecular and cellular tissue composition contributes to declined fertility [1]. Fibrosis, mediated through increased collagen and decreased hyaluronan (HA) content, is a hallmark of ovarian ageing, contributing to tissue stiffening and the development of an inflammatory microenvironment [2]. High levels of pro-inflammatory cytokines and changes in macrophage ontogeny and polarisation in aged tissue suggest a crucial role for macrophages in age-associated inflammation in the ovary [3]. Our research investigates the roles of matrix stiffness (biophysical signalling cues) and composition (biochemical signalling cues) for macrophage function during ovarian ageing (Fig. 1 A). We hypothesise that macrophages of different ontogeny vary in their response to the changes in matrix stiffness and composition upon ovarian ageing and that the aged tissue provides a restrictive niche for ovarian tissue-resident macrophages (TRMs).

Methods: We used a photocrosslinkable hydrogel composed of methacrylated gelatin (mimicking the collagen content) and HA to create a bioengineered microenvironment with tuneable composition and stiffness mimicking the stiffness and composition of young and aged ovarian tissue in vitro. Altering the light exposure time for hydrogels of different gelatin:HA ratios provided matrices of various biochemical and biophysical cues for macrophage culture (Fig. 1 B).

Results: Primary macrophages were successfully isolated from mouse ovarian tissue and bone marrow, and the expression of pan-macrophage marker F4/80 was confirmed post-isolation and upon culture in the gelatin:HA hydrogels (Fig. 1C). In a preliminary study comparing the RAW264.7 cell line and TRMs we observed that culture in an HA-rich stiff matrix (~10 kPa) resulted in decreased cell proliferation (Fig. 1D).

Discussion & Conclusions: We have successfully isolated and cultured tissue-specific ovarian and monocyte-derived macrophages. Preliminary results indicate that macrophage proliferation is affected by matrix stiffness. We are currently investigating the cellular responses of ovarian and monocyte-derived macrophages to matrix properties with a focus on cell survival, migration, polarisation and expression of genes related to cell-ECM interactions. Dissecting how matrix characteristics affect macrophages of different ontology will provide novel insights into cell-matrix interactions during ovarian ageing, possibly paving the way to identify novel biomarkers and targets for alternative therapeutic interventions to control ovarian fibrosis.

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Peptide-Glycyrrhizin Conjugate as Dual Actions Agent for Obesity Therapeutic Application

Ms. Priscilla Lia¹, Mr. Jaehong Min¹, Dr. Dong Yun Lee¹,²,³
¹Hanyang University, Seoul, South Korea, ²Institute of Nano Science and Technology & Institute for Bioengineering and Biopharmaceutical Research, Seoul, South Korea, ³Elixir Pharmatech Inc, Seoul, South Korea

Introduction:
Long thought to be metabolic disorders, obesity and its comorbidities have recently been linked to low-grade chronic inflammation caused by metabolic dysregulation and are becoming recognized as inflammatory diseases [1]. However, current commercially available anti-obesity medications have only focused on how to treat obesity, ignoring the main culprit, inflammation. Glycyrrhizin (GL), the most active chemical compound of licorice, has been used for its anti-inflammatory effect since ancient times. Further, recent studies demonstrate GL potential anti-obesity effect. Nevertheless, GL high liver targetability hampers its obesity therapeutic application because of the off-targeting concern. Therefore, we conjugated a white adipose tissue-targeting peptide into the carboxyl groups of the GL with the help of heterobifunctional polyethylene glycol linker (thiol-PEG-amine), anticipating the dual actions of peptide-GL conjugate (PGC).

Methods:
PGC was prepared by EDC-NHS coupling GL with thiol-polyethylene glycol-amine (PEG), which then conjugated with adipose-homing peptide (AHP) through a disulfide bond with the help of Ir(ppy)3 photocatalyst. Moreover, its cytotoxicity and efficacy on the differentiated 3T3-L1 cell line, murine fibroblast, alone or in co-culture with RAW 264.7 cell line, murine monocyte/macrophage-like cells, were investigated in vitro.

Results:
PGC was successfully prepared by EDC-NHS coupling reaction and Ir(ppy)3 photocatalyst. PGC was non-cytotoxic up to 400μM GL equivalent concentration. Differentiated 3T3-L1 cells effectively outperformed undifferentiated preadipocytes in terms of PGC uptake. Also, at optimal treatment condition (pretreatment with 200μM GL equivalent concentration) lipid content of differentiated 3T3-L1 cells was decreased by up to 78% and TNF-α secretion of differentiated 3T3-L1 cells was reduced by up to 50%. Moreover, treatment with the PGC increased the ATP binding cassette transporter A1 expression, which could be successfully blocked by treatment with a liver receptor (LXR) inhibitor.

Discussion and conclusion:
PGC effectively selectively targeted mature adipocytes by targeting the membrane protein, prohibitin. The anti-obesity and anti-inflammatory properties of PGC came from the LXR-ABCA1 pathway in vitro. The current study showed that PGC has strong potential as a dual-action (anti-obesity and anti-inflammatory effects) agent for obesity therapeutic application. In the future, PGC performance in the high-fat diet-induced obesity mouse model will be investigated

References:

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Aurozyme: A Revolutionary Nanozyme in Colitis, Switching Peroxidase-like to Catalase-like Activity

Ms. Lim Gayoung¹, Ms. Lee Sieun¹, Mr. Kim Hyung Shik¹, Mr. Lee Dong Yun¹,²
¹Hanyang University, Seoul, South Korea, ²Elixir Pharmatech Inc., Seoul, South Korea

1. Introduction
Inflammatory bowel disease (IBD) is a refractory disease instigated by several factors such as disrupted intestinal barrier functions, elevated levels of reactive oxygen/nitrogen species (ROS/RNS), and high-mobility group box 1 (HMGB1) because these hazard signals dysregulate mucosal immune responses, which triggers the severity of colitis.

2. Subjects and Methods
Aurozyme, a novel nanomedicine composed of gold nanoparticles (AuNPs) and glycyrrhizin (GL) with a glycol chitosan coating layer, represents a promising therapeutic approach for colitis of multiple etiologies.

3. Results
It effectively scavenges reactive oxygen/reactive nitrogen species (ROS/RNS) and damage-associated molecular patterns (DAMPs), neutralizing hazardous signals involved in colitis. Aurozyme's unique ability to switch the harmful peroxidase-like activity of AuNPs to beneficial catalase-like activity enables it to promote sustained anti-inflammatory effects, restore intestinal function, and increase the abundance and diversity of beneficial probiotics essential for gut microbial homeostasis.

4. Discussion and Conclusion
To our knowledge, this is the first biocompatible instance to switch peroxidase-like activities to catalase-like activities using AuNPs. Collectively, our findings suggested that mucoadhesive gold nanoparticle could be used as a potential therapeutic for IBD treatment.

5. Acknowledgement
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Protein X Knockout on Tendon-derived Stem Cell Functions and Histopathology of Tendon

Ms. Angel Yuk Wa Lee¹, Dr. Chi Ming Wong², **Prof. Jerry Jiankun Xu¹**, Prof. Patrick Shu Hang Yung¹,³, Dr. Po Yee Pauline Lui¹,³

¹Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, , Hong Kong SAR, ²Department of Health Technology and Bioinformatics, The Hong Kong Polytechnic University, , Hong Kong SAR, ³Center for Neuromusculoskeletal Restorative Medicine Ltd., Hong Kong Science Park, , Hong Kong SAR

Title: Protein X Knockout on Tendon-derived Stem Cell Functions and Histopathology of Tendon

Authors: Angel Yuk Wa Lee¹, Chi Ming Wong², Jerry Jiankun Xu¹, Patrick Shu Hang Yung¹,³, Pauline Po Yee Lui¹,³*

Affiliations: 1 Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China. 2 Department of Health Technology and Bioinformatics, The Hong Kong Polytechnic University, Hong Kong SAR, China. 3 Center for Neuromusculoskeletal Restorative Medicine Ltd., Hong Kong Science Park, Kong SAR, China. (*Correspondence)

Category: Tissue engineering and regeneration

Introduction: Chronic tendinopathy is a prevalent and disabling musculoskeletal problem affecting both athletes and sedentary individuals. Its underlying pathogenesis is poorly understood, and treatment is usually symptomatic. Recent studies have suggested the roles of inflammation and excessive apoptosis in the pathogenesis of tendinopathy. Inflammation has been reported to compromise tenogenic differentiation and upregulate chondro-osteogenic differentiation potential of tendon-derived stem cells (TDSCs). Obesity-induced local inflammation is an important risk factor associated with the development of tendinopathy. However, the pathological changes and the underlying mechanisms of obesity-associated tendinopathy remain unclear. Protein X is an anti-inflammatory and anti-apoptotic factor. Its expression has been shown to increase in clinical samples of tendinopathy in our unpublished results. It has been shown to promote the proliferation and tenogenic differentiation of TDSCs. Except this study, there has been no other study investigating the effect of Protein X deficiency on the pathological changes in tendon and functions of TDSCs. This study therefore aimed to investigate the effect of Protein X knockout on the functions of TDSCs and histopathology of tendons of mice fed with high-fat diet (HFD) or standard chow (SC).

Subjects and Methods: Protein X knockout (KO) mice and wild-type (WT) mice were fed with SC (10% kcal fat) or HFD (60 kcal% fat, 20 kcal% protein, and 20 kcal% carbohydrate) ad libitum for 12-16 weeks. Achilles tendons were harvested for histopathological analysis. The expression of Protein X in mouse Achilles tendons under SC and HFD conditions was examined by immunohistochemical staining. The mRNA expression of tenogenic marker, inflammatory cytokines, and matrix-remodelling enzymes in WT-TDSCs and KO-TDSCs under SC and HFD conditions were examined by qRT-PCR.

Results: Our results showed that Protein X KO induced early histopathological changes resembling tendinopathy in tendons of mice, with hypercellularity, rounding of active tendon cells, cell malalignment, presence of chondrocyte-like cells, fat accumulation, and blood vessel ingrowth under both SC and HFD conditions. Protein X was expressed in healthy Achilles tendons. However, its expression was higher in tendons of mice under HFD treatment, especially in the abnormal round cells. KO-TDSCs showed lower expression of tenogenic marker Scx compared to WT-TDSCs under both SC and HFD conditions (all p<0.05). Compared to WT-TDSCs under SC condition, KO-TDSCs also showed lower mRNA expression of anti-inflammatory cytokines Il10 and Il33 under SC condition and lower expression of Il10 under HFD condition (all p<0.05). There was significant lower expression of Mmp3, Timp1 and Mmp3/Timp1 ratio in KO-TDSCs compared to WT-TDSCs under SC condition (all p<0.05).
However, the expression of Mmp3 and Mmp3/Timp1 ratio increased in KO-TDSCs under HFD condition compared to WT-TDSCs under SC condition (all p<0.05).

Discussion and Conclusion: Protein X was expressed in healthy tendons and Protein X KO induced early tendon histopathology under both SC and HFD conditions, supporting its role in maintaining tendon homeostasis. Protein X KO altered the functions of TDSCs, with lower tenogenesis, lower expression of anti-inflammatory cytokines and lower expression of matrix-remodelling enzymes. The expression of Protein X increased in tendons of HFD. Together with the significant increased expression of matrix-degrading enzyme in KO-TDSCs under HFD condition, our data therefore supported the beneficial effect of Protein X in reducing matrix degeneration in tendons of obese individuals. In conclusion, Protein X may be a novel target for the treatment of degenerative tendinopathy.

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Disclosure: Protein X is a code and not the real name of the protein under investigation.
DOCK5 Inhibition Promotes Osteoblast Differentiation and BMP2-Induced Bone Regeneration via Rac1 inhibition and TAK1 Signaling Pathway

Dr. Ju Ang Kim¹, Ms Soomin Lim¹, Dr. Jiwon Lim¹, Dr. Su Jeong Lee¹, Dr. Hye Jung Ihn², Professor Jong-Sup Bae³, Professor Jung-Eun Kim⁴, Prof. Eui Kyun Park¹

¹Department of Pathology and Regenerative Medicine, School of Dentistry, IHBK, Kyungpook National University, Daegu, South Korea, ²Cell and Matrix Research Institute, Kyungpook National University, Daegu, South Korea, ³Research Institute of Pharmaceutical Sciences, College of Pharmacy, Kyungpook National University, Daegu, South Korea, ⁴Department of Molecular Medicine, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University, Daegu, South Korea

Authors: Ju Ang Kim¹, Soomin Lim¹, Jiwon Lim¹, Su Jeong Lee¹, Hye Jung Ihn², Jong-Sup Bae³, Jung-Eun Kim⁴ and Eui Kyun Park¹*

Affiliations: ¹Department of Pathology and Regenerative Medicine, School of Dentistry, IHBK, Kyungpook National University, Daegu 41940, Republic of Korea. ²Cell and Matrix Research Institute, Kyungpook National University, Daegu 41944, Republic of Korea. ³Research Institute of Pharmaceutical Sciences, College of Pharmacy, Kyungpook National University, Daegu 41566, Republic of Korea. ⁴Department of Molecular Medicine, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University, Daegu 41944, Republic of Korea. (* Correspondence)

Category: Tissue Engineering and Regeneration

Background: DOCK5 (Dedicator of cytokinesis 5), a guanine nucleotide exchange factor (GEF) protein, is a member of the DOCK-A subfamily in DOCK family and plays a critical role in intracellular signaling networks. DOCK5 has been shown to regulate osteoclast differentiation by inhibiting the activity of Rac1 and Cdc42, but its role in osteogenesis is unknown.

Methods: To investigate the direct effect of DOCK5 on bone formation, we generated mice lacking DOCK5. The mice exhibited elevated bone mass, enhanced mineral apposition, and increased osteoblast differentiation. We created calvarial defects in both wild type and knockout mice to assess in vivo bone regeneration. Additionally, we examined synergistic effects of BMP2 with DOCK5 inhibition in a calvarial defect model. To evaluate ectopic bone formation, we used C21, a DOCK5 inhibitor, and verified the mechanism of DOCK5 inhibition using C21 in vitro.

Results: Bone mass was significantly increased in DOCK5 knockout mice, as confirmed by micro-CT and histomorphometric analysis. Using mouse BMSCs, it was found that DOCK5 KO promoted osteoblast differentiation, indicating that DOCK5 inhibition may enhance osteoblast differentiation independently of its function in osteoclasts. Further experiments using DOCK5 KO mice in calvarial bone defect model and ectopic bone formation models revealed a significant induction of bone regeneration compared to WT mice. Additionally, inhibiting DOCK5 with C21 in WT mice significantly increased BMP2-induced ectopic bone formation. The mechanism of DOCK5 inhibition was investigated using the mouse osteoblast cell line MC3T3-E1 and human bone marrow mesenchymal stem cells (BMSCs), and osteoblast differentiation was significantly increased by C21 during BMP2-induced differentiation as assessed by ALP, Alizarin-red staining, and RT-PCR analysis. Furthermore, phosphorylation assays revealed that DOCK5 inhibition significantly increased the activation of MKK3/6 and p38 under TAK1. Additionally, inhibiting DOCK5 significantly reduced the activity of Rac1 induced by BMP2.

Discussion and Conclusion: In summary, our findings suggest that inhibiting DOCK5 promotes osteoblast differentiation, particularly by suppressing Rac1 activity during BMP2-induced osteoblast differentiation, and activating TAK1, MKK3/6, and p38 pathways. DOCK5 inhibition has been reported...
to inhibit osteoclast differentiation, and our study has now uncovered its potential to promote osteoblast differentiation, and highlighting its potential as a target for efficient bone regeneration.

Acknowledgement: This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (2017R1A5A2015391 and 2021R1C1C2093188) and Basic Science Research Program funded by the Ministry of Education (RS-2023-00241045).
Microparticle-containing Cell Spheroid System as a Tool for Optimal Concentration of TGF-β1 in Chondrogenic Differentiation

Ms. Minji Kim

Introduction: Osteoarthritis (OA) is a degenerative disease that causes a significant deterioration in patients’ quality of life. In recent years, cell therapy has been raised as a promising regenerative therapy for OA and cell spheroid system has been frequently adopted. However, conventional cell spheroids, which are densely aggregated cells, have limited supply of growth factor (GF) into the central region for chondrogenic differentiation of cells. Therefore, the appropriate GF concentrations to induce differentiation into chondrogenic cells are controversial.

Methods: In this study, we developed cell spheroids with microparticles with leaf-stacked structure (LSS; cell/LSS spheroid) using an agarose concave well. The morphology of the cell/LSS spheroid was observed by optical microscope and H&E staining. Through the structural characteristics of the cell/LSS spheroid, infiltration behavior of cell culture medium and viability of the cell/LSS spheroid was estimated. Then, chondrogenic differentiation of the cell/LSS spheroid according to the concentration of TGF-β1 was analyzed.

Results: The LSS particles are evenly distributed among cells and provide space for supply of cell culture medium that prevent apoptosis of the cell/LSS spheroid. Also, the GF was penetrated into central region of cell/LSS spheroid, and thus effectively induce chondrogenic differentiation even at significantly lower GF concentration than the conventionally used.

Discussion and conclusion: Based on the findings, we suggest that the cell/LSS spheroid can provide an appropriate environment to supply GF uniformly to the cells throughout the spheroid, and thus can be a platform system for determining the optimal GF concentration for cell differentiation into specific cells.

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Multi-functional membrane for effective tendon regeneration by supply of growth factor and tissue adhesion prevention

Mr. Ho Yong Kim

1Dankook University, , South Korea

Introduction: Flexor tendon ruptures are often occurring in sports, exercising, or daily activity with high mechanical loads. However, it is difficult to fully restore the function of a damaged tendon because of insufficient regeneration of tendon and inevitably occurred tissue adhesion with surrounding tissue during the healing process. To overcome these problems, various techniques including delivery of bioactive molecules (e.g., growth factors, and drugs), cell therapy, surgical barrier, and physical rehabilitation training have been adopted in clinical practices. However, there are still few tendon repair strategy that satisfy clinicians and patients.

Methods: Multi-functional leaf-stacked structure membrane (MLSS) was fabricated by a heating-cooling procedure with tetruglycol in contact with one side of a dense PCL film and coating of alginate on the other side of a film (opposite side of the solvent treatment). The morphology, platelet-derived growth factor-BB (PDGF-BB) release behavior, tenogenic differentiation (on bioactive layer; PDGF-BB-immobilized LSS layer), cell adhesiveness (on anti-adhesion layer; alginate layer) and animal study (using rabbit flexor tendon defect model) were investigated.

Results: It was observed that the leaf-stacked structure layer and alginate layer were stably formed on both side of the membrane. From in vitro and in vivo experiments, it was observed that the alginate layer effectively prevents cells/tissue adhesion and LSS layer allows enhanced tenogenic differentiation and tendon regeneration.

Discussion and Conclusion: On the basis of our findings, we suggest that the multi-functional MLSS (supply of growth factor for tendon regeneration & prevention of tissue adhesion) can be an alternative for sufficient tendon regeneration.

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Development of a kinetic model for individual cell potency in culture of human mesenchymal stem cells

Ms. Aya IMAI¹, Dr. Kei SASAKI¹,², Prof. Masahiro KINO-OKA¹,³
¹Graduate School of Engineering, Osaka University, Suita, Japan, ²Global Center for Medical Engineering and Informatics, Osaka University, Suita, Japan, ³Research Base for Cell Manufacturability, Techno Arena, Graduate School of Engineering, Osaka University, Suita, Japan

Title: Development of a kinetic model for individual cell potency in culture of human mesenchymal stem cells

Authors: Aya Imai¹, Kei Sasaki¹,², Masahiro Kino-oka¹,³
Affiliations: ¹Graduate School of Engineering, Osaka University. ²Global Center for Medical Engineering and Informatics, Osaka University. ³Research Base for Cell Manufacturability, Techno Arena, Graduate School of Engineering, Osaka University

Category: Enabling technologies, SYIS

Introduction: In recent years, with the development of regenerative medicine, there is a need to establish a stable large-scale culture technique for human mesenchymal stem cells. However, there are disorders of cell potency in cell culture[1]. It is necessary to elucidate the causes of disorder of cell potency to achieve stable stem cell culture. In this study, we focused on cell potency, and developed a kinetic model that represents histone methylation, which affects the cell potency[2]. This model represents the disorder of histone methylation caused by the cells during culture.

Methods: We have developed a kinetic model in which each cell moves autonomously according to rules[3]. In this experiment, the area of the culture vessel was set to be a 1 cm², the seeding density was 3.0×10³ cells/cm², and 100 calculations were performed under homogeneous seeding. The initial values of the cell positions are all defined to be the same.

Results and Discussion: In this study, we hypothesized a kinetic model in which the degree of histone methylation accumulates due to mechanical stimulus applied to the cells. The results of the 100 culture calculations with the same initial values and homogeneous seeding showed that the cell density and methylation level varied among the culture vessels. Compared to cell density, the variation in methylation level was larger. This indicates that the behavior of the individual cells and the influence of surrounding cells on cell potency are significantly different even when homogeneous seeding with the same initial cell position is performed. We also focused on the cultures with the highest and lowest maximum methylation levels, and showed how the methylation levels of all cells in those vessels changed over time. The results suggest fluctuations in methylation levels within the culture vessels.

Conclusion: In conclusion, this study has developed a kinetic model to express fluctuation of histone methylation which affect cell potency that may lead to variation in cell potency.


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Application of antibody-conjugated small intestine submucosa to capture urine-derived stem cells for bladder repair in a rabbit model

Ms. Yuting Song¹, Ms. Yan Qing Li¹, Mr. Mao Xuan Tian¹, Prof. Hui Qi Xie¹
¹West China Hospital, Sichuan University, Chengdu, China

Introduction: The need for bladder reconstruction and side effects of cystoplasty have spawned the demand for the development of alternative material substitutes. Biomaterials such as submucosa of small intestine (SIS) have been widely used as patches for bladder repair, but the outcomes are not fully satisfactory. To capture stem cells in situ has been considered as a promising strategy to speed up the process of re-cellularization and functionalization.

Subjects and Methods: In this work, we have developed an anti-CD29 antibody-conjugated SIS scaffold (AC-SIS) by Traut’s Reagent and Sulfo-SMCC, which are bladder repair patch, and evaluated the biocompatibility, urine-derived stem cells (USCs) capture capacity and bladder defect repair ability in vitro and in vivo.

Results: In vitro assays demonstrate that AC-SIS scaffold has good mechanical properties and biocompatibility, which could promote adhesion and proliferation of the USCs. Furthermore, AC-SIS scaffold has satisfied antibody graft rate, which exhibited effective capture capacity under static and dynamic conditions models. The in vivo evaluation in rabbit full-thickness bladder defect model showed that the scaffold could promote rapid urothelium, smooth muscle regeneration and vascularization in bladder reconstruction.

Discussion and Conclusion: In this study, based on the principle of antigen-antibody binding, we fabricated a bladder repair patch (AC-SIS), which is capable of specifically capturing autologous USCs in situ, thereby resulting in hence facilitate bladder repair and regeneration in vivo. The current work opened a new revenue for the design and application of bladder reconstruction scaffolds based on capture of particular stem cells for tissue regeneration.

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Cell-adaptable Hierarchical Hydrogel with High Stiffness and Fast Relaxation

Mr. Zhinan Yang¹, Dr. Boguang Yang, Dr. Zhaoli Gao
¹The Chinese University Of Hong Kong, , Hong Kong SAR

Introduction: Hydrogels are ideal synthetic matrices to emulate extracellular matrix (ECM) for the 3D culture of cells. The hydrogels should recapitulate the seemingly contradictory requirements of microscopic dynamics and macroscopic strength of ECM, and achieving these two properties simultaneously is challenging. Natural ECM achieves this balance through hierarchical structures consisting of robust fibrous components (e.g., collagen) and loose fillings (e.g., glycosaminoglycans), inspiring the design and fabrication of our double network (DN) hydrogels as the ECM mimics. Our hydrogel demonstrates high mechanical stiffness while possessing the network adaptability to allow rapid stellate spreading of the encapsulated cells.

Methods: Regenerated silk fibroin (SF) was obtained following a reported protocol (D Rockwood. Nat Proto. 2011; 6:1612-1631). Hyaluronic Acid (HA) was modified with the guest molecule of 4-tert-butylphenylacetic acid (TP) to obtain HA-TP, and acryloyl beta-cyclodextrin (Ac-CD) was used as the host molecule (B Yang. Nat Comm. 2021;3514). The DN hydrogel was fabricated by ultrasound-induced self-assembly of SF to form the first network, followed by the photo-polymerization of HA-TP@Ac-CD (host@guest complexation between CD and TP) to form the second network (Fig. 1a).

Results: Scanning electron microscopy (SEM) was used to observe the microstructure of the DN hydrogel, and irregular interconnected porous structures were observed (Fig. 1b). To characterize the rheological and mechanical properties of DN hydrogel, single network (SN) hydrogels that are solely composed of SF (S-SF) or HA (S-HA) were fabricated as control groups. The S-HA hydrogel exhibited frequency-dependent and weak storage modulus (G’) and loss modulus (G’’). In contrast, S-SF hydrogel and D-SF&HA hydrogel showed frequency-independent and strong G’ and G’’ (Fig. 1c). The toughness and Young’s modulus of the hydrogels also followed the same trend, and D-SF&HA showed toughness as high as 10.7 kPa and Young’s modulus as high as 18.0 kPa due to the presence of SF (Fig. 1d). The D-SF&HA hydrogel could relax half of the stress within 29 seconds, a typical feature of viscoelastic materials with dynamic structures (Fig. 1e). Human mesenchymal stem cells (hMSCs) encapsulated in S-SF hydrogel did not spread due to the high stiffness of SF hydrogel. In contrast, hMSCs in the S-HA hydrogel and D-SF&HA hydrogels had significant spreading because of the highly dynamic network HA network(Fig. 1f, 1g).

Conclusion: The DN hydrogel with the self-assembly of SF as the first network and dynamically crosslinked HA as the second network possesses both macroscopic strength and microscopic dynamics, thereby providing valuable insights into the design of hydrogels to better mimic natural ECM in tissue engineering applications.
Preparation of tumor-associated macrophages (TAMs)-educated macrophages for wound healing

Ms. Rong Yong¹, Professor Chunming Wang
¹University Of Macau, , Macao SAR

Background: Diabetes can lead to chronic wounds which are difficult to heal and are accompanied by persistent inflammation and vascular damage. And during tumor development, tumor-associated macrophages (TAM) produce large amounts of immunosuppressive and proliferative factors. Inspired by this, normal macrophages were educated with TAM conditioned medium (TAM-CM) to generate TAM-educated macrophages (TAME). TAME encapsulates the repair capacity of TAM to repair diabetic wounds.

Methods: We prepared TAME by collecting TAM supernatants. We tested the function of TAME on wound healing and tissue healing by bioinformatic analysis, in vivo and in vitro experiments. To improve safety, we analysed the TAM supernatant. TAME-C was obtained by culturing macrophages using nine recombinant proteins instead of TAM supernatant. And then tested for its effect on wound healing and tissue repair.

Results: Analysis of bioinformatic data indicates that TAME has an anti-inflammatory and healing-promoting phenotype. In vitro studies have shown that TAME can mediate the reduction of inflammation, stimulate angiogenesis and promote the growth of fibroblasts. In vivo studies have shown that TAME promotes diabetic wound healing, resolves wound inflammation and reconstructs the damaged vascular system in both type I and type II diabetic mice models. Subsequently, we evaluated the similarity between TAME-C and TAME through bioinformatic data, in vivo and in vitro experiments, demonstrating that it has similar repair functions to TAME.

Discussion and Conclusion: We demonstrate that TAME can regulate inflammation, stimulate angiogenesis and promote fibroblast growth, thereby repairing diabetic wounds. Our study shows the possibility of a new regenerative medicine cell therapy option that recapitulates TAM function in normal macrophages. And considering the oncogenicity of TAM, we analysed its composition and used recombinant proteins to induce TAME-C which is similar to TAME. It provides a safe basis for its subsequent clinical translation.
Graphene Polylactic Acid Laser-printing Technology to Enhance Properties of 3D Printed Prosthetics and Medical Devices

Mr. Alberto Augello1,2, Dr. Silvia Gentilini3, Dr. Andrea Gnoli3, Dr. Valentina Palmieri1,2,3, Prof. Wanda Lattanzi2,4, Mr. Diego Sibilia4, Dr. Giordano Perini1,2, Ms Ginevra Friggeri1,2, Dr. Flavio De Maio5,6, Dr. Francesca Bugli5,6, Dr Riccardo Torelli6,7, Prof. Giovanni Delogu5,6, Prof. Maurizio Sanguinetti6,7, Prof. Claudio Conti3,7, Prof. Marco De Spirito1,2, Prof. Massimiliano Papi1,2

1Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, Roma, Lazio, 2Fondazione Policlinico Universitario “A. Gemelli” IRCCS, Rome, Lazio, 3Istituto dei Sistemi Complessi, CNR, Rome, Lazio, 4Istituto di Anatomia umana e Biologia cellulare, Università Cattolica del Sacro Cuore, Rome, Lazio, 5Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario "A. Gemelli", Rome, Lazio, 6Dipartimento di Scienze biotecnologiche di base, cliniche intensivologiche e perioperatorie, Università Cattolica del Sacro Cuore, Rome, Lazio, 7Dipartimento di Fisica, Università Sapienza, Rome, Lazio

Title: Graphene Polylactic Acid Laser-printing Technology to Enhance Properties of 3D Printed Prosthetics and Medical Devices
 Authors: A. Augello 1,2, S. Gentilini 3, A. Gnoli 3, V. Palmieri * 1,2,3, W. Lattanzi 2,4, D. Sibilia 4, G. Perini 1,2, G. Friggeri 1,2, F. De Maio 5,6, F. Bugli 5,6, R. Torelli 6,7, G. Delogu 5,6, M. Sanguinetti 6,7, C. Conti 3,7, M. De Spirito 1,2, M. Papi 1,2

Affiliations: (* Correspondence)

*Correspondence: Valentina Palmieri valentina.palmieri@cnr.it

• Introduction
Graphene-family materials are capable of reinforcing the mechanical, optical, and thermal properties of 3D-printed medical devices. In particular, graphene nanoplatelets have antibacterial properties and can adsorb visible and infrared light with high efficiency giving innovative properties to 3D printed scaffolds.

• Subjects and Methods
Fused deposition method to 3D print PLA additioned with graphene (PLA-G) here is combined with an innovative technology that allows to laser print complex surfaces such as CAD obtained from medical image. Several biological entities (i.e. cells, viruses and bacteria) are tested for their interactions with the PLA-G surface.

• Results
Here we demonstrate that the addition of graphene nanoplatelets to PLA filaments allows the creation of 3D-printed devices that can be sterilized by near-infrared light exposure at power density analog to sunlight. This method has been used to kill SARS-CoV-2 viral particles on the surface of 3D printed PLA-G by three minutes of exposure. Furthermore, the addition of graphene nanoplatelets to the PLA allow for laser printing the surface of 3D scaffolds creating nanopatterns that increase mesenchymal stem cell adhesion and limit the S. aureus growth.

• Discussion and Conclusion
3D-printed PLA-G scaffolds are highly biocompatible and can represent the ideal material for the production of sterilizable personal protective equipment and daily life objects intended for multiple users. Laser-printing of PLA-G surfaces increases the osteoconductivity of graphene scaffolds.

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Enhancing Biophysical and Biological Properties of Polycaprolactone 3D Scaffolds: Graphene Oxide Integration for Customizable Peptide Functionalization via Phage Display Technology

Dr. Valentina Palmieri1,2,3, Dr. Francesca Sciandra4, Dr. Stella Garcia Colomboroli4, Dr. Simona Viscuso4, Ms Irene Moretti1, Mr Alberto Augello1,2, Dr. Giordano Perini1,2, Ms. Ginevra Friggeri1,2, Prof. Marco De Spirito1,2, Prof. Massimiliano Papi1,2

1Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, Rome, Lazio, 2Fondazione Policlinico Universitario "A. Gemelli" IRCSS, Rome, Lazio, 3Istituto dei Sistemi Complessi, CNR, Rome, Lazio, 4Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" - SCITEC (CNR), Rome, Lazio

Title: Enhancing Biophysical and Biological Properties of Polycaprolactone 3D Scaffolds: Graphene Oxide Integration for Customizable Peptide Functionalization via Phage Display Technology

Authors: V. Palmieri*, F. Sciandra, S. G. Colomboroli, S. Viscuso, I. Moretti, A. Augello, G. Perini, G. Friggeri, M. De Spirito, M. Papi

*Correspondence: Valentina Palmieri valentina.palmieri@cnr.it Category: Design and Application of Biomaterials

• Introduction

Polycaprolactone (PCL) is a biocompatible material extensively studied as tissue regenerative scaffold for its easy 3D printability (low-temperature melting point). Application of PCL includes bone regeneration scaffolds, muscle, nerve and skin patches. However, PCL can have poor mechanical properties and the lack of specific protein interaction and antibacterial effects can limit its applicability in vivo. Graphene Oxide (GO), a bidimensional nanomaterial with antibacterial and light absorptive properties can be added to polymers to improve their intrinsic properties and modify the interaction with proteins in vivo. The knowledge of the affinity of adsorbed proteins on 3D PCL scaffolds will greatly increase the chance to control the cellular and generally biological interaction.

• Subjects and Methods

Fused deposition method was performed with Cellink BioX 3D Bioprinter to 3D print PCL or PCL-GO 1%. Mechanical and light absorptivity of scaffolds has been characterized. The viability of different cell lines has been tested on 3D printed scaffolds using Celltiterglo and fluorescence microscopy. The effect on E.coli bacteria has been quantified with CFU experiments. Plasma protein corona on scaffolds has been evaluated. Phage display technology has been performed to evaluate specific peptides capable of binding PCL or PCL-GO scaffolds.

• Results

Here we demonstrate that the addition of 1% GO to PCL pellets changes dramatically the affinity of peptides and the interaction with biological cells on 3D scaffolds. PCL-GO scaffolds show increased mechanical properties (+20% tensile strength) and are capable of absorbing infrared light and increasing local temperature. The viability of eukaryotic cells was influenced by GO addition. Murine RAW 264.7 cell line (macrophages) are less sensitive compared to other cell line tested. A series of peptides have been selected with phage display and analyzed to discover specific charge/hydrophobicity affinity to the surface. Amplified bacteriophages on the surface exhibit excellent biocompatibility and have been used to demonstrate the delivery of antibacterial phages from scaffolds.

• Discussion and Conclusion

3D-printed PCL and PCL-GO are both highly biocompatible, the addition of GO increases the mechanical properties and can confer scaffolds antibacterial properties. Furthermore, the affinity of specific peptides to the surface can be exploited to functionalize the surface with phages and increase the antibacterial effect/deliver specific genes.

• References


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Positive effects of p53 on osteogenic phenotype induced by BMP-9 in human periosteum-derived cells.

Ms. HyeSeong Oh1,2, Eun-Byeol Koh1,2, Young-Jin Seo1,2, Jin-Ho Park1, June-Ho Byun1,2

1Department of Oral and Maxillofacial Surgery, Gyeongsang National University School of, Jinju, South Korea, 2Department of Convergence Medical Science, Gyeongsang National, Jinju, South Korea

Authors: Hye-Seong Oh1,2, Eun-Byeol Koh1,2, Young-Jin Seo1,2, Jin-Ho Park1, June-Ho Byun1,2

Affiliations: 1Department of Oral and Maxillofacial Surgery, Gyeongsang National University School of Medicine and Gyeongsang National University Hospital, Institute of Health Sciences, Gyeongsang National University, Jinju, Republic of Korea.
2Department of Convergence Medical Science, Gyeongsang National University, Jinju, Republic of Korea

Category: Stem Cells and Cell-Based Therapies

Background: Bone morphogenetic protein (BMP) is very widely known as a factor that can induce bone formation. In particular, osteogenic studies related to BMP-2, 4, 7, and 9 have been verified through numerous papers. Recent studies have confirmed that BMP-9 is a particularly potent inducer of osteogenic differentiation in mesenchymal stem cells. Therefore, in this study, we aimed to investigate the role of BMP-9 compared to BMP2 in osteoblast differentiation of human periosteal-derived cells (hPDC).

Methods: In this work, we examined BMP-2 and 9, candidates for bone formation drugs. We tested their effects on osteogenesis in hPDCs by assay through ALP activity and calcium content. By phospho-kinase array, p53 was identified as the most significantly up-regulated protein in the BMP-9 treated group. As there is currently controversy about the PI3K/Akt pathway for p53 in osteogenesis, we investigated the role of p53 in hPDC on osteoblast differentiation.

Results: BMP-9 induces osteogenic differentiation of hPDCs better than BMP-2. The phosphorylation array suggested the highest expression of p53 in the BMP-9 group. Additionally, we showed that Runx2 expression was higher in the BMP-9 group than in the BMP-2 group, and the group treated with BMP-9 with Pfα (p53 inhibitor) was higher than the group treated with BMP-9 alone at the first week of differentiation. Among the microRNAs regulating p53, miRNA-34a/b/c and 145 were identified by qRT-PCR. As a result, it was observed that miRNA34a/b significantly was increased in the BMP-9-induced group than in the control group.

Discussion and Conclusion: Our findings demonstrate that BMP-9 has a potent osteogenesis-inducing ability compared to BMP-2. We established that the PI3K/Akt/MDM2 signaling pathway, known to involve p53, is activated in hPDCs treated with BMP-9. Furthermore, we confirmed that the activation of the PI3K/Akt/MDM2 axis promotes osteoblast differentiation through a feedback mechanism involving p53. It is suggested that BMP9-mediated signal transduction may induce osteoblast differentiation differently from previously known
mechanisms. These results imply that BMP-9 can be investigated for clinical applications in severe bone defects.

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A Non-degradable Glycan Material Replacing the ECM to Alleviate Intervertebral Disc Degeneration

Mr. YU LIU¹, Prof. Chunming Wang
¹University of Macau, , Macao SAR

Introduction: Intervertebral disc degeneration (IDD), an aging-degenerative disease, is the leading cause of low back pain in 80% of patients. Although surgery could alleviate pain partly, invasive treatment cannot reverse the degenerative process of IDD effectively. The main changes in the IDD process are the reduction of the extracellular matrix (ECM) and loss of phenotype of the nucleus pulposus cells (NPCs). Therefore, intervening deterioration process and promoting tissue regeneration are key strategies for low back pain.

Subjects and methods: Inspired by aliphatic analogs in metabolic glycoengineering, we designed and synthesized a non-degradable glucomannan with methylene modification (GMOC). In vitro, NPCs were co-cultured with GMOC to observe NPCs’ morphology and functional changes and identified potential mechanisms through transcriptomics and protein mass spectrometry tests. In vivo, GMOC nanoparticles were injected into the rat IDD model, and GMOC gel was filled into the rabbit intervertebral disc defect model to investigate the therapeutic and intervention effects.

Results: GMOC demonstrated resistance to degradation by enzymes associated with the IDD process. The NPCs have shown colony shape and more Col2 expression on the GMOC coating surface. Transcriptome analysis confirmed that GMOC could effectively maintain the juvenile phenotype of NPCs. GO analysis suggested that cell adhesion function was regulated by GMOC. Furthermore, mass spectrometry revealed that GMOC significantly enriches Mfge8 as a critical mediator of the interaction between CMOC and NPCs. In vitro, various treatments improved the height of the intervertebral space, increased the water content of the tissue, and maintained the integrity of the nucleus pulposus tissue.

Discussion and conclusion: This glycan material could effectively replace the ECM and avoid secondary degradation after treatment. In addition, GMOC could improve the function of NPCs by mimicking the metabolic glycoengineering process. This work represents an effective and safe treatment strategy for IDD. The treatment methods of different models highlight potential translational possibilities.
Enhancing Mettl14 stability promoted CST regeneration by increasing Trib2 mRNA methylation after spinal cord injury

Dr. Chengjun Li1,2, Tian Qin1,2, Yuxin Jin1,2, Yiming Qin1,2, Yong Cao1,2, Hongbin Lu2,3, Jianzhong Hu1,2
1Department of Spine Surgery and Orthopaedics, Xiangya Hospital, Central South University, Changsha, China, 2Key Laboratory of Organ Injury, Aging and Regenerative Medicine of Hunan Province,, Changsha, China, 3Department of Sports Medicine, Xiangya Hospital, Central South University, Changsha, China

Background: Acute spinal cord injury (ASCI) usually causes axon rupture, which is difficult to regenerate. N6-methyladenosine (m6A) modifications, as the most common form of epigenetic regulations at the RNA level, have been proven to play an essential role in biological processes. Whereas, whether m6A modifications participated in cortical neuron activity and corticospinal tract (CST) regeneration after SCI has never been reported.

Methods: In this work, the m6A modification level of mice’s locomotor cortex (M1) after complete spinal cord transection was detected using an m6A dot blot. m6A modification-related proteins were evaluated by immunofluorescence, qRT-PCR, and western blot. By constructing Mettl14 interfering lentivirus (sh-Mettl14), we explored the effect of Mettl14 on axonal regrowth both in vivo and in vitro. Through molecular docking, we identified the potential mettl14 stabilizer and explored its role in SCI treatment.

Results: Through bioinformatics analysis and MeRIP-qPCR, we found Mettl14 promoted neuronal apoptosis and inhibited axon regrowth by promoting the expression of tribbles homolog 2 (Trib2) in an m6A-regulated manner, thereby activating the MAPK/JNK pathway. Using molecular docking, we discovered that a naturally occurring small compound, syringin, stabilizes the Mettl14.

Discussion and Conclusion: Our study demonstrated that Mettl14 regulated CST regeneration through Trib2-MAPK/JNK pathway after SCI. Treatment of SCI mice with syringin improved neural functional recovery, which may provide a potential therapeutic approach for SCI.
Enhancement of Therapeutic Potential in Wharton's Jelly Mesenchymal Stem Cells via Priming with Interferon-gamma and Hypoxia

Ms. Yu Ling Tan1, Dr. Sue Ping Eng2, Prof. Dr. Mohamad Nasir SHAFIEE3, Dr. Jia Xian LAW1, Assoc. Prof. Dr. Min Hwei NG1

1Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Cheras, Malaysia, 2NK Biocell Sdn. Bhd., Unit 1-22A, 1st Floor Pusat Perdagangan Berpadu (United Point), No.10, Jalan Lang Emas, Kepong, Malaysia, 3Department of Obstetrics & Gynaecology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Cheras, Malaysia

Introduction: MSCs are important in regenerative medicine due to their regenerative abilities, immunomodulatory properties, and trophic factor secretion. However, their survival and functionality are influenced by their environment. Previous research has shown that subjecting MSCs to hypoxia and pro-inflammatory substances like interferon-gamma (IFN-γ) can improve their functionality, survival rate, and therapeutic effectiveness, as well as reduce variations in MSC immunomodulation caused by donor differences. Therefore, this study aims to evaluate how hypoxia and IFN-γ priming impact the immunosuppressive and angiogenic properties of MSCs, with the goal of maximizing their therapeutic potential.

Methods: Wharton’s Jelly-derived mesenchymal stem cells (WJ-MSCs) isolated from umbilical cords were subjected to in vitro priming with 50 ng/mL IFN-γ and 1% oxygen level (hypoxia). We then probe the effects of these priming conditions on MSC in terms of the upregulation of genes related to immunomodulation, angiogenesis, and regeneration, and MSC’s ability to induce macrophage polarization.

Results: Our findings indicate that only IFN-γ upregulated the gene expressions of immunomodulatory factors including IDO (by ~300fold), TGF-β and PGE2, and angiogenic factor (VEGF). Furthermore, when MSCs were primed with either IFN-γ or hypoxia, macrophages displayed a preference for the M2 phenotype (anti-inflammatory), as evidenced by the increase in CD36+ macrophages. Subsequently, we investigated the combined effect of these priming strategies but found no synergistic impact from such a combined approach.

Conclusion & Future recommendation: Based on our findings, both IFN-γ priming and hypoxia show potential for improving the therapeutic characteristics of WJ-MSCs. IFN-γ priming enhances immunosuppressive factors, while hypoxia promotes angiogenic potential. Therefore, different priming approaches can be tailored to specific treatment needs. It has been reported that IFN-γ priming increases aerobic respiration, whereas hypoxia promotes anaerobic respiration. It would be interesting to investigate the implications of such metabolic shifts on the therapeutic function of MSCs.

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Keywords: Mesenchymal stem cell, IFN-γ, hypoxia, immunosuppressive, priming
UTX deletion promoted functional recovery by epigenetically regulating microvascular endothelial cells senescence after spinal cord injury

Dr. Chengjun Li1,2,3, Tian Qin1,2, Jinyun Zhao1,2, Yuxin Jin1,2, Yiming Qin1,2, Yong Cao1,2, Hongbin Lu2,3, Jianzhong Hu1,2

1Department of Spine Surgery and Orthopaedics, Xiangya Hospital, Central South University, Changsha, China, 2Key Laboratory of Organ Injury, Aging and Regenerative Medicine of Hunan Province, Changsha, China, 3Department of Sports Medicine, Xiangya Hospital, Central South University, Changsha, China

Background: Spinal cord injury (SCI) is a traumatic disease of the central nervous system, which often leads to motor, sensory and autonomic dysfunction of patients below the injury level. Until now, there is still no effective treatment. SCI usually causes destruction of microvascular network, and the limited regeneration ability of micro-vessels impair the recovery of neurological function after SCI. Whether endothelial cell senescence was involved in this process and the internal mechanism need to be further explored. The epigenetic factor ubiquitous transcribed tetratripeptide repeat, X chromosome (UTX) is widely expressed in the eukaryotic nucleus, and plays an indispensable role in a variety of physiological and pathological processes. However, it is not clear whether UTX is involved in microvascular endothelial cell senescence after SCI. This study aims to explore the role and mechanism of UTX in regulating endothelial cell senescence, which may provide potential therapeutic targets for the treatment of SCI.

Methods: Here, a modified SCI model was established to explore the senescence phenotype of vascular endothelial cell at different time points after SCI. Hydrogen peroxide (H2O2) was used induce cellular senescence in primary brain microvascular endothelial cells (BMECs) and bEnd.3 cell lines. We applied transgenic mice to investigate the effects of H2K27 demethylase UTX on endothelial cell senescence. RNA-sequencing (RNA-seq) and chromatin immunoprecipitation-qPCR (ChIP-qPCR) were performed to screen downstream genes regulated by UTX. The adeno-associated virus (AAV) and lentivirus (LV) of target gene were constructed to analyze the potential mechanism of UTX influencing endothelial cell senescence.

Results: Our results showed SCI induced spinal cord microvascular endothelial cell (SCMECs) senescence, and the number of senescent cells reached a peak on the 14th day after injury. In vitro experiments indicated that senescent bEnd.3 cells released a large amount of senescence-associated secretory phenotype (SASP), with decreased ability of tube formation and migration. UTX was elevated after SCI, while conditional deletion of UTX reduced endothelial cells senescence and promoted neurological recovery after SCI. RNA-seq and ChIP-qPCR suggested that calponin 1 (CNN1) was the downstream target gene of UTX. By local injection of adeno-associated virus (AAV-CNN1), the inhibitory effect of UTX deletion on endothelial cells senescence was significantly reduced.

Discussion and Conclusion: We demonstrated that SCI induced vascular endothelial cells senescence. Conditional deletion of UTX reduced endothelial cell senescence and promoted neurological function recovery after SCI. Furthermore, through high-throughput sequencing and AAV application, we revealed the internal molecular mechanism of UTX regulating vascular endothelial cell senescence. Our findings may provide potential therapy targets for the treatment of SCI.
Identification and characterization of human skeletal stem cell-like cells derived from infrapatellar fat pad

Dr. Jianzhong Hu\textsuperscript{1,2,3}, Dr. Yan Xu\textsuperscript{2,3}, Dr. Jinrui Xun\textsuperscript{2,3}, Dr. Xin Shi\textsuperscript{2,3}, Dr. Xinzhu Qiu\textsuperscript{2,3}, Dr. Tao Zhang\textsuperscript{2,3}, Yong Cao\textsuperscript{1,2}, Dr. Jianzhong Hu\textsuperscript{1,2}, Prof. Hongbin Lu\textsuperscript{2,3}

\textsuperscript{1}Department of Spine Surgery and Orthopaedics, Xiangya Hospital, Central South University, Changsha, China, \textsuperscript{2}Key Laboratory of Organ Injury, Aging and Regenerative Medicine of Hunan Province, , China, \textsuperscript{3}Department of Sports Medicine, Xiangya Hospital, Central South University, , China

Background
Skeletal stem cells (SSCs) have attracted extensive attention for their crucial role in bone accrual and therapeutical values. The substantial unmet cellular need of regenerative medicine and tissue engineering calls for identification of a novel source for human SSC isolation, or even skeletal stem cell-like cells (SSCLCs).

Methods
hSSCLCs were isolated through enzyme-digestion and fluorescent-activated cell sorting (FACS) from human tissues including placenta, cord blood, Wharton’s Jelly and various adipose depots. Proportion of hSSCLCs in all those tissues were compared through flow cytometry. For adipose tissue, immunofluorescent staining was also employed to substantiate our flow results. In vitro CFU-F assay, chondrogenic and osteogenic assays were performed to assess self-renewal and multipotency for differentiation of hSSCLCs. Transcriptomic profiling of adipose-derived hSSCLCs was achieved through scRNA-seq.

Results
Here, we illustrated that adipose tissues, but not fetal tissues, contain a satisfying abundance of hSSCLCs, especially infrapatellar fat pad (IPFP). Moreover, we discovered IPFP-derived hSSCLCs display intact self-renewal and a marked elevation in chondrogenic and osteogenic differentiation. Transcriptomically comparing IPFP-hSSCLCs and dorsal adipose depot (DSAT)-derived hSSCLCs through scRNA-seq, we further demonstrated IPFP-hSSCLCs are less differentiated but more motivated in expressing transcriptomes related to chondrogenic and osteogenic differentiation.

Discussion and Conclusion:
Our study first identified infrapatellar adipose tissue as an encouraging source for isolating hSSCLCs with intact SSC properties which might be promising in treating diseases related to bone and/or cartilage defects.
The effect of local sympatholysis on bone-tendon interface healing in a murine rotator cuff repair model

Tingmo Huang1,2, Liyang Wan1,2, Chengjun Li2,3, Jianjun Huang1,2, Jianzhong Hu2,3, Prof. Hongbin Lu1,2
1Department of Sports Medicine, Xiangya Hospital, Central South University, Changsha, China, 2Key Laboratory of Organ Injury, Aging and Regenerative Medicine of Hunan Province, Changsha, China, 3Department of Spine Surgery and Orthopaedics, Xiangya Hospital, Central South University, Changsha, China

Background: Although neuroregulation plays an important role in tissue healing, the key neuroregulatory pathways and related neurotransmitters involved in bone-tendon interface (BTI) healing are still unknown. It is reported that sympathetic nerves can regulate cartilage and bone metabolism, which are the basic aspects of BTI repair after injury, through the release of norepinephrine (NE). Thus, the purpose of this study was to explore the effect of local sympatholysis (LS) on BTI healing in a murine rotator cuff repair model.

Methods: Specifically, C57BL/6 mice underwent unilateral supraspinatus tendon (SST) detachment and repair was established on a total of 174 mature C57BL/6 mice (12 weeks old): 54 mice were used to examine the sympathetic fibers and its neurotransmitter NE for the representation of sympathetic innervation of BTI, while the rest of them were randomly allocated into LS group and control group to verify the effect of sympathetic denervation during BTI healing. The LS group were intervened with fibrin sealant containing 10ng/ml guanethidine, while the control group received fibrin sealant only. Mice were euthanized at postoperative 2, 4 and 8 weeks for immunofluorescent, qRT-PCR, ELISA, Micro-computed tomography (CT), histology and biomechanical evaluations.

Results: Immunofluorescence, qRT-PCR and ELISA evaluations indicated that there were the expression of tyrosine hydroxylase (TH), NE and β2-adrenergic receptor (β2-AR) at the BTI site. All the above showed a trend of increasing at the early postoperative stage and they started to decrease with the healing time after a significant peak. Meanwhile, local sympathetic denervation of BTI was achieved after the use of guanethidine as shown in the NE ELISA outcomes in two groups. QRT-PCR analysis revealed that the healing interface in the LS group expressed more transcription factors, such as Runx2, Bmp2, Sox9, and Aggrecan, than the control group. Further, radiographic data showed that the LS group significantly possessed higher bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and lower trabecular spacing (Tb.Sp) than the control group. Also, histological test results showed that there was more fibrocartilage regenerated at the healing interface in the LS group compared with the control group. Mechanical testing results demonstrated that the failure load, ultimate strength and stiffness in the LS group were significantly higher at postoperative week 4 (P < 0.05), but not at postoperative week 8 (P > 0.05), compared to the control group.

Conclusion: The regulation of sympathetic innervation was involved in the healing process of injured BTI, and local sympathetic denervation by using guanethidine was beneficial for BTI healing outcomes.

The translational potential of this article: This is the first study to evaluate the expression and specific role of sympathetic innervation during BTI healing. The findings of this study also imply that the antagonists of β2-AR could serve as a potential therapeutic strategy for BTI healing. Also, we firstly successfully constructed a local sympathetic denervation mouse model by using guanethidine loaded fibrin sealant, which provided a new effective methodology for future neuroskeletal biology study.
Rotator cuff healing is regulated by the lymphatic vasculature

Dr. Xiaopeng Tong\textsuperscript{2,3}, Dr. Tao Zhang\textsuperscript{2,3}, Dr. Shengcan Li\textsuperscript{2,3}, Dr. Xin Shi\textsuperscript{2,3}, Dr Yang Chen\textsuperscript{2,3}, Dr. Yan Xu\textsuperscript{2,3}, Dr. Chao Deng\textsuperscript{1,2}, Dr. Jianzhong Hu\textsuperscript{1,2}, \textbf{Prof. Hongbin Lu}\textsuperscript{2,3}

\textsuperscript{1}Department of Spine Surgery and Orthopaedics, Xiangya Hospital, Central South University, Changsha, China, \textsuperscript{2}Key Laboratory of Organ Injury, Aging and Regenerative Medicine of Hunan Province, Changsha, China, \textsuperscript{3}Department of Sports Medicine, Xiangya Hospital, Central South University, Changsha, China

Background: Despite great advances in surgical techniques for rotator cuff tear (RCT) over the past decades, the postoperative failure rate of RCT is still high due to the poor healing competence of bone-tendon interface (BTI). The lymphatic vasculature plays a regulatory role in inflammatory disease and affects tissue healing. However, whether lymphangiogenesis and the role of lymphatic vasculature in the physiopathological process of rotator cuff (RC) injury remains unknown.

Methods: In this study, we constructed a mouse RC injury model and the BTI samples were collected for measurement. Firstly, immunofluorescence was used to investigate the temporal and spatial distribution of lymphangiogenesis in BTI area at different post-injury time points. Subsequently, the mice of experimental group were gavaged with the lymphatic inhibitors (SAR131675) on the first postoperative day to inhibit lymphangiogenesis, while the control group was treated with the vehicle. At postoperative week 2 and 4, the samples were collected for immunofluorescence staining to evaluate lymphatic angiogenesis inhibition. At postoperative week 4 and 8, The supraspinatus (SS) tendon-humeral complexes were collected for bone morphometric, histological and biomechanical tests to assess the healing outcome of the BTI.

Results: Immunofluorescence results showed that the lymphatic proliferation in the BTI injury area and increased in consistence with the healing time, and the lymphatic hyperplasia area significantly diminished at postoperative week 4. The lymphatic hyperplasia area in the SAR group was significantly lower than that in the control group both at 2 and 4 weeks postoperatively. Moreover, the administration of SAR131675 significantly impeded RC healing, as evidenced by lower histological scores, lower bone morphometric parameters, and worse biomechanical properties in comparison with that in control group at postoperative weeks 4 and 8.

Conclusion: Lymphangiogenesis plays a positive role in RC healing, and targeting the lymphatic drainage at healing site may be a new therapeutic approach to promote RC injury repair.
Polyvinyl Alcohol Nanocellulose Sponge: A Promising Scaffold for Nasal Mucosal Regeneration

Ms. Nattha Suwanprakorn¹, Enkhzaya Davaa, Eunbin Ji, Dahee Ryu, Hosun Jung, Su-Geun Yang
¹Department of Biomedical Science, BK21 FOUR Program in Biomedical Science and Engineering, Inha University College of Medicine, Incheon, South Korea

Title: Polyvinyl Alcohol Nanocellulose Sponge: A Promising Scaffold for Nasal Mucosal Regeneration
Authors: Nattha Suwanprakorn, Enkhzaya Davaa, Eunbin Ji, Dahee Ryu, Hosun Jung, Su-Geun Yang*
Affiliations: Department of Biomedical Science, BK21 FOUR Program in Biomedical Science and Engineering, Inha University College of Medicine, Incheon 22212, Korea (* Correspondence)
Category: Design and Application of Biomaterials

Background: Nanocellulose has emerged for a wide range of applications in biomedical engineering because of its water absorption capacity, appropriate elasticity. We investigated the hemostatic and regenerative abilities of an expanding polyvinyl alcohol (PVA)-nanocellulose sponge on nasal mucosal defects.

Methods: A 3 mm-diameter nasal defect was made in experimental rabbits. Rabbits were divided into four groups with control, vaseline, PVA and PVA-nanocellulose packing groups. After the defect was created, bleeding times and amounts were monitored. Packing materials were removed on experimental day (ED) 2. On ED 3, 7 and 14, histological analysis and immunohistochemical study for neutrophils were performed. Inflammatory cells were counted and epithelial thicknesses were evaluated.

Results: Bleeding amounts and times in the vaseline packing group were smaller than in the PVA groups. PVA-nanocellulose group showed less neutrophils than in the other groups on ED 7. Average epithelium thickness in the PVA-nanocellulose group was significantly smaller than in the control group at ED 7, but at ED 14, there was no significant intergroup difference. PVA-nanocellulose group had a significant lower inflammatory cell count than the control group on ED 7.

Discussion and Conclusion: PVA-nanocellulose sponge applied to nasal mucosal defects can significantly enhance mucosal regeneration during early wound healing.

Acknowledgement: This research was supported by the Basic Science Research Program and the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MOE and MSIT) (2018R1A6A1A03025523, 2021H1D3A2A02045561 and RS-2023-00208587). This study was also supported by the BK21 Four program through the National Research Foundation (NRF) funded by the Ministry of Education of Korea.
Superior Low-immunogenicity of Tilapia Type I Collagen by Inhibiting Activation of Dendritic Cells

Prof. Jiao Sun

Introduction: Collagen is widely used in regenerative medicine and plastic surgery. Although collagen from terrestrial mammals exhibits good biological effects, frequent clinical reports of varying degrees of allergy remain. Recently, collagen from aquatic organisms, such as tilapia, has been recognized for its bio-efficient, low immunogenicity, and excellent biosafety. However, it has always been unclear why fish collagen induces a lower degree of immune response than collagen derived from terrestrial mammals. In our study, using bovine or porcine type I collagen (BCI or PCI) as a control, we investigated in depth the effects of tilapia type I collagen (TCI) on dendritic cell (DC) and T cell functions, focusing on the impact and potential mechanisms of the differences in immunogenicity of different collagens.

Subjects and Methods: Beginning with initiators of immune responses, we conducted a series of in vitro studies on the expression of surface markers, the release of cytokines, and changes in the ability of migration and phagocytosis to investigate the influence that three collagens exerted on DCs. The effects on differentiation and release of cell factors of naive T cells were also studied. Additionally, bio-informatic methods were employed to analyze variations of collagens in their structure to explore the relationship between secondary structure and DC maturation. Finally, the immunogenicity of tilapia type I collagen was evaluated using the humoral and cellular responses of C57BL/6 mice implanted with collagen subcutaneously.

Results and Discussion: Firstly, the in vitro results indicate that in contrast to BCI and PCI, DCs co-cultured with TCI will not be activated and will not promote Th1 differentiation. With the help of bio-informatic technologies, we may successfully explain the phenomenon, that is, aspartic acid on the 1262nd position in TCI α1 chain inhibits the formation of calcium-binding motifs. As a result, there is only one calcium-binding motif in TCI while three in BCI or PCI, whose corresponding position in primary structure is asparagine. Consequently, BCI and PCI show a higher ability to take Ca2+ and increase intracellular calcium concentration, ultimately activating the STIM1-Orai1/NF-κB signaling pathway, DC maturation, and Th1 polarization. A series of self-amplifying immune responses will also be observed.

Conclusion: In contrast to BCI or PCI, TCI exhibits extremely low immunogenicity, and our findings have important implications for the promotion and widespread application of TCI in biomedicine.

Upregulation of UCHL1 in Periodontal Ligament Mesenchymal Stem Cells Promotes Angiogenesis in Periodontitis

Dr. Lu Lin¹, Dr. Weijun Yu¹, Dr. Guanglong Li¹, Dr. Yuting Gu¹, Prof. Min Jin¹, Prof. Eryi Lu¹
¹Renji Hospital, Shanghai Jiao Tong University School Of Medicine, Shanghai, China

Background: Periodontitis is a chronic inflammatory disease of periodontium destruction, including alveolar bone and soft tissues. In periodontitis, chronic inflammation despairs the osteogenesis of periodontal ligament mesenchymal stem cells (PDLSCs), and these PDLSCs also regulate periodontal microenvironment, such as pro-angiogenesis-contributed abnormal vascularization, which in turn influences inflammation and osteogenesis. The behaviors of PDLSCs have been considered critical for the impaired alveolar bone regeneration in periodontitis. Our previous study has demonstrated that the deubiquitinating enzyme UCHL1 inhibition could promote the osteogenesis of PDLSCs and alveolar bone regeneration in periodontitis. However, whether UCHL1 affected pro-angiogenesis of PDLSCs and abnormal vascularization in periodontitis was still unclear.

Methods: The pro-angiogenesis effects and UCHL1 expression of PDLSCs, isolated from healthy individuals and periodontitis patients or then treated with proinflammatory cytokines in vitro, were evaluated. And the effects of UCHL1 inhibition on the pro-angiogenesis of PDLSCs and its underlying mechanisms were explored. The therapeutic effects of UCHL1 inhibition on periodontitis were then assessed in the ligature-induced murine periodontitis models.

Results: PDLSCs from periodontitis patients or treated with proinflammatory cytokines in vitro, held stronger ability of pro-angiogenesis and higher UCHL1 expression. And the higher UCHL1 expression in PDLSCs was positively associated with abnormal vascularization in periodontium form periodontitis patients. Functionally, the pro-angiogenesis effects of PDLSCs in periodontitis could be inhibited by UCHL1 knockdown, which was due to the downregulation of YAP/TAZ signaling pathway. Moreover, the therapeutic effects of UCHL1 inhibition on ameliorating periodontitis were verified, as indicated by less bone loss and reduced inflammation.

Discussion and Conclusion: We proved UCHL1 to be a key positive regulator of the pro-angiogenesis of PDLSCs and abnormal vascularization in periodontitis, and demonstrated a promising target for alleviating abnormal vascularization in periodontium, which might provide a better microenvironment for alveolar bone regeneration in periodontitis.
Enhancing recellularization and thrombosis prevention of the kidney dECM through albumin coating and rotating culture system

Ms Boyun Kim¹, Ms Hyunwoo Jo Jo¹,², Ms Bo Young Choi¹, Dr Jina Ryu¹
¹R&D Center, ROKIT Healthcare, Inc, Seoul, South Korea, ²Department of Biomicrosystem Technology, Korea University, Seoul, South Korea
Title: Enhancing recellularization and thrombosis prevention of the kidney dECM through albumin coating and rotating culture system

Authors: Boyun Kim¹, Hyunwoo Jo1,2, Bo Young Choi¹, Jina Ryu¹*

Affiliations: 1R&D Center, ROKIT Healthcare, Inc., Seoul, Korea. 2Department of Biomicrosystem Technology, Korea University, Seoul, Korea. (*Correspondence)

Category: Tissue Engineering and Regeneration

Background: Successful recellularization and prevention of thrombosis are crucial for the effective implantation of biomaterials in various therapeutic applications. This study introduces a novel approach utilizing albumin coating and a rotating culture system to enhance recellularization and prevent thrombosis in biomaterial implantation.

Methods: We optimized to decellularized of porcine kidney by whole organ perfusion or slice. Then, the decellularized kidney tissue was coated with 20% of albumin and cultured in rotating culture system. Cell proliferation was measured with ELISA, and cell penetration into tissue was analyzed by live cell staining.

Results: In this study, we applied two approaches to evaluate the efficacy of albumin coating and the rotating system. Albumin coating showed the prevention of blood clot formation, and significantly enhanced cell adhesion and proliferation on Kidney dECM. Cell culture with roller bottle enabled to infiltrate cells into tissue and enhanced cell proliferation.

Discussion and Conclusion: Albumin coating of dECM scaffolds enhanced recellularization by promoting cell adhesion, proliferation. Additionally, albumin coating effectively prevented thrombus formation. These findings contribute to the development of improved dECM-based scaffolds with enhanced recellularization potential and reduced risk of thrombosis, ultimately advancing the fields of regenerative medicine and transplantation.

Acknowledgement: This research was supported by the Korean Fund for Regenerative Medicine (KFRM) grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Health & Welfare) (code: KFRM RS-2023-00216159).
Mg-1Ca alloys and High-purity Mg: Comparative Assessment of the Merits regarding Degradation, Osteogenesis, and Biosafety for Orthopedic Applications in vivo

Ms. Hua LU¹, Mr. Baiyan SUI¹

¹Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Title: Mg-1Ca alloys and High-purity Mg: Comparative Assessment of the Merits regarding Degradation, Osteogenesis, and Biosafety for Orthopedic Applications in vivo

Authors: Hua Lu, Baiyan Sui, Xin Liu, Jiao Sun

Affiliations: Department of Dental Materials, Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine; College of Stomatology, Shanghai Jiao Tong University; National Center for Stomatology; National Clinical Research Center for Oral Diseases; Shanghai Key Laboratory of Stomatology, Shanghai 200011, China (* Correspondence)

Background: Mg-1Ca alloys and High-purity magnesium (HP Mg), as representations of Mg-matrix implants produced by purifying and alloying, are employed in biomedical applications primarily because of their bioactivity and degradability. The superiority of both degradation properties, the match between degradation and osteogenesis in vivo, and biosafety are critical problems that will decide future purifying or alloying to construct Mg-based implants and promote clinical translation.

Methods: In this work, we investigated the benefits and limitations of degradation behavior and biosafety of Mg-1Ca alloy and HP Mg according to bone implantation assay in vivo.

Results: The results indicated that Mg-1Ca alloy and HP Mg both showed a noticeable degradation at 4 weeks, and they then went into a steady degradation period within 26 w according to the quantitative analysis results. There were no apparent aberrant histological alterations. The hematological and hemochemical indices of the three groups have shown insignificant. These findings imply that Mg-1Ca and HP Mg do not have systemic adverse effects on the organism due to their local degrading activity. Additionally, the implantation and degradation of Mg-1Ca and HP Mg do not result in major organ dysfunction or pathological abnormalities.

Discussion and Conclusion: Mg-1Ca alloy and HP Mg exhibit a favorable match between their degradation and the surrounding osteogenesis, resulting in no significant difference in degradation for 26w in vivo. The current work demonstrated that the implantation and degradation of Mg-1Ca alloy and HP Mg neither cause hematological nor hemochemical abnormalities and major organ pathological reactions, thus exhibiting long-term biosafety in vivo.

Acknowledgment: This work was supported by grants from the Science and Technology Commission of Shanghai Municipality (18DZ2201500, 19DZ2203900).
Small molecule-assisted assembly of multifunctional ceria nanozymes for synergistic treatment of atherosclerosis

Zhangyou Yang¹, Xiaoxue Fu¹, Chao Yu¹
¹Chongqing Key Laboratory for Pharmaceutical Metabolism Research, Chongqing Pharmacodynamic Evaluation Engineering Technology Research Center, College of Pharmacy, Chongqing Medical University, Chongqing, China

Title: Small molecule-assisted assembly of multifunctional ceria nanozymes for synergistic treatment of atherosclerosis

Authors: Xiaoxue Fu¹, Zhangyou Yang¹*, Chao Yu¹*

Affiliations: 1Chongqing Key Laboratory for Pharmaceutical Metabolism Research, Chongqing Pharmacodynamic Evaluation Engineering Technology Research Center, College of Pharmacy, Chongqing Medical University, 400016, Chongqing, P. R. China.

Category: Design and Application of Biomaterials

Background: Considering that intravascular reactive oxygen species (ROS) and inflammation are two characteristic features of the atherosclerotic microenvironment, developing an appropriate strategy to treat atherosclerosis by synergistically regulating ROS and inflammation has attracted widespread attention.

Methods: A new strategy for assembling a specific small-molecule ligand, zoledronic acid (ZOL), with cerium ions to obtain multifunctional CZ NCs. Moreover, the platelet membrane-modified nanodrugs, PCZ@PB NCs, were developed as a bionic nanozyme platform for treating AS.

Results: The proposed PCZ@PB NCs specifically accumulate at inflammatory atherosclerotic lesions, synergistically regulate ROS levels and inflammation, and efficiently inhibit foam cell formation. In vivo evaluation in ApoE⁻/⁻ mice showed that the bionic PCZ@PB NCs can effectively inhibit the progression of atherosclerosis (AS).

Discussion and conclusion: The functionalized ceria-ZOL nanocomposites (CZ NCs) exhibit the advantages of traditional ceria nanozymes and Ce-MOFs. The reported biomimetic nanozymes act therapeutically by modulating the AS inflammatory microenvironment through multi-effect synergy. This work might provide new perspectives in designing precision medicine for treating oxidative stress and inflammatory diseases.

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A robust intracellular expansion of super-functioning mitochondria for osteoarthritis mitotherapy

Mr. Xuri CHEN¹, Dr. Hongwei OUYANG¹,²
¹Zhejiang University, School of Medicine, Hangzhou, China, ²Liangzhu Laboratory, Hangzhou, China

Introduction: Osteoarthritis (OA) is a common degenerative joint disease characterized by the breakdown of joint cartilage. Mitochondrial dysfunction of the chondrocyte is a risk factor for OA progression. Mitotherapy enhances ATP synthesis, oxygen consumption, and cell viability, thereby improving systemic function. However, the acquisition of active mitochondria remains a major challenge for tissue regeneration.

Subjects and Methods: Mt-MSCs were cultured in the customized medium for mitochondria expansion. We tested the function and effects of Mt-MSCs-derived mitochondria on OA therapy.

Results: Mt-MSCs were efficient for mitochondria expansion under a customized medium. Mitochondria could be easily prepared because of the exhibited strong proliferative and self-renewal abilities of Mt-MSCs. Mt-MSCs exhibited higher mitochondrial bioenergetics and its mitochondria showed higher activities. Transcriptome revealed that enhanced cell proliferation and mitochondrial biogenesis of Mt-MSCs were through the upregulated AMPK pathway. At last, mitotherapy corrected energy imbalance and restored cellular metabolism to improve cartilage homeostasis and protect against the pathological progression of OA.

Discussion and Conclusion: We constructed a robust and efficient intracellular mitochondrial expansion system in need of tissue engineering and regenerative medicine. This engineering strategy was universal and could be applied to a variety of mitochondrial disorders. In this study, we showed the great potential of mitochondrial therapy in the treatment of OA.
Development of photo-click alginate hydrogels for tissue engineering and biofabrication

Mr. Matthew Mail¹, Mr. Callum VIDLER¹, Professor Andrea O’CONNOR¹, Associate Professor Daniel HEATH¹
¹Department of Biomedical Engineering, Graeme Clark Institute, The University of Melbourne, Victoria 3010, Australia

Title: Development of photo-click alginate hydrogels for tissue engineering and biofabrication

Authors: Matthew Mail1, Callum Vidler1, David Collins1, Andrea J. O’Connor1, Daniel Heath1

Affiliations: 1Department of Biomedical Engineering, Graeme Clark Institute, The University of Melbourne, Victoria 3010, Australia

Category: Design and Application of Biomaterials

Background: 3D bioprinting is a fabrication process that enables precise control over the architecture of three-dimensional tissue scaffolds and models. Typically, this technique requires a cell-laden bioink that provides structure and shape fidelity to the construct1. To accomplish this goal, bioinks are often polymeric solutions with shear-thinning properties that can be rapidly cured into crosslinked hydrogels in a cytocompatible manner. Additionally, it is desirable to design the inks to exhibit appropriate biological motifs to the encapsulated cells to provide anchorage sites and other biological cues. Sodium alginate is a biopolymer that has seen extensive use as a bioink due to its cytocompatibility, non-fouling properties, and readily functionalisable carboxylic acid side-groups2. Leveraging these properties with light-based click chemistry allows for an adaptable bioink that can be used in multiple printing modalities.

Methods: In this work, an alginate-based hydrogelation system was developed to photocrosslink via thiol-ene click chemistry. Norbornene groups were covalently attached to the alginate backbone using well-established carbodiimide/hydroxysuccinimide chemistry. Norbornene-functionalised Alginate (AN) was crosslinked into hydrogels via thiol-ene reaction with the di-thiol crosslinker 2,2’-(Ethylenedioxy)diethanethiol (EDDT) upon exposure to 405 nm light, mediated by the photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP). For printing experiments acid yellow was added as a photoabsorber.

Results: AN hydrogels demonstrated a wide range of mechanical properties from ~5 to 110 kPa based on the degree of crosslinking, as verified by uniaxial compressive testing. Gelation occurred within 2 s on exposure to 405 nm light with intensity of 31 mW/cm², as confirmed by photocrosslinking rheometry. Bioinks were formulated with 5 w/v% AN, 0.1 w/v% LAP, a 1:1 Norbornene:EDDT molecular ratio, and 0 - 0.1 w/v% acid yellow. The bioink mixture was used in different light-based print modalities to both print and pattern structures with features down to 10 µm in size.

Discussion and Conclusion: We have demonstrated that thiol-ene click alginate hydrogels can be used in different printing modalities for patterning and printing. The combination of cell-inert but readily functionalizable sodium alginate, orthogonal covalent crosslinking, and visible-light initiated hydrogelation make a powerful tool for 3D bioprinting applications and adherent cell studies.

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Electrospun PVA/Gelatin nanofiber with electrical stimulating film for diabetic wound healing

Mr. Jeong-Uk Kim¹, Mr. Young-Hyeon An², Mr. Nathaniel Suk-Yeon Hwang¹,²,³
¹School of Chemical and Biological Engineering, Institute of Chemical Process, Seoul, Republic of Korea, ²BioMax/N-Bio Institute, Seoul National University, Seoul, Republic of Korea, ³Institute of Engineering Research, Seoul National University, Seoul, Republic of Korea

Background: Skin is the body’s outermost protective barrier but is susceptible to injury. In conditions like diabetes, skin wounds become chronic and do not heal with conventional dressings. In addition, existing dressings lack specific functionalities for chronic wound recovery. Thus, there is an urgent need to develop functional patches for effective healing of chronic wounds.

Methods: In order to treat diabetic wounds, we have created an electro-stimulating membrane combined with electrospun nanofiber (M-sheet). We added numerous alternative patterns of Zn and AgCl using the screen printing technique on a polyurethane substrate, which produced redox-mediated electrical fields.

Results: By simulating different metal patterns, we manufactured the M-sheet by selecting the most effective pattern that generated electric fields. The M-sheets we produced successfully generated both short and long-term electric fields. Furthermore, our in vitro experiments further confirmed the beneficial impact of electric fields on cell migration. Moreover, the electric field exhibited antimicrobial properties and stimulated macrophage polarization. Building upon these in vitro findings, we applied M-sheet patches on diabetic mouse models for wound healing, yielding remarkably effective results in wound recovery.

Discussion and Conclusion: Our study has successfully developed the M-sheet, a nanofiber dressing incorporating an electrically stimulating redox membrane designed explicitly for healing wounds in individuals with diabetes. Our in vitro experiments showed that the M-sheet significantly enhanced cell migration, exhibited antibacterial properties, and demonstrated excellent biocompatibility. Furthermore, when applied to diabetic mice in our in vivo experiments, the M-sheet displayed remarkable outcomes regarding wound recovery. This research emphasizes the potential of utilizing external electrical stimulation, without the need for any additional equipment, to effectively treat chronic wounds. The findings also showcase the advancements in developing cutting-edge patches offering special wound recovery effects.

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A Novel Role of ADAMTS13 in Regulating Angiogenic Markers in Human Mesenchymal Stem Cells under Serum-Deprivation Stress

Ms Srishti DUTTA GUPTA¹, Dr Malancha TA¹

¹Indian Institute of Science Education and Research Kolkata, KOLKATA, India, ²Indian Institute of Science Education and Research Kolkata, KOLKATA, India

Title: A Novel Role of ADAMTS13 in Regulating Angiogenic Markers in Human Mesenchymal Stem Cells under Serum-Deprivation Stress

Authors: Srishti Dutta Gupta and Malancha Ta

Affiliations: Department of Biological Sciences, Indian Institute of Science Education and Research, Kolkata (IISER Kolkata), India.

Category: Stem Cells and Cell-Based Therapies

Introduction: The immunomodulatory and regenerative properties possessed by mesenchymal stem cells (MSCs) play a vital role in facilitating their widespread application in cell-based therapies. They secrete many trophic and bio-active factors which enable them to promote angiogenesis and neo-vascularization. However, post-transplantation, MSCs often encounter adverse micro-environmental conditions like nutrient-deprivation, hypoxia or an inflammatory milieu, which hamper their efficacy. Our study involved understanding the molecular mechanisms underlying angiogenesis in human umbilical cord-derived Wharton’s Jelly MSCs (WJ-MSCs) under serum-deprivation stress condition by identifying novel factors influencing this process. ADAMTS13, a protease responsible for cleaving the Von-Willebrand factor (vWF) and also known for its pro-angiogenic functions, was found to be a key player in our study.

Methods: MSCs, isolated from the WJ of human umbilical cord by explant culture method, were subjected to serum-deprivation stress. mRNA and protein expression levels of ADAMTS13 and some key angiogenic markers were determined by qRT-PCR and Western blotting experiments, respectively. Immunofluorescence staining was used to determine localization and expression patterns of ADAMTS13 and vWF. siRNA and inhibitor-based studies were conducted to investigate the role and regulation of ADAMTS13 in controlling some key angiogenic markers.

Results: We found that ADAMTS13 was upregulated under serum-deprivation stress in WJ-MSCs. The increase was also functionally validated by efficient disruption of vWF multimers in serum-deprived WJ-MSCs via immunofluorescence studies. Correspondingly, potent pro-angiogenic markers like VEGF, PDGF, IL-6 and TNF-α were also seen to be upregulated. Moreover, the p38 and JNK signaling pathways were found to be the negative and positive regulators of ADAMTS13 expression, respectively, in serum-deprived WJ-MSCs. Interestingly, when the WJ-MSCs were exposed to a more stringent nutrient stress condition comprising both glucose and serum starvation, a further increased expression for ADAMTS13, VEGF and PDGF was observed, while the regulation pattern remained the same. Further, siRNA-mediated knockdown of ADAMTS13 in serum-deprived WJ-MSCs led to considerable reversal in the expression of the angiogenic markers, suggesting that ADAMTS13 was playing a role in regulating their expression levels. Additionally, the results obtained from our study indicated that the Notch pathway and p53 could be the other probable factors controlling the expression of ADAMTS13 under serum-deprivation stress. Finally, our study also demonstrated that ADAMTS13 acted via the EphrinB2/EphB4 receptor tyrosine kinase axis, followed by ERK signaling, to modulate the expression patterns of the downstream angiogenic markers.

Discussion and Conclusion: Overall, our study highlighted ADAMTS13 as one of the key players regulating the expression of angiogenic markers in WJ-MSCs under serum-deprivation stress condition. Our observations lay the foundation of facilitating the prospects of ADAMTS13-based MSC therapy in treating cardiovascular and ischemic disorders.

Acknowledgements: This work was funded by IISER, Kolkata. We thank UGC, India for the fellowship of Ms Srishti Dutta Gupta.
Magnetically Assembled Endothelial Cell-Coated Spheroid for Vascularization

Ms. Dayeon ROO¹, Mr. Hodong SEOK², Ms. Kyoung-Ha SO³,⁴, Mr. Nathaniel Suk-Yeon HWANG¹,²,³,⁴

¹Interdisciplinary Program in Bioengineering, Seoul, Republic of Korea, ²Interdisciplinary Program in Stem Cell Biology, Seoul, Republic of Korea, ³School of Chemical and Biological Engineering, Seoul, Republic of Korea, ⁴Bio-MAX/N-Bio Institute, Institute of Bio-Engineering, Seoul, Republic of Korea

3D spheroids are often used as a therapeutic strategy and transplanted in vivo owing to their ability to better mimic the microphysiological environment. However, if the transplanted spheroids do not engraft into the host tissue and do not vascularize, long-term survival and functionalization of the transplanted spheroids are not guaranteed. Therefore, it is crucial for the transplants to be able to engraft and vascularize. Among various fabrication methods of a spheroid, such as the uses of a microwell, a microfluidic device, a spinner, and hanging drop, the use of magnetic force enables rapid cell aggregation and spatial regulation. Encapsulating a magnetic spheroid with endothelial cells paves the way toward vascularization. Here, we magnetically bioprinted vascular assembly by coating magnetic spheroids with the magnetic nanoparticles (MNPs)-internalized vascular endothelial cells. MNPs extracted from a bacteria strain called Magnetospirillum sp. AMB-1 were incubated with C2C12 murine myoblast cells and human umbilical vein endothelial cells (HUVEC) for internalization. MNPs-internalized C2C12 were first magnetically bioprinted by placing a neodymium magnet under a well plate. For these magnetic spheroids to be representative of a general transplant, their properties are eliminated by fixing spheroids. MNPs-internalized HUVEC were then added to encapsulate the fixed C2C12 spheroid. Internalization of MNPs did not hinder cell viability and vascularization-related gene expression. When transplanted, the vascular assemblies could sprout and engrafted into the host tissue within three days. In addition, they could reduce the necrosis level of the hindlimb ischemic model when transplanted intramuscularly. As these vascular assemblies are easily fabricated by coating spheroids with MNPs-internalized endothelial cells and controlled by a magnetic force, they may be used for engraftment and vascularization in various applications with spheroids with different functions.

Keywords: Magnetic Nanoparticles, Magnetosome, Cellular assembly, 3D spheroid, Engraftment, Vascularization, Transplantation, Hindlimb ischemia

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Enhancing the Functions of Vascular Endothelial Cells in vitro

Rikuto Sato¹, Katsuhisa SAKAGUCHI, Yusuke TOBE, Jun Homma, Hidekazu SEKINE, Kiyotaka IWASAKI, Tatsuya SHIMIZU
¹Waseda University, , Japan

Introduction: Functional vessel formation is essential for transplanting the engraftment of regenerated tissue and organ. However, the angiogenetic and antithrombotic functions of vascular endothelial cells in the regenerated tissues in culture remain largely unidentified. In this study, we aimed to identify whether increasing the passage number or altering the hardness of the culture surface affects the function of endothelial cells.

Subjects and Methods: To investigate whether the antithrombotic ability of human umbilical vein endothelial cells is maintained in culture, we evaluated changes in the antithrombotic ability of the cells after repeated subcultures. We also used culture dishes with different hardness to evaluate the effect of substrate hardness on antithrombotic activity. Thrombomodulin, tPA, and PAI-1 levels were analyzed using RT qPCR and ELISA.

Results: The thrombomodulin expression was measured using RT qPCR, and the expression decreased to less than half in P10 compared with that in P1. The P10 endothelial cells were cultured on the PDMS substrate (32 kPa) and a normal culture dish (3 GPa). The endothelial cells cultured on the PDMS substrate exhibited an approximately four-fold increase in thrombomodulin expression levels compared with those cultured on the normal culture dish.

Discussion and Conclusion: Our results suggested that the anti-thrombogenic potential of the cells decreases in culture depending on the gradual changes in each substance. The soft substrate tended to maintain the anti-thrombogenic potential of the cells. Collectively, we have proposed a novel method to enhance transplantation efficiency by preserving and enhancing the functional properties of functional endothelial cells in regenerated tissue.
Unique Good Manufacturing Practice (GMP) challenges for Advanced Therapy Products (ATP)

Dr Maggie Chow, Dr Gina Jiang

1Hong Kong Institute of Biotechnology, Hong Kong, Hong Kong SAR, 2Chinese University Institute of Biotechnology, Hong Kong, Hong Kong SAR, 3Office of Strategic Development, CUHK, Hong Kong, Hong Kong SAR

Advanced therapy products (ATPs) are rapidly expanding area of the biopharmaceutical industry that includes gene and cell therapies, immunotherapies and human tissue engineering. Producing ATPs in compliance with good manufacturing practice (GMP) guidelines poses unique challenges that require careful attention. Our ATP GMP Centre at the Hong Kong Institute of Biotechnology (HKIB) is designed according to the PIC/S GMP Standards to ensure quality, consistency and safety of ATP products.

As the ATP GMP biopharmaceutical industry is relatively new in Hong Kong, manufacturing ATPs under GMP standards presents several unique challenges, including (1) Complexity of the manufacturing process: The development and production of ATPs is a complex multi-steps process that involves manipulation of living cells, gene editing and tissue engineering. This complexity can lead to challenges in scaling up and maintaining consistency in quality and stability. (2) Need for specialized equipment and facilities with quality management system: The cleanroom facilities for production has to be designed according to regulatory requirements and continuously monitored to eliminate potential particles and microbiological contaminations. All production equipment must be validated and qualified for ATP manufacture. Quality control strategies are executed by trained operators to ensure the ATPs produced meet specifications. Hence, the cost associated with research and development, clinical trials and manufacturing can be significant. (3) Limited availability of trained personnel: There is currently a limited pool of trained personnel with specialized knowledge and skill in the manufacture of ATPs. This can be challenging to recruit and retain a highly-skilled team that can translate basic research into a commercialized ATP in the market.

To successfully address these challenges, it is essential to implement robust quality control measures, maintain facility cleanliness, operate strictly according to procedures and use validated and qualified manufacturing equipment. Future collaboration and support from industry, funding agencies, academia, clinicians and regulatory department will certainly promote and accelerate the development and production of safe and effective ATPs to benefits patients and their families in needs.
A “Nonsolvent Quenching” Strategy for 3D Printing of Polysaccharide Scaffolds with Immunoregulatory Accuracy

Mr. Zhencheng Liao¹, Chunming Wang¹,²

¹State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medicine & Department of Pharmaceutical Sciences, Faculty of Health Science, University of Macau, Macau SAR, , ²Zhuhai UM Science & Technology Research Institute (ZUMRI), Hengqin, China

Title: A “Nonsolvent Quenching” Strategy for 3D Printing of Polysaccharide Scaffolds with Immunoregulatory Accuracy

Authors: Zhencheng Liao1, Chunming Wang1,2 *

Affiliations: 1State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medicine & Department of Pharmaceutical Sciences, Faculty of Health Science, University of Macau, Taipa, Macau SAR. 2Zhuhai UM Science & Technology Research Institute (ZUMRI), Hengqin, Guangdong, China (*Correspondence)

Category: Design and Application of Biomaterials

Background: Polysaccharides are a major biomaterial category with inherent immunomodulatory activities; but without modification, they cannot form stable 3D structures upon extrusion-based printing, which renders their immune-regulatory potential underexploited for biomaterial design. Inspired by “quenching” from metal processing, we speculated that when the polysaccharide solution is extruded in filaments into a nonsolvent, the outer layer of the filament may immediately become supersaturated and solidify, forming a framework to lock the water inside the filament. As such, the nonsolvent “quenches” the polysaccharide filament, utilizing the force of water within the filament to maintain the structure, so that the filament can continue to stack into the pre-designed shape without collapse.

Method: In this study, we first screened the appropriate non-solvent for different polysaccharides, optimized the printing conditions, and finally assessed how this method could endow polysaccharides with precise modulation of host immune responses in wild-type and knockout mice models.

Results: A “Nonsolvent Quenching” (NSQ) strategy enables the polysaccharides scaffolds into sophisticated structures with high shape fidelity at organ-relevant scales and a longer shelf-life. Furthermore, using a linear immunoactive polysaccharide (konjac glucomannan, GM) as an example, NSQ fabricated scaffolds with different grid spacing (1.5 and 2.5 mm, respectively) revealed distinct immuno-regulatory effects, mediated by differential activations of carbohydrate receptors (notably TLR2) and inflammatory cascades in mice.

Discussion and Conclusion: We have demonstrated a facile and generic strategy for high-fidelity 3D printing of polysaccharides with varying physicochemical characteristics without any chemical modification or physical blending, thereby potentially unmasking their accurate immunomodulatory activities for future biomaterials design. In addition, we found NSQ may also be an ideal strategy to prepare sacrificial templates of channel networks.
Regeneration of Gastrointestinal Fistulas Using Adipose-Derived Stromal Vascular Fraction Cells and 3D-Bioprinted Scaffold: A Promising Approach

Prof. Massimiliano Papi1,2, Dr. Ivo Boskoski1,2, Dr. Angelo Trivisonno1,2, Dr. Dania Nachira1,2, Dr. Giordano Perini1,2, Dr. Valentina Palmieri1,2,3, Mr. Alberto Augello1,2, Prof. Marco De Spirito1,2, Dr. Venanzio Porziella1,2

1Università Cattolica del Sacro Cuore, Roma, Lazio, 2Fondazione Policlinico Universitario "A. Gemelli" IRCSS, Rome, Lazio, 3Istituto dei Sistemi Complessi, CNR, Rome, Lazio

Title: Regeneration of Gastrointestinal Fistulas Using Adipose-Derived Stromal Vascular Fraction Cells and 3D-Bioprinted Scaffold: A Promising Approach

Authors: Massimiliano Papi1,2 *, Ivo Boskoski1,2, Angelo Trivisonno1,2, Dania Nachira1,2, Giordano Perini1,2, Valentina Palmieri1,2,3, Alberto Augello1,2, Marco De Spirito1,2, Venanzio Porziella1,2

Category: Tissue Engineering and Regeneration

• Introduction: Gastrointestinal fistulas (GIFs) are abnormal communications between the digestive tract or the adjacent organs that lead to severely reduced health-related quality of life and short survival. Therapy mainly relies on endoscopic surgical interventions, but patients often require prolonged hospitalization and may develop complications. Furthermore, surgery leads to inflammatory events that strongly affect efficiency of tissue regeneration and often cause production of reactive oxygen species (ROS), thus creating a harsh surrounding environment. Therefore, novel therapeutic strategies aimed at improving the quality of life and survival rate of patients are needed. Within this context, regenerative medicine and bioprinting techniques could have a remarkable impact on the management of GIFs. In recent times, the field of regenerative medicine has shown strong interest in Stromal Vascular Fraction (SVF). We propose a 3D-Bioprinting tissue engineering approach that combines polymeric scaffolds able to release a controlled amount of N-acetylcysteine (NAC) and SVF to restore the injured tissue.

• Subjects and Methods: Adipose SVF was obtained by mechanical emulsification of autologous adipose tissue, the adipose tissue was disrupted, with a consequent release of stromal cellular elements. A flexible copolyester filament was used for 3D printing. The scaffold was designed with a slight curvature to adapt better to the fistula's morphology and with holes to enable anchoring of the cellular bioink. Bioink was prepared by mixing SVF with alginate. To prepare solution of PVA-NAC, PVA was used at a fixed concentration (10 % w/v), while NAC was dissolved to reach 5.7 mM or 22.8 mM concentrations.

• Results: To evaluate release of NAC over time, we observed the percentage of mass loss on PVA-NAC scaffold and NAC released in solution (Figure f, g). Our data indicate no evident toxicity on cellular components of SVF both in terms of viability and cytotoxicity even at a relatively long-time exposure to scaffolds. Since NAC is known for its antibacterial properties, we tested the effect of NAC released from PVA-NAC on a gastrointestinal pathogen, E.coli. A significant inhibition in bacteria growth was observed after incubation with PVA-NAC, which reached 82 ± 3.3%. We tested the reduction of the reactive oxygen species on bioprinted SVF scaffolds. After bioprinting, SVF scaffolds had an increase in ROS production up to 161.6 ± 8.65% after 30 days. Differently scaffolds with an infill of PVA-NAC demonstrated a dose-dependent reduction in ROS production. We investigated the capability of scaffolds with PVA-NAC infill to induce migration of endothelial cells. Supernatant of SVF scaffolds with in fill of PVA-NAC induced a significantly dose-dependent increased migration of endothelial cells, up to 145.3 ± 13.1%.

• Discussion and Conclusion: Polymeric scaffolds modified with the addition of the water-soluble PVA, with the bioactive compound NAC and SVF represent a non-immunogenic and innovative method characterized with multifunctional effects. Enrichment of PVA with NAC significantly reduce production of ROS by ADSCs during differentiation in the measured timespan of one month. In addition, it allowed to significantly modulate ROS production following induced oxidative stress which mimics the inflammatory state of the post-surgical harsh environment. Finally, we measured an increased migration rate of endothelial cells towards SVF scaffolds with an infill of PVA-NAC. This
augmented migration resulted significant with respect to control SVF scaffolds particularly on PVA enriched with NAC at the highest tested concentration. These findings could promote reconstitution of physiological tissues by potentially increasing formation of novel blood vessels in injured regions.

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D-Galactose Induced Ageing Model In C57BL/6 Mice

Ms. Vick Key Tew¹, Mr Elancheleyen MAHINDRAN¹, Dr Jia Xian LAW¹, Dr Fazlina NORDIN¹, Dr Jen Kit TAN², Prof Dr Kalavathy RAMASAMY³, Assoc Prof Dr Angela Min Hwei NG¹

¹Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Cheras, Malaysia, ²Biochemistry Department, Faculty of Medicine, Universiti Kebangsaan Malaysia, Cheras, Malaysia, ³Faculty of Pharmacy, Universiti Teknologi Mara, Bandar Puncak Alam, Malaysia

Authors: Vick Key Tew¹, Elancheleyen Mahindran¹, Jia Xian Law¹, Fazlina Nordin¹, Jen Kit Tan², Kalavathy Ramasamy³, Angela Min Hwei Ng¹*

Affiliations: ¹Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Malaysia.
²Biochemistry Department, Faculty of Medicine, Universiti Kebangsaan Malaysia, Malaysia.
³Faculty of Pharmacy, Universiti Teknologi Mara, Malaysia.
*Corresponding author’s email: angela@ppukm.ukm.edu.my

Category: Tissue Engineering and Regeneration

Introduction: The increasing number of elderly individuals worldwide has prompted numerous research studies aimed at understanding the ageing process and enhancing the quality of life for older people. This surge in research on therapeutic interventions for anti-ageing purposes has created a demand for cost-effective animal models of ageing. To simulate the physiological changes associated with ageing, researchers commonly use D-galactose, a substance that induces ageing-like effects in animals when administered over an extended period. Elevated levels of D-galactose in the body contribute to the accumulation of reactive oxygen species (ROS), resulting in the onset of oxidative stress, inflammation, and apoptosis. These effects can manifest in symptoms resembling the aging process. (1)

Methods: In our study, we administered 150mg/kg of D-galactose (n=24) or saline (n=8) (as control group) via subcutaneous injection to C57BL/6 mice on a daily basis for 8 weeks. Subsequently, the dosage of D-galactose was increased to 200mg/kg for an additional 4 weeks. We evaluated the condition of the mice using the Morris water maze, grip test, and rotarod performance test.

Results: Contrary to expectations, the mice injected with D-galactose did not exhibit decline in cognitive and motor functions compared to the mice injected with saline. Our findings indicate that the subcutaneous injection of 150mg/kg followed by 200mg/kg of D-galactose over a total of 12 weeks did not induce ageing-like effects in our C57BL/6 mice.

Discussion: We aimed to investigate whether the elevated metabolism rate in our C57BL/6 mice allowed them to efficiently process the high levels of D-galactose in their bodies, thereby preventing the manifestation of aging-like symptoms. Consequently, we are interested in determining whether administering a higher dosage of D-galactose would induce aging-like effects in C57BL/6 mice. In future studies, we intend to explore the impact of D-galactose on the aging process of C57BL/6 mice by increasing the dosage to 300mg/kg. Additionally, we will include additional analyses such as novel object recognition, cytokine measurements, and faeces testing. These additional measures will provide further insights into the impact of D-galactose on the ageing process in C57BL/6 mice.

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Acknowledgement: This work is supported by the research grant from Regena Asia Sdn Bhd (FF-2021-030) and Universiti Kebangsaan Malaysia (GUP-2020-024).
Plasma Enhanced-CVD Deposited Nanodiamonds Coated Ti-Implants for Preventing Implant-Associated Infections and Better Host Cell Response

Mr. Sumanta Ghosh, Mr. Shuyu Bu, Dr. Wenjun Zhang, Dr. Prasanna Neelakantan, Dr. Will Wei Qiao

1Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, 2Center of Super-Diamond and Advanced Films (COSDAF), Department of Materials Science and Engineering, City University of Hong Kong, Hong Kong SAR, 3Center of Super-Diamond and Advanced Films (COSDAF), Department of Materials Science and Engineering, City University of Hong Kong, Hong Kong SAR, 4Department of Endodontics, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, USA, 5Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR

Background: Implant-associated infection, namely peri-implantitis, is one of the most prominent factors for dental implant failure. In our previous study, we demonstrated that the nanodiamonds exhibited excellent antimicrobial activity against both bacterial and fungal cells in planktonic as well as pre-formed biofilms. Herein, this study aims the potential of Plasma Enhanced Chemical Vapor Deposition (PECVD) technique as a facile strategy for creating the in-situ nanodiamonds (NDs) coating over conventional titanium (Ti) for eliciting an antifouling, antibiofilm, and biocompatible coating for better cell-to-implant integration.

Methods: NDs-coated Ti specimens with different methane (CH4) and hydrogen (H2) ratios were prepared through the PECVD technique. After that, the surface and physiochemical properties of NDs-deposited Ti-implants were characterized by different techniques such as Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), contact angle, Raman and X-ray photoelectron Spectroscopy (XPS) and bioactivity assessment. Next, the antifouling, antimicrobial, and antibiofilm efficiency of the NDs-coating was tested against both mono-species and dual-species cross-kingdom biofilm models using various in-vitro assays such as ATP bioluminescence, Colony Forming Unit (CFU) Counting, Reactive Oxygen Species (ROS) quantification, XTT reduction and also imaged using Confocal Laser Microscopy and SEM. Furthermore, we have also evaluated the adhesion and proliferation of osteoblasts on NDs-coated Ti substrate compared to non-coated controls.

Results: XPS and Raman spectroscopy data revealed that the sp³/sp² hybridized carbon atom ratio and crystallinity were maintained after the coating. SEM and AFM data showed NDs were coated uniformly over the implant surface. The deposition of NDs contributed to a significant increase in surface roughness of around 126.34 nm relative to nearly 160 nm in the control group. This is also confirmed by water contact angle (WCA) analysis, as WCA decreased approximately from 84° to 66° after the NDs deposition. Compared with the non-treated control, NDs-coated Ti specimens have significantly higher microbial killing efficiency against mono-species C. albicans and P. gingivalis, respectively. Nonetheless, ~2.45 CFU log reduction has been observed on the NDs-Ti against the C. albicans mono-species biofilm, and XTT analysis confirmed the significant reduction against the dual species, cross kingdom biofilm. Lastly, the increased number of MC3T3-E1 osteoblast cells and enhanced F-actin production over the NDs-coated implants indicate biocompatibility, better cell proliferation and integration than the non-coated controls.

Discussion and Conclusion: This current study confirmed PECVD as a facile manufacturing process for the in-situ, uniform deposition of NDs over Ti-based medical implants, which can act as an antifouling, antibiofilm, biocompatible, and promote host cell proliferation for the prevention of peri-implantitis disease. Thus, the current study suggests PECVD deposited NDs can serve as a promising multifunctional coating for medical implants.
Folate-Modified Poly-beta-cyclodextrin Complexing with Indocyanine Green for Targeted Cellular Imaging

**Dr. Yuting WEN**¹ ² ³ *, Dr. Jianfeng WANG¹, Professor Zhiwei HUANG¹, Professor Jun LI¹ ² ³ ⁴

¹Department of Biomedical Engineering, National University of Singapore, Singapore, Singapore, Singapore, ²National University of Singapore (Suzhou) Research Institute, Suzhou, China, ³National University of Singapore (Chongqing) Research Institute, China, ⁴NUS Environmental Research Institute (NERI), National University of Singapore, Singapore, Singapore

**Title:** Folate-Modified Poly-β-cyclodextrin Complexing with Indocyanine Green for Targeted Cellular Imaging

**Authors:** Yuting Wen¹ ² ³ *, Jianfeng Wang¹, Zhiwei Huang¹, Jun Li¹ ² ³ ⁴

**Affiliations:** ¹ Department of Biomedical Engineering, National University of Singapore, Singapore 119276, Singapore. ² National University of Singapore (Suzhou) Research Institute, Suzhou, Jiangsu 215000, China. ³ National University of Singapore (Chongqing) Research Institute, Chongqing 401120, China. ⁴ NUS Environmental Research Institute (NERI), National University of Singapore, Singapore 117411, Singapore (* Correspondence)

**Category:** Design and Application of Biomaterials

**Background:** Indocyanine green (ICG) is an FDA-approved diagnostic agent that is widely used as a near-infrared (NIR) fluorescent imaging probe due to its low toxicity and excellent NIR absorption and emission properties [1]. However, the use of ICG in imaging and detection is limited due to its instability in aqueous solutions and lack of targeting properties [2]. Its relatively low photostability could affect image quality and limit long-term imaging experiments. Furthermore, ICG has limited tissue specificity and could accumulate in non-targeted areas, leading to reduced imaging specificity. It may also exhibit non-specific binding to proteins, potentially interfering with the interpretation of imaging results. To address these issues, there is an urgent need to develop a novel carrier that is simple to prepare, can stabilize ICG in an aqueous state, improve its photostability, and enhance its targeting properties.

**Method:** In this study, we develop a tumor-targeted ICG encapsulated cyclodextrin (CD)-based inclusion complex rationally assembled from ICG and folate-modified poly-β-CD (ICG@PCD-FA) for enhanced targeted cell imaging. The absorbance, stability, tumor-targeted imaging capacity of encapsulated ICG is evaluated.

**Results:** The results show that the absorbance and stability of ICG are significantly improved after encapsulation in PCD-FA. The CD in the carrier plays a crucial role as a protective cave to prevent aggregation and improve the water and optical stability of ICG. The conjugated ligands FA in the system endow ICG with tumor-targeted ability. Specifically, ICG@FA-PCD exhibits enhanced performance in cell permeation in three-dimensional spheroids and highlights folate receptor positive cells under NIR endoscopic observations.

**Discussion and Conclusion:** In this study, a tumor-targeted ICG encapsulated CD-based inclusion complex was successfully developed for enhanced targeted cell imaging. The use of CD in the carrier as a host molecule for ICG can protect it from aggregation and improve its water and optical stability. The inclusion complex with folate-modified PCD demonstrated increased tumor-targeted ability due to the presence of the conjugated ligand FA, which enhances the specificity and sensitivity of the imaging agent for cancer cells. These findings suggest that ICG loaded in folate-modified PCD complex has significant potential in tumor NIR imaging and diagnosis. The developed system provides an innovative approach to improve the performance of ICG for biomedical applications and may open...
up new avenues for the development of other stable and targeted imaging agents. Further research can focus on optimizing the system to enhance the imaging contrast and sensitivity and expand its clinical applications.

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Thermo conductive carbon nanotube framed 3D network biomaterials for smart drug delivery system

Ms. Ji-hye Kang¹, Mr Ueon sang Shin

¹Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Choenan, South Korea

Introduction: Biomaterials that achieve drug release in a controlled manner have been intensively developed over the last few decades. Among them, various external (e.g., thermal or electrical) responsive materials have pharmacological advantages that include improved precise drug delivery by simply switching the stimulus on or off. However, achieving highly uniform distribution of the hydrophobic conductive materials remains a challenge, and is essential in achieving a well-connected electrical and thermal conducting path.

Subjects and Methods: Thermally and electrically responsive biomaterials were developed for application in a smart drug delivery system. Membranes and hydrogels were prepared by self-assembly based on carbon nanotubes (CNTs) as the thermal/electrical conductor and core unit, chitosan (Chit) as a hydrophilic glue and shell unit, and then by chemical integration of temperature-responsive copolymer (pNIBIm) as a drug carrier and additional outer shell.

Results: This study investigated a three-dimensional interwoven porous nanostructure for controllable drug delivery, showing efficient network exhibited high surface area and high storage modulus, uniform heat distribution, and high electrical conductivity. Uniform distribution and connectivity of biomaterials were improved by formulating the triple core-shell structure, which clearly showed the thermo- and electric- responsive swelling and deswelling characteristics of the triple core–shell structured CNT-frames. And these drug carriers exhibited excellent controllable and switchable drug delivery with remarkable thermal and electrical stimulus-responsive properties.

Discussion and Conclusion: These varied core-shell type approaches were each able to improve drug efficiency and all went beyond the limit in the smart control of drug release including recognition and decision concerning the drug release timing and amount under different conditions. The physiochemical, mechanical, electrical, thermal, and biocompatibility characteristics of biomaterials made of core-shell type led to remarkable electrical- and thermal-responsive properties. And that allowed for the development of an excellent controllable and switchable drug delivery platform for biomedical engineering and medicine applications.
Generation Of Myocardial Infarction Model Using Human iPSC-derived Cardiac Organoids For Drug Efficacy Testing

Mrs. Su-Jin Lee¹, Ms. Eunji Kim¹, Mrs. Hyang-Ae Lee¹
¹Korea Institute of Toxicology (KIT), Deajeon, South Korea

Background: Myocardial infarction (MI) is a significant cardiac condition characterized by fibrotic replacement of myocardial cells following stress-induced heart damage. Due to their limited regenerative capabilities, myocardial cells are considered to have restricted regenerative potential. Animal models used to study MI have inherent limitations in replicating human physiological functions. In recent years, there has been growing interest in developing efficient disease models using human induced pluripotent stem cells (iPSCs).

Methods: In this study, we successfully generated an MI model using iPSC-cardiac organoids (iPSC-hCOs) through the manipulation of pathophysiological factors, including oxygen levels and fibrosis-inducing factors. We comprehensively assessed the morphological, functional, and genetic changes in the generated MI model through morphology analysis, measurement of Ca²⁺ transients, contractility analysis, field potentials (FP) measurement using multi-electrode arrays (MEA), and gene expression analysis.

Results: A significant decrease in FP amplitude was observed in the MI model compared to the normal model. Quantitative PCR (qPCR) analysis revealed a notable downregulation of major ion channel expression and impaired expression of maturation-related genes in the MI model. Moreover, the evaluation of verapamil, a calcium channel blocker, and ranolazine, a late sodium activator, in the MI model confirmed their efficacy in promoting functional recovery, as evidenced by the modulation of fibrosis-related gene expression, contractility improvement, and analysis of Ca²⁺ transients.

Discussion and conclusion: We successfully established and characterized an MI model using iPSC-hCOs. By employing ranolazine and verapamil, we were able to enhance myocardial cell functionality and promote regeneration. The developed model holds great potential for studying myocardial infarction and represents a valuable tool for screening potential therapeutic agents targeting MI.

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HIF1A knockdown in MSCs affects the regulation of histone H3K9 methylation under hypoxia in vitro

Ms. Polina BOBYLEVA, Ms. Ekaterina TYRINA

1Institute of Biomedical Problems, RAS, Moscow, Russia

Authors: Polina Bobyleva, Ekaterina Tyrina

Affiliations: Institute of Biomedical Problems, RAS, Moscow, Russia

Category: Stem Cells and Cell-Based Therapies

Background: Realization of the regenerative potential of MSCs is associated with their functioning under conditions of ischemia, while a reduced level of O2 modifies a number of properties of MSCs due to the HIF-1-dependent mechanism. At the same time, other oxygen-sensitive molecules, particularly histone methylases and demethylases, including EHMT2/G9A, KDM3 and KDM4, which regulate the degree of chromatin accessibility by the mean of histone methylation, also control MSC functions. The study aims to reveal the patterns of functioning of the oxygen-dependent regulators HIF-1α and histone methylases/demethylases (EHMT2/G9A, KDM3 and KDM4) in MSCs, which is essential for understanding the fundamental mechanisms of cellular response to hypoxic conditions.

Methods: The experiments were performed in vitro using MSCs from human umbilical cord. In order to evaluate the effects of HIF-1α, HIF1A mRNA was inhibited by RNA interference technique. Then, the MSCs were exposed to hypoxia (1% O2) for 1 h and 24 h. After that quantitative PCR was performed to assess the effects of HIF1A inhibition and hypoxic conditions on the expression of genes encoding epigenetic regulatory factors, such as KDM3A, KDM4A, EHMT2. The levels of KDM3A, KDM4A, EHMT2/G9a proteins and mono-/dimethylated forms of histone H3K9 were analyzed using flow cytometry.

Results: The transcriptional response of umbilical cord MSC genes involved in epigenetic regulation through H3K9 methylation was more pronounced after 24 h incubation at pathological hypoxia (1% O2) compared with acute (1 h) hypoxic exposure, with KDM4A and EHMT2 but not KDM4A regulated HIF-1-dependently, both in hypoxia and in normoxia. The levels of KDM3A, KDM4A, and EHMT2/G9a proteins determining H3K9 methylation were HIF-1-dependently upregulated in MSCs during 24 h exposure to pathological hypoxia and were less regulated during acute hypoxia. HIF-1-dependent regulation of KDM3A, KDM4A, and EHMT2/G9a in MSCs also took place under normoxia.

Pathological hypoxia stimulated mono- and dimethylation of H3K9 in MSCs, in the case of monomethylation it was HIF-1-dependent and more pronounced at 24 h of hypoxic exposure than at acute (1 h) hypoxia. HIF-1 also regulated mono- and dimethylation of H3K9 in MSCs from the umbilical cord under normoxic conditions.

Discussion and Conclusion: Thus, oxygen-dependent regulation involving HIF-1 mediates the control of umbilical cord MSC transcriptional activity by engaging in the regulation of H3K9 repressive methylation through modification of the levels of mRNA and the corresponding methylase and demethylase proteins. Along with this, the HIF-1-independent mechanism regulating H3K9 dimethylation plays a significant role. For the examined cells, HIF-1-dependent regulation of methylation occurs not only under hypoxic conditions, but also in normoxia.

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A HPLC Method for Quantifying Active Molecules in Drug-Loaded 3D Printed Porous Composite Scaffold

Mrs. Ling Li¹, Mrs Cui Shan Huang, Ms Xiao Yan Lai, Dr. Xiang Bo Meng, Dr. Ling Qin, Dr. Xin Luan Wang
¹Shenzhen Institute Of Advanced Technology, Chinese Academy Of Sciences, , China

Background: Bioactive molecules play an essential role in tissue engineering as they promote tissue regeneration as growth factors. Low temperature 3D printing technology allows for the fabrication of drug-loaded porous composite scaffolds. The content and release rate of bioactive molecules in these tissue engineering materials are crucial factors to consider. This study aimed to establish a high-performance liquid chromatography (HPLC) method for quantifying active molecules in drug-loaded PLGA/TCP composite scaffolds made by low-temperature 3D printing.

Methods: Puerarin was used as the model drug in this study. It was mixed with poly (lactic-co-glycolic acid)/β-calcium phosphate to form a porous PLGA/TCP/Puerarin (PTP) composite scaffold by 3D printing, which facilitated bone defect repair by promoting angiogenesis and osteogenesis [1]. 50 mg of the scaffolds were weighed and dissolved in 2 ml of DMF. Then, 2 ml of methyl alcohol was added to the tube, mixed, centrifuged, and transferred the supernatant to a 10 ml volumetric flask. The quantitative analysis was performed using an LC-20A HPLC system.

Results: Puerarin separation and analysis were conducted on an Agilent Zorbax Eclipse XDB-C18 column (4.6×250 mm, 5 μm) with a flow rate of 1 ml/min at 25 °C. The mobile phase consisted of solvent A (glacial acetic acid water) and solvent B (methyl alcohol). A gradient of 23% B from 0-30 min, 100% B from 30.01-40 min, and 23% B from 40.01-55 min was established. The selectivity, LLOQ (0.15 μg/ml), linearity, extraction recovery, and matrix effect of this method met the actual sample test requirements of the Chinese Pharmacopoeia 2020 (Table 1).

Conclusion: A quantitative method was established and validated for quantifying active molecules in drug-loaded scaffolds. This method is crucial in ensuring the safe and effective translation of these scaffolds into clinical applications.

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References:
Bioactive hydrogel facilitates the seamless integration of grafts for osteochondral defects after autologous mosaicplasty

Dr. Hongwei Wu¹ ², Mr. Wei Sun¹ ², Prof. Hongwei Ouyang¹ ²
¹Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative Medicine, and Department of Orthopedic Surgery of the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China; ²Zhejiang University-University of Edinburgh Institute, Zhejiang University School of Medicine, and Key Laboratory of Tissue Engineering and Regenerative Medicine of Zhejiang Province, Zhejiang University School of Medicine, Hangzhou, China

Title: Bioactive hydrogel facilitates the seamless integration of grafts for osteochondral defects after autologous mosaicplasty.

Authors: Hongwei Wu¹ ², Wei Sun¹ ², Hongwei Ouyang¹ ²

Affiliations: ¹Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative Medicine, and Department of Orthopedic Surgery of the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China; ²Zhejiang University-University of Edinburgh Institute, Zhejiang University School of Medicine, and Key Laboratory of Tissue Engineering and Regenerative Medicine of Zhejiang Province, Zhejiang University School of Medicine, Hangzhou, China

Category: Tissue Engineering and Regeneration

Background: Autologous mosaicplasty is frequently used in treating osteochondral defects. Gap integration is one of the key factors influencing the surgical outcome which requires seamless integration of bone and hyaline cartilage regeneration between the host and transplanted plus. In order to improve the integration and achieve the repaid restoration of osteochondral tissue, we developed an effective system that could significantly enhance the integration of the grafts and host tissue post-mosaicplasty.

Methods: Injectable gelatin methacryloyl (GelMA) hydrogel loaded with bioactive supramolecular nanofiber (BSN-GelMA) was designed to seal the junction rapidly. New Zealand rabbit osteochondral defect model was used to demonstrate the efficacy of the BSN-GelMA system.

Results: We found that the BSN-GelMA system could achieve rapid and seamless healing in the gap region between plugs of osteochondral defects following mosaicplasty in six weeks. We further evaluated the histology score, glycosaminoglycan content, bone volume, and collagen II expression level, in which the BSN-GelMA system showed a significantly better outcome than GelMA and blank group indicating that these improved results were caused by bio-interactive materials, which acted as tissue fillers to bridge the gap, prevent cartilage degeneration, and promote cartilage survival and migration of bone marrow mesenchymal stem cells by releasing bioactive supramolecular nanofibers from the GelMA hydrogel.

Discussion and Conclusion: This study provides a powerful and applicable approach to improve gap integration after autologous mosaicplasty. It is also a promising off-the-shelf bioactive material for cell-free in situ tissue regeneration.

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Glycosaminoglycans composition affects outcomes of multiple differentiation of hMSCs

Dr. Xingxing YANG1, Prof. Barbara CHAN1,2,3
1 Tissue Engineering Lab, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China, 2 Advanced Biomedical Instrumentation Centre, Hong Kong, China, 3 School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China

Title: Glycosaminoglycans composition affects outcomes of multiple differentiation of hMSCs

Authors: Xingxing Yang1, Barbara Pui Chan1,2,3 *
1 Tissue Engineering Lab, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China
2 Advanced Biomedical Instrumentation Centre, Hong Kong, China
3 School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China

* Email: bpchan@cuhk.edu.hk

Category: Tissue Engineering and Regeneration

Background: Mesenchymal stem cells (MSCs) are clinically important cell sources as they have the advantages of immunosuppressive potential, easy harvesting, and a strong capacity to self-renewal and differentiation potential that allow differentiation into various types of cells, such as osteoblasts, chondrocytes, and nucleus pulposus cells. Glycosaminoglycans (GAGs) play a vital role in binding and organizing water and regulating cell proliferation and differentiation, mimicking the GAG composition is expected to benefit to outcomes of hMSCs differentiation.

Methods: In this work, scaffolds with different GAGs compositions (GAG/HYP ratio 0-20:1) were fabricated. The effects of these GAG-incorporated materials on the differentiation of human mesenchymal stem cells (hMSCs) into osteogenic, chondrogenic, and discogenic lineage were compared.

Results: The Col group without any GAG incorporation, showed higher calcium (Ca) deposition, and osteogenic phenotypic expression. The aCol-GAG, which has similar GAG content to the cartilage, showed the lacuna structures and intensive GAGs and higher expression of chondrogenic markers. The aCol-aHA-GAG (GAG/HYP 20:1) showed intensive GAG deposition and higher phenotypic expression of NPCs.

Discussion and Conclusion: This work demonstrated the importance of GAGs in the differentiation of MSCs and the scaffolds compositionally simulate the native tissue can promote the differentiation of hMSCs into specific lineage.

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Development Of A 3D Organ-on-chip Model Of The Collecting Duct For The Study Of Kidney Diseases

Dr. Alessandra Grillo¹, Miss Chutong Zhong¹, Dr Keith Siew¹, Prof Stephen Walsh¹
¹London Tubular Centre, Department of Renal Medicine, London, United Kingdom

Introduction: Kidney basement membrane components play a crucial role in the function of different segments of the nephron. An example of this mechanism is hensin, an ECM protein that has shown to promote transition between α and β intercalated cells, stimulating different mechanisms of action in the collecting duct (CD). Current models using organ-on-chip systems mainly use collagen I as scaffold, without considering segment-specific compositions. Additionally, most kidney models reproduce proximal tubule systems, with no current 3D model of the collecting duct to study physiological mechanisms in vitro. Therefore, the aim of the study is to develop a 3D model of the collecting duct using organ-on-chip system by integrating more biomimetic ECM components and physiological cues.

Methods: M1-CCD cells from collecting duct were cultured on the top channel of a three-lane organ-on-chip systems (OrganoPlate, Mimetas) to produce tubular structures, where the middle channel was filled with a permeable ECM formed by collagen I. Different combinations of collagen I, collagen IV and laminin I were used as ECM scaffold and the newly-formed tubule was tested for typical CD markers (pendrin, NKCC1, ENaC) to investigate their expression in relation to the ECM changes. Human umbilical vein endothelial cells (HUVEC) were seeded on the bottom channel to mimic the renal vascular system in contact with the CD.

Results: M1-CCD showed intrinsic ability to form 3D structures when seeded on low-attachment plates. M1-CCD were then cultured on the OrganoPlate system including different combinations of basement membrane proteins, such as collagen IV and laminin, to the recommended collagen I, in order to create a more biomimetic environment for collecting duct epithelial cells. M1-CCD cells successfully formed a tubular structure as shown in Figure. The bottom channel was cultured with endothelial cells and transport of small ions was evaluated between the two channels.

Discussions and Conclusions: we successfully developed and characterised a 3D in vitro model of the collecting duct using the organ-on-chip technology and M1-CCD cell line. Further steps include the incorporation of patient urine-derived epithelial and blood-derived endothelial cells to create a more accurate and personalised platform for disease modelling and drug testing.

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Surface Modification of Lipid Nanoparticles by Poly(2-methacryloyloxyethyl phosphorylcholine) for Improved Cellular Uptake in Gene Delivery

Ms. Chitinart Thedrattanawong¹, Dr. Yuting Wen¹,²,³, Prof. Jun Li¹,²,³,⁴

¹Department of Biomedical Engineering, National University of Singapore, Singapore, Singapore, ²National University of Singapore (Suzhou) Research Institute, Suzhou, China, ³National University of Singapore (Chongqing) Research Institute, Chongqing, China, ⁴NUS Environmental Research Institute (NERI), National University of Singapore, Singapore, Singapore

Title: Surface Modification of Lipid Nanoparticles by Poly(2-methacryloyloxyethyl phosphorylcholine) for Improved Cellular Uptake in Gene Delivery

Authors: Chitinart Thedrattanawong¹, Yuting Wen¹,²,³, Jun Li¹,²,³,⁴ *

Affiliations: ¹Department of Biomedical Engineering, National University of Singapore, Singapore 119276, Singapore. ²National University of Singapore (Suzhou) Research Institute, Suzhou, Jiangsu 215000, China. ³National University of Singapore (Chongqing) Research Institute, Chongqing 401120, China. ⁴NUS Environmental Research Institute (NERI), National University of Singapore, Singapore 117411, Singapore (* Correspondence)

Category: Design and Application of Biomaterials

Background: Lipid nanoparticles (LNPs) are a type of nanocarrier system that has gained significant attention in the field of gene delivery. LNPs consist of a lipid bilayer structure composed of cationic or ionizable lipids, along with other components such as phospholipid, cholesterol, and polyethylene glycol (PEG). Each phospholipid has a unique geometrical packing characteristic that influences the shape of the lipid molecule. For example, DOPE has this parameter close to one, resulting in a cylinder shape, but DSPC has the parameter value less than one, resulting in an inverted cone structure. The shape of phospholipid also has the effect on the cellular uptake of LNPs. In the context of COVID-19, LNPs have been extensively utilized in the development of mRNA-based vaccines, including the Pfizer-BioNTech and Moderna COVID-19 vaccines. These vaccines employ LNPs to deliver synthetic mRNA encoding the spike protein of the SARS-CoV-2 virus into cells. LNPs have demonstrated several advantages in gene delivery, including high biocompatibility, efficient cellular uptake, and low immunogenicity. Their versatility and relatively easy manufacturing process have made them an attractive option for rapid vaccine development, as demonstrated during the COVID-19 pandemic. However, it still has some limitations including lack of stability and quick removal from blood circulation. PEG, which was used in the Pfizer-BioNTech and Moderna COVID-19 vaccines, is widely used for modifying surface of LNPs to enhance stability and protect them from protein absorption. Nonetheless, PEGylated LNPs are cleared rapidly from blood circulation upon repeated injections because the immune system produced anti-PEG antibodies during the first dosage of PEGylated LNPs injection, a process known as accelerated blood clearance (ABC) which leads to low efficiency of cellular uptake. To overcome the limitation of PEG, the alternative polymer was found to replace PEG. Poly(2-methacryloyloxyethyl phosphorylcholine) (pMPC) is a zwitterionic non-fouling polymer that has gained significant attention in the field of biomaterials. When used as a surface coating for nanoparticles, pMPC offers several important functions [1], including non-fouling property, reduced immunogenicity, enhanced circulation time, and improved stability. Most importantly, it could improve the cellular uptake of LNPs due to its cellular membrane-like structure. To solve the problem of PEGylated LNPs, pMPC could be used to develop LNPs to enhance the cellular uptake and transfection efficiency.

Methods: In this study, we compared pMPC modified LNP formulations developed in our research with those of Pfizer-BioNTech. The Pfizer-BioNTech LNPs, referred to as DSPC/lipid(C14)-PEG2k LNPs, comprised of ALC-0315, lipid(C14)-PEG 2kDa, DSPC, and cholesterol. Our developed LNPs, termed
DOPC/palmitoyl(C16)-pMPC4.5k LNPs, incorporated ALC-0315, palmitoyl(C16)-pMPC 4.5 kDa, DOPC, and cholesterol. The palmitoyl(C16)-pMPC component was synthesized by atom transfer radical polymerization (ATRP) utilizing palmitoyl-Br as initiator. Genetic materials, including pDNA, mRNA and siRNA, were encapsulated into LNPs through the following process. The lipid components were dissolved in an ethanol solution, which was then injected into an aqueous buffer solution containing nucleotides to form the LNPs [2]. The cellular uptake of the LNPs was accessed using confocal microscopy and flow cytometry, while transfection efficiency was evaluated through fluorescence microscopy.

Results: In vitro transfection of LNPs was conducted using HeLa cells to assess the efficacy of LNPs. Cells were treated with different LNPs in incomplete DMEM medium for 6 hours and observed under a fluorescence microscope. The results showed that pMPC-modified LNPs exhibited superior transfection efficiency compared to PEG-modified LNPs for all tested genetic materials. To evaluate in vitro cellular uptake, the DOPC/palmitoyl(C16)-pMPC4.5k LNPs, identified as the most effective formulation based on transfection efficiency, were compared with Pfizer formula, DSPC/lipid(C14)-PEG2k LNPs. The particles were incubated with HeLa cells for 1, 2, and 6 hours. Consistent with the transfection results, the data indicated pMPC-modified LNPs exhibited higher cellular uptake compared to PEG-modified LNPs, suggesting pMPC could be enhance the cellular uptake and transfection efficiency of LNPs.

Discussion and Conclusion: The DOPC/palmitoyl-pMPC4.5k LNPs showed superior performance in terms of both transfection and cellular uptake compared to DSPC/lipid-PEG2k LNPs. This might be attributed to chemical similarity between pMPC and the phosphorylcholine group, which is a constituent of cell membrane and known to enhance cellular binding [1]. This study highlights pMPC as a promising alternative polymer for LNP modification, offering increased efficiency in cellular uptake.

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Reference:
3D-printed implant-derived versatile Mg function as favorable biochemical cues for endogenous stem cells-mediated dual-lineage regeneration of osteochondral defect

Mr. Liangbin Zhou¹², Prof. Jiankun Xu¹, Dr. Wenxue TONG¹, Dr. Lizhen Zheng¹, Prof. Chunyi Wen², Prof. Yuxiao Lai³, Dr. Kevin Ki-wai Ho¹, Prof. Ling Qin¹³
¹Musculoskeletal Research Laboratory of Department of Orthopaedics & Traumatology, Innovative Orthopaedic Biomaterials and Drug Translational Research Laboratory, Li Ka Shing Institute of Health Sciences, Faculty of Medicine, The Chinese University of Hong Kong, , Hong Kong SAR, ²Department of Biomedical Engineering, Faculty of Engineering, The Polytechnic University of Hong Kong, , Hong Kong SAR, ³Centre for Translational Medicine Research and Development, Shenzhen Institute of Advanced Technology, The Chinese Academy of Sciences, Shenzhen, China

Osteochondral defects (OCD) frequently occur in the knee joints because of trauma and arthritis. However, due to cartilage’s limited self-healing capability, OCD repair is still a great challenge despite various approaches that have been proposed and developed. Here, we designed a one-step strategy to induce in situ OCD repair using acellular 3D-printed Mg microparticles-functionalized scaffolds, thereby creating an appropriate microenvironment for initiating and magnifying the healing roles of endogenous stem/progenitor/stem cells (ESPCs) with an easy surgical application. The well-designed Mg-based scaffolds exhibited a porous structure with 82.9 ± 1.9% porosity, 100% connectivity, and 553.5 ± 52.64 μm in mode pore diameter. The C, O, P, Ca, and Mg elements of the 3D-printed scaffolds were evenly distributed. The incorporation of Mg microparticles could significantly improve Young's modulus (82.8 ±14.1 MPa) and compressive strength (3.1 ± 0.2 MPa) of the PTM scaffolds compared with the PT scaffolds (45.7 ± 5.4 MPa, and 1.5 ± 0.1 MPa respectively). For both PT and PTM scaffolds, no in vitro cytotoxicity effect was observed with MC3T3-E1 cells and BMSCs, and in vivo biosafety was confirmed by hematological analysis (serum Ca, P, Mg ions, and Cre, Urea, ALB, GLB, ALT, and AST levels) and histopathological analysis of the systematic toxicity of major organs. Compared with the control group, after 4, 8, and 12 weeks of implantation, the PTM scaffolds showed pain-alleviating effects and could markedly improve the gross appearance of defect sites, regenerate surface area (%), ICRS macroscopic scores, and Oswestry macroscopic scores. A similar trend was found in high-frequency ultrasound imaging and IAUS assessment. The micro-CT imaging and quantitative data of BV/TV, BMD, and Tb.N indicated that PTM scaffolds could dramatically promote subchondral bone regeneration. Besides, the histological staining and IHC analysis revealed that the cartilaginous layer and subchondral bone layer were notably restored and repaired by the PTM scaffolds compared to the control group. Additionally, the pro-regenerative roles of implant-derived Mg as a favorable pro-proliferation factor, pro-recruitment factor, pro-differentiation factor, and anti-inflammation factor were fully elucidated and confirmed both in vitro and in vivo. In conclusion, we have 3D-printed a novel porous Mg-based bioactive composite scaffold with desirable biomechanical and biochemical properties. The PTM scaffolds could regenerate the articular cartilage and subchondral bone simultaneously via guiding the ESPCs, holding great potential as a one-step surgery strategy for challenging OCD repair.
Development of biomass-based bioinks through modifying okara for extrusion-based 3D printing of hydrogels

Dr Jingling ZHU¹,², Prof Jun Li¹,²
¹NUS Environmental Research Institute, National University of Singapore, Singapore, Singapore, Singapore,
²Department of Biomedical Engineering, National University of Singapore, Singapore, Singapore

Title: Development of biomass-based bioinks through modifying okara for extrusion-based 3D printing of hydrogels

Authors: Jingling Zhu,¹,² and Jun Li ¹,²*

Affiliations: ¹NUS Environmental Research Institute, National University of Singapore, 5A Engineering Drive 1, Singapore 117411, Singapore. ²Department of Biomedical Engineering, National University of Singapore, 15 Kent Ridge Crescent, Singapore 119276, Singapore (* Correspondence)

Category: Enabling Technologies

Background: Extrusion-based bioprinting is a rapid and effective method for three-dimensional (3D) printing of hydrogels with intricate shapes and functionalities for tissue engineering applications. However, the lack of suitable bioinks with good printability, shape retention, and mechanical strength has hindered the progress in 3D bioprinting of hydrogels. Advancements in materials that offer superior printability are crucial for overcoming this challenge and advancing the field.

Methods: In this work, we chemically modified biomass okara to produce a novel macromonomer to be formulated into photocurable bioinks. The rheological properties and printability of the bioinks were evaluated for extrusion-based 3D printing of okara-derived hydrogels.

Results: The bioinks formulated with the chemically modified okara demonstrated improved shear-thinning behavior and enhanced printability. Moreover, the mechanical strength of the UV-cured hydrogels was significantly increased with the incorporation of the chemically modified okara. Various 3D models, including grid models, cylinder models, and human ear models, were successfully printed using an extrusion-based 3D bioprinter.

Discussion and Conclusion: We demonstrated that the successfully modification of okara. The okara-derived inks exhibited higher viscosity and more pronounced shear-thinning behavior. The addition of the chemically modified okara improved the ink printability, enhanced the mechanical strength and shape stability of the printed materials. The current work demonstrated a promising strategy to design bioinks for printing objects with potential application in bioengineering.

Acknowledgement: This work was supported by the National Research Foundation, Singapore under its Intra-CREATE Thematic Grant “Science of Sustainable Cities (Food)”– NRF2020-THE003-0005.
Stable Mechanical Fixation of Osteochondral Scaffold Enhanced Overlying Cartilage Regeneration

Prof. Chaozong Liu

University College London, United Kingdom

Background: The treatment of osteochondral lesions has become a major concern in Orthopaedics because predominantly these defects do not heal spontaneously which make the joints susceptible to “early onset” secondary osteoarthritis [1]. Tissue engineering approaches have emerged for the repair of cartilage defects and damages to the subchondral bones and have shown potential in restoring joint’s function. However, tissue engineering scaffolds often fail to satisfactorily regenerate the bone and the native hyaline cartilage and result in the formation of inferior (in terms of mechanical properties) fibrocartilage [2], affecting the durability of the regenerated tissue. We have developed a functionally graded multi-layered scaffold to address large osteochondral defects, with focus on improving bone ingrowth and cartilage quality. This study investigated the efficacy of this scaffold in vivo following implantation in sheep knee.

Materials and Methods: The scaffold was fabricated using a combination of additive manufacturing and freeze-drying/critical drying techniques. Three layers of Ti matrix, PLA and collagen-PLGA were created to mimic subchondral bone, calcified cartilage and cartilage in native tissue, respectively. Multi-layered collagen/hydroxyapatite scaffolds were used as control. Ten sheep were operated on and either the scaffold (n=6) or the control (n=4) was implanted in the left medial condyle (Figure 1). The tissue was retrieved 12 weeks post-operation. Bone ingrowth into the titanium matrix and quality of the cartilage was assessed macroscopically, histologically and with the use of uCT.

Results and Discussion: It was observed that gross morphological appearance of regenerated cartilage was superior in osteochondral scaffold group compared to the control group. Collagen 2 and Safranin-O stainings confirmed formation of a hyaline-like cartilage. The pQCT examination revealed that the BV/TV ratio in the surrounding subchondral bone was significantly higher (p=0.01) in the osteochondral scaffold group (~40%) than that in the control group (~15%). The bone-scaffold contact analysis revealed the bone-implant contact achieved 61%. It is believed that the new bone growth into the Ti matrix at bone section provided a stable mechanical fixation providing a strong support to the overlying cartilage layer leading to an improved cartilage formation (Fig 1)

Conclusion: We have demonstrated from the in vivo study that bone ingrowth in the bone section of osteochondral scaffold resulted in a stable mechanical fixation of the osteochondral scaffold. This provided a strong support for the overlying cartilage layer and enhanced the cartilage regeneration.

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References:
The regulator of the synovial lymphatic system in acute ACL injury and repair: synovial fibroblast-derived IL-6 promotes the lymphatic endothelial cell barrier dysfunction through STAT3/ICAM1

Mingde Cao1,2, Xueyou Zhang1,2, Michael TY Ong1,2, Patrick SH Yung1,2,3, Yangzi Jiang1,2,3

1Department of Orthopaedics & Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, 2Center for Neuromusculoskeletal Restorative Medicine, Hong Kong Science Park; 3Institute for Tissue Engineering and Regenerative Medicine, School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR

Authors: Mingde Cao1,2, Xueyou Zhang1,2, Michael TY Ong1,2, Patrick SH Yung1,2,3, Yangzi Jiang1,2,3*

Affiliations: 1. Department of Orthopaedics & Traumatology, Faculty of Medicine, The Chinese University of Hong Kong; 2. Center for Neuromusculoskeletal Restorative Medicine, Hong Kong Science Park; 3. Institute for Tissue Engineering and Regenerative Medicine, School of Biomedical Sciences, The Chinese University of Hong Kong; Hong Kong, SAR (*Correspondence)

Category: Tissue Engineering and Regeneration

Introduction: The synovial lymphatic system (SLS) is a key drainage structure within the joint and its function impairment can contribute to inflammation imprint and the progression of arthritis. Lymphatic endothelial cells (LECs) are the main constituent of the SLS and their function abnormality following acute ACL injury has been previously demonstrated1, 2. However, the exact cellular molecular mechanism is not yet clear.

Subjects and Methods: Single-cell sequencing results of the whole synovium from ACL rupture mice knee joint and sham knee joint were analyzed3. LECs population was identified, and potential cellular interactions and patterns were illustrated by Cell-chat and Nichenet. Transwell co-culture systems were used to study the effect of inflamed synovial fibroblasts (SFs) on the junction function of LECs. Potential mediators were analyzed by cytokine array (Cat. No. ARY028). Functional assays of LEC permeability by Tran-epithelial/trans-endothelial electrical resistance (TEER) and dextran leakage were examined to reveal the effect of the trans-activation of IL-6 signaling on LECs barrier function and related downstream changes.

Results: Lyve1+/Prox1+ LECs population were identified in ACL rupture mice synovium. We extracted LECs and compared their transcriptomic changes after ACL rupture and control. Several associated up-regulated genes (Postn, Serpine1, etc.) and down-regulated genes (Apod, Adm, etc.) were identified. Pathway enrichment analysis revealed that the ECM organization and ECM-receptor interaction are the enriched functional pathways. Cell-communication analysis revealed that SFs have the most significant interactions with LECs and IL6/Il6st is one of the key interaction patterns from SFs to LECs. The co-culture system identified the primary SFs from ACL rupture rather than normal knee joint stimulate the junction impairment of the LECs. Cytokine array identified the IL-6 as the significant differentiate expressed secretome of SFs derived from ACL rupture knee joint. IL-6/sIL-6R (20 ng/ml) induces junction dysfunction and promotes the permeability and loss of junctional localization of VE-cadherin and ZO-1 in vitro; meanwhile (100 ng/ml) intra-articular injection of IL-6/sIL-6R induced SLS draining dysfunction and the disassembly of the junction of LECs in vivo. IL-6/sIL-6R activates Y705 STAT3 phosphorylation and activates ICAM1 transcription in LECs.

Discussion and Conclusion: SFs-derived IL-6 could induce the trans-activation of IL6 signaling in LECs and contribute to the dissociation of junction protein through the STAT3/ICAM1 axis. Pharmacological inhibition of IL-6 in the early stages post ACL rupture could promote SLS function and provide favorable conditions for intra-articular tissue regeneration.

References:
Acknowledgment: This work was supported by the National Key R&D Program (grant No. 2019YFA0111900 to Y.J.), and the Center for Neuromusculoskeletal Restorative Medicine (CNRM, Health@InnoHK to P.S.H.Y. and Y.J.).
Towards Microdroplet Printing of Collagen Scaffold.

Ms. Isha Sikri

Introduction: Collagen dressings have been shown to be effective in wound healing in diabetes mellitus. The attraction of collagen is that it not only provides a structural support and framework at the wound bed but also assists in controlling many cellular activities including cell differentiation, migration and protein synthesis that are vital in the process of wound healing. The challenge of using collagen is the development of precision scalable manufacturing processes. To further augment the efficacy of printing technology, microdroplet dispensing tool is used to eject collagen droplets of picolitres volume. This technology facilitates multi material printing with minimal cross contamination. It is essential to overcome the problems of wettability and interfacial forces when working with liquids of ultra-low volumes. This study aims to develop a methodology for separately printing collagen and Mesenchymal Stromal cells (MSC) using microdroplet to construct a scaffold as a potential advanced therapy for diabetic wounds.

Methods: Collagen ink was characterized by performing a rheometric study of the collagen solutions used for printing to compare the dynamic viscosities of the neutralized solutions at different concentrations (ranging from 2 mg/ml to 6 mg/ml). This determines how much the viscosity varied with the solution temperature (4°C, 21°C). A disposable dispensing cartridge of 70 µm orifice size was used to print different structures. The printing parameters such as print speed, separation distance between the drops, amplitude, width and frequency were optimized. The distribution of collagen filaments on the surfaces were analyzed using optical microscopy after collagen deposition.

Results: The characterization of the collagen solution revealed that viscosity decreases with increase in temperature. A viscosity ranging between 5-15 cP was chosen for printing. Soluble neutralized collagen with 2 mg/ml concentration was printed onto a glass substrate at repetition rate of 200Hz. A stage speed of 8 mm/s was chosen to overlap the drops to get the collagen filaments together. The mean diameter and volume of each drop was 47.33 ± 0.03 µm and 55.51 ± 0.10 pl respectively. The pattern observed in figure 1a showed visible indications of collagen drops at precise location followed by drops being overlapped to form a single line of 20 mm (figure 1b). A 20 mm X 20 mm square grid was printed with each unit measuring 0.5mm (figure 1c) on glass substrate. Arrays of square were separated by 155 µm over a large area of 20 mm (figure 1d).

Discussion and Conclusion: Data to date suggests that collagen printing using microdroplet dispensing technique has been demonstrated. The precise selective placement of microquantity of drop on the surface can be useful for printing low viscosity, ultra-low volume liquids with high resolution and accuracy. The printing process of patterned collagen structures was demonstrated on clean glass slide with optimization of temperature and pH. The amount of collagen deposited in each drop needs to be investigated. The current work establishes a promising strategy to design MSC-Collagen bio-construct scaffold with therapeutic potential for diabetic wounds and could potentially be used for patients with diabetic foot ulcers in the future.

Acknowledgement: This work was supported by Health Research Board (HRB) Ireland through the HRB Collaborative Doctoral Awards under the grant CDA-PA-2019-011.
Biphasic scaffold with epimedin C promotes osteochondral regeneration in osteoarthritic rats

Xiangbo Meng¹, Cuishan Huang¹, Ling Li¹, Jiming Li¹, Bimin Gao¹, Ling Qin¹,², Xinluan Wan¹,²
¹Translational Medicine R&D Center, Institute of Biomedical and Health Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China, ²Musculoskeletal Research Laboratory, Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Hong Kong SAR

Title: Biphasic scaffold with epimedin C promotes osteochondral regeneration in osteoarthritic rats

Authors: Xiangbo Meng¹, Cuishan Huang¹, Ling Li¹, Jiming Li¹, Bimin Gao¹, Ling Qin¹,², Xinluan Wang¹,²,*

Affiliations: ¹Translational Medicine R&D Center, Institute of Biomedical and Health Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China, ²Musculoskeletal Research Laboratory, Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, China (* Correspondence)

Category: SYIS Tissue engineering and regeneration

Introduction: Repairing osteochondral defects (OCD) in the context of osteoarthritis (OA) becomes especially difficult due to the arthritic environment that impairs osteochondral regeneration. To address this challenge, we designed and fabricated a biphasic, porous, and degradable scaffold that incorporates anti-inflammatory and anabolic molecules epimedin C (Epi C) [1] using low-temperature rapid prototyping technology.

Methods: We fabricated a biphasic scaffold (PTP-1%Epi C) using poly(lactic-co-glycolic acid) (PLGA) with Epi C for cartilage repair, and PLGA and β-tricalcium phosphate (PT) with Epi C for subchondral bone repair. The scaffolds’ porosity and mechanical properties were thoroughly tested. To establish an OA-OCD model in rats, we conducted medial meniscectomy and induced a bone-cartilage defect in the femoral groove with a diameter and depth of 2mm. We then assessed the effectiveness of the biphasic, bioactive scaffold in promoting osteochondral regeneration in this model after 12 weeks of implantation.

Results: The pore sizes of the macro-pores in PLGA and PT layer were 180-220 m and 450-550 m, respectively. The E-modulus of the PLGA and PT layer were 46.93 MPa and 16.05 MPa, respectively. Epi C within biphasic scaffold could be released in a controlled and sustained mode for up to 3 weeks, and the cumulative release rate was approximately 80%. The in vivo evaluation of 12-weeks post-implantation in critically sized OA-OCD rat models showed that the bone mass in PTP-Epi C group was higher than the control group (Figure 1).

Discussion and conclusion: Our results demonstrated that the biphasic scaffold with Epi C was highly biocompatible and exhibited promising results in promoting subchondral bone regeneration. These findings provide a promising potential strategy for clinical application for the treatment of patients with OA-OCD.

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Reference:
Title: PEGylation of Thrombin Inhibitor Peptide Ultravariegin for Longer Circulation Half-Life and Greater Antithrombotic Effect

Authors: Xia Song¹ ², Yuting Wen¹, Aaron Wei Liang Li³, Jingling Zhu², Cho Yeow Koh³, R. Manjunatha Kini⁴⁵, Mark Yan Yee Chan³⁶ and Jun Li¹ ² *

Affiliations: ¹Department of Biomedical Engineering, National University of Singapore, Singapore, Singapore. ²NUS Environmental Research Institute (NERI), National University of Singapore, Singapore, Singapore, ³Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, ⁴Department of Biological Sciences, National University of Singapore, Singapore, Singapore, ⁵Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, ⁶Cardiac Department, National University Heart Centre, Singapore, Singapore (* Correspondence)

Category: Design and Application of Biomaterials

Background: Anticoagulant therapy is widely used to prevent and treat arterial and venous thrombosis in patients with cardiovascular disease, cerebrovascular disease and cancer. There remains a major need for efficacious anticoagulant therapy that incurs the lowest possible bleeding risk. We have previously reported a high-affinity, high-specificity bivalent direct thrombin inhibitor (DTI) with non-covalent binding to thrombin, i.e. variegin and its variants, among which the most outstanding variant is ultravariegin (UV). UV has shown an affinity for thrombin at 4 pM, 445× better than the commercial DTI bivalirudin. UV achieved greater antithrombotic efficacy with far less bleeding than unfractionated heparin and bivalirudin in a series of experiments in small and large animals. However, short half-life of UV limits its clinical applications. PEGylation, the conjugation of poly(ethylene glycol) (PEG) polymer chains onto peptides/drugs, can provide shielding for proteins/drugs to reduce nonspecific interaction with blood components. This widely used strategy is a promising option to improve blood circulation time of UV.

Methods: In this work, we conducted PEGylation of UV via cysteine-specific conjugation with maleimide-PEG5k and maleimide-PEG10k. The PEGylated UV was purified via reversed phase HPLC. Inhibition of thrombin amidolytic activity by the PEGylated UV was tested in vitro using active site-directed substrate S2238. Ex vivo antithrombin efficacy of inhibitors was also analysed in rat and rabbit plasma. Eventually, in vivo evaluation of pharmacokinetics profiles of PEGylated UV was carried out in rats and rabbits after intravenous (IV) or subcutaneous (SC) injection.

Results: UV was successfully conjugated to PEG. The PEGylated UV showed a slight decrease in in vitro inhibition of thrombin amidolytic activity and a lower ex vivo antithrombin efficacy than unmodified UV in rat and rabbit plasma. However, PEGylation could significantly enhance the half-life
of UV in both rats and rabbits. In addition, SC injection could prolong the circulation time of UV or PEGylated UV than IV injection.

Discussion and Conclusion: We demonstrated that PEGylation of UV could enhance the half-life of UV in animals. The improved half-life of UV may reduce its injection frequency, making UV an ideal choice for future clinical applications.

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Infrapatellar Fat-Pad Derived MSCs for Osteochondral Tissue Engineering

Mr. Ho Kwan Jeffrey Leung¹, Dr. Chun Hoi Yan², Professor Barbara P Chan¹

¹ Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong SAR, ²Department of Orthopaedics and Traumatology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR

Title: Infrapatellar Fat-Pad Derived MSCs for Osteochondral Tissue Engineering

Authors: Ho Kwan Jeffrey Leung¹, Chun Hoi Yan², Barbara P Chan¹

Affiliation: ¹ Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Pokfulam Road, HKSAR. ² Department of Orthopaedics and Traumatology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam Road, HKSAR

Category: Tissue engineering and regeneration

Introduction: Osteoarthritis (OA) is a progressive degenerative musculoskeletal condition that affects an estimated 25 million individuals globally, causing articular cartilage and subchondral bone degeneration within synovial joints, resulting in chronic joint pain and movement limitations. Cell senescence and age-dependent cell proliferative decline are major issues with bone marrow derived mesenchymal stem cells (BM-MSCs) retrieved from aged osteoarthritis patients to derive enough MSCs for OA-related osteochondral tissue engineering, furthermore, the procedure of bone marrow aspiration remains to be an undesirably painful process for aged patients to endure and recover from. Infrapatellar fat pad derived stem cells (IFP-MSCs) was selected for investigation as a possible alternative cell source because of its lack of age-dependent cell proliferation reduction, and substantial quantities of MSC could be easily obtained by minimally invasive OA diagnostic or surgical procedures. The differentiative capabilities of both chondrogenic and osteogenic lineages of IFP-MSCs isolated from aged OA patients and New Zealand white rabbits utilizing our proprietary collagen microencapsulation platform technology were investigated in this study, The development of cartilage and bone microtissues differentiation was examined, and potential of fabricating a complex tri-layered engineered osteochondral tissue (eOCT) was explored.

Methods: Human IFP-MSCs (hIFP-MSCs) were selectively isolated from excised osteochondral cartilage specimens of aged OA patients of 60 years of age or above who underwent total knee replacement surgery. Rabbit IFP-MSCs (RbIFP-MSCs) were isolated from New Zealand white rabbits (Age 6 months+). IFP-MSCs were expanded and differentiated into chondrogenic or osteogenic lineages in chemically defined culture medium for 21 days after collagen microsphere encapsulation. Chondrogenic and osteogenic differentiation were examined using collagen microencapsulation concentration at 0.05-2mg/ml and 0.5-4mg/ml respectively. Both chondrogenic and osteogenic differentiated microtissues were evaluated by histological, mechanical, and biochemical assays of various markers.

Results: Both hIFP-MSCs and RbIFP-MSCs microencapsulated with collagen at a concentration of 0.05mg/ml and 0.1mg/ml resulted in optimal chondrogenic differentiation in forming cartilage microtissue with extensive cartilage formation visible from Safranin O staining, positively stained collagen type-II and aggrecan in immunohistochemical stainings, and upregulation of chondrogenic expressions such as Sox9 in qPCR. Whereas microencapsulation with collagen at a concentration of 2mg/ml resulted in optimal osteogenic differentiation and bone microtissues formation with prominent calcium mineralization in Von Kossa staining, and upregulation of osteogenic gene markers such as osteocalcin in qPCR.

Discussion and Conclusion: Our data suggests IFP-MSCs are a viable alternative MSC cell source, proliferating a considerable number of cells while also being capable of fully differentiating into both chondrogenic and osteogenic lineages via collagen microencapsulation platform in forming cartilage and bone microtissues. The current work shows promising results of using IFP-MSCs for osteochondral tissue engineering and future osteoarthritis therapeutic applications.

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Fabrication of the biomimetic glycosaminoglycans (GAGs)-rich scaffold

Dr. Xingxing YANG1,2, Dr. Hongcai Li1,2, Prof. Barbara Chan1,2,3
1 Tissue Engineering Lab, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China
2 Advanced Biomedical Instrumentation Centre, Hong Kong, China
3 School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China

Title: Fabrication of the biomimetic glycosaminoglycans (GAGs)-rich scaffold

Authors: Xingxing Yang1,2, Hongcai Li1,2, Barbara Pui Chan1,2,3*
1 Tissue Engineering Lab, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China
2 Advanced Biomedical Instrumentation Centre, Hong Kong, China
3 School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China
* Email: bpchan@cuhk.edu.hk

Category: Design and Application of Biomaterials

Background: Glycosaminoglycan (GAG) is a major extracellular matrix (ECM) component with important structural and mechanical functions. During tissue degeneration, cells could not maintain a GAG-rich ECM, resulting in significant depletion of GAG and hence subsequent adverse changes in tissue structure and function. To mimic the GAG content of the GAG-rich tissue, we have developed a biomimetic scaffold, consisting of ECM including GAGs, hyaluronic acid, and collagen. In this work, we are aimed to fabricate GAG-rich scaffolds with different amination reagents, collagen, and GAG types, and explain the mechanism of the biomimetic scaffold.

Methods: In this work, the biomimetic scaffold namely aminated collagen-aminated hyaluronic acid-glycosaminoglycan (aCol-aHA-GAG) was fabricated. The ultrastructure and EDX analysis of aCol-aHA-GAG scaffolds with different amination reagents and different types of collagen, such as Col1 and Col2, and GAGs, such as CS and HS, was investigated. To verify the mechanism of the bottlebrush structure, aCol-aHA-GAG was stained with the aggrecan (ACAN) antibody and co-stained with immunogold nanoparticles.

Results: The aCol-aHA-GAG scaffold showed a nanobead and bottlebrush structure, which is similar to the native tissue. The TEM results demonstrated that the HA/aHA was the backbone of the bottlebrush structure, and aCol but not the Col was the necessary component of the bottlebrush structure, and different types of collagen and GAGs showed a different length of bottlebrush. The S element was distributed along the brush of the bottlebrush structure. The immunogold staining showed that the bottlebrush in aCol-aHA-GAG was recognized as aggrecan.

Discussion and Conclusion: This work demonstrates a biomimetic scaffold that ultrastructural simulates the aggrecan, facilitating their potential applications in GAG-rich tissues.

Acknowledgement

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Induction Of Hypertrophic Cardiomyopathy From Human iPSC-derived Cardiomyocytes Using Mechanical Stress And ET-1

Ms. Eunji KIM¹, Ms. Su-Jin LEE¹, Mr. Sunho KIM¹, Ms. Hyang-Ae LEE¹
¹Korea Institute of Toxicology (KIT), Daedeon, Republic of Korea

Background: Hypertrophic cardiomyopathy (HCM) is a heart disease that increases the risk of heart failure, arrhythmia, and sudden cardiac death. Pathological hypertrophy in HCM can result from prolonged or excessive mechanical stress, gene mutations, or altered expression of endogenous substances. Endothelin-1 (ET-1), a potent vasoconstriction peptide, is an important endogenous substance produced not only by endothelial cells but also by cardiomyocytes. Elevated levels of ET-1 have been observed in plasma concentration in HCM patients. In this study, we investigated the induction of an HCM disease model using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) through mechanical stress and/or ET-1 stimulation, aiming to faithfully replicate the hypertrophic ventricular environment observed in vivo.

Methods: Prior to HCM model construction, we conducted cardiac differentiation of hiPSCs by modulating signaling pathways involved in cardiac development using small molecules and growth factors. The characterization of the hiPSC-CMs included an assessment of their beating activity and expression of cardiac-specific markers. Following confirmation of the hiPSC-CMs, we successfully constructed an HCM model by subjecting the hiPSC-CMs to mechanical stress and treating them with ET-1. Then, we comprehensively validated the HCM model through morphological analysis, contractility analysis via calcium transient measurements, and electrophysiological analysis.

Results: In the HCM model, we observed a notable increase in cardiomyocyte size relative to the normal control group, accompanied by the upregulation of genes associated with hypertrophy. Comprehensive functional analysis of the cardiomyocytes, including assessment of contractility and Ca2+ transients, revealed a reduction in contractile force and impaired Ca2+ transient dynamics compared to the normal control group.

Discussion and Conclusion: In this study, we successfully constructed an HCM model by subjecting hiPSC-CMs to pathophysiological conditions such as mechanical stress and ET-1. Our findings provide valuable insights into the pathogenesis of HCM and show the potential for serving as a promising testbed for the identification and development of drug candidates for HCM patients.

Acknowledgments: This work was supported by the Technology Innovation Program (#20009774) funded by the Ministry of Trade, Industry, and Energy (MOTIE, Korea) and by a grant from the National Research Foundation of Korea (NRF-2022M3A9H1015784). This research was also supported by the Korea Institute of Toxicology (#1711133839).
PINCH1 of cancer-associated fibroblasts promotes collagen type 1 and fibronectin expression and tumorigenesis in pancreatic cancer

Ms. Rong Wang1,2, Dr. Wong Yu Hin1,4, Dr. Ling Guo2, Professor chuanyue Wu2, Professor Barbara.P. Chan1,3,4

1The University of Hongkong, Hongkong, China, 2The Southern University of Science and Technology, Shenzhen, China, 3The Chinese University of Hongkong, Hongkong, China, 4Advanced Biomedical Instrumentation centre, Hongkong, China

Background: Pancreatic ductal adenocarcinoma (PDAC) is a malignant disease with high mortality and low 5-year survive rate worldwide. Dense stroma, is a hallmark of PDAC, which provides structural integrity and signaling for cancer cell survival, proliferation, metastasis; also makes PDAC a hard solid tumor and create physical barriers for anti-tumor drug delivery. Cancer-associated fibroblasts (CAF), an important player in tumor stroma, functioned as a niche for cancer cell to promote tumor progression by ECM remodeling and deposition, growth factor release. Various makers of CAF like paladin and CD146 is showed function as prognostic role in pathophysiology of PDAC; but lack of true, unique marker for CAF is still a challenge in treatment; besides, excessive extracellular matrix such as collagen type I and fibronectin usually cause fibrosis in tumor progression, therefore, it is very important for us to understand the distinct molecular mechanism of CAF modulation of matrix protein. PINCH1 (also called LIMS1), is a widely and conserved expressed focal adhesion protein, plays a vital role in cell-ECM adhesion, cell survive, proliferation, and migration. PINCH1 expression is upregulated in various types of cancer, including breast cancer, lung cancer, prostate cancer, and colon cancer and tumor stromal cells. Recent study shows that PINCH1 ablation can reduce collagen type I synthesis in lung cancer, CAF is mainly responsible for collagen type I and fibronectin synthesis among tumor stroma cells, we next sought to investigate the role of PINCH1 in CAF modulating matrix protein expression in tumor microenvironment (TME), and explore the underlying molecular mechanisms of PINCH modulating tumor matrix, which could be provide new thoughts for intervene the progression of PDAC in clinical.

Methods: In this work, we use cell derived matrice (CDM) to explore PINCH1 regulation of extracellular matrix. Besides, molecular, cellular, and genetic biology approaches to examine the function of PINCH1 in CAF in modulation of collagen type I and fibronectin expression and tumorigenesis. Furthermore, a subcutaneous mice model is employed to explore the PINCH1 in pancreatic CAF in regulation of tumor growth and related signaling.

Results: Ablation of PINCH1 from pancreatic CAF by lentivirus encoding shPINCH1 diminish collagen1a1 mRNA level and protein level, and this process is dependent on NOTCH1, the distinct molecular mechanism is that PINCH1 interact with NOTCH1 and prevent the later protein degradation, and thus regulation of collagen1a1 expression, as collagen 1a1 is one target gene of NOTCH1. On the other hand, knockdown of PINCH1 in pancreatic CAF reduce fibronectin protein level rather than mRNA level, PINCH1 regulate Fibronectin probably by ILK and integrin beta1. In subcutaneous mice model, we show that PINCH1 deficient CAF reduce tumor growth by AKT signaling in a way.

Discussion and conclusions: In this study, we firstly demonstrate that an important protein-PINCH1 was involved in regulating collagen and fibronectin expression in TME, and identify NOTCH1 is a new binding partner of PINCH1 after a known series of interactions including ILK, NOTCH2, RSU1, Myoferlin and smurf1. What’s more, PINCH1 of CAF is important to tumor growth in mice model and cancer cells proliferation and migration, which provide new perspective for therapeutic control of PDAC treatment, especially for handing with difficulties in drug delivery.

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Ultrafast Self-assembled Magnesium-Nanocomposite Hydrogels as Minimally Invasive Approach for Mandible Regeneration

Ms. Jiaxin Guo1,2, Hao Yao1,2, Liang Chang1,2, Yuantao Zhang1,2, Yuxiong Su3, Prof. Jiankun Xu1,2, Ling Qin1,2

1Musculoskeletal Research Laboratory, Department of Orthopedics & Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, China
2Innovative Orthopedic Biomaterial and Drug Translational Research Laboratory, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China
3Division of Oral and Maxillofacial Surgery, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR

Authors: Jiaxin Guo, Hao Yao, Liang Chang, Yuantao Zhang, Yuxiong Su, Jiankun Xu, Ling Qin

Affiliations:
1Musculoskeletal Research Laboratory, Department of Orthopedics & Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, China
2Innovative Orthopedic Biomaterial and Drug Translational Research Laboratory, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China
3Division of Oral and Maxillofacial Surgery, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China (*Correspondence)

Category: SYIS (Student and Young Investigator Section) plus Tissue Engineering and Regeneration

Background: Mandibular defect becomes a prevalent maxillofacial disease resulting in mandibular dysfunctions and huge psychological burdens. Recently, a critical side effect observed in patients undergoing long-term bisphosphonate (BPs)-administration may predispose to BPs-related osteonecrosis of the jaw (BRONJ), this is an area of necrotic bone in the jaw with impaired healing capacity, reduced angiogenesis, and inevitable infections. The osteo- and angio-promotive properties of Magnesium (Mg) implants and Mg2+ in vivo have been widely recognized by our previous study. Magnesium-oxide nanoparticle (MgO-NP) is a preferable option for the Mg2+ supplement, which has been approved as safe by the Food and Drug Administration (FDA). Moreover, the introduction of NPs into the hydrogels is thought to improve structure diversity, mechanical strength, and stimuli response. Additionally considering the special aesthetic restoration of facial structures, we aim to fabricate a Mg-nanocomposite hydrogel as minimally invasive therapy for challenging mandible regeneration and further explore the healing mechanism.

Methods: In this work, we assembled a biomimetic nanocomposite hydrogel (PBR@MgO hydrogel) by Amidation reaction and optimized based on Polyethylene glycol (PEG) hydrogel by BSA and RGD involvement. PBR@MgO hydrogel was implanted to mandible defects and donor-site iliac crest defects induced in porcine to introduce the minimally invasive applicable use. Then, we successfully established a rat BRONJ model and assessed the therapeutic efficacy of PBR@MgO hydrogel by radiography and histology methods, and further explored the underlying mechanism by RNA bulk Sequencing.

Results: The PBR@MgO hydrogel achieved a controllable ultrafast crosslinking by MgO-NPs, it exhibited well injectability and adhesive filling in the bone defect areas in porcine. In vitro, The introduction of MgO NPs and the crosslinked RGD peptide improved the mechanical strength of the PBR@MgO hydrogels while exhibiting better cell adhesion, infiltration, and osteogenic differentiation than conventional PEG hydrogels. In vivo, PBR@MgO hydrogel implantation augmented mandible formation in BRONJ with enhanced osteogenesis-angiogenesis coupling, which could be attenuated by local VEGF receptor-2 inhibitor injection.
Discussion and Conclusion: The biomimetic PBR@MgO hydrogel displays improved mechanical strength and osteopromotive properties, making it a potential candidate for minimally invasive surgery and complex maxillofacial tissue reconstruction. PBR@MgO hydrogels have been shown to promote angiogenesis-mediated mandible regeneration in cases of BPs-related mandibular osteonecrosis. Controllable crosslinked and strong osteoinductive ability provide the PBR@MgO hydrogel with promising clinical translation potential in complex maxillofacial tissue reconstruction.

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References:
Cell-Derived Matrix As An Extracellular Reservoir Of Bioactive Wnt3a

Dr. Yu Hin WONG1,2, Prof. Barbara Pui CHAN1,2,3
1Tissue Engineering Lab, Department of Mechanical Engineering, The University of Hong Kong (HKU), Hong Kong SAR, 2Advanced Biomedical Instrumentation Centre (ABIC), Hong Kong Science and Technology Park, Hong Kong SAR, 3School of Biomedical Sciences, The Chinese University of Hong Kong (CUHK), Hong Kong SAR
Authors: Yu Hin Wong1,2; Barbara Pui Chan1,2,3*

Affiliations: 1. Tissue Engineering Lab, Department of Mechanical Engineering, The University of Hong Kong (HKU), Hong Kong; 2. Advanced Biomedical Instrumentation Centre (ABIC), Hong Kong Science and Technology Park, Hong Kong; 3. School of Biomedical Sciences, The Chinese University of Hong Kong (CUHK)
*Corresponding address: bpchan@cuhk.edu.hk

Introduction: The Wnt signaling pathway is an important pathway for cellular processes such as proliferation, differentiation and embryogenesis. Wnt proteins are secreted by the cells into the extracellular space where they can bind to the Wnt receptors on the cell surface membrane. Proteomics studies have identified their presence in the extracellular matrix, however little is known about their interaction with the extracellular matrix. Human mesenchymal stem cells (hMSCs) can secrete cell-derived matrix (CDM) that is composed of extracellular matrix (ECM) proteins and a host of growth factors. In fact, it has been shown that the ECM can act as a reservoir for growth factors. We hypothesize that Wnt proteins such as Wnt3a, one of the 19 Wnt proteins in human, can bind to the ECM.

Subjects and Methods: In this work, we first screened for colocalization of Wnt3a with common ECM proteins found in the CDM, which is secreted by confluent hMCs under the presence of ascorbic acid. ECM proteins that colocalize with Wnt3a are further screened for any possible interaction and binding. The bioactivity of the ECM-bound Wnt3a in terms of beta-catenin signaling and cell proliferations were investigated.

Results: Fibronectin in the CDM was found to colocalize with Wnt3a. Moreover, fibronectin can also interact and bind directly to Wnt3a. Loss of functions of CDM-bound Wnt3a was found to inhibit hMSCs proliferation.

Discussion and Conclusion: The current work reveals the novel interaction and direct binding of Wnt3a with fibronectin and that it is possible to alter cell function such as stem cell proliferation through hMSCs-secreted CDM-bound growth factor Wnt3a. This demonstrates the possibility of using ECM-bound growth factors to influence cell activities as a tissue engineering approach.

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Engineering a biomimetic immune microenvironment using Multiphoton Microfabrication and Micropatterning (MMM) technology for natural killer cell niche studies

Dr. Xinna WANG¹, Miss Hoi Ying MAN¹, Prof. Barbara P. CHAN¹,²

¹Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong, China, ²School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China

Introduction: Natural killer (NK) cells are promising in cancer immunotherapy due to their excellent anti-cancer effects. Interleukin (IL) cytokines (e.g., IL-15, IL-18) are essential soluble factors for maintaining NK cells and boosting their anti-cancer actions in vitro. Nevertheless, the short lifetime of these ILs in the culture media limited their feasibility for the long-term maintenance of NK cells. Multiphoton microfabrication and micropatterning (MMM) is a direct laser writing technology that has been applied in engineering artificial soluble niches able to sustain and potentiate the bioactivity of the soluble factors of interest. In the present work, multiple ILs (e.g., IL-15, IL-18) will be immobilized via MMM to reconstitute an immune microenvironment suitable for maintaining NK cells.

Subjects and Methods: Protein micropillar arrays were fabricated by two-photon initiated photo crosslinking of bovine serum albumin (BSA) in the presence of photosensitizer Rose Bengal (RB), followed by functionalizing the surface of the BSA micropillars with a thin layer of biotin-binding protein neutravidin (NA) via the same photo-crosslinking process. The home-made biotinylated IL15 (bIL15) and IL18 (bIL18) were then immobilized on the NA-functionalized BSA micropillars through biotin-NA binding. NK cell viability was evaluated after being cultured on the IL18 immobilized micropillars for 7 days and the cell viability maintained by the immobilized IL18 and by the free IL18 at different concentrations was compared.

Results: bIL15 and bIL18 can be immobilized on the NA substratum in a spatial- and quantity-controlled manner. In addition, NK cells can be maintained viably on the IL18 micropillar arrays for at least 4 days post-cell seeding. Moreover, the cell viability in the immobilized IL18 groups is comparable to that in the free IL18 groups.

Discussion and Conclusion: The current work demonstrates that MMM technology is capable to spatially and quantitatively control the ILs, and NK cells can be maintained on the ILs-immobilized micropillar arrays. Further investigations on signaling events activated in the NK cells maintained by the immobilized ILs and functionalities (e.g., secretion of IFNγ and cancer cell killing effect) of such NK cells are warranted. This biomimetic in vitro immune microenvironment would pave the way for NK cell niche studies and further evaluation of the efficacy of NK cell-related cancer immunotherapy.

Acknowledgement: This work was financially supported by RGC GRF (17126122), Research Grant Council, HKSAR government.
Reconstituting the Cell-cell Interaction Niche with Multiphoton Microfabrication and Micropatterning (MMM) technology – E-cadherin as an example

Dr. Abigail Dee CHEN¹, Dr. Nan HUANG¹, Dr. Chi Hung YIP¹, Prof. Barbara Pui CHAN¹,²

¹Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China, ²School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China

Introduction:
Reconstituting the complex cell microenvironment in vitro is important to understand the effects of multiple niche factors individually and in combination with each other. The Multiphoton Microfabrication and Micropatterning (MMM) Platform [1] has previously been developed to reconstitute the stiffness and modulus [2], matrix niche [3], topographical niche, and soluble factor niche, with high spatial and quantitative control. Cell-cell interaction is another essential component of the cell niche, involved in morphogenesis and homeostasis of healthy tissues, as well as in disease development such as cancer. In this work, we developed a method for reconstituting the cell-cell interaction niche, using E-cadherin as an example. Furthermore, using our platform, we investigated the effects of cell modulus and stiffness on epithelialization and the Epithelial-to-Mesenchymal Transition (EMT).

Methods:
Using MMM technology, both direct crosslinking and indirect immobilization of E-cadherin were investigated. E-cadherin pattern density was controlled via laser power and reagent concentrations, and was quantified using immunofluorescence staining. Bioactivity was verified via analysis of adhesion and adherens junction markers of MDCK and MCF7 cells on the patterns. To investigate cell modulus and stiffness on EMT and epithelialization, MDCK cells were seeded onto basally-coated E-cadherin structures with different moduli and stiffnesses, and laterally-coated E-cadherin structures with different moduli combined with a laminin-coated base. Cell shape, EMT, and epithelialization markers were stained and evaluated after 3 days.

Results:
E-cadherin pattern density was spatially and quantitatively controllable with the MMM platform with both direct crosslinking and indirect immobilization methods, while bioactivity was retained. Moreover, lower moduli and stiffnesses of basally-coated E-cadherin structures led to poorer epithelialization, decreased junctional integrity, and increased expression of EMT markers. Cells on low stiffness E-cadherin structures also showed increased aspect ratio and elongated cell morphology. The combination of laterally-coated E-cadherin structures with a laminin-coated base showed cells at the leading edge of low modulus conditions with more elongated morphologies.

Discussion and Conclusion:
This work provides a method for reconstituting the in vivo cell-cell interaction niche, using E-cadherin as an example. Moreover, using the MMM platform, the individual and combinatorial effects of cell-cell interaction with elastic modulus, stiffness, and extracellular matrix proteins can be accurately investigated, in order to mimic different cell modulus, stiffnesses, and the basolateral presentation of matrix and cellular cues of cells in vivo, in particular, epithelial cells. Our results investigating the E-cadherin-coated structures’ modulus and stiffness on epithelialization and EMT suggest that cadherin-mediated mechanosensing may also play a role in EMT progression. Additionally, our results underline the importance of studying the effects of the cell-cell interaction-mediated mechanosensing in parallel with matrix mechanosensing studies.

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Acknowledgements:
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CaP nanocomposite-based cfDNA scavenger alleviates periodontitis-related alveolar bone resorption

Mr. Yifan Wang

Introduction: Alveolar bone resorption caused by periodontitis is a common inflammatory bone resorption disease. Orthodontic treatment changes the position of teeth through the absorption and reconstruction of alveolar bone, which carries the risk of inducing or exacerbating periodontitis. Therefore, periodontitis is a contraindication for orthodontic treatment. To address this challenge, this study constructed a composite nanomaterial that alleviates periodontal inflammation and alveolar bone resorption by scavenging cell-free DNA (cf-DAN).

Subjects and Methods: The cationic polyamide amine dendrimer (PAMAM-G3) was coated on selenium-doped hydroxyapatite nanoparticles (SeHANs) to construct nano cf-DNA scavengers for periodontitis, G3@SeHANs. G3@SeHANs were then compared with soluble PAMAM-G3 to evaluate their effectiveness in regulating inflammation and remitting alveolar bone resorption and explore the underlying mechanism.

Results: Both G3@SeHANs and PAMAM-G3 inhibited the pro-inflammatory response related to periodontitis in vitro by scavenging cfDNA, significantly alleviating inflammatory bone resorption in a ligated periodontitis mouse model. In addition, G3@SeHANs regulated the mononuclear phagocyte system in the periodontitis environment, promoting the M2 polarization of macrophages. The efficacy of G3@SeHANs was superior to PAMAM-G3 in scavenging pro-inflammatory cf-DNA and reducing inflammatory alveolar bone resorption in vivo.

Discussion and Conclusion: This study reveals the importance of cf-DNA in periodontitis and suggests that CaP nanocomposites based on SeHANs and PAMAM-G3 have important potential as cf-DNA scavengers for the treatment of inflammatory alveolar bone resorption.

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Hanyao Huang1; Weiyi Pan1; Yifan Wang1; Hye Sung Kim; Dan Shao; Baoding Huang; Tzu-Chieh Ho; Yeh-Hsing Lao; Chai Hoon Quek; Jiayu Shi; Qianming Chen; Bing Shi; Shengmin Zhang; Lei Zhao; Kam W. Leong; Nanoparticulate cell-free DNA scavenger for treating inflammatory bone loss in periodontitis, Nature Communications, 2022, 13:5925
Co-culture of adipose-derived stem cells (ADSCs) and ADSCs spheroids improves cartilage differentiation of ADSCs.

Ms. Eunji LEE¹, Ms. Su Jin LEE¹, Ms. Yeon Hee RYU¹, Prof. Suk-Ho MOON²

¹Department of biomedicine and Health Science, College of Medicine, The Catholic University of Korea, Banpo-daero Seocho-gu, Republic of Korea, ²Department of Plastic and Reconstructive Surgery, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Banpo-daero Seocho-gu, Republic of Korea

Background: Stem cell research is known to be used in tissue engineering. Adipose-derived stem cells (ADSCs) has self-renewal, angiogenesis, differentiation, cytokine secretion, paracrine effect, and immunomodulatory effects, and they are pluripotent cells that can be differentiated into various cell systems including osteoblasts, chondrocytes, and adipocytes. It is known that the growth factor and cytokine can control the growth potential ADSCs, and the spheroid environment is known to successfully induce cartilage formation in ADSCs through the considerable possibility of chondrocytes in ADSCs. Spheroids are known to promote cell-cell and cell-substrate interactions and provide a similar environment to the body, and Stem cell spheroids promote stem cell marker expression and promote the secretion of cytokines, chemokines, and angiogenesis factors. Spheroid is known to mimic the biological microenvironment more accurately, so it was investigated whether adipose-derived stem cells (ADSCs) affect cartilage differentiation of ADSCs when injected as cell therapy. Therefore, we hypothesized that it affects ADSCs’ the cartilage differentiation by mimicking the body’s microenvironment through the co-culture of spheroids and ADSCs.

Methods: To confirm characterizing the spheroids, we performed the nucleus and ECM of the cells of the spheroids though H&E, MT Stain and to confirm the stemness by immunofluorescence staining of stem cell markers (Oct4, Sox2, Nanog). We conducted 4 days of co-culture to confirm spheroids affect apoptosis and survival in ADSCs through flow cytometry and Live/Dead stain, and a cytokine array to confirm the difference in cytokines secreted from ADSCs and spheroids. And then cartilage differentiation was performed in an environment where spheroids and ADSCs were co-cultured.

Results: We identified cell nuclei and ECMs in spheroids through H&E and MT Stain and confirmed that they were ADSC spheroids by confirming that they have stemness through immunofluorescent staining. Compared to single cultured ADSCs, co-cultured ADSCs did not affect apoptosis, viability and cytokine array analysis confirmed that the expression of MIF and MIP-3α, which are responsible for regulating macrophage function, was higher in the co-culture group. It was shown that differentiation was promoted in the co-cultured group when the degree of differentiation was confirmed through an alcin blue stain after inducing a single cultured ADSCs and an ADSCs group co-cultured with spheroids in a cartilage differentiation medium.

Discussion and Conclusion: Therefore, we confirm that spheroids affect the differentiation of ADSCs through co-culture, suggesting that cartilage differentiation is promoted when ADSCs are injected into the body environment. Cytokine array analysis confirmed that MIF and MIP-3α expression, which are responsible for regulating macrophage function, were expressed higher in co-culture groups than in ADSCs, which is expected to be the basis for future studies on immunity, immune control, and inflammation.

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Reference


Regulation of Head and Neck Squamous Cell Carcinoma Migration and Invasion Behaviors by Mild Reduction of Cell Surface

Ms. Laurensia Danis Anggradita¹,², Sung Sik Hur¹, Jae Hong Park³, Myung Jin Ban³, Yongsung Hwang¹,²
¹Soonchunhyang Institute of Medi-bio Science (SIMS), Soonchunhyang University, Rep. of Korea,
²Department of Integrated Biomedical Science, Soonchunhyang University, Rep. of Korea,
³Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, Soonchunhyang University, Cheonan, Republic of Korea

Title: Regulation of head and neck squamous cell carcinoma migration and invasion behaviors by mild reduction of cell surface

Authors: Laurensia D. Anggradita¹,², Sung Sik Hur¹, Jae Hong Park³, Myung Jin Ban³,*, and Yongsung Hwang¹,²,*

Affiliations: ¹Soonchunhyang Institute of Medi-bio Science (SIMS), Soonchunhyang University, Republic of Korea. ²Department of Integrated Biomedical Science, Soonchunhyang University, Republic of Korea. ³Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, Soonchunhyang University, Cheonan, Korea (*Co-correspondence).

Category: SYIS (Student and Young Investigator Section) / Tissue Engineering and Regeneration

Background: Highly migratory and metastatic potential are two of the hallmarks of head and neck squamous cell carcinoma (HNSCC), which could increase the patient’s mortality rate. The properties of membrane surface, as the outermost layer of the cell, have been shown to modulate various cellular behaviors, including cell adhesion, migration, and invasion.

Methods: This study aimed to elucidate the role of the cell surface free thiol groups induced by mild reduction of the cell surface proteins using a tris(2-carboxyethyl) phosphine hydrochloride (TCEP) in the formation of focal adhesion (FA) complexes and their subsequent contribution to modulating cellular interactions during cancer cell migration and invasion. We further evaluated the potential of free thiol groups as new therapeutic strategies in HNSCC by traction force microscopy, intracellular force microscopy, and monolayer stress microscopy under physiological conditions (2.55 kPa) and tumor microenvironment (49.4 kPa).

Results: Our finding revealed that TCEP could generate free thiol groups on the membrane of HNSCC, which could change the cell morphology from cuboidal or epithelial to elongated mesenchymal-like cell shapes dependent upon matrix stiffness. This was achieved by the upregulation of integrin-mediated phospho-FAK signaling pathway, which led to the activation of mesenchymal to epithelial transition (MET) and consequently suppressed MET markers in both mRNA and protein levels, as well as cell migration.

Conclusions: This study provides a proof-of-principle that cell surface-free thiol groups can be a novel therapeutic target to engineer cancer adhesion, inhibiting cancer metastasis or reduce the aggressive properties of HNSCC by regulating the cell-matrix and cell-cell interaction during adhesion and migration.

Acknowledgement: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (MICT) (grant no. 2019R1A5A8083404)
Nanosubstrate engineered for modulating stem cell adhesion and endocytosis

**Dr. Bohan YIN**, Dr. Siu Hong Dexter WONG, Prof. Mo YANG

1Department of Biomedical Engineering, The Hong Kong Polytechnic University, Kowloon, Hong Kong SAR

Background: Human mesenchymal stem cells (hMSCs) are a great potential source for regenerating damaged/injured tissue, and guiding hMSCs toward specific lineage is of utmost importance in the field of tissue engineering. Nanosubstrate engineering can be a biomechanical approach for modulating hMSC differentiation in tissue engineering. However, the study of the effect of clathrin-mediated processes on manipulating this behavior is unexplored.

Methods: We fabricated nanosubstrates by conjugating gold (Au) nanosphere with an aspect ratio (AR) of 1 (AR1-S) and Au nanorod with an AR of 4 (AR4-S), respectively, with further modification of Arg-Gly-Asp (RGD) peptides on the Au surface. The particle surface area, density, and ligand density were precisely controlled. We utilized these integrin-binding nanosubstrates at confined nanogeometries that regulate clathrin-mediated adhesion- or endocytosis-active signaling pathways for modulating stem cell fates.

Results: Isotropically presenting ligands at the nanoscale enhances the expression of clathrin in cells, thereby facilitating the uptake of dexamethasone-loaded nanoparticles (NPs) to boost the osteogenesis of stem cells. In contrast, anisotropic ligand nanogeometry suppresses this clathrin-mediated NP entry by strengthening the association between clathrin and adhesion spots to reinforce mechanotransduced signaling, which can be abrogated by pharmacological inhibition of clathrin. We manipulate the feature of ligand nanogeometry on AR1-S to maximize osteogenic differentiation outcome by the enhanced uptake of dexamethasone-loaded NPs via robust endocytic activity, regardless of their weak mechanical engagement between integrin and ligand.

Discussion and Conclusion: We elucidate the roles of clathrin in hMSCs for mediating endocytosis or participating in integrin-mediated mechanotransduction by nanosubstrate engineering. Our findings not only highlight the dual functions of clathrin in CME and integrin-mediated mechanosensing by tuning the anisotropic ligand nanogeometry, but also provide design rules of nanosubstrates for improving hMSC osteogenesis or other potential types of differentiations (e.g., adipogenesis) for tissue engineering.

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Red blood cell extracellular vesicles incorporated into 3D printed scaffolds for spinal cord injury regeneration

Mr. Chongquan Huang¹, Mr. Migara Kavishka JAYASINGHE², Dr. Kieran Lau¹, Dr. Thi Nguyet Minh Le³, Dr. Sing Yian Chew¹,³,⁴

¹School of Chemistry, Chemical Engineering and Biotechnology, Nanyang Technological University, Singapore, Singapore, ²Neuroscience@ NTU, Interdisciplinary Graduate Programme, Nanyang Technological University, Singapore, Singapore, ³School of Medicine, Department of Pharmacology, National University of Singapore, Singapore, Singapore, ⁴Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore, ⁵School of Material Science and Engineering, Nanyang Technological University, Singapore, Singapore

Chongquan Huang¹, Migara Kavishka JAYASINGHE², Kieran Lau¹, Thi Nguyen Minh Le², Singyian Chew¹,³,⁴

Introduction
Spinal cord injury, as a typical central nervous system injury, always leads to in severe morbidity and permanent disability. Among variety of treatment methods, gene therapy, especially RNA interference, is a promising treatment method with great potential to improve the cell regeneration capacity and to deplete detrimental factors in the injury sites. However, efficient delivery system is required for practical use. Theoretically, an ideal delivery system should be able to 1) help gene material to pass through the cell membrane and allow downstream function; 2) keep the gene material from degradation by endogenous enzymes; 3) enable long-term and sustained release of RNA to cover the process of tissue regeneration and 4) be biocompatible in physical and chemical properties to allow tissue regrowth. In this study, we extracted red blood cell extracellular vesicles (RBCEVs) and loaded siRNA/miRNA into them as the primary gene carrier to achieve 1) and 2). Thereafter, the RNA-loaded RBCEVs were incorporated into gelatin meth acryloyl (GelMA) solution for 3D printing, which could permit physical support and guidance for neural regeneration and act as a reservoir for gene materials.

Method
Transfection efficiency of RNA-RBCEVs was first tested in different types of glia cells and neurons. Thereafter, microRNA cocktail was loaded into RBCEVs, which was then mixed into GelMA solution for photo crosslinking-based 3D printing. Before transplantation into the rodent spinal cord injury model, the printed scaffolds were also tested in vitro using oligodendrocytes progenitor cell (OPCs) to investigate the function of RNA-RBCEVs on OPCs differentiation and myelination. Finally, in a total transection spinal cord injury model on rats, the scaffold was transplanted to evaluate the regeneration promoting effect after 2 and 4 weeks.

Results
RNA-RBCEVs/GelMA scaffold showed great potential in gene delivery for SCI treatment. Both OPCs and astrocytes could uptake fluorescent-RBCEVs efficiently with a dosage-dependent trend. At an ideal ratio, most of the OPCs and astrocytes successfully took up RNA-RBCEVs. Additionally, GAPDH
gene expression in all types of neural cells (astrocytes, OPCs, microglial, and neurons) could be downregulated by siGAPDH-RBCEVs treatment. When compared with commercial transfection reagent, TKO, similar knockdown efficiencies were observed. Furthermore, siGAPDH-RBCEVs/GelMA scaffold could successfully knock down GAPDH expression when cultured with OPCs in a trans-well, which outperformed TKO-siGAPDH-incorporated scaffolds. Importantly, miRNA-RBCEVs/GelMA scaffold could successfully support OPCs and induce OPC differentiation and myelination. At day 3 of culture, Olig2 average intensity in the OPCs cocultured with miRNA-RBCEVs/GelMA scaffolds showed significance when compared to negative RNA control group.

Discussion and conclusion
To summarize, RBCEVs is a promising RNA vector for neural cell gene delivery, which is still functional after incorporated into GelMA scaffolds to induce downstream cellular response. Further investigation of this study will focus on animal SCI model to investigate the therapeutic effects of this miRNA-RBCEVs/GelMA scaffolds in nerve regeneration and remyelination.

Acknowledgements
This research was partially supported by the National Research Foundation, Prime Minister’s Office, Singapore under its Campus for Research Excellence and Technological Enterprise (CREATE) programme (IntraCREATE grant award number: NRF2019-THE002-0001), and the MOE Tier 2 grant (MOE-T2EP30220-0002).
The Mechanism of Bone Marrow Mesenchymal Stem Cell-Derived Exosomes Carrying siRNA target HOTAIR on the Differentiation of Bone Marrow Mesenchymal Stem Cells in Steroid-Induced Femoral Head Necrosis

Dr. Min YI, Dr. Fei Fei LIN, Dr. Qing Yu WANG

1Department of Orthopedics of the Second Hospital of Jilin University, Changchun, China

Background: Steroid-induced osteonecrosis of the femoral head (SONFH) is a frequently encountered and challenging condition in orthopedics, characterized by a dismal prognosis. The development of hormonal osteonecrosis is significantly influenced by the abnormal differentiation of bone marrow mesenchymal stem cells (BMSCs) towards osteogenic or lipogenic pathways. Through transcriptome expression profiling of lesioned BMSCs, our team has confirmed a significant association between IncRNA-HOTAIR and disorders related to osteogenic-lipogenic metabolism. Exosomes play a crucial role in the modulation of stem cell differentiation and possess the potential to enhance the therapeutic effectiveness of mesenchymal stem cells derived from bone marrow.

Methods: In this study, human bone marrow mesenchymal stem cells (BMSCs) were subjected to culture, followed by the extraction of exosomes (BMSC-exo) through differential centrifugation. The identification of exosomes was carried out using exosome marker proteins, electron microscopy, and nanoparticle tracking analysis. Furthermore, HOTAIR-siRNA was loaded into exosomes derived from BMSCs using ultrasound. The resulting BMSC-Exo was found to carry the HOTAIR-siRNA. The study involved incubating BMSC-exo with SONFH-BMSCs, followed by co-cultured for varying durations (3, 7, 10, 14, 18, 21D). The samples were then subjected to alizarin red staining, alkaline phosphatase staining, and quantitative analysis, as well as evaluation of the expression of osteogenic and lipogenic differentiation markers and their immunohistochemical staining analysis. The aim was to investigate the impact of BMSC-exo with HOTAIR-siRNA on osteogenic and lipogenic differentiation and to assess the expression of key molecules of the Wnt signaling pathway.

Results: Under electron microscopy, the BMSC-exo exhibited a cup-shaped morphology that was either round or elliptical. The periphery of the structure displayed a high degree of brightness, while the central region appeared gray. The particle size distribution of the BMSC-exo ranged between 60 and 100 nm. The induction of osteogenic and lipogenic differentiation was achieved through the coincubation of exosomes containing HOTAIR-siRNA with SONFH BMSCs. The results indicate that ARS staining and quantitative analysis of ALP were elevated in comparison to the control group. The study utilized qRT-PCR to identify elevated expression levels of BMP2, OPG, and RUNX2, and the results of immunohistochemistry to observe the osteogenic markers RUNX2 and Osterix, indicating an augmentation in osteogenic potential. The results of the staining and quantitative analysis of oleocanthal indicated a decrease in the lipogenic differentiation potential of SONFH-BMSCs. The study utilized qRT-PCR to identify a decrease in the expression of PPARγ, C/EBPα, and Adipsin. Additionally, the results of immunohistochemistry analysis of PPARγ and C/EBPα indicated a reduction in lipogenic differentiation ability. The expression of Oct4 was observed to have decreased while the expression of β-catenin was significantly increased in SONFH-BMSCs. Both protein expressions exhibited corresponding modifications.

Discussion and Conclusions: Exosomes derived from human bone marrow mesenchymal stem cells were utilized to deliver small interfering RNA targeting the long non-coding RNA HOTAIR. This approach resulted in decreased expression of HOTAIR and targeted regulation of the low expression of DKK1. Additionally, activation of the Wnt/β-catenin signaling pathway was observed, leading to the promotion of osteogenic differentiation and reduction of lipogenic differentiation in patients with steroid-induced osteonecrosis of the femoral head. Ultimately, this intervention led to the improvement of femoral head necrosis.
Acknowledgment: This work was supported by the Natural Science Foundation of Jilin Province #3D5222868429.
Investigation of biological effect of cold plasma on adipose stem cells

Dr. Chih-Hsun Lin

Taipei Veterans General Hospital, Taiwan

Introduction:
Enhancing chronic wound healing remains a challenge in clinical practice. This study investigates the feasibility of optimizing plasma conditions to activate adipose stem cells (ASCs) and improve their therapeutic potential for wound healing.

Subjects and Methods:
Rat ASCs (rASCs) were isolated from abdominal and inguinal fat. Stem cell characteristics were assessed through CD marker analysis and differentiation assays. Different plasma treatments, including direct and indirect exposure, as well as variations in duration and frequency of treatment, were applied using a dielectric barrier discharge (DBD) patch on rASCs and culture medium. The biological effects of plasma treatment were evaluated by measuring rASC proliferation using a CCK-8 assay and assessing cell migration under a microscope. Changes in the expression levels of cell surface antigens were analyzed using flow cytometry.

Results:
Direct plasma treatment significantly increased rASC proliferation compared to the control group. Notably, exposure times within 10 minutes demonstrated a significant enhancement in cell proliferation, while extended exposure to 20 minutes inhibited cell growth. Furthermore, exposing rASCs to plasma for 1 minute daily over two days resulted in a 15% increase in CD34 expression.

Discussion and Conclusion:
Short-term plasma treatment shows potential for improving rASC proliferation and stemness. The observed biological effects of plasma on ASCs necessitate further investigation to enhance functional outcomes in cell therapy applications.
Collagen-based Biomimicking Material for Generating: Drug Screening Platform for Personalized Medicine

Mr. Lok Him Ko², Professor Barbara Pui Chan¹,²,³
¹Tissue Engineering Lab, Department of Mechanical Engineering, HKU, Hong Kong, China, ²Advanced Biomedical Instrumentation Centre, Hong Kong, China, ³School of Biomedical Science, the Chinese University of Hong Kong, Hong Kong, China

Collagen-based Biomimicking Material for Generating Drug Screening Platform for Personalized Medicine

Ko Lok Him, Barbara Pui Chan*
Department of Mechanical Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong, China
*Email: bpchan@hku.hk

INTRODUCTION: Cell resides in a complex environment which experience unique mechanical topological stress and biological stimulus to maintain its own phenotype. In cancer, Extracellular matrix (ECM) has great contribution for disease progression, tendency of metastasis and drug resistance[1,2]. In this study, we aim to develop a collagen based sophisticated biomaterial which crosslink multiple ECM component encapsulating patient derived cells to form collagen sphere to generate in vitro tumour model. The model will then be screened with drug with different dosage and undergo viability testing for giving good reference to aid clinician judgement for better treatment design.

EXPERIMENTAL METHODS: Collagen was aminated using Ethylenediamine (EDA), as to increase its positive charge and hence able to conjugate with the negatively charged GAG and aminated hyaluronic acid or different ECM component to form biomaterial like αCol-HA, Col-LM using a crosslinker EDC [3]. The cancer cells and stromal cells was cultured and expanded to a sufficient number for gel encapsulation. Crosslinked collagen co-precipitate with GAG were used to encapsulate cell mixture and incubated for gel formation and suspend in culture medium.

RESULTS AND DISCUSSION

αCol-GAG, Col-LM is used to encapsulate cancer cells and stromal cells with a seeding density of 1E5 per well. For tissue and cells recognition, the tissue is stained with safranin O, haematoxylin & Eosin; for viability testing, Live-dead staining dye is used. In this study, HEPG2 a liver cancer cell line and MIA-PACA2 a pancreatic cancer cell line is utilized Addition of stromal cells in HEPG2 give better contraction to form rigid and tumour like tissue, cells and matrix is tightly packed. Col-LM in PDAC-CAF model provide good viability on both day 3 and day 7 with structure indicate the present of glycosaminoglycan.

CONCLUSIONS: This work demonstrates the capability of collagen based synthetics material in generating scaffold for cancer cells and stromal cells in maintaining cell growth with good viability and produce tumour like tissue structure, proving the potential of the material is generating or mimicking the complex nature of tumour microenvironment.

REFERENCE


ACKNOWLEDGEMENT: This work was supported by the Mid-Stream Research Program (MRP/047/21) and the Health@InnoHK program from the Innovation and Technology Commission, of the Hong Kong Special Administrative Region government.
Hydrogel with photothermal/photoelectricity effect mediates interactive regulation of dual-signal of lab-on-paper biosensor to detection human papillomavirus

Dr. Lin Li¹, Dr. Haihan Yu¹, Prof. Lina Zhang¹, Dr. Yuwen Wang², Dr. Hongmei Yang¹, Dr. Shanshan Li¹, Dr. Xiang Zhang³, Prof. Jinghua Yu¹
¹School of Chemistry and Chemical Engineering, University of Jinan, Jinan, China, ²Shandong Provincial Key Laboratory of Preparation and Measurement of Building Materials, University of Jinan, Jinan, China
Authors: Lin Li¹, Haihan Yu¹, Lina Zhang², Yuwen Wang², Hongmei Yang¹, Shanshan Li¹, Xiang Zhang², Jinghua Yu¹*
Affiliations: 1 School of Chemistry and Chemical Engineering, University of Jinan, Jinan, Shandong province, China. 2 Shandong Provincial Key Laboratory of Preparation and Measurement of Building Materials, University of Jinan, Jinan, Shandong province, China. (* Correspondence)
Category: Design and Application of Biomaterials

Background: High-risk human papillomavirus (HPV) persistence is the culprit of cervical cancer. Point-of-care in vitro testing is the principal way to prevent cervical cancer and improve cure rates, which is of vast significance for the early diagnosis of cancer.

Methods: In this work, the cascaded CuInS2-Mn/AgBiS2 nanocomposite polysaccharide hydrogel is assembled in miniature interface of flexible lab-on-paper biosensor to realize an interactive photothermal photoelectric dual-signal feedback mechanism for point-of-care testing of HPV-16 in body fluids.

Results: The conductive thermostable CuInS2-Mn polysaccharide hydrogel paper-electrode with unexceptionable photoexcitation activity can be act as an energy converter. In the presence of the target, the photothermal and photoelectric signal were self-switched by triple-helix modified with AgBiS2, which is beneficial to the separation and transmission of photoexcitation carriers. Furthermore, the designed triple-helix molecule additionally contains a special inhibitory fragment of Cas12a, which can regulate the AgBiS2 content at the hydrogel electrode surface according to the HPV-16 concentration. During the process, the photoelectric signal rapidly decays and the photothermal signal climbs, forming interactive regulation of dual-response. The limit-of-detection of the lab-on-paper biosensor were 0.21 fM for photoelectricity and 10.93 fM for photothermal.

Discussion and Conclusion: Thanks to the ingenious design of cascaded nanocomposite polysaccharide hydrogel, the versatility lab-on-paper biosensor can not only materialize thermal-change of preliminary screening, but also photoelectric-response of precise quantification. The current work broadens new horizons for the manufacture of low-cost, high patient compliance and less invasive home intelligent diagnosis biosensor.

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In Situ Grown COFs Containing Single Fe Sites on CdS for Electrochemical Detection of NO Released from Living Cells

Dr. Shanshan Li¹, Dr. Meiling Xu², Dr. Haihan Yu¹, Prof. Lina Zhang², Dr. Lin Li¹, Dr. Xiang Zhang², Prof. Peihua Zhu¹, Prof. Jinghua Yu¹*, Prof. Chaomin Gao¹
¹School of Chemistry and Chemical Engineering, Jinan, China, ²Shandong Provincial Key Laboratory of Preparation and Measurement of Building Materials, University of Jinan, Jinan, China

Title: In situ grown COFs containing single Fe sites on CdS for electrochemical detection of NO released from living cells

Authors: Shanshan Li¹, Meiling Xu², Haihan Yu¹, Lina Zhang², Lin Li¹, Xiang Zhang², Peihua Zhu¹, Jinghua Yu¹*, Chaomin Gao¹

Affiliations: 1School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, China. 2Shandong Provincial Key Laboratory of Preparation and Measurement of Building Materials, University of Jinan, Jinan, 250022, China (* Correspondence)

Category: Design and Application of Biomaterials

Background: In situ detection of nitric oxide (NO) released from living cells has become very important in studies of some critical physiological and pathological processes including blood vessel dilation, anti-coagulation, neurotransmission, and anti-inflammation, but it is still much challenging due to the low concentration and fast decay of NO.

Methods: In this work, we prepared the Fe-Bpy-COF@CdS by in situ growth of Fe-Bpy-COF on amino-functionalized CdS. The Fe-Bpy-COF bearing Fe active sites was fabricated by chelating coordination of dipyridyl units in Bpy-COF, in which the Bpy-COF was synthesized by the acid-catalyzed imine condensation of 1,3,5-tris(4-aminophenyl) triazine and 2,2″-bipyridine-5,5″-dicarboxaldehyde.

Results: The Fe-Bpy-COF@CdS was drop-cast onto glassy carbon substrate surface to construct the Fe-Bpy-COF@CdS-based biosensing platform. The prepared biosensor displayed a fast and ultrasensitive response toward NO in wide dynamic range of 0.09 to 400 μM with a high sensitivity of 8.9 μA·μM⁻¹·cm⁻² and a low detection limit of 16 nM. Furthermore, the biosensor was used to capture molecular signals immediately after NO released from human umbilical vein endothelial cells (HUVECs) under drug stimulation.

Discussion and Conclusion: The Fe-Bpy-COF@CdS exhibited the highly dispersed Fe active sites and highly specific electrocatalysis of Fe-Bpy-COF, and promoted charge separation and transfer, thereby resulting in a superior performance with low detection limit, fast response time and excellent selectivity for NO biosensing. The current work demonstrated a hopeful strategy to construct a composite with the COF containing single metal sites and inorganic materials for real-time analysis of NO released from HUVECs in a biological system.

Acknowledgement: This work was supported by National Natural Science Foundation of China (21874055, 22104043).
Nerve Growth Factor-Derived Peptides Accelerate Osteogenesis Of Human Bone Marrow Mesenchymal Stem Cells

Ms. Xiao Li1,2, Ms. Yau Tsz CHAN1,2, Ms. Jing SUN1,2, Professor Sung Chi Rocky TUAN1,2, Professor Yangzi JIANG1,2

1Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China, 2School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

Authors: Xiao Li 1,2, Yau Tsz, CHAN 1,2, Jing SUN 1,2, RS. TUAN 1,2, Yangzi JIANG 1,2*

Affiliations: 1 Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China. 2 School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China (* Correspondence)

Category: SYIS (Student and Young Investigator Section), Enabling Technologies

Introduction: As a skeletal neurotrophin, nerve growth factor (NGF) participates in the maintenance of skeletal pain, and NGF signal pathway involves in bone formation and fracture healing. Bone loss occurs as a result of traumas and injuries, and poor bone healing leads to functionally debilitating conditions of patients, such as loss of self-sufficiency and deterioration in life quality. Human bone marrow mesenchymal stem cells (BMSC) contribute to the homeostasis of the skeletal system under the regulation of different cytokine profiles. Also, NGF can promote BMSC survival in transplanted tissue during BMSC transplantation therapy. The NGF-derived peptides (1,2) were investigated here because of the advantages of small molecular weight, low immunogenicity, high bioactivity, and specific binding to tropomyosin receptor kinase A (TrkA, high-affinity receptor for NGF) in our earlier study (3). In this study, we firstly used bioinformatic tools to simulate the site mutations of specific amino acids of NGF-derived peptide and screened the biological effects of peptide candidates on osteogenesis with hBMSCs.

Materials and Methods: We reviewed the reported NGF-derived peptides with biological functions, and a prototype peptide, NGF(1-14), was selected as prototype because of its specific binding with TrkA. This peptide was modified and the interaction with TrkA was predicted using molecule docking platforms of PEP-FOLD Peptide Structure Prediction Server and High Ambiguity Driven protein-protein DOCKing system based on crystal structures of NGF and TrkA (AlphaFold/UniProt-P01138, Gene NGF; AlphaFold/UniProt-P04629, Gene NTRK1; Protein Data Bank, PDB 1www). Two NGF(1-14) derivatives with high binding affinity during computation simulation were selected. They were then synthesized and the binding affinity with TrkA was tested by surface plasmon resonance (SPR).

Human primary BMSCs were isolated from hip femoral heads obtained from patients who underwent total hip arthroplasty, cells were collected and characterized by flow cytometry with hBMSC markers CD73, CD90, CD105, CD34, and CD45. To study the biological effects of the designed peptides, cells were cultured in an osteogenic medium with NGF or NGF-derived peptides for 4 weeks. The osteogenic calcification of hBMSCs was evaluated by Alizarin red and von Kossa staining, and the degree of calcification was quantified by ColonyArea in ImageJ 1.5s.

Results: The molecule docking platform predicted eight NGF(1-14)-derived peptides, and two NGF(1-14) derivatives presented higher binding efficacy with the TrkA receptor based on the root-mean-square-deviation value. SPR results showed one NGF(1-14) derivative bound to TrkA successfully, while the other cannot bind to TrkA. The hBMSCs used in this study was characterized with surface marker expression of CD73 (85.20%), CD90 (76.69%), CD105 (94.50%), CD34 (0.11%) and CD45 (0.10%). Alizarin red and von Kossa staining intensity revealed that NGF-derived peptides significantly enhanced the calcification of hBMSC in a degree of 13% compared to 3% of the control group.
Discussion and Conclusion: This study demonstrated that the NGF-derived peptides improved osteogenesis in human primary BMSC in vitro, which provided a small molecule tool to regulate NGF signaling in osteogenic cells. In the near future, animal models with bone defects will be used to detect the bioactivity of NGF-derived peptides on bone formation in vivo.

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Reference
Effect of Collagen IV and Laminin Cross-gradient on Epithelialization Studies

Ms. Maitraee MISTRY¹,², Dr. Abigail Dee CHEN¹, Prof Barbara CHAN¹,²,³
¹Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China, ²Advanced Biomedical Instrumentation Centre, Hong Kong, China, ³School of Biomedical Science, The Chinese University of Hong Kong, Hong Kong, China

Title: Effect of Collagen IV and Laminin Cross-gradient on Epithelialization Studies

MISTRY M ¹,², CHEN A ¹, CHAN B ¹,²,³
¹ Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong, China
² Advanced Biomedical Instrumentation Centre, Hong Kong, China
³ School of Biomedical Science, The Chinese University of Hong Kong, Hong Kong, China
*Email: bpchan@cuhk.edu.hk

Category: Design and Application of Biomaterials

Background: Collagen IV and Laminin are two of the major extracellular matrix (ECM) protein constituents in the native basement membrane of epithelial cells. In vivo, the basement membrane plays a key role in cell adhesion, proliferation, and function. One of the key functions of epithelial cells is to form the epithelial barrier to regulate the transport of nutrients and foreign pathogens. This study aims to investigate the effect of a cross gradient of Collagen IV and Laminin on the barrier integrity of epithelial cells.

Methods: Varying ratios of Collagen IV and Laminin were combined to form chemically defined gelated basement membranes with a cross-gradient of the two ECM proteins. A fixed concentration of Collagen I was used as the gelation agent. Optimal concentrations of epithelial cells were added onto the gelated ECM and cultured to form sub-confluent epithelial cell models. MDCK, MCF7 and Caco2 cells were used to form in-vitro epithelial models of the kidney, breast and intestine respectively. Barrier integrity of the epithelial models were then evaluated morphologically by immunofluorescence staining of tight junction marker ZO1, and adherens junction marker e-cadherin. Functional barrier integrity of the invitro epithelial models was analysed through Transepithelial electrical Resistance (TEER) and Dextran permeability measurements.

Results: Quantification of the junctional proteins showed that the Co4-LN cross-gradient had varying effects on the three epithelial cell models. However, in most cases, a 50:50 or 30:70 ratio of Co4 and LN respectively gave the best results in terms of the barrier integrity formed.

Discussion and Conclusion: We demonstrated the effect of varying concentrations of Co4-LN on the barrier integrity of invitro epithelial cell models. Optimizing the ideal ratio of Collagen IV and Laminin in the basement membrane can pave the way for engineering a chemically defined bio-functional basement membrane that can be used to model more accurate bio-mimetic invitro epithelial models for drug screening and drug discovery.

Acknowledgement: This work was supported by the Mid-Stream Research Program (MRP/047/21) and the Health@InnoHK program from the Innovation and Technology Commission, and the Collaborative Research Fund (CRF) - Group Research Project(C7139-20G) from the Research Grant Council, of the Hong Kong Special Administrative Region government.
Development of a chemically defined Co4-LN Crosslinked basement membrane for Epithelialization studies

Ms. Maitraee MISTRY1,2, Dr. Xingxing YANG1,2, Dr. Abigail Dee CHEN1, Prof Barbara Chan1,2,3
1Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China, 2Advanced Biomedical Instrumentation Centre, Hong Kong, China, 3School of Biomedical Science, The Chinese University of Hong Kong, Hong Kong, China

Title: Development of a chemically defined Co4-LN Crosslinked basement membrane for Epithelialization studies

MISTRY M 1,2 , YANG X 1,2 , CHEN A 1 , Chan B 1,2,3
1 Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong, China
2 Advanced Biomedical Instrumentation Centre, Hong Kong, China
3 School of Biomedical Science, The Chinese University of Hong Kong, Hong Kong, China
*Email: bpchan@cuhk.edu.hk

Category: Design and Application of Biomaterials

Background: Epithelial disease modelling, drug discovery and drug screening relies heavily on invitro models that are biomimetic and can accurately model the extracellular matrix (ECM) microenvironment. In current invitro epithelial models Engelbreth-Holm-Swarm (EHS) mouse sarcoma derived Matrigel is used as the gold standard to model the basement membrane. However, while Matrigel is physiologically relevant, it is tumorigenic, limiting its application to mostly diseased epithelial models. It also varies batch to batch and often contains a lot of non-defined growth factors which can act as confounding variables in the interpretation of the results obtained. Thus, we have developed a Chemically Defined Gelated non-tumorigenic Basement Membrane. The developed basement membrane can be used for 3D modelling of the native epithelial layer for both healthy and diseased models.

Methods: Optimum ratio of collagen IV is crosslinked to Laminin via EDC. The Crosslinked ECM protein mixture is gelated with a fixed concentration of Collagen I to form a chemically defined basement membrane. Healthy epithelial model of the kidney was formed by culturing MDCK cells, and diseased epithelial model of the intestine and breast cancer was formed by seeding Caco2 and MCF7 cells respectively. The morphological barrier integrity of the resultant epithelial models was evaluated via immunofluorescence staining of junction markers z01 and ecadherin, while Transepithelial electrical Resistance (TEER) Measurements and Dextran Permeability assay was conducted to quantify the functional barrier integrity of the models. The results obtained were compared to that of similar epithelial models formed on Matrigel.

Results: Comparative analysis of the barrier integrity of the epithelial models formed in our chemically defined basement versus that of Matrigel, showed significantly similar results, if not better.

Discussion and Conclusion: We developed a chemically defined Collagen IV – Laminin crosslinked basement membrane gelated with Collagen I. The engineered membrane is chemically defined, physiologically relevant and non-tumorigenic. It can be used as an alternative to Matrigel to form both healthy and diseased 3D invitro epithelial cell models for various applications such as drug screening, disease modelling and drug discovery.

Acknowledgement: This work was supported by the Mid-Stream Research Program (MRP/047/21) and the Health@InnoHK program from the Innovation and Technology Commission, and the
Collaborative Research Fund (CRF) - Group Research Project(C7139-20G) from the Research Grant Council, of the Hong Kong Special Administrative Region government.
Chemical modified composite hydrogels enhance engineered cartilage formation

Mr. Zheyuan Zhou, Dr. Fengjie Zhang, Prof. Chao Wan

Key Laboratory for Regenerative Medicine, Ministry of Education, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China. 2 Center for Neuromusculoskeletal Restorative Medicine, Hong Kong Science Park, Hong Kong, Hong Kong SAR, China, 2Center for Neuromusculoskeletal Restorative Medicine, Hong Kong Science Park, Hong Kong, Hong Kong SAR, China, 3Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong, Hong Kong SAR, China

Title: Chemical modified composite hydrogels enhance engineered cartilage formation

Authors: Zheyuan Zhou, Fengjie Zhang, Chao Wan*

(* Correspondence: cwan@cuhk.edu.hk)

Category: Tissue Engineering and Regeneration

Background: Osteoarthritis (OA) is a type of degenerative joint disease that is characterized by gross loss of articular cartilage and alterations of other joint tissues. OA has emerged as a significant clinical and public health issue affecting the elderly globally. Being a tissue of avascular, aneural and lack of lymphatic drainage, articular cartilage has very limited self-regeneration capacity. Current therapies, such as non-steroid anti-inflammatory drugs (NSAIDs) and joint replacement are unsatisfactory for different stages of OA pathology. Discovery of novel therapies remains a significant clinical demand. Recently, 3D bioprinting based tissue engineering has been considered as a promising approach to facilitate articular cartilage repair or regeneration. Generation of optimized bioinks that facilitate chondrogenic cell incorporation, adhesion, growth, and differentiation is an essential step for functional tissue engineering of cartilage tissue. In this study, we aim to develop a composite bioink with alginate and gelatin (A-G) using carbodiimide chemistry crosslinking method and examine the conditions for incorporating chondrocytes in the 3D printable bioink to fabricate engineered cartilage tissue formation for articular cartilage repair.

Methods: A type of composite bioink containing A-G was generated by a reaction with 1% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 0.25% N-hydroxysuccinimide (NHS) to form chemical networks with amide bonds, a type of covalent bond, between alginate and gelatin. To accomplish the printability, three groups of bioinks including 6% alginate (A6), 3% alginate-6% gelatin (A3G6), and 4% alginate-8% gelatin (A4G8) were incorporated with chondrocytes derived from C57BL/6 new-born mice, 3D printed into porous disks and subsequently crosslinked in 100mM calcium chloride solution. Fourier-transform infrared spectroscopy (FTIR), mechanical testing and bicinchoninic acid (BCA) assay were performed to characterize the physical and chemical properties. Cell proliferation was assessed by MTT assay following 1, 3 and 7 days of culture. Histological, histochemical and immunohistochemical staining (IHC) analyses were conducted to analyze the extracellular matrix (ECM) components and chondrogenic markers including SOX 9, Col II, and MMP 13 in the in vitro 3D culture or in the subcutaneous transplantation model.

Results: FTIR analysis revealed that the peaks observed around 1620 and 1560 cm⁻¹ were attributed to the presence of Schiff’s base in the EDC/NHS treated group, whereas only a single peak presenting the carboxylic acid group in the non-EDC treated group. BCA assay showed that the concentration of degraded gelatin in the EDC-treated A-G bioscaffolds was significantly lower than that of the non-EDC treated ones. The Young’s modulus in EDC-treated groups of A3G6 and A4G8 were significantly higher (23.43 ± 0.28 and 28.03 ± 2.44kPa) than that of non-EDC treated ones (9.98 ± 0.75 and 10.86 ± 0.22 kPa), respectively, which was well matched to the mechanical environment of chondrocytes’
pericellular matrix (PCM). Chondrocytes maintained viability in these bioscaffolds, with higher proliferation rate in the 3D printed A-G chondrocytes/hydrogel constructs compared with that of the purified alginate control. Moreover, in both in vitro and in vivo experiments, chondrogenic markers including SOX 9 and Collagen type II exhibited enhanced positive ratio in the A-G printed bioscaffolds, while the expression of MMP 13 was decreased in the A-G bioscaffolds compared with the pure alginate group.

Discussion and Conclusion: Our results suggest that the EDC/NHS treatment (carbodiimide chemistry) facilitates the formation of a covalent bond (Schiff’s base) between alginate and gelatin. The slow degradation rate and higher Young’s modulus are exhibited in the EDC-treated group, indicating that modified A-G bio-scaffolds keep well fidelity at physiological temperature and possess a similar Young’s modulus with that of the chondrocyte PCM (24.0 ± 8.9 kPa). Compared with the purified alginate control, A-G groups showed increased potential to support chondrocyte proliferation, which might be attributed to the addition of the adhesive ligands and enhancement of cell aggregation in the 3D bioscaffolds. The enhanced chondrogenesis in the printed A-G chondrocytes/hydrogel constructs in vitro and in vivo indicates that the newly generated 3D bioscaffold may serve as a promising platform for cartilage tissue engineering and its potential therapeutic applications.

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Phosphorylated focal adhesion kinase by mild reduction of cell surface proteins inhibits a RhoA/ROCK2-dependent adipogenic differentiation

**Mr. Ji hoon Jeong**¹,², Mr. Sung Sik HUR¹, Mr. Yongsung HWANG¹,²

¹Soonchunhyang Institute of Medi-bio Science (SIMS), Soonchunhyang University, Cheonan, Korea, ²Department of Integrated Biomedical Science, Soonchunhyang University, Asan, Korea

Title: Phosphorylated focal adhesion kinase by mild reduction of cell surface proteins inhibits a RhoA/ROCK2-dependent adipogenic differentiation

Authors: Ji Hoon Jeong 1,2, Sung Sik Hur 1 and Yongsung Hwang 1,2,*

Affiliations: 1 Soonchunhyang Institute of Medi-bio Science (SIMS), Soonchunhyang University, Cheonan 31151, Korea. 2Department of Integrated Biomedical Science, Soonchunhyang University, Asan 31538, Korea (*Correspondence)

Category: SYIS (Student and Young Investigator Section) / Tissue Engineering and Regeneration

Backgrounds: Although cell surface engineering has enormous potential to regulate various cellular functions, such as cell adhesion, morphology, migration, invasion, differentiation, and in vivo engraftment, less progress has been made in modulating cell fate determination through mild reduction of cell surface proteins.

Methods: By harnessing the tris(2-carboxyethyl)phosphine (TCEP), a mile reducing agent with an ability to break disulfide bonds within and between proteins, we examined a molecular mechanism through which cell surface modification induces cell adhesion, spreading, and fate determination with an emphasis on the adipogenic potential of murine preadipocytes (3T3-L1). Furthermore, by using cell traction and intracellular force microscopy, we evaluate cell-extracellular matrix (ECM) traction forces and intracellular tensions.

Results: Our findings revealed that through mild reduction, free active thiol groups on the cell surface of preadipocytes were successfully exposed and cells exhibited increased cell size, elongation, number, and area of focal adhesions, leading to the inhibition of adipogenic differentiation of 3T3-L1 cells. Furthermore, by harnessing cell traction and intracellular force microscopy, we reported that cell-ECM traction force and intracellular tension were significantly increased by mild reduction of cell surface.

Conclusions: Our findings show that a mild reducing agent breaks the disulfide bond of the cell surface protein, which increases cell spreading, cell adhesion, cell-ECM traction force, and intracellular tension, and eventually inhibits adipogenesis. Collectively, these results highlight the role of mild reduction of the cell surface in adipogenic differentiation, which may be exploited to regulate cell fate determination.

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Transplantation Device for Regenerative Therapy Using Biodegradable Nonwoven Fabrics with Strongly Oriented Fiber

Dr. Hiroshi SUNAMI¹, Ms Naoko FUTENMA², Mr Hitoshi NAKASONE², Ms Junko DENDA¹, Dr Yusuke SHIMIZU²

¹Faculty of Medicine, University of the Ryukyus, Nishihara-cho, Nakagami-gun, Japan, ²University of the Ryukyus Hospital, Nishihara-cho, Nakagami-gun, Japan

Introduction: We recently developed a system for extracting adipose-derived stem cells (ADSCs) from adipose tissue slices and rapidly culturing them without enzymatic treatment by using a biodegradable nonwoven fabric. [1] The new system was developed to solve three problems facing the use of ADSCs in regenerative medicine. The first is that production of ADSCs is costly. The second is the difficulty in filling a tissue volume with cells when the affected part is deformed or large. The third is the difficulty of ensuring that injected cells are targeted accurately and remain viable for long periods of time. The system described above is expected to address these three problems.

Results and discussion: We report a biodegradable nonwoven fabric that can be used to extract adipose-derived stem cells (ADSCs) from adipose tissue slices and to quickly culture them without enzymatic treatment. The nonwoven fabric is made of highly oriented fibers consisting of biodegradable poly(lactic-co-glycolic acid) and hydroxyapatite (Figure 1). The nonwoven fabric facilitates ADSC extraction and culture from adipose tissue slices, which in this study were induced to differentiate into vascular endothelial-, chondrocyte- and adipocyte-like cells. Thick sheets of ADSCs supported by the nonwoven fabric were cut to size and transplanted into the cheeks of rats. X-ray computed tomography imaging showed that the implanted fabric biodegraded over 12 weeks. These results demonstrate that not only can ADSCs in the sheet be induced to differentiate into several types of cells, but also that the cell sheet can be successfully transplanted into animals, after which the implanted fabric biodegrades. We expect that this transplantation device will solve the three problems and promote development of simple regenerative therapy that only involves seeding tissue slices on a nonwoven fabric.


Woochan Kim, Yonghyun Gwon, Sunho Park, Jangho Kim

1Chonnam National University, Gwangju, South Korea

Here, we present an analysis of nanoscale deformation of PDMS molds in response to heat and pressure during the repetitive molding process of thermoplastic polymers. The width and height of the nano-sized ridges of PDMS molds decreased as the number of replications of thermoplastic polymers increased. Using the precisely controlled deformation of nanostructures in PDMS molds, we demonstrated that nanostructures of different sizes can be fabricated on representative thermoplastic and UV-curable polymers consistently. Using the precisely tunable methodologies of nanoscale structures, we propose a methodology to fabricate hierarchical multiscale scaffolds with controlled hydrophilic and hydrophobic properties by employing capillary force lithography in combination with oxygen plasma modification. In response to multiscale nanotopographic and chemically modified surface cues, the O-FMN patch enhanced regeneration of the mineralized fibrocartilage tissue of the tendon–bone interface and the calvarial bone tissue in vivo in rat models.

Keywords: bioinspired scaffold, multiscale topography, nanotopography, plasma modification, tissue engineering

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Chondrocyte-targeted synergistic delivery of siRNA and therapeutic chondroitin sulfate to alleviate osteoarthritis

Ms. Hang Su, Ms. Qiuwen Zhu, Prof. Hongwei Ouyang
Zhejiang University, Hangzhou, China

Background: Osteoarthritis (OA) is a chronic degenerative joint disease characterized by cartilage loss due to an imbalance between anabolism and catabolism. The high expression of catabolic enzymes and low expression of extracellular matrix in osteoarthritis leads to severe cartilage wear and tear. One of the emerging therapeutic methods is inhibiting the overexpression of catabolic enzymes by intra-articular delivery of siRNA to alleviate cartilage damage. However, traditional carriers can only assist in siRNA delivery and cannot promote extracellular matrix synthesis.

Methods: In this study, we designed an innovative carrier-free strategy that can not only inhibit metabolic enzyme secretion but also increase extracellular matrix synthesis, achieving synergistic therapeutic effects. The system consists of a cationic polymer that can promote chondrocyte secretion and enrichment of GAGs (glycosaminoglycans), adsorbing and stabilizing siRNA by electrostatic adsorption, and the therapeutic chondroitin sulfate as exogenous supplementation of GAG.

Results: Interestingly, we found that the addition of chondroitin sulfate can not only reduce the size of cationic polymer-RNA nanoparticles but also significantly reduce cell toxicity. After co-cultivation with human OA cartilage implants for 7 days, the synergistic treatment system can effectively decrease the expression of ADAMTS5. In vivo, the synergistic treatment system can reduce ADAMTS5 expression and effectively alleviate osteoarthritis progression after 8 weeks of injection in DMM mice.

Discussion and Conclusion: Synergistic delivery of siRNA and therapeutic chondroitin sulfate may be an effective strategy for the treatment of matrix loss in early OA.
Chondroitin sulfate-modified cerium oxide nano-particles targeting chondrocyte’s Golgi for the treatment of osteoarthritis

Mr. Yongrui Cai

1Department of orthopaedics/Orthopaedic institute, Westchina hospital, Sichuan University, Chengdu City, China

Introduction

Osteoarthritis (OA) is one of the most common chronic and progressive joint diseases. It is pathologically characterized by degeneration and destruction of articular cartilage, subchondral bone sclerosis, and marginal osteophyte formation. The pathogenesis of OA is still unclear. Despite living in a hypoxic environment, chondrocytes still possess abundant and active mitochondria to provide sufficient ATP. The disruption of mitochondrial function leading to the increase of intracellular ROS levels is considered to be one of the reasons that disrupt the homeostasis of cartilage and lead to cartilage damage in OA. At the same time, ROS is widely distributed in inflammatory chondrocytes and has a serious impact on cell function. As the center of cartilage anabolism, Golgi apparatus will show structural damage, decreased synthesis function and abnormal increase of apoptotic molecules under oxidative stress stimulation.

In addition to the classical functions of protein processing and transport, it has been suggested that GA contributes to the regulation of a wide range of higher-order cellular processes, including cell polarization, stress response, directed migration, mitosis, metabolism, autophagy, apoptosis, DNA repair, and inflammation. However, the presence of ROS not only impairs Golgi-related protein sorting and vesicle trafficking steps, but also affects the normal glycosylation function. In addition, some studies have found that excessive activation of ROS can lead to the destruction of Golgi structure and the activation of Golgi-involved apoptotic signaling pathways.

Based on the above research background, we can propose the following hypothesis: during the progression of OA, excessive ROS interferes with the REDOX state of Golgi apparatus, thereby affecting the metabolite state of chondrocytes; After targeted improvement of oxidative stress in the Golgi, the synthesis function of chondrocytes is restored and the progression of OA is slowed down.

Subjects and Methods

OA is a common chronic and progressive joint disease. During the progression of OA, articular chondrocytes produce a large number of ROS, which are widely distributed in inflammatory chondrocytes and have a serious impact on cell function. As the center of cartilage anabolism, Golgi apparatus will show structural damage, synthesis and secretion function decline under oxidative stress stimulation. The aim of this study is to construct Chondroitin sulfate (CS)-modified Cerium dioxide nano particulars (CeNPS), and to explore its physical and chemical properties and Golgi targeting function. To verify its therapeutic effect on OA.

CeNPS were synthesized by the microemulsion method, and CS was attached to the CeNPS surface with an amide bond. The morphology and structure of the materials were observed and characterized by transmission electron microscopy, infrared spectroscopy, and nuclear magnetic resonance. The Catalase (CAT) and Superoxide dismutase (SOD) activities of each material were measured at the same time. Inflammatory and oxidative stress models were constructed in vitro to study the therapeutic effect and protective effect of the materials on chondrocytes. The rat OA model was established by ACLT+DMM method in vivo, and the materials were injected into the articular cavity to study their therapeutic effects on OA.

Results

The successful modification of cerium oxide by chondroitin sulfate was confirmed by the formation of amide II band and the presence of S=O absorption peak in IR spectrum, the H atom signal of sugar ring skeleton and the acetyl methyl hydrogen proton in 1H NMR spectrum, and the sulfur element detected in XPS and EDS. The modified material (CS@CNPS) had a higher Ce3+/Ce4+ ratio, and the CAT and SOD activity tests showed that CS@CeNPS had a better reactive oxygen species scavenging ability.
CCK8 results showed that CeNPS were significantly toxic at levels above 10ug/ml, while CS@CeNPS showed significant toxicity at levels above 50ug/ml. Fluorescence co-localization showed that the red fluorescence of the material and the green fluorescence of the Golgi apparatus were both seen in the perinuclear region and arranged in a triangle, and the region became yellow after merging. It was demonstrated that CS@CeNPS was successfully aggregated in the Golgi apparatus. In the inflammation and oxidative stress model, each material group had a certain therapeutic effect, up-regulating the cartilage anabolic indicators (COL2/ACAN/SOX9), down-regulating the catabolic indicators (MMP13/ADAMTS5), and reducing the level of ROS (P < 0.05). Among them, CS@CeNPS significantly enhanced the above effects compared with the single drug group (P < 0.05), and fluorescence showed that the morphology of Golgi apparatus was relatively normal and intact under the treatment of this drug.

In the OA model, CS, CeNPS and CS@CeNPS all had lower knee pathological scores than the control group (P < 0.05), and CS@CeNPS significantly improved the therapeutic level compared with the single drug group (P < 0.05). No abnormal changes were found in pathological sections of other tissues and organs in each group.

Discussion and Conclusion
Chondroitin sulfate modified cerium oxide nanoparticles showed better SOD and CAT activities and lower cytotoxicity. The sulfate group endowed the nanoparticles with good Golgi targeting ability, and greatly upregulated the level of anabolism, alleviated the catabolic process to the greatest extent, and effectively protected the Golgi apparatus in the cell model. In animal experiments of osteoarthritis, all three groups of materials showed certain therapeutic effects, among which cerium oxide nanoparticles modified with chondroitin sulfate showed the best therapeutic effect. Although limited by technical means, this study cannot further elucidate how nanoparticles interact with the Golgi apparatus to affect the function of the latter, the present experimental results at least demonstrate the important role of the Golgi apparatus in the pathological process of OA, and also suggest that the Golgi apparatus can be used as a new target for the treatment of arthritis. It provides a new path for the related research of OA.
Direct micropatterning of Wnt5a gradient using a multiphoton microfabrication platform

Mrs. Mina Razaghzadeh Bidgoli¹, Dr. Xinna Wang¹, Prof. Barbara Pui Chan¹,²

¹Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China, ²School of Biomedical Science, The Chinese University of Hong Kong, Hong Kong, China

Title: Direct micropatterning of Wnt5a gradient using a multiphoton microfabrication platform

Authors: Mina Razaghzadeh Bidgoli1, Xinna Wang1, Barbara Pui Chan1,2*

Affiliations: 1Department of Mechanical Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong, Hong Kong SAR, P. R. China, (*correspondence email: bpchan@hku.hk)

2 School of Biomedical Science, The Chinese University of Hong Kong, Hong Kong, China

Categories: Design and application of Biomaterials

Introduction: It has been well known that the gradient of secreted signaling molecules, specially Wnt ligands, could provide quantitative information to trigger specific cell behavior and response. Wnt ligands can transduce their signals through canonical or non-canonical Wnt pathways. In the non-canonical Wnt-PCP pathway, it appears that Wnt molecules act as an important global cue for planar cell polarity (PCP) orientation since they can bind to the Frizzled (Fz) receptor, a core PCP protein, in a gradient manner of distribution[1]. However, there is currently no in-vitro system to directly study the effect of Wnt5a gradient on cell behaviors. Here, we aim to engineer a synthetic platform using multiphoton microfabrication technology to directly print Wnt5a in a gradient concentration manner.

Multiphoton microfabrication and micropatterning (MMM) technique is a promising technology to fabricate three-dimensional (3D) structures in micron and submicron scale range through photochemical crosslinking. This technique has been widely used to fabricate different 3D protein microstructures and micropatterns with various mechanical properties and gradients of growth factors in a controllable manner[2]. Our preliminary results show that a gradient concentration of Wnt5a molecules could be directly crosslinked on the fabricated micropatterns.

Methods: Multiphoton microfabrication and micropatterning (MMM) has previously been developed by our group to fabricate 3D protein-based micropatterns with high controllability. The bovine serum albumin (BSA) micropattern can be fabricated on the modified glass using methylene blue (MB) as the photosensitizer. Thereafter, sulfo-SANPAH will be applied on the pattern to chemically bind to the BSA. Sulfo-SANPAH as a linker can bind to the amine group of Wnt5a through two-photon irradiation. Hence, it would be possible to directly coat Wnt5a on the top of the BSA pattern dose-dependently. Finally, the presence of coated Wnt5a will be evaluated.

Results: As shown in Figure 1, immunofluorescence imaging and quantitative results suggest that Wnt5a could be coated directly on the BSA substrates in a gradient manner. The local density of crosslinked Wnt5a on the matrix is positively associated with the scan cycle and IR laser power. Accordingly, a gradual increasing the scan cycles resulted in an improvement in the fluorescence signal of Wnt5a in linear trend; however, the series reaches the saturation level and becomes nonlinear for higher powers.

Figure 1. Characterization of Wnt5a gradient. (a) Immunofluorescence staining of crosslinked Wnt5a. SC stands for scan cycle. (b) Quantification of crosslinked Wnt5a.

Discussion and Conclusion: The MMM platform has been shown to successfully and directly engineer a gradient of Wnt5a on the surface of a protein substrate in one step without using toxic and organic reagents. The Wnt5a gradient may contribute to the development of an in vitro PCP model in the long run.
References:

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Functionalized multi-walled carbon nanotube platforms for enhanced gliotransmission and astrocyte proliferation

**Mrs. Tanza Baby**¹,², Mr Ueon sang Shin¹,²

¹Department of Nanobiomedical Science, BK21 FOUR NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan, Republic of Korea, ²Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan, Republic of Korea

Title: Functionalized multi-walled carbon nanotube platforms for enhanced gliotransmission and astrocytic proliferation

Authors: Tanza Baby¹,², Ueon Sang Shin¹,²*

Affiliations: ¹Department of Nanobiomedical Science, BK21 FOUR NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan 31116, Republic of Korea. ²Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan 31116, Republic of Korea. (*Correspondence)

Category: Design and Application of Biomaterials

Introduction: Astrocyte modulates neuronal activity and excitatory/inhibitory balance via gliotransmission, which has recently been identified as a novel target for neurological disorders. CNT-based nanomaterials can accelerate astrocytic proliferation and gliotransmission-related functions, thus rendering it an improved platform for neuron-glia interaction.

Subjects and Methods: CNT platforms with various nano-topographies and features like hydrophilicity and conductivity were fabricated to provide a non-invasive microenvironment for cells. Adhesion and subsequent proliferation when cells contact with nanomaterials were also discovered. Furthermore, by in vitro and ex vivo the effect of CNT on gliotransmission and glutamate uptake ability based on astrocyte intracellular resting Ca2+ levels were tested.

Results: We studied the influence of CNT on astrocyte functions involved in synaptic transmission by releasing gliotransmitters using CNT platforms. Astrocytes on CNT platforms performed better. Cell adhesion and proliferation were enhanced by increased integrin and GFAP expression. Furthermore, intracellular GABA and glutamate levels in astrocytes were increased using CNT platforms. We also found that ex vivo CNT incubation enhanced gliotransmitters in brain slices. Furthermore, TRPV1 raised intracellular resting Ca2+ levels, which are critical for gliotransmission on CNT substrates.

Discussion and Conclusion: This work demonstrates that CNT platforms promote proliferation by developing persistent astrocyte adhesion. The electrical and nano-topographic characteristics of CNTs improved astrocyte functions associated with gliotransmission through intracellular Ca2+ and synaptic clearance. Furthermore, gliotransmitters from the astrocytes were controlled when functionalized CNTs were co-incubated ex vivo with astrocytes in the cerebellum. As a result, this study implies that CNTs could be effective nanomaterials for therapeutic approaches in the brain by employing gliotransmitters from astrocytes and grafting cells on CNT platforms.
The Development of 3D Cell Printing Model of Blood-Brain Barrier for Studying White Matter Hyperintensity

Mr. Kingston King-Shi Mok, Dr. Gerald Wai-Yeung Cheng, Mr. Sunny Hoi-Sang Yeung, Ms. Iris Wai-Ting Ma, Dr. Kai-Hei Tse

1The Hong Kong Polytechnic University, , Hong Kong SAR

Introduction

White matter hyperintensity (WMH) is a common pathology in sporadic Alzheimer’s disease (AD) resulting from blood-brain barrier (BBB) dysfunction. Apolipoprotein variant APOE4 is known to be the strongest genetic risk factor for AD and is associated with WMH. Human APOE4 expression by astrocytes was shown to disrupt the lipid transportation to oligodendrocytes, possibly leading to WMH. The development of a BBB model in vitro is essential for understanding the pathogenesis of WMH formation. To develop the optimal BBB model, it is essential to evaluate the biomaterials and the compatibility. This project assessed the biocompatibility of cell components in the BBB and especially evaluated the lipid transport function of astrocytes in an alginate-based hydrogel model. We aim to test if astrocytes survive and support oligodendrocytes in the cell printed construct.

Subjects and Methods

The CELLINK INKCREDIBLE+ bioprinter was utilized to establish a BBB model through the process of extrusion-based cell printing. Alginate-based hydrogel was mixed with the cell lines within the BBB, including endothelial cells, astrocytes and oligodendrocytes, using a sterile 22-gauge nozzle. The proliferation rate of the cells in the hydrogel was examined by AlamarBlue Cell Viability assay. Two-dimensional cell culture was served as control. The alginate-based hydrogel was mixed with the cells at different cell density (5000 cells/μL to 100000 cells/μL) and examined at 1, 3 and 7 days in vitro. A transwell culture was used to determine if alginate printed hAPOE expressing astrocytes can mediate lipid transport and support the oligodendrocytes differentiation.

Results

The findings indicated that all cell lines (bEND3, U87 and Oli-Neu) were able to survive and undergo proliferation within alginate-based hydrogel. Specifically, the alginate construct with Oli-Neu (100,000 cells/μL) and the alginate construct with astrocytes (50,000 cells/μL) demonstrated a three-fold increase in the rate of proliferation from day 1 to day 7. On the other hand, the alginate-U87 construct (50,000 cells/μL) exhibited a 1.25-fold increase in the proliferation rate during the same period. Important, the alginate printed primary astrocytes remained to express hAPOE and support differentiation of Oli-Neu in the transwell system.

Discussion and Conclusion

Our findings suggested that alginate-based cell printing is suitable for BBB model for studying WMH pathogenesis. In particular, the APOE expression and function is retained by astrocytes and the model is suitable for studying APOE or other genetic risk factor associated pathogenesis in WMH.

Acknowledgement

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Anti-Angiogenin-Like Protein 4 Antibody Alleviates Rheumatoid Arthritis By Inhibiting Joint Destruction And Inflammation

Dr. Wenxiang Cheng

Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, China

Introduction:
The pathogenesis of rheumatoid arthritis is complex and currently incurable. The discovery of rheumatoid arthritis therapeutic targets and the development of therapeutic drugs are of great importance. Antibody drugs have shown unparalleled advantages over other types of drugs in the treatment of rheumatoid arthritis because of their high specificity and high affinity. Angiopoietin-like protein 4 (ANGPTL4) is involved in the inflammatory response and has been found to correlate with disease activity and bone destruction in rheumatoid arthritis.

Subjects and Methods:
In this work, we measured ANGPTL4 levels in the sera of patients with rheumatoid arthritis and healthy individuals. The effect of anti-mouse ANGPTL4 (anti-ANGPTL4) monoclonal antibody (mAb) treatment on joint destruction was investigated using a collagen-induced arthritis (CIA) model. In vitro experiments were performed to observe the promotion of inflammation and osteoclast differentiation by recombinant ANGPTL4 protein and to verify the therapeutic effect of anti-ANGPTL4 mAb.

Results:
Serum levels of angiopoietin-like protein 4 were significantly increased in patients with rheumatoid arthritis compared to healthy subjects. In vivo experiments showed that anti-ANGPTL4 mAb inhibited the pro-inflammatory cytokine interleukin-6 (IL-6) in serum. micro-CT and histological experiments confirmed that anti-ANGPTL4 mAb significantly improved the extent of bone destruction in CIA. In vitro studies showed that recombinant ANGPTL4 protein promoted inflammatory responses and osteoclast differentiation, which was effectively inhibited by anti-ANGPTL4 mAb.

Discussion and Conclusion:
ANGPTL4 promotes inflammatory responses and osteoclastic differentiation, thereby exacerbating disease progression in rheumatoid arthritis. Anti-ANGPTL4 mAb notably ameliorates arthritis and joint destruction in the CIA model. These results suggest that targeted inhibition of angiopoietin-like protein 4 may be a novel strategy for the treatment of synovitis and joint destruction in rheumatoid arthritis.

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Multiphoton-based Cell Niche Screening for Intestinal Epithelial Culture

Mr. Chun Hin Chris CHAN¹, Dr. Abigail Dee CHEN¹, Dr. Nan HUANG¹, Prof. Barbara Pui CHAN¹,²
¹Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China, ²School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China

Title: Multiphoton-based Cell Niche Screening for Intestinal Epithelial Culture

Authors: Chun Hin Chris Chan1, Abigail Dee Chen1, Nan Huang1, Barbara Pui Chan1,2

Affiliations:
1 Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China
2 School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China
* Email: bpchan@cuhk.edu.hk

Category: Design and Application of Biomaterials

Introduction: The behaviours of cells are highly influenced by their native microenvironment known as the cell niche which consists of complex biochemical and mechanical components. The Multiphoton Microfabrication and Micropatterning (MMM) technology developed by our lab is a powerful tool in biomimicking the native cell niche, with the ability to control and decouple individual niche factors. We aim to utilize the MMM technology in the area of intestinal epithelial culture. The development of in vitro intestinal epithelial cells (IECs) culture models has various applications such as drug testing, virus screening and personalized treatment. The traditional intestinal organoid model has obvious limitations including an “inside out” enclosed lumen and the negligence of geometrical, mechanical and biochemical components present in the native tissues. This study utilized the MMM platform and the developed niche factors library to carry out cell niche screening and determine the optimal niche conditions to achieve IECs monolayer culture.

Methods: The MMM platform was used to fabricate various cell niche factors in a 61-well cell niche biochip for cell niche screening purpose. A total of 23 niche factors were used for single niche factor analysis as follows: 6 topological factors (Concave, Convex, Grating width 1um, Grating width 5um, Grating hierarchy perpendicular, Microwell pattern), 5 growth factor coatings (EGF, TGFβ3, BMP2, Wnt3A, FGF), 5 ECM coatings (Collagen Type-IV, Fibronectin, Laminin, Vitronectin, Fibrinogen), 3 Elastic moduli (15kPa, 30kPa, 45kPa) and 4 stiffnesses (Micropillar arrays of height 5um, 10um, 15um, 20um). ECM were crosslinked to the structures by direct laser crosslinking. The growth factors were coated to the structures using an indirect immobilization method with the use of NeutrAvidin (NA) being crosslinked to the structures in advance. CACO-2, an epithelial cell line of human colorectal adenocarcinoma cells, were seeded on the biochips with the niche factors for 1, 3 and 6 days. The phenotypes of the seeded cells are evaluated and analysed based on cell attachment, monolayer formation and immunofluorescence images of epithelial marker E-Cadherin and tight junction marker ZO-1 captured by a confocal microscope and their respective signal levels. Optimal single niche factors were shortlisted for further optimization.

Results and Discussion: Differences in cell phenotypes are identified upon one day of culture. Among the ECM proteins groups, Collagen Type IV gives the best cell attachment and monolayer formation. Continuous cell-cell junctions are well-formed as observed from the fluorescent signals of ZO1. Among the Growth Factors groups, EGF, BMP2 and WNT3a give better cell attachment and more continuous ZO1 junctions than other growth factors. Without any coatings, signals of cell junction markers are generally weak. Cell attachment is higher on 5um micropillar arrays than on 10um micropillar arrays, while the Convex and Grating width 5um topology give better cell attachment than...
other topology niche factors. Attachment is poor on uncoated flat surfaces of different elastic modulus, but best on 30 kPa flat surfaces. The MMM platform is capable of fabricating diversified niche factors. Optimal single niche factors will be selected and shortlisted for fabrication of combinatorial and multiplex niche biochip defining the optimal niche for intestinal cell cultures in the future.

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References:
Fabrication of aligned collagen structures using dehydration-assisted bioprinting system

Dr. JaeYoon Lee¹, Dr. JaeYoon Lee², Dr. Dajeong Yoon³, Prof. Geun Hyung Kim¹,²
¹Department of Biomechatronic Engineering, Suwon, Republic of Korea, ²Biomedical Institute for Convergence at SKKU, Suwon, Republic of Korea, ³Burn Institute, Seoul, Republic of Korea

Background: Biomaterials made using collagen are successfully used as a three-dimensional (3D) substrate for cell culture and considered to be promising scaffolds for creating artificial tissues. Although there are various collagen crosslinking methods, representative methods such as photo-crosslinking, chemical crosslinking, and thermal crosslinking are commonly used. To address the main issue of low mechanical properties of collagen bioink, there is still a need for the development of a bio printing system that can mimic the physical and morphological properties of the tissue for restoration or replacement, while also solving the low mechanical properties of collagen bioink.

Methods: In the present study, we developed a new method to use low-concentration collagen (2wt%) 3D structure using poly-ethylene glycol (PEG) as dehydration agent. The method was optimized using different processing conditions, such as PEG molecule weight, PEG concentration, moving speed, and bioink flow rate. The cellular activities including cell viability, orientation, and proliferation were also evaluated. To further enhance mechanical property and mimic the alignment of native tissue.

Results: Dehydrated collagen structure showed highly increased mechanical properties compared to collagen group with conventional fibrillation. Collagen structures were crosslinked using PEG with various molecular weights and concentrations, and the viability and proliferation were confirmed using live/dead and DAPI/phalloidin staining. In addition, collagen fiber formation and alignment were confirmed using SEM images under various crosslinking and printing conditions.

Discussion and Conclusion: We propose that the newly developed a novel method to fabricate low-concentration collagen structure with enhanced mechanical properties and topographical cues. In addition, we also suggest that the developed bioprinting system could be a new strategy for tissue engineering, enabling the rapid fabrication of 3D cell-constructs for tissue regeneration and repair.

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Targeting Engineered Exosomes Loaded with Immune Activator for Osteosarcoma Treatment

Ms. Naping Xiong¹, Jiankun Xu¹, Prof. Wenxue Tong¹, Prof. Ling Qin¹
¹Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong SAR

Title: Targeted engineered exosomes loaded with immune activator for osteosarcoma treatment

Authors: Naping XIONG¹, Jiankun XU¹, Wenxue TONG¹*, Ling QIN¹*

Affiliations: ¹Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China.

(*Correspondence, twxleo@cuhk.edu.hk; lingqin@cuhk.edu.hk)

Category: Design and Application of Biomaterials

Background: Osteosarcoma (OS) is a highly aggressive primary malignant bone tumor that predominantly affects children and adolescents. The current standard care for OS includes extensive surgical resection, chemotherapy and neoadjuvant therapy. However, neoadjuvant therapy still faces the challenges of high metastasis and high recurrence. In recent years, immunotherapy has made a breakthrough in oncotherapy, such as metastatic non-small cell lung cancer, leukemia and liver cancer. However, immunotherapies for OS patients have shown poor prognosis due to the dynamic immunogenic features and low targeting property. Therefore, there is an urgent need to develop a highly targeted delivery system to mediate the immunotherapeutic drugs. Exosomes are widely found in many tissues and bodily fluids, including blood, ascites fluid, saliva and adipose tissue. Due to the highly engineerable nature of exosomes, and their engineered specificity to cells and tissues, exosomes have been widely reported as drug-delivery vehicles. In this study, we report the use of OS-targeting exosomes as vehicles for the delivery of immune activators into tumor cells as a potential new therapeutic strategy for OS.

Aims: Targeted delivery to the tumor cells is crucial for the success of OS immunotherapy. In this study, we utilized the phage display technique to screen the OS affinity peptide (OAP). Phage display is a selection technique in which a peptide variant library is expressed on the external surface of a bacteriophage virion, while the genetic material encoding each variant resides internally. After OAP screening by the osteoblast cells (MC3T3) excluding and tumor cells (K7M2) adhering, we integrate the OAP with lysosome-associated membrane glycoprotein 2b (Lamb2b) on the surface of exosomes to produce the OAP-exosomes that can specifically target OS cells. In the tumor microenvironment, OAP-exosomes burst release of immune activator, and activate innate immune activities, such as macrophage polarization, dendritic cell maturation and activation to enhance tumor-specific antigen presentation cells. This cascade will lead to CD8+ T cell differentiation and activation, which effectively eliminates OS cells and inhibits recurrence rates. To verify the targeting property and anti-tumor recurrence ability of our designed materials, we conducted an in vivo mouse model of OS.

Methods: Phage display was used to screen the OS affinity peptide (OPA) based on an established M13 phage vector that peptides are exposed on the external surface for adhering to the potential targets. OS exhibits significant heterogeneity with osteogenic characteristics. Therefore, we firstly incubated the M13 phage library with the MC3T3 osteoblast cell line, and removed the phages that showed adhere to osteoblasts. We then collected the supernatant and incubated it with the K7M2-luc tumor cell line. The OPA peptide was successfully screened after seven times repeats (Figure A). The OAP-Lamp2b plasmid was transfected into bone marrow drives dendritic cells by Lipofectamine 3000 according to the manufacturer’s instructions. (Figure D). The K7M2 cells expressing firefly luciferase (K7M2-luc) were implanted in a collagen mesh into the femur of balb/c mice, and mice reliably developed orthotopic tumors and lung metastases as judged by bioluminescence imaging and histopathological analysis. (Figure E). Immune activators were loaded by the electroporation technique. (Figure F)

Results: The OS affinity peptide (OAP) (Figure B, C) has been successfully screened that targeting the OS cells specifically after seven times selection.

Discussion and Conclusion: Although immunotherapies are great promising strategies for treat cancers, the dynamic immunogenic features and low targeting in OS limit their efficacy and further
implementation. In this study, we demonstrated a novel approach for anti-OS immunotherapy using a targeting exosome delivery system to mediate an immune activator, which enables precision immunotherapy for OS. Encapsulating of immune activator into the engineered exosomes, which may have improved permeability and retention effects that exert a targeting function and accurately delivery drugs into the tumor cells. We hope that this project will improve the clinical immunotherapy for OS via inhibiting OS, and preventing metastasis and recurrence.

Acknowledgements: This work is supported by the Mainland-Hong Kong Joint Funding Scheme (MHP/030/20), Health and Medical Research Fund (HMRF, 09203786), and Areas of Excellence Scheme (AoE/M-402/20).

(A) Schematic illustration of M13 phage display screening design for osteosarcoma affinity peptide (OAP). (B) The results of 7 rounds of OAP screening. (C) The amino acid (AA) frequency logos illustrate that the prevalence of each amino acid is represented by its size, while its color indicates its properties. These results show that 7-peptide sequences are gradually becoming more consistent, transitioning from disorder to order. (D) The OAP-Lamp2b engineered exosomes as a targeted delivery system for immune activator to tumor cells to develop a potential treatment for OS. (E) Orthotopic implantation technique for OS mouse model and its treatment. (F) Schematic representation of the plasmid constructs containing Lamp2b, OAP-Lamp2b, and OAP-GFP-Lamp2b.
Development of A Gelatin-Based Double Network Dynamic Hydrogel for Vascularized Tissue Engineering

Mr. Runze Xu¹, Liliang Ouyang¹, Wei Sun¹
¹Department of Mechanical Engineering, Tsinghua University, Beijing, Hong Kong SAR

Title: Development of A Gelatin-Based Double Network Dynamic Hydrogel for Vascularized Tissue Engineering

Authors: Runze Xu¹, Liliang Ouyang¹*, Wei Sun¹*

Affiliations: 1Department of Mechanical Engineering, Tsinghua University, Beijing 100084, China

Category: Design and Application of Biomaterials

Background: Tissue vascularization is a fundamental process to establish functional tissue. Hydrogel materials could mimic vascular microenvironment by providing physical and biochemical cues to stimulate vascular networks formation, and could also be used as bioinks to engineer vascularized tissue. Static covalently crosslinked hydrogels have excellent printability, shape fidelity and stability. However, the cells have to actively degrade the hydrogel to spread and to remodel the hydrogel, that may delay and hamper the subsequent cellular processes. Reversibly crosslinked dynamic hydrogel could promote endothelial cell spreading into network through enhanced matrix-cell interaction. Nonetheless, dynamic hydrogels are in the lack of stability to support 3D culture. Double network dynamic hydrogel (DNDH) strategy has been applied to develop a bioink to stimulate vascular morphogenesis, as well as supporting 3D culture stability of the printed structure.

Methods: In this work, we synthesized and characterized the DNDH hydrogel components, i.e., GelMA, GelADH and DexCHO. The stability and printability of the hydrogel, as well as the effects of hydrogel components concentrations to vascular morphogenesis were screened.

Results: The modification of the hydrogel backbone is confirmed by 1H NMR test and TNBS assay. Upon addition of GelMA network, The DNDH is shown to maintain hydrogel stability and have suitable printability for extrusion based bioprinting. The DNDH hydrogel was shown to support endothelial vascular morphogenesis under both mono-culture and co-culture conditions.

Discussion and Conclusion: We demonstrated that the gelatin-based DNDH have suitable printability, that could support stable 3D culture after printing, and stimulate vascular morphogenesis. The DNDH hydrogel could be used as a general bioink to establish various vascularized tissues.
Recombinant humanized type III collagen improves vaginal laxity

Ms. Lina Hu

Introduction
Vaginal laxity is a symptom of pelvic floor dysfunction that is attracting increasing attention, at least due to the advent of laser vaginoplasty. A standardized definition of vaginal laxity or ‘looseness’ does not exist, but it is generally assumed that pregnancy and childbirth play a role. With the change of national fertility policy and the increase of vaginal delivery, postpartum vaginal laxity is becoming more common in clinical obstetrics and gynecology. Vaginal laxity is the result of a combination of pathogenic factors, the content and function of collagen and elastin in the ECM determine the supporting function of pelvic floor organs. Studies have shown that a correlation between the treatment of vaginal laxity and collagen and elastin content. Collagen is the most important structural macromolecule protein in the human body and is the framework structure in the Extracellular matrix (ECM). During pregnancy, the collagen breakdown of the pelvic floor, cervix, vagina and vulva is greater than the synthesis, and collagen deficiency is also a cause of postpartum atrophy of the vaginal epithelium. It has been demonstrated that supplementation of type III collagen in the genital tract mucosa can regulate the ratio of matrix metalloproteinases/metalloproteinase inhibitors (MMPs/TIMPs) and improve the ECM of mucosal tissue, thus providing a better microenvironment for mucosal cells. Recombinant humanized collagen (rhCol) developed by biosynthesis technology and featured by tandem repeat of amino acid unit, which was encoded by a specific segment of human collagen gene. A recombinant humanized collagen type III (rhCol III) was used in this study. The amino acid sequence of the repeat unit in rhCol III was exactly same as that in human type III collagen (Gly483–Pro512). Therefore, the supplement of rhCol III might promote the structure and remodel ECM to improve the vaginal environment and restoring the vaginal function.

Subjects and Methods
This is a multicenter, randomized, controlled, statistically superior clinical trial to evaluate the efficacy and safety of recombinant type III humanized collagen lyophilized fibers in improving vaginal laxity. The trial design: 84 subjects with vaginal laxity were enrolled in the study. A randomized, controlled trial design was used to observe and analyze the subjects before and 4 weeks after treatment. Selection Criteria 1. age ≥18 years. 2. history of at least one vaginal delivery (≥28 weeks of gestation) prior to enrollment, and last delivery more than 3 months before more than 3 months after enrollment. 3. meet the diagnostic criteria for vaginal laxity, with a quantitative score of moderate or severe vaginal laxity. 4. normal gynecological pelvic examination and normal vaginal discharge during the screening period. 5. willing to have vaginal intercourse or use a masturbator at least once a month. 6. Patients have no plans to have children or donate eggs during the study period or within 6 months of study completion and voluntary use of effective contraception. 7. sign an informed consent form and be willing to comply with the study protocol.

Research Subgroups: Experimental group: rhCol III lyophilized fiber + medical type III collagen vaginal gel. Control group: medical type III collagen vaginal gel, rhCol III lyophilized fiber: intravaginal injection treatment with 2ml sterile saline dissolved into 2mg/ml, injected by sterile syringe, single dose of 12mg, once every two weeks, 2 times. Medical type III collagen vaginal gel: vaginal placement at bedtime, 2g once, once every other day, 10 times 10 times in total.

Evaluation Indicators: Main evaluation indicators, improvement rate of quantitative vaginal laxity score
Secondary evaluation indexes. 1. Female Sexual Function Index (FSFI) score; 2. Vaginal Laxity Questionnaire (VLQ) score; 3. pelvic floor muscle assessment (electrodiagnostic) score. Safety evaluation indicators: adverse events, serious adverse events, SUSAR, vital signs (blood pressure, pulse, respiration, temperature), physical examination (including gynecological examination), laboratory tests (routine blood, urine, liver function, renal function, coagulation, immunoglobulin), ECG, and early withdrawal for safety or tolerability reasons.
Results
Under the FAS and PPS sets, the improvement rate of the quantitative score of the main evaluation index of vaginal laxity decreasing ≥1 grade after treatment was higher in the test group than in the control group, and the P value of the chi-square test was less than 0.0001 (<0.05). The efficacy of "recombinant type III humanized collagen lyophilized fiber" (specification: 4 mg/bottle) developed by Shanxi Jinbo Biomedical Co. The efficacy of the product "recombinant type III humanized collagen lyophilized fiber" (size: 4 mg bottle) combined with "medical type III collagen vaginal gel" (size: 2 g/stem) for the treatment of vaginal laxity basically meets the requirements of the program, and has the effectiveness, safety and operability in clinical use.

Discussion and Conclusion
Vaginal laxity is a common clinical condition that seriously affects life quality. While, the treatment of vaginal laxity is controversial and lacks standardization. As a large protein closely related to vaginal laxity and a major component of skin mucosa, collagen can be the most ideal material for vaginal laxity repair. However, the molecular weight of collagen is large and external supplementation through the mucosa is ineffective. Intramucosal injection directly into the vaginal wall can provide physical padding and support to the tissue, which can alleviate the symptoms of vaginal laxity. Previous studies have shown that rhCol III injection can reduce local collagenase, increase local type I and type III collagen content, improve extracellular matrix, and have excellent effects on vaginal epithelial mucosal damage repair, which can provide a new clinical method to reduce vaginal laxity. rhCol III n has a unique 164.88° triple helix structure, which is more conducive to cell adhesion. The charged amino acid residues of collagen and the charge on the surface of the cells are physically attracted to each other for cell adhesion, which provides a good external scaffold for the cells and thus supports the local tissue cells. rhCol III fibrils in the extracellular matrix through self-crosslinking and self-assembly, which contributes to tissue remodeling and repair of tissue structure. In conclusion, rhCol III could promote the clinical manifestation of vaginal laxity effectively and safely through vaginal injection, which could be an novel treatment for vaginal laxity.
Spatiotemporal transcriptomic landscape of developing mouse tendon

Mr. Tingyun Lei¹, Mr. Ruifu Lin¹, Mr. Jie Han¹, Prof. Xiao Chen¹, Prof. Zi Yin¹
¹Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative Medicine, School of Medicine, Zhejiang University, Hangzhou, China
Authors: Tingyun Lei 1; Ruifu Lin 1; Jie Han 1; Xiao Chen 1*; Zi Yin 1*
Affiliations: 1 Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative Medicine, School of Medicine, Zhejiang University, Hangzhou, 310058, China (* Correspondence)
Category: Stem Cells and Cell-Based Therapies

Background:
Tendon injuries cause prolonged disability and hardly recover completely. Developmental biology is the template for investigating tissue regeneration. Understanding tendon development is therefore of considerable clinical relevance. Some recent progress has been made in this field through single-cell RNA sequencing (scRNA-seq). However, there is still a lack of topographical transcriptomic information that can help understand cellular trajectories and interactions during tendon development.

Methods:
Spatial enhanced resolution omics-sequencing (Stereo-seq), a spatial transcriptomic technology, achieved finer resolution, higher sensitivity, and larger view. In this study, P7 and P14 of mouse Achilles tendon-bone were subjected to scRNA-seq and Stereo-seq for generating the spatiotemporal transcriptomic data. The single-cell and spatial transcriptomics were integrated for spatial cellular deconvolution, pseudotime trajectory analysis and cell-cell communication analysis.

Results:
Unsupervised clustering of scRNA-seq and stereo-seq data revealed various subpopulations and we identified spatial modules corresponding to specific tissue organizations. Next, we performed an integrated analysis of the scRNA-seq and stereo-seq data. By cell type deconvolution, we identified the distribution of tendon cell subpopulations and found a tendon stem cell population existed at tendon sheath and persisted throughout tendon development and maturation. By pseudotime analysis, we constructed the hierarchical differentiation relationship among tendon cell subpopulations. Finally, we investigated the spatial ligand-receptor pairs and identified potentially crucial interactions during tendon development. Furthermore, by using Fgf7 knockout mice, we identified FGF7 signaling as a crucial regulator for tendon maturation.

Discussion and Conclusion:
In this study, we have combined single-cell and spatial transcriptomics to explore the developing mouse tendon at a high spatial resolution. We identified a tendon sheath stem cell population and constructed the spatiotemporal hierarchical relationship of tendon cell differentiation. Additionally, we dissected the microenvironment signalings and demonstrated that FGF7 signaling plays an essential role in tendon maturation. In general, this work establishes a fundamental reference for further dissecting the mechanisms governing tendon development.

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Establishment of a coculture system with bovine osteochondral and synovial explants as an ex vivo inflammatory osteoarthritis model

Dr Kaihu Li¹,²,³, Prof Yong Zhu³, Prof Mauro Alini¹, Prof Martin Stoddart¹, Dr Sibylle Grad¹, Dr Zhen Li¹
¹AO Research Institute Davos, Davos Platz, Switzerland, ²Department of Orthopaedics, The Second Xiangya Hospital of Central South University, Changsha, China, ³Department of Orthopaedics, Xiangya Hospital of Central South University, Changsha, China

Background: Osteoarthritis (OA) is a whole-joint disorder involving inflammation in cartilage, synovium, and subchondral bone. An ex vivo inflammatory OA model incorporating multiple intra-articular tissues could better mimic the native joint milieu and reduce animal studies.

Methods: In this study, an inflammatory ex vivo coculture system with osteochondral explants (OCEs) and synovial explants (SEs) was induced using 1 ng/mL interleukin one beta (IL-1β) and tumor necrosis factor alpha (TNF-α). Meanwhile, OCE and SE monoculture groups served as controls to test the effects of coculture on cartilage and synovium tissues. Furthermore, 5-aminosalicylic acid (5-ASA), a promising OA drug reported previously, was assessed in the coculture system to evaluate the effects of 5-ASA and the potential of the system to be applied in drug screening for OA treatment.

Results: Under inflammatory stimulation, cartilage and synovium tissues in monoculture displayed a strong inflammatory response transcriptionally, biochemically, and histologically. Under non-inflammatory conditions, coculture markedly downregulated the gene expression of aggrecan (ACAN) and collagen type II (COL2) in cartilage tissue compared with monocultured cartilage. Under inflammatory conditions, coculture elevated the gene expression of interleukin 6 (IL-6) in synovium tissue compared with monocultured synovium. Furthermore, the synovium tissue in the coculture inflammatory group showed the highest synovitis score. At protein level, the interleukin-8 (IL-8) release in the coculture medium was significantly higher than the additive amount in the OCE and SE monoculture groups under inflammatory microenvironment. For drug assessment, 20 mM 5-ASA-treated group could promote the gene expression of CD163/CD86 (M2/M1) and inhibit CD86 (M2) expression in synovium compared with inflammatory group. Moreover, the cumulative release of IL-6 into the medium was mitigated upon 20 mM 5-ASA treatment.

Conclusions: This study revealed the interaction between OCEs and SEs in an ex vivo coculture system, which further validated the anti-inflammation effects of 5-ASA. This preclinical coculture system could be used not only to evaluate the effects of novel OA drugs on both cartilage and synovium, but also to explore the crosstalk mechanisms between intra-articular tissues, bridging the gap between basic research and clinical translation.
Bioinspired Cyclic Compression Enhances Chondrogenesis In A 3D Printed Composite Bioscaffold

Mr. Hon Son Ooi

1The Chinese University of Hong Kong, Hong Kong SAR

Background: Osteoarthritis (OA) has been a prevalent degenerative joint disease and public health issue affecting the aging population. The onset of OA is largely associated with increased cyclic compressive stress due to the increase in human life expectancy and body weight. Both compressive and tensile stresses, applied statically or cyclically, have been shown to advance the progression of OA. Although heavy mechanical loading is considered to induce articular cartilage degeneration, mild mechanical loading is positively correlated with chondrogenesis. Studies involving cyclic loading have been conducted on the 3-dimensional (3D) chondrocyte laden constructs, including chondrocyte pellets, chondrocyte embedded hydrogels and cell sheets to facilitate engineered cartilage formation. However, the efficiency and molecular mechanisms of the chondrocyte fate control under the cyclic mechanical stimuli remain unclear. In this study, the role of bioinspired cyclic compressive loading in controlling the fate of chondrogenic cells and engineered chondrogenesis in a 3D printed composite bioscaffold is examined systemically.

Methods: Chondrocytes derived from new born C57BL/6 mice were embedded into 12% (w/v) alginate-dialdehyde-gelatin (A-D-G) composite hydrogel at a concentration of 5 107 cells/ml. The chondrocyte containing bioink was then used to fabricate a composite bioscaffold using 3D bioprinting technique (Scheme 1). All the samples were cross-linked with 100mM CaCl2 and kept under routine culture conditions for 3 days, placed under a tailor-made pneumatic controlled cyclic compression system with the defined compressive pressures (100 kPa and 200 kPa) at 0.25 Hz, with none-compression as the control. Following up to 7 days culture, the samples were processed for histological, histochemical and immunofluorescent staining analyses. Cell proliferation in the 3D bioscaffold was examined by MTT assay. The chondrogenic marker gene expression was detected by real-time PCR.

Results: The fabricated 12% (w/v) A-D-G bioscaffold possesses a Young’s Modulus of 28kPa, which is comparable to that of the pericellular matrix (PCM) of chondrocytes in the native articular cartilage. Chondrocytes in the 3D printed bioscaffold respond to the cyclic compressive stimuli by increased proteoglycan synthesis in the extracellular matrix (ECM) of the chondrocytes as indicated by the positive Alcian blue staining, when compared with that of the none-compression control. H&E staining reveals that the cyclic compression (100kPa and 200kPa) restrains cell size of chondrocytes from hypertrophy following 7 days culture. The number of cells exhibiting a flattened morphology similar to that of the superficial zone chondrocytes in the articular cartilage is increased under the cyclic compression than that of the control, suggesting that the cyclic compression is inducive to acquire superficial articular chondrocyte morphology by mimicking the cyclic compressive stress of gait. Real-time PCR analysis reveals that chondrocytes under compressive stimuli show upregulation of mRNA levels of SOX9, Col2α1 and aggrecan, while the hypertrophy markers RUNX2, ADAMTS4, ADAMTS5 and MMP13 are downregulated than that of the none-compression control. Immunofluorescent staining indicates that SOX9, the key transcription factor of chondrocyte, is elevated under the cyclic compression than that of the control. In addition, the level of RUNX2, a key regulator of hypertrophic chondrocyte, is decreased in chondrocytes under the cyclic compression. This is accompanied by an increase in collagen type II production and a decrease in MMP13 expression in the cyclic compression group than that of the none-compression control. This indicates that the cyclic compression suppresses chondrocyte hypertrophy in the 3D printed bioscaffold while enhances chondrocyte ECM production. Intriguingly, lubricin, a key ECM component of the superficial articular cartilage, is significantly upregulated under the cyclic loading, suggesting that the cyclic loading facilitates the functional modeling of the articular cartilage surface like structures.
Discussion and Conclusion: Our data suggest that the cyclic compressive loading may serve as a promising strategy to enhance engineered chondrogenesis in the 3D printed bioscaffold. The cyclic compression on the A-D-G bioscaffold facilitates the establishment of a suitable biomechanical and biological microenvironment for chondrocyte differentiation and phenotype maintenance. The combination of the 3D bioprinting technique and the cyclic compression system may serve as a promising approach for engineered cartilage formation and its potential therapeutic applications.

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Influencing neurotrophic factor expression using electric fields and scaffold fibre alignment

Ms. Quy-susan Huynh¹, Dr Damian Holsinger¹

¹Laboratory of Molecular Neuroscience and Dementia, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Australia

Neurotrophic factors (NT) such as brain-derived neurotrophic factor (BDNF) are highly effective proteins that aid neural regeneration and survival. However, the introduction of NT into injury sites is impeded by the process of diffusion and the limited quantity that can be introduced. These issues could be addressed by inducing cells to produce NT in situ. Cells respond to both electrical and mechanical stimuli and hence it is possible to utilise the effects of these stimuli to modulate protein expression.

SH-SY5Y cells were electrically stimulated using platinum electrodes to deliver a 100mV/mm electric field (EF), generating currents of 20-27µA. Cells were stimulated for 3hr whilst growing on aligned and non-aligned, electrospun polycaprolactone fibre scaffolds and were stained for BDNF, β-tubulin (cytoskeletal protein), and DAPI (nuclear staining), 17 hr following stimulation to investigate the effects of fibre orientations, the presence of the EF or a synergistic effect of both on the expression of BDNF and nuclear morphology.

We found that fibre orientations alone influenced nuclear morphology and had some effect on BDNF expression. The addition of the EF did not significantly increase BDNF expression in different fibre orientations except for cells found in the middle of the EF, where electrospun fibres were orientated perpendicular to the direction of the EF. This would suggest that there is a synergistic influence between fibre orientation and EF in modulating BDNF expression. Both fibre orientation and the EF effected nuclear morphology, where fibre orientation had a larger effect. Cells are morphologically different due to variations in fibre orientation, and hence may experience the effect of the EF differently, leading to altered levels of BDNF expression. These results demonstrate the potential of electrical and mechanical cues to help improve nerve regeneration by promoting BDNF expression in cells surrounding the injury site.
Promotion of peripheral nerve system remyelination (and underline mechanism) by cell therapy

Ms. Pei-Yi Ou Yang1, Chia-Ching Wu1
1Institute of Cell Biology and Anatomy, National Cheng Kung University, Tainan, Taiwan

Title: Promotion of peripheral nerve system remyelination (and underline mechanism) by cell therapy

Authors: Pei-Yi Ou Yang1, Chia-Ching Wu1*

Affiliations: 1Institute of Cell Biology and Anatomy, National Cheng Kung University, Tainan, Taiwan
(* Correspondence)

Category: Stem Cells and Cell-Based Therapies

Background: Peripheral nerve injury (PNI) is a prevalent form of nervous system damage that leads to dysfunction or damage. During myelin degeneration, Schwann cells (SCs) convert into repair SCs. Previous studies have shown that repair SCs clean up myelin debris, promoting axonal regrowth, and remyelinate regenerated axons. Cell therapy is a technique that utilizes the physiological functions of cells to treat or repair damaged or diseased tissues. It involves the injection of specific cells into a living body to act at the site of injury and provide a therapeutic effect. The current surgical method for cell therapy, which involves using silicone conduits, is an emerging treatment that promotes tissue repair and regeneration by transplanting cells into damaged tissues through silicone channels. However, the use of silicon conduits may be limited by their stability and biocompatibility, as well as the challenge of controlling cell transport and localization.

Methods: In this work, we first tested the feasibility of using different cells such as adipose stem cell (ASC), ASC-derived SCs, rat Schwann cell (RT4), mouse myoblast (C2C12), and C2C12-derived myotube, then double-confirmed our findings using western blot or PCR. We created animal models of sciatic nerve injury and utilized transmission electron microscope (TEM) and focused ion beam (FIB) techniques to analyze the structures of both healthy and injured nerves. We also stained the tissue with several neuron markers, including MPZ, S100β, and p75NTR, to evaluate nerve regeneration. Finally, we co-cultured RT4 cell with rat dorsal root ganglion (rDRG) or C2C12-derived myotube with mouse dorsal root ganglion (mDRG) to investigate the role of different types of cells in remyelination.

Results: We are going to demonstrate the feasibility of using specific cells for cell therapy by forming cells into cell sheet, as evidenced by the results of Western blot and PCR analyses. In animal models, tissue sectioning, IHC staining, and transmission electron microscopy (TEM) will be performed to observe the ultrafine structure of the myelin sheath in both healthy and injured nerves. Next, axonal regeneration is affected by co-culturing rDRG with RT4 cells or mDRG with C2C12-derived myotube, ex vivo. We will validate the applicability of this cell therapy to peripheral nerves in animals and the ability to control the nerve remyelination.

Discussion and Conclusion: Based on the results, we demonstrated that cell therapy improved neural regeneration by increasing myelination processes. However, it is possible to develop a neuroma, which is a benign tumor of nerve tissue that can cause pain and other specific symptoms when a nerve is injured. Furthermore, the extent of neural repair achievable in human clinical applications remains unknown. If cell therapy is applied in human clinical trials, additional factors that may affect the repair process will need to be addressed. In the future, further research and refinement of the cell therapy will be necessary to ensure its safety and effectiveness in human patients.
3D printed marine-based biocomposite for skin tissue regeneration

Prof. Geun Hyung Kim¹, Ms. Seoyul Jo¹
¹Sungkyunkwan University School of Medicine, , South Korea

Background: Commonly, decellularized extracellular matrix (dECM) derived from mammalian has been accepted as an outstanding biomaterial for tissue engineering. However, there are some problems such as auto-immune responses, increased risk of viral and bacterial disease transmission, rejection by patients for religious reasons, and cost-related issues. As an alternative to the mammal dECM (M-dECM), fish-derived dECM (F-dECM) showed potentials to overcome those limitations.

Methods: In this study, we developed a new composite bioink using two types of F-dECM. The composition of the mixture was carefully selected to obtain a mechanically stable and highly bioactive artificial cell construct. In addition, the bioink used in this work was synthesized with methacrylate (Ma) for UV crosslinking. The F-dECM composite bioink was applied to 3D printing technique to control the structural features of scaffolds. To confirm skin tissue regeneration, evaluation of in vitro cellular activity (cytotoxicity, wound healing ability, and angiogenesis) was performed.

Results: A mechanically stable and highly bioactive cell-laden scaffold was successfully obtained using the F-dECM composite bioink with optimized composition and 3D bioprinting technique. The cellular results showed that the F-dECM biocomposite exhibited much higher cellular activities compared to M-dECM. After air-liquid culture for 28 days, the gene expression of each layer (the epidermal layer and epidermis layer) was significantly upregulated compared to M-dECM.

Discussion and Conclusion: The results indicated that F-dECM biocomposite has better wound healing ability and skin tissue regeneration than M-dECM. These results suggest that fish-skin-based dECM can be considered as a potential biomaterial for skin tissue regeneration.
Construction of Polycaprolactone Electrospun-Decellularized Human Umbilical Arterial Scaffold as Small Diameter Vascular Graft

Dr. Chih-Hsun Lin¹
¹Taipei Veterans General Hospital, , Taiwan

Introduction:
Developing small diameter vascular grafts for clinical applications poses significant challenges. This study focuses on creating a vascular graft by combining a decellularized human umbilical arterial scaffold with an electrospun polycaprolactone (PCL) outer layer.

Subjects and Methods:
Human umbilical arteries from full-term deliveries were used as scaffold material. The outer layer was removed using collagenase, followed by a decellularization protocol involving 1% SDS perfusion for 24 hours and DNase-containing serum agitation for 8 hours. Heparin was added to prevent thrombus formation. An electrospun fiber layer replaced the outer layer, allowing control over pore size by manipulating the fiber diameter. Mechanical properties were evaluated using a custom-built mechanical tester with digital image correlation. In vivo implantation of the bilayer scaffolds assessed aneurysmal dilatation after 60 days. Histological examination evaluated cellular repopulation in the middle layer.

Results:
Both electrospinning conditions improved the mechanical properties of the umbilical arterial scaffolds. In vivo implantation showed no aneurysmal dilatation after 60 days. Histological examination revealed cellular repopulation in the middle layer.

Conclusion:
Combining a decellularized human umbilical arterial scaffold with a PCL electrospun outer layer holds promise for small diameter vascular graft fabrication. The enhanced mechanical properties observed, absence of aneurysmal dilatation, and cellular repopulation in vivo suggest the potential of this hybrid scaffold for vascular tissue engineering.
A single cell niche engineering platform controls asymmetric cell division of stem cells

Dr. Nan Huang1,2, Prof. Barbara Pui Chan1,2
1Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China, 2School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China

Title: A single cell niche engineering platform controls asymmetric cell division of stem cells

Authors: Nan Huang, Barbara Pui Chan*
* Correspondence

Category: Design and Application of Biomaterials

Background: The cell niche is a complex microenvironment, which includes biochemical signals, biophysical signals, and diffusive signals. All these cell niche signals orchestrate in a well-organized manner to direct stem cell behavior and fate, such as asymmetric cell division (ACD) during early development. However, our understanding of how local niche signals influence stem cell behavior and fate is not enough, owing to the lack of good in vitro technology that is able to precisely, quantitatively, spatially, and independently manipulate individual local niche signals.

Methods: In this work, we used a multiphoton microfabrication and micropatterning (MMM) technology to fabricate protein-based 3D single cell micro-niche (3D-SCμN) with multiple cell niche signals to study their effects on the control of cell division direction and asymmetric cell division orientation.

Results: (a) Microarrays of protein-based 3D-SCμNs with spatially controlled biophysical and biochemical niche signals were successfully fabricated. (b) The tensile force axis generated within a single mESC, upon functional binding to the opposing bio-functionalized micropillar pairs, was found to predominantly determine the cell division direction. (c) Asymmetrically presenting FN and E-Cad on the opposing micropillar pair to a single mESC entrapped in a 3D-SCμN, rather than their symmetric counterparts, resulted in polarity formation in the mESC and triggered a preferred ACD orientation, as demonstrated by the asymmetric expression of a series of polarity, stemness, and differentiation markers.

Discussion and Conclusion: This work reported a novel method to engineer the 3D-SCμN with controllable biophysical and biochemical niche signals. And our asymmetric 3D-SCμN represented a powerful biological tool for the study of the biological mechanism of asymmetric cell division in the future.

Acknowledgment: This work was financially supported by the General Research Fund (17128518 and 17126122) from the Research Grant Council and Innovation and Technology Fund Tier 3 (ITS-408-18) from the Innovation and Technology Commission, of the HKSAR government; Platform Technology Fund (102009742) and Seed funding for basic science (11159131), from the University Research Committee (URC), and the University of Hong Kong (HKU).
Fibrin-based myocardial sheet stacking generates three-dimensional cardiac tissue with high transplantation efficiency

Prof. Sakaguchi Katsuhisa1, Mr. Yusuke Tobe2, Dr. Jun Homma3, Prof. Hidekazu Sekine3, Prof. Katsuhisa Matsuura3, Prof. Tatsuya Shimizu3

1Department of Medical Engineering, Tokyo City University, Tokyo, Japan, 2Department of Medical Engineering, Tokyo City University, Tokyo, Japan, 3Department of Modern Mechanical Engineering, Waseda University, Tokyo, Japan, 3Institute of Advanced Biomedical Engineering and Science, Tokyo Women’s Medical University, Tokyo, Japan

Title: Fibrin-based myocardial sheet stacking generates three-dimensional cardiac tissue with high transplantation efficiency.

Authors: Katsuhisa Sakaguchi1*, Yusuke Tobe2, Jun Homma3, Hidekazu Sekine3, Katsuhisa Matsuura3, Tatsuya Shimizu3

Affiliations: 1 Department of Medical Engineering, Tokyo City University, Tokyo, Japan. 2 Department of Modern Mechanical Engineering, Waseda University, Tokyo, Japan. 3 Institute of Advanced Biomedical Engineering and Science, Tokyo Women’s Medical University, Tokyo, Japan. (*Correspondence)

Background: The development of three-dimensional (3D) tissues may potentially facilitate remarkable advancements in myocardial therapeutic and pharmaceutical research. However, cardiac tissue engineering for cardiomyoplasty necessitates scale-up and vascularization. In this study, we utilized cell sheet technology and an angiogenesis-stimulating adhesive to construct a 3D myocardial tissue and assess its transplantation efficiency.

Methods: We designed a 3D cardiac tissue with high engraftment efficiency that can be rapidly generated by stacking cardiomyocyte sheets using fibrin as an adhesive. Cell sheets were fabricated by peeling confluent cultured cells from a polymer-coated culture dish subjected to low temperatures. The high engraftment rate is attributable to the retention capacity of the adhesive protein. These tailored 3D myocardial tissues were transplanted into the backs and infarction sites of rat models of myocardial infarction (MI) for evaluation.

Results: A multi-layered cell sheet constructed using fibrin was transplanted into the subcutaneous tissues and sites of infarction in rat MI models to generate 10 layers of multi-layered myocardial tissue with a thickness of approximately 500 µm. The subsequent assessment demonstrated myocardial functional recovery in the transplant recipients.

Discussion and Conclusion: Transplantation of this novel fibrin-based multilayered cardiomyocyte sheet twice at 1-week intervals successfully generated 3D myocardial tissue, implying its efficacy in aiding tissue-engineered transplantation therapy. This study focused on engineering the myocardium, but we believe that this strategy can be applied to diverse cell transplantation experiments regardless of whether the cells are polarized.

Acknowledgment: This study was supported by the JSPS KAKENHI (Grant Number 18K18838, 20K20986) and Terumo Life Science Foundation.
A tumor-on-chip platform for immunotherapy based on lung cancer assembloids

Mrs. zixuan wang\textsuperscript{1}, Yanmei Zhang\textsuperscript{1}, Zhuo Xiong\textsuperscript{1}
\textsuperscript{1}Biomanufacturing Center, Department of Mechanical Engineering, Tsinghua University, Beijing, Hong Kong SAR

Title: A tumor-on-chip platform for immunotherapy based on lung cancer assembloids

Authors: Zixuan Wang\textsuperscript{1}, Yanmei Zhang\textsuperscript{1}, Zhuo Xiong\textsuperscript{1}\ast

Affiliations: 1Biomanufacturing Center, Department of Mechanical Engineering, Tsinghua University, Beijing 100084, People’s Republic of China

Category: Enabling Technology

Background: Immunotherapy is a powerful therapeutic approach able to re-educate the immune system to fight cancer. A key player in this process is the tumor microenvironment (TME), which is a dynamic entity characterized by a complex array of tumor and stromal cells as well as immune cell populations trafficking to the tumor site through the endothelial barrier. Recapitulating these multifaceted dynamics in vitro is critical for studying the intimate interactions between cancer and the immune system and to assess the efficacy of emerging immunotherapies.

Subjects and Methods: In this work, we construct a tumor-on-chip platform by microfluidic technique to mimic the 3D TME structure and simulate the process of in vivo tumor immunotherapy. First, we develop a patient-specific lung cancer assembloid (LCA) model by using a droplet microfluidic technology based on a microinjection strategy. Second, we create a tumor-on-chip platform. LCAs are loaded in the central channel. One of the lateral channels is seeded with HUVECs, the other channel enables nutrients for the assembloids.

Results: First, the LCAs presents better consistency in drug response profiling compared to the corresponding cancer organoids and accurately replicates the clinical outcomes of patients, suggesting the potential of the LCA model to predict personalized treatments. Second, by adding patient-derived immune cells and PD-1 in this vascular channel, it is possible to accurately simulate the in vivo immunotherapeutic cell targeted migration and infiltration of tumors.

Discussion and Conclusion: We provide a method for the high-throughput generation of uniform cancer assembloids and a personalized preclinical model that replicates patient-specific TME for immunotherapy. This model and future adaptations may drive clinical-translational efforts to develop novel combinations and personalized immunotherapy for lung cancer.
Development And Characterization Of Exosome-loaded Gelatine Hydrogel Cross-linked With Genipin For Vocal Fold Regeneration In Glottic Insufficiency

Ms. Zarqa Iffah ZAMLUS, Professor Marina MAT BAKI, Associate Professor Dr Mawaddah AZMAN, Associate Professor Dr Yogeswaran LOKANATHAN

1Department of Otorhinolaryngology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Cheras, Malaysia, 2Centre for Tissue Engineering & Regenerative Medicine (CTERM), Faculty of Medicine, Universiti Kebangsaan Malaysia, Cheras, Malaysia

Introduction: Glottic insufficiency is commonly due to the injury on laryngeal nerve which affects the quality of voice and difficulty in breathing. Currently, injection augmentation using hyaluronic acid is done as treatment but it requires re-injection every 3-6 months. Exosomes are vesicles that carry variety of biomolecules for cell signaling. Gelatin hydrogel are natural biopolymer that usually use as drug delivery. To enhance biomechanical properties of scaffold, gelatin hydrogel is cross-linked with genipin and use as drug delivery system for exosomes to target area.

Methods: This study uses exosomes derived from umbilical cord and incorporate them in gelatin hydrogel cross-linked with genipin (Exo-GCGH). These scaffolds are then test on vocal fold fibroblasts (VFF) to see its effectiveness.

Results: Exo-GCGH was shown to express low cytotoxicity after culture them together with VFF for 7 days. Moreover, Exo-GCGH also exhibits better cell proliferation on VFF compared to GCGH. The experiments are still ongoing as there are test to observe the specific protein that corresponds with Exo-GCGH.

Discussion and Conclusion: From the tests, it shows that Exo-GCGH is non-toxic and give positive results on VFF growth. The current work gives a promising result that exosomes play a part on importance of cell growth and proliferation.
3D Bio-printed Bi-layer Endometrial Construct for Functional Regeneration of Uterine Endometrium

Ms. Nanfang Nie¹, Dr Lin Gong, Dr Xiaohui Zou
¹Department of Gynaecology, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

Introduction: The endometrium is essential for embryo implantation and pregnancy maintenance. Once the endometrium is severely damaged, it will cause endometrial dysfunction, including intrauterine adhesions (IUAs), infertility, pregnancy-related complications, etc. Current clinical treatments are not satisfied. Therefore, it is important to develop a new strategy to reconstruct the natural structure of the endometrium and restore pregnancy function.

Subjects and Methods: A sodium alginate-hyaluronic acid composite hydrogel that has similar mechanical properties to the uterus was chosen to meet the needs of uterine volume expansion in late pregnancy. Then we fabricated the biomimetic bilayer EC with primary endometrial epithelial and stromal cells using 3D bio-printing technology to mimic the structure of a dense epithelial layer and a loose stromal layer of the endometrium. Then the bi-layer EC was applied to a partial full-thickness excision rat model and the therapeutic effect was assessed by hematoxylin and eosin staining, Masson staining, immunohistochemistry staining, and pregnancy function test.

Results: 30 days and 90 days after implantation, the histological analysis showed that the 3D bio-printed bi-layer EC restored the morphology and structure of the endometrial wall, including organized luminal/glandular epithelium, stroma, vasculature, and the smooth muscle layer. It also restored the hormone-response of the regenerated endometrium. Additionally, in the 3D bio-printed group, the regenerated endometrium was sufficient to support embryo implantation and development. The embryo implantation rate in the surgical area was as high as 75% (12/16).

Discussion and Conclusion: In summary, the 3D bio-printed bi-layer EC effectively promotes the recovery of the morphological structure and pregnancy function of the damaged uterus. This study showed a promising therapeutic in the field of endometrial regenerative medicine.

Acknowledgement: This work was supported by the National Natural Science Foundation of China (Grant 81871127, 92049117).
Hyaluronic Acid Methacrylate Contained Biomimetic Interphotoreceptor Matrix Bioink Supports Bioprinting and in situ Differentiation of Neural Stem Cells

Mr. Xiao Luo

Tsinghua University, China

Introduction: The major difficulty in treating Age-related Macular Degeneration (AMD) is that traditional drugs and instruments are inadequate to repair damaged photoreceptors. Constructing tissue-engineered biomimetic retina tissue as a transplant in replace of damaged photoreceptors is a potential method for treating AMD. Bioprinting is an ideal method for constructing such biomimetic retina tissue because it can precisely arrange cells, which meets with the highly polarized cell arrangement pattern in retina. Preparing interphotoreceptor matrix (IPM)-based hydrogel as a component of bioink is benefit for enhancing the survival and function of photoreceptors.

Methods: In this work, we prepared a biomimetic IPM hydrogel containing hyaluronic acid methacrylate (HAMA), gelatin, alginate and fibrinogen, as a component of the bioink. The mechanical properties of the hydrogel are tested. A neural stem cell bioprinted 3D construct is created and the ability of in situ differentiation of neural stem cells is examined.

Results: The elastic modulus of the biomimetic bioink resembles IPM. Fluorescence staining results showed that the survival rate of neural stem cells was over 100%, and the neural stem cell mark of Nestin was highly expressed, indicating that the printing method did little harm to the survival and function of neural stem cells. After adding induced medium, the expression of Nestin was replaced by neuron mark NeuN, showing that neural stem cells differentiated in situ into neurons.

Discussion and Conclusion: We prepared a bioink that supports the division and in situ differentiation of neural stem cells in a bioprinted construct. The gelatin-alginate-fibrinogen component showed great performances in the bioprinting of primary neural cells in our previous work[1], while HA is a key component of IPM. The ratio of adding HA in bioink system is discussed and optimized, and further improvement would be implemented when biomimetic design is precisely considered. The success of maintaining neural stem cells constructs for more than one month supports further integrity of HA in our bioink system, and our current work has demonstrated that the bioink showed great potential in bioprinting photoreceptors.
Rapid Printed Double-layer Scaffold With Ultra-small Nano-oligomers For Osteochondral Regeneration

Ms. Yuqing Gu1, Ms. Shufang Zhang1
1Zhejiang University, Hangzhou, China

Introduction: Avascular articular cartilage has poor self-regeneration capacity. If damage on cartilage is untreated, it might extend further into the subchondral bone, causing pain and osteoarthritis (OA). Due to structural and biological heterogeneity of articular cartilage and subchondral bone, it is still challenging to develop an integrated scaffold that synchronously fulfills the requirements for osteochondral defects regeneration. Nano-hydroxyapatite (HANP), although widely used for bone regeneration, could be "isolated" by the bone matrix because of large size, so we need to develop smaller subnanometer bone apatite crystals.

Methods: Here, we use gelatin methacrylate (GelMA) hydrogel to simultaneously develop a bilayer scaffold with different structures via digital light processing (DLP) technology. The upper layer hydrogel has both lotus- and radial-distribution pores to facilitate the migration and distribution of cells, while the lower hydrogel has lotus-like pores to guide the migration of bone marrow mesenchymal stem cells (BMSCs) to the upper space. In the lower layer hydrogel, the incorporation of 1nm-sized conformational precursor oligomers (CPO) of calcium carbonate endowed the GelMA hydrogel with a higher osteogenic capacity.

Results: Biocompatibility of the composite CPO/GelMA hydrogel was also verified. Additionally, in both subcutaneously and in situ implanted scaffolds, cells infiltrated into both lotus-like and radical pores, which is conducive for the repair of cartilage defect. 16 weeks post implantation in the rabbit osteochondral defect model, simultaneous cartilage and subchondral bone regeneration were achieved in CPO/GelMA group. The BV/TV value of the CPO/GelMA group (47.28%±5.932%) was significantly improved compared with the blank group (21.52%±5.845%) (P=0.0027). Moreover, SEM results showed that the regenerated cartilage of the CPO/GelMA group was more similar to normal with a smoother microsurface.

Discussion and Conclusion: It is expected that this rapid printed bilayer scaffold with ultra-small nano-oligomers can become a new strategy for osteochondral regeneration.
Calcium carbonate coating of PLA scaffolds by pressure-assisted and heat-induced method

Prof. Mario Monzón1, Dr. Ricardo Donate1, Dr. Rubén Paz1, Álvaro Quintana1, Dr. Pablo Bordón1

1Mechanical Engineering Department, University of Las Palmas de Gran Canaria, Edificio de Fabricación Integrada, parque tecnológico de la ULPGC, Tafira Baja, Spain

Title: Calcium carbonate coating of PLA scaffolds by pressure-assisted and heat-induced method

Authors: Mario Monzón*, Ricardo Donate, Rubén Paz, Álvaro Quintana, Pablo Bordón

Affiliations: Mechanical Engineering Department, University of Las Palmas de Gran Canaria, Edificio de Fabricación Integrada, parque tecnológico de la ULPGC, Tafira Baja. 35017. Las Palmas de Gran Canaria. Spain

Category: Design and Application of Biomaterials

Background: By embedding ceramic particles as a coating, the functionality improvement of the polymeric scaffolds can be concentrated on the cell-surface interface, thus creating a more favourable environment for the adhesion and proliferation of cells. Additive Manufacturing (AM) techniques constitute a powerful tool for bone tissue engineering, allowing 3D porous structures with great control over pore size, to mimic the function of native bone tissue.

Methods: In this work a pressure-assisted method is developed to coat PLA scaffolds, made by additive manufacturing, with calcium carbonate (CaCO3) particles for bone tissue engineering. CaCO3 has the capacity of buffering agent to reduce the acidic degradation byproducts of PLA. The 3D printed PLA scaffolds, after placed into a mould, were coated with 1 mL of a 60% w/w CaCO3 solution in distilled water, poured into the cavity. A controlled maximum pressure of 10 MPa was applied, allowing the slurry to infiltrate the internal porous structure. After the compression step, the coated scaffold was extracted from the mould and placed into an oven, kept at 155°C for 45 min. The heated and softened surface enables the CaCO3 particles to be embedded, providing a good adhesion to the polymeric matrix. The surface coating was evaluated under optical and SEM microscopy, water contact angle (WCA), EDX. Also, the compression mechanical property was tested and an enzymatic degradation analysis was carried out.

Results: A good level of porosity was kept after the coating, from an average initial value of pore size of 399±20 µm to 350±13 µm. So the estimated thickness of the coating was in the range 10-30 µm. The WCA decreased from 87.7±1.4° to 82.6±4.2° after coating.

The incorporation of CaCO3 particles significantly increased the compressive modulus (from 131±7 MPa to 150±2 MPa) and the yield strength (from 18±2 MPa to 25±1 MPa). In terms of the enzymatic degradation, the coated scaffold showed similar weight loss to the one without coating but the PH of their surrounding media was kept almost constant, confirming the buffering effect of the CaCO3 to counteract the pH decrease of the media caused by the presence of PLA acidic by-products.

Discussion and conclusion: The pressure-assisted and heat-induced coating method presented in this work (infiltration of 60% w/w CaCO3 solution in AM PLA scaffolds by compression at up to 10 MPa load, followed by a subsequent heat treatment at 155°C for 45 min) has shown to be effective for embedding CaCO3 particles on the surface of PLA scaffolds applied for bone tissue engineering, increasing mechanical properties, increasing hydrophilicity and controlling the acidic effect of the degradation of PLA.

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10-hydroxy-2-decenoic Acid Protects against Osteoarthritis through targeting Aspartyl β-hydroxylase

Mrs Nana GENG, Mrs Mengtian Fan, Mr. Biao Kuang, Miss Menglin Xian, Miss Lin Deng, Prof. Fengjin GUO

1Department of Cell Biology and Genetics, School of Basic Medical Sciences, Chongqing Medical University, Chongqing, China

Osteoarthritis (OA) is a degenerative joint disease with joint pain as the main symptom, caused by fibrosis and loss of articular cartilage. Because of the complexity and heterogeneity of patients, there is a lack of effective individualized treatment methods in clinical practice. Chondrocyte senescence leads to the occurrence and progression of OA. However, treatments aiming to inhibit chondrocyte senescence in OA are still rare. 10-hydroxy-2-decenoic acid (10-HDA) is a drug and food homologous compound. Undoubtedly, targeting the cellular senescence of chondrocytes is considered a promising approach for the treatment of OA. Despite this, studies on inhibiting chondrocyte senescence in OA are still rare. Concurrently, the underlying mechanisms of chondrocyte senescence in OA have not been fully elucidated.

10-hydroxy-2-decenoic acid (10-HDA) is a natural drug and food homologous compound originating from royal jelly (RJ). As a unique medium-chain fatty acid, 10-HDA comprises approximately 40% of the total fatty acids in RJ. Interestingly, 10-HDA has been reported to prevent and treat skin photodamage through inhibiting UVA-induced cellular senescence and the generation of reactive oxygen species (ROS). The pharmacological and biological activities of 10-HDA have also been reported against other diseases, such as rheumatoid arthritis (RA), colon cancer, colorectal carcinoma, neuroinflammation, skeletal muscle atrophy, bacterial infections, colitis, various solid tumors, and osteoporosis.

In the current study, we aimed to explore whether and how 10-HDA administration can alleviate chondrocyte senescence and prevent articular cartilage degradation. This study broadens the therapeutic prospect of natural medicines derived from food in OA, and provides a new idea for the individualized treatment of OA. Here, we revealed that 10-HDA suppressed cartilage degeneration in cartilage explants from patients with OA, and progression and pain in OA mice model. Using DARTS, mass spectrometry, and Western blotting we found that 10-HDA targeted aspartyl β-hydroxylase (ASPH) in OA. We further confirmed that 10-HDA alleviated senescence by targeting the ASPH/ERK/p53/p21 and ASPH/GSK3β/p16 pathways in human chondrocytes. Overall, our findings indicated that 10-HDA regulates cartilage metabolism in OA through inhibiting chondrocyte senescence. Therefore, the nutritional product 10-HDA might be a novel and promising therapeutic drug against OA in the future.
Development of methacrylated gelatin-based traction and intracellular stress measurement techniques to understand mechanotransduction of stem cells during single-cell and collective migration

Dr. Sung Sik HUR1,2, Ji Hoon JEONG1, KyungMu NOH1,2, Dr. Jin Kwon CHUNG3, Dr. Jae Hong PARK4, Dr. Yongsung HWANG1,2
1Soonchunhyang Institute of Medi-Bio Science (SIMS), Soonchunhyang University, , Republic of Korea, 2Department of Integrated Biomedical Science, Soonchunhyang University, , Republic of Korea, 3Department of Ophthalmology, Soonchunhyang University Seoul Hospital, , Republic of Korea, 4Department of Otorhinolaryngology-Head and Neck Surgery, Soonchunhyang University Cheonan Hospital, , Republic of Korea
Title: Development of methacrylated gelatin-based traction and intracellular stress measurement techniques to understand mechanotransduction of stem cells during single-cell and collective migration
Authors: Sung Sik Hur1,2,†, Ji Hoon Jeong1,2,†, KyungMu Noh1,2, Jin Kwon Chung3, Jae Hong Park4,#, and Yongsung Hwang1,2,#
Affiliations: 1Soonchunhyang Institute of Medi-Bio Science (SIMS), Soonchunhyang University, 2Department of Integrated Biomedical Science, Soonchunhyang University, 3Department of Ophthalmology, Soonchunhyang University Seoul Hospital, 4Department of Otorhinolaryngology-Head and Neck Surgery, Soonchunhyang University Cheonan Hospital, Republic of Korea († Equal contribution, #Co-correspondence)
Category: Enabling Technologies

Background: Cell-generated cell-matrix (traction) and intracellular (cell-cell) stresses in the physiological and pathological microenvironments have been of great interest. Although these mechanical stresses exerted by live cells have been conventionally analyzed under polyacrylamide (PAAm) hydrogel-based method due to its good linear elastic and transparent optical properties, PAAm hydrogel has limitations, including difficulties in encapsulating cells in three-dimension (3D) microenvironment.

Methods: First, we compared viscoelastic behaviors of methacrylated gelatin (GelMA) and PAAm, including Young’s modulus, dynamic modulus (G’, G’’), and tanδ, stress relaxation, and strain recovery. Next, we evaluated the spatiotemporal manner of cell-generated traction and intracellular tension during single-cell and collective cell migration of human tonsil-derived mesenchymal stem cells (hT-MSCs) cultured on both GelMA and PAAm matrices using traction force microscopy (TFM), intracellular force microscopy (IFM), and monolayer stress microscopy (MSM).

Results: Our results showed that viscoelastic and elastic properties of both PAAm and GelMA-based hydrogels were equivalent, with stiffnesses ranging from 3.4 kPa to 60.6 kPa. Our findings indicate that our GelMA-based TFM, IFM, and MSM methods were able to measure cell-generated forces during single-cell and collective cell migration. The mechanotransduction behaviors of hT-MSCs were modulated by matrix stiffness (soft and stiff) in a spatiotemporal manner.

Conclusion: Our results suggest GelMA can be a good candidate for a cell-ECM and intracellular stress measurement platform. This technique can offer insight into the important roles of dynamic cell-matrix and intracellular stresses in regulating various stem cell behaviors, such as adhesion, proliferation, migration, and lineage commitment within 3D microenvironments.
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Development of analysis system for in vitro drug sensitivity with genetic LQTS and BrS patient derivatives

Mr. Yun-gwi Park1, Ph.D Soon-Jung Park2, Prof. Sung-Hwan Moon1
1Department of Animal Science and Technology, Chung-Ang University, Anseong-si, South Korea, 2Stem Cell Research Institute, T&R Biofab Co. Ltd, Siheung-si, South Korea

Title: Development of analysis system for in vitro drug sensitivity with genetic LQTS and BrS patient derivatives

Authors: Yun-Gwi Park1, Soon-Jung Park2, Sung-Hwan Moon1*

Affiliations: 1Department of Animal Science and Technology, Chung-Ang University, Anseong-si, Gyeonggi-do, South Korea. 2Stem Cell Research Institute, T&R Biofab Co. Ltd, Siheung-si, Gyeonggi-do, South Korea (* Correspondence)

Category: Stem Cells and Cell-Based Therapies

Background: Drug-induced cardiotoxicity can differ among individuals, particularly those with underlying diseases. Here, we tried to develop a system to confirm the cardiotoxic sensitivity based on Comprehensive in vitro Proarrhythmia Assay (CiPA), a new cardiotoxicity evaluation method using human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (hiPSC-CMs).

Methods: The hiPSC was generated from normal and two types of genetic arrhythmia patients, Long-QT syndrome (LQTS) and Brugada syndrome (BrS). Then, they were differentiated to hiPSC-CMs. After the confirmation of disease-specific phenotype by electrophysiology, the cardiotoxicity was evaluated using bisphenol A (BPA), COVID-19 drug candidates, CiPA 28 drugs.

Results: In the normal and patient iPSC-CMs, 1) Exposure to 10 μM BPA caused abnormal action potential and mitochondrial deformation; 2) Treatment of 30 μM remdesivir, one of the COVID-19 drug candidates, reduced Na+ peak amplitude and prolonged QT interval; 3) Most of CiPA 28 drugs, the cardiotoxic sensitivity was higher in the iPSC-CMs from LQTS and BrS patients than normal ones.

Discussion and Conclusion: These results demonstrate that hiPSC-CMs derived from normal and genetic disease patients may be a good cellular resource compared to conventional systems. Furthermore, these findings could provide a database for patient-specific prescription and treatment.

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Paintable Decellularized-ECM Hydrogel for Enhancing Cardiac Repair and Angiogenesis in Myocardial Infarction

Mr. Jaewoo LEE1, Mr. Seul-Gi Lee2, Prof. Nathaniel Suk-Yeon Hwang1, Prof. C-Yoon Kim3

1School of Chemical and Biological Engineering, Seoul National University, Seoul, Republic of Korea,
2Department of Stem Cell Biology, School of Medicine, Konkuk University, Seoul, Republic of Korea,
3College of Veterinary Medicine, Konkuk University, Seoul, Republic of Korea

Title: Paintable Decellularized-ECM Hydrogel for Enhancing Cardiac Repair and Angiogenesis in Myocardial Infarction

Authors: Jaewoo Lee¹, Seul-Gi Lee², C-Yoon Kim³ *, Nathaniel S. Hwang¹ *

Affiliations: ¹School of Chemical and Biological Engineering, Seoul National University, Seoul, 151-742, Republic of Korea
²Department of Stem Cell Biology, School of Medicine, Konkuk University, Seoul, 143-701, Republic of Korea
³College of Veterinary Medicine, Konkuk University, Seoul, 05029, Republic of Korea (*Correspondence)

Category: Design and Application of Biomaterials

Introduction: Myocardial infarction (MI), caused by coronary artery blockage, results in ventricular wall thinning, fibrosis, and loss of cardiac function. Currently developed patches for MI treatment have shown a lack of adhesiveness, requiring additional treatments such as sutures or light irradiation, which can cause secondary damage to the infarcted region. This challenge can be solved by the catechol-based adhesive hydrogel, which presented stable adhesion to the wet tissue surface. Moreover, with painting, the adhesive hydrogel can also be simply applied with fabricating patches in situ. Heart decellularized-ECM (hdECM) has been proven to be an effective material for reducing cardiac fibrosis and inducing angiogenesis after in vivo implantation. Overall, a hdECM-contained paintable adhesive hydrogel is a potential option for improving MI curing.

Methods: We fabricated tyramine-conjugated hyaluronic acid (HA_t) hydrogel and decellularized cardiac tissue with the optimal decellularization method. We analyzed characteristics of hdECM-contained paintable HA_t hydrogel (pdHA_t), including swelling ratio, mechanical properties, and controllable wet adhesiveness. Moreover, MI regeneration and angiogenesis abilities were evaluated in vivo for 28 days.

Results: Decellularized cardiac tissue preserved ECM compounds while reducing cellular components to safe levels for implantation, with no rejection observed. Compared to HA_t hydrogel without hdECM, pdHA_t demonstrated an appropriate swelling ratio to avoid cardiac tamponade along with improved mechanical properties. pdHA_t exhibited stable and long-term wet adhesion when painted on the infarcted heart surface. Furthermore, pdHA_t could lose its adhesion through a simple washing step, thereby avoiding unnecessary adhesion to nearby organs. After 28 days of in vivo analysis, pdHA_t prevented fibrosis, ventricular wall thinning, and heart malfunction. Additionally, pdHA_t promoted angiogenesis as evidenced by the endothelial cell and smooth muscle cell staining.

Discussion and Conclusion: We demonstrated that hdECM can improve the characteristics of HA_t paintable hydrogels. Furthermore, pdHA_t, delivered through a simple painting method, can effectively regenerate infarcted cardiac tissue and promote angiogenesis. This study highlighted both the simplified hydrogel application system with controllable wet adhesion and the promising abilities of hdECM, including angiogenesis.

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Epigenetic regulation and gene profiles of direct reprogramming of human adipose-derived stem cells into neurospheroids

Dr. Ming-Min Chang1,2,3,4,5, Professor Chia-Ching Wu1,2,3,4,5

1Department of Cell Biology and Anatomy, College of Medicine, National Cheng Kung University, Tainan, Taiwan ROC, 2Medical Device Innovation Center, National Cheng Kung University, Tainan, Taiwan ROC, 3Department of Biomedical Engineering, College of Engineering, National Cheng Kung University, Tainan, Taiwan ROC, 4Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan ROC, 5International Center for Wound Repair and Regeneration, National Cheng Kung University, Tainan, Taiwan ROC

Background: Adipose-derived stem cells (ASCs), known as multipotent mesenchymal stromal cells, are widely used in clinical application in recent years. Adipose tissues have many advantages, e.g. easily harvested and isolated, abundant and easy to culture, as an adult stem cell source compared to bone marrow. ASCs can be differentiated into most mesenchymal cells including adipocytes, osteocytes, and chondrocytes, and have the potential to differentiate into non-mesenchymal cells, such as the neuronal cells. This cell fate conversion without transitioning through an intermediary pluripotent state is termed direct reprogramming. Our previous work demonstrated chitosan induced spheroid formation and differentiation of human ASCs into a mixed population of neural lineage-like cells containing nestin- and neurofilament heavy chain (NFH)-positive cells in the inner sphere, and glial fibrillary acidic protein (GFAP)-positive glial cells in the surface of sphere. The administration of ASCs into a chitosan-coated nerve conduit induced neurospheroids and facilitated nerve regeneration in rat sciatic nerve injury models. However, the underline mechanisms of ASC transdifferentiation into neurospheroids is still unknown. The development and repair of the nervous system requires the coordinated expression of several specific genes. Epigenetic factors, including histone modifications, DNA methylation, and non-coding RNA-mediated regulations, are known to play a pivotal role in controlling stem cell fate and differentiation. Here, we investigated the epigenetic regulation and gene profiles of direct reprogramming

Methods: 10x Genomics 3’ CellPlex multiplexing platform is used to construct single-cell RNA sequencing (scRNA-seq) libraries of two samples from single cells of hASCs and neurospheroids direct reprogramming from chitosan induced hASC. The scRNA-seq dataset was analyzed by Cell Ranger, R studio/R language, Surat, Monocle 2 and Ingenuity Pathway Analysis (IPA) software. Inhibitors of histone trimethyltransferases (SNDX-5613, chateocin, and GSK126) were used to confirm which genes were regulated by epigenetic regulatory enzymes. Western blotting analysis and qPCR assays were performed.

Results: H3K4me3, H3K9me3, and H3K27me3 marks play a crucial role in direct reprogramming of hASC into neurospheroids, respectively. The scRNA-seq results showed that there were 8 distinct clusters in hASC and neurospheroids. hASCs mainly contained the cluster 0 (32%), cluster 1 (26%), cluster 2 (20%), and cluster 5 (10%), whereas the spheroids is mainly comprised of the cluster 3 (30%), and cluster 4 (21%), and cluster 6 (8%). These results characterized the heterogeneity of hASCs and also indicated that the chitosan-induced neurospheroids composed of multiple populations. The trajectory analysis revealed a continuum of cells with one branch point and three distinct fates. Cluster 1 cells from ASCs were found at first two end of the trajectory, cluster 3 cells from spheroids were found at the third end, and cluster 6 cells from spheroids lied between the branch point and the cluster 3 end. Overlaying the cluster trajectory distribution and the pseudotime tree map indicated that the cluster 3 cells of spheroids were direct reprogrammed from cluster 1 cells of ASCs. The cluster 6 cells might be the transient subpopulation during the spheroid formation. In these study we also screen the genes regulated by histone trimethylation at H3K4, H3K9, and H3K27, and established gene profiles for direct reprogramming hASCs into neurospheroids.

Discussion and conclusion: Revealing the underlying mechanisms of direct reprogramming of hASCs into neurospheroids with neurogenic potentials can help us to develop a potential clinical therapeutic strategy for injured peripheral nerve regeneration and healing.
Acknowledgement: This study was supported by grants from National Science and Technology Council (NSTC 111-2320-B-006-029-MY3, and NSTC111-2320-B-006-069-MY2).
Design, development and pre-clinical testing of articulated robotic in situ bioprinter for skin wound healing

Dr. Vladimir Mironov¹, Alex Levin, Stanislav Petrov, Elizaveta Koudan, Alexei Kovalev, Fedor Senatov
¹Center for Biomedical Engineering, National University of Science and Technology “MISIS”, Moscow, Russia, ²Priorov Central National Institute of Traumatology and Orthopedics, Moscow, Russia
Title: Design, development and pre-clinical testing of articulated robotic in situ bioprinter for skin wound healing
Authors: Alex Levin, Stanislav Petrov, Elizaveta Koudan, Alexei Kovalev¹, Fedor Senatov, Vladimir Mironov *
Affiliations: Center for Biomedical Engineering, National University of Science and Technology “MISIS”, Moscow, Russia, 1Priorov Central National Institute of Traumatology and Orthopedics, Moscow, Russia (*Correspondence)
Category: Enabling Technologies
Background: In situ bioprinting is one of the most clinically relevant techniques in the emerging bioprinting technology because it could be performed directly on the human body in the operating room and it does not require bioreactors for post-printing tissue maturation. However, commercial in situ bioprinters are still not available on the market.

Methods: In this study, we demonstrated the benefit of the originally developed first commercial articulated collaborative in situ bioprinter for the treatment of full-thickness wounds in rat and porcine models. We used an articulated and collaborative robotic arm from company KUKA and developed original printhead and correspondence software enabling in situ bioprinting on curve and moving surfaces.

Results: The results of in vitro and in vivo experiments show that in situ bioprinting of bioink induces a strong hydrogel adhesion and enables printing on curved surfaces of wet tissues with a high level of fidelity. The in situ bioprinter was convenient to use in the operating room. Additional in vitro experiments (in vitro collagen contraction assay and in vitro 3D angiogenesis assay) and histological analyses demonstrated that in situ bioprinting improves the quality of wound healing in rat and porcine skin wounds.

Discussion and Conclusion: The absence of interference with the normal process of wound healing and even certain improvement in the dynamics of this process (Fig. 1) strongly suggests that in situ bioprinting could be used as a novel therapeutic modality in wound healing.
Therapeutic tissue regenerative nanohybrids self-assembled from bioactive inorganic core chitosan shell nanounits

Mr. Ye Sung Lee

1Institute of Tissue Regeneration Engineering (ITREN), Dankook University, South Korea,
2Department of Nanobiomedical Science & BK21 NBM Global Research Center for Regenerative Medicine, Dankook University, South Korea, 3Department of Biomaterials Science, College of Dentistry, Dankook University, South Korea, 4Cell & Matter Institute, Dankook University, Republic of Korea, UCL Eastman-Korea Dental Medicine Innovation Centre, Dankook University, South Korea, 5Department of Biochemistry, College of Medicine, Inha University, Republic of Korea, UCL Eastman Dental Institute, University College London, London, United Kingdom

Title : Therapeutic tissue regenerative nanohybrids self-assembled from bioactive inorganic core chitosan shell nanounits


Affiliations : Institute of Tissue Regeneration Engineering (ITREN), Dankook University, South Korea, Department of Nanobiomedical Science & BK21 NBM Global Research Center for Regenerative Medicine, Dankook University, South Korea, Department of Biomaterials Science, College of Dentistry, Dankook University, South Korea, Cell & Matter Institute, Dankook University, Republic of Korea, UCL Eastman-Korea Dental Medicine Innovation Centre, Dankook University, South Korea, Department of Biochemistry, College of Medicine, Inha University, Republic of Korea, UCL Eastman Dental Institute, University College London, London

Category : Tissue Engineering and Regeneration

Background : Natural inorganic/organic nanohybrids are a fascinating model in biomaterials design due to their ultra microstructure and extraordinary properties. Here, we report unique-structured nanohybrids through self-assembly of biomedical inorganic/organic nanounits, composed of bioactive inorganic nanoparticle core (hydroxyapatite, bioactive glass, or mesoporous silica) and chitosan shell - namely Chit@IOC. The inorganic core thin-shelled with chitosan could constitute as high as 90%, strikingly contrasted with the conventional composites.

Methods : The Chit@IOC nanohybrids were highly resilient under cyclic load and resisted external stress almost an order of magnitude effectively than the conventional composites. The nanohybrids, with the nano-roughened surface topography, could accelerate the cellular responses through stimulated integrin-mediated focal adhesions.

Results : The nanohybrids were also able to load multiple therapeutic molecules in the core and shell compartment and then release sequentially, demonstrating controlled delivery systems. The nanohybrids compartmentally-loaded with therapeutic molecules (dexamethasone, fibroblast growth factor 2, and phenamil) were shown to stimulate the anti-inflammatory, pro-angiogenic and osteogenic events of relevant cells. When implanted in the in vivo calvarium defect model with 3D-printed scaffold forms, the therapeutic nanohybrids were proven to accelerate new bone formation.

Discussion and Conclusion : Overall, the nanohybrids self-assembled from Chit@IOC nanounits, with their unique properties (ultrahigh inorganic content, nano-topography, high resilience, multiple-therapeutics delivery, and cellular activation), can be considered as promising 3D tissue regenerative platforms.
Acknowledgement: This work was supported by the grants (2020R1F1A1072210, 2018 R1A2B3003446, 2018K1A4A3A01064257, 2019R1A6A1A11034536, 2015K1A1A2032163), National Research Foundation, Republic of Korea, and also supported in part from Dankook University (Priority Institute Support Program in 2021).
Effect And Mechanism Of YaoBiTong Capsule On Rat Puncture-induced Intervertebral Disc Degeneration Model

Dr. Wenxiang Cheng¹, Songlin Liang¹,², Jiawen Zhan³,⁴, Hongyan Guo³,⁴, Nianhu Li², Peng Zhang¹

¹Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China,
²Shandong University of Traditional Chinese Medicine, Jinan, China,
³Wangjing Hospital, China Academy of Chinese Medical Sciences, Beijing, China,
⁴CapitalBio Corporation, Beijing, China

Title: Effect and Mechanism of YaoBiTong capsule on rat Puncture-induced intervertebral disc degeneration model

Authors: Songlin Liang¹,², Jiawen Zhan³,⁴, Hongyan Guo³,⁴, Nianhu Li², Peng Zhang¹, Wenxiang Cheng¹

Affiliations: ¹Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China
²Shandong University of Traditional Chinese Medicine, Jinan, China
³Wangjing Hospital, China Academy of Chinese Medical Sciences, Beijing, China
⁴CapitalBio Corporation, Beijing, China

Background:
Repairing damaged intervertebral discs (IVDs) is a challenging issue in the field of spine, particularly after intervertebral disc degeneration (IDD). Nucleus pulposus (NP) damage causes the abnormal IVD environment, and the abnormal environment further aggravates NP damage, which is a common factor in the exacerbation of IDD patients. Therefore, maintaining the normal metabolism and repair of the nucleus pulposus as much as possible is the key to treatment after nucleus pulposus injury.

As one of the most commonly used Chinese patent medicines, YaoBiTong capsule (YBTC) is a summary of Professor Sun Shuchun's clinical treatment experience and recorded in the Chinese Pharmacopoeia(2015). It consists of 8 traditional Chinese drugs, Panax notoginseng (Sanqi, Chinese) of principle medicine, Ligusticum striatum DC. (Chuanxiong, Chinese) known as chuanxiong in Chinese, Corydalis yanhusuo W.T.Wang (Yanhusuo, Chinese) and Cynanchum otophyllum C.K.Schneid (Baishao, Chinese) of minister medicines, Angelica pubescens Maxim.f.biserrata Shan et Yuan (Duhuo, Chinese) of assistant medicine, Rheum palmatum L. (Dahuang, Chinese), Achyranthes bidentata BL (Niuxi, Chinese) and Cibotium barometz (L.).J.Sm. (Gouji, Chinese) of envoy medicines has shown good efficacy for IDD, however, the mechanism of YBTC in treating IDD is not clear at present.

Applying modern scientific methods to clarify the efficacy as well as pharmacological mechanisms of YBTC will provide a potentially effective way to repair NP and IVD.

Methods: Male SD Rats aged 8-10 weeks were used in this study and divided into 6 Groups according to the different operation: Sham-4 weeks, IDD-4 weeks, YBT-4 weeks, Sham-8 weeks, IDD-8 weeks and YBT-8 weeks (n=10). Puncture were performed at the C5/6 and C7/8 levels of the caudal spine of the rats in IDD and YBT groups with using a gauge size hypodermic needle(25G). After surgery, the rats in the YBT group were administrated with 340 mg/kg/day YBTC by gavage over a 4-week and 8-week. MRI examination was performed every week to observe the medullary nucleus. At the end point, rats were euthanized and the caudal segment (C5/6 and C7/8) were harvested and fixed in 10% neutral formalin fixative buffer. Then the samples had undergone microo-CT scan. The HE staining and IHC clarify the possible pathway and potential mechanism of YBTC therapy.

Results: Radioactivity evaluation showed that the decrease rate of NP height, volume and average signal value of rats in YBTC group was reduced under MRI T2 sequence, and the time point of low NP signal appeared later than that in IDD group. CT showed that rats in YBT group have a reduced swelling of the tissue around the puncture site; Histological observations showed that YBTC was observed to reduce the morphological changes of nucleus pulposus cells and annulus fibrosus cells caused by NP injury; promote the repair of injured nucleus pulposus and reduce the disordered arrangement of injured annulus fibrosus.

Discussion and Conclusion:
This work revealed the good efficacy of YBTC in the treatment of IVD and the repair of IVD-related tissues, and also suggested the potential of traditional Chinese medicine in the field of bone repair.
Although attempts have been made to clarify this conclusion from a number of different perspectives and indicators, it must be made clear that histological and imaging results are more subjective than biological results and there are some differences between the RNA sequence results in rats and those in humans. In addition, the mechanism of YBTC and the transformation of TCM in vivo remains to be explored, in the future. However, thanks to the development of tissue engineering, it is possible for TCM compounds, medicinal materials, monomers and other derivatives to be combined with biological materials. In the future, as more mechanisms of TCM regulating diseases are revealed, TCM will be more closely related to tissue engineering, bringing hope to more diseases in more fields.

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Smart Hydrogel Biomaterials as Drug Carriers for Corneal Wound Healing

Prof. Jui-Yang Lai¹
¹Department of Biomedical Engineering, Chang Gung University, Taoyuan, Taiwan

Title: Smart hydrogel biomaterials as drug carriers for corneal wound healing

Author: Jui-Yang Lai¹*

Affiliation: ¹Department of Biomedical Engineering, Chang Gung University, Taoyuan, Taiwan (* Correspondence)

Category: Design and Application of Biomaterials

Background: In clinical ophthalmology, corneal abrasion is a condition characterized by inflammation, scar formation, and visual impairment. Current treatments remain constrained by complex and sequential conditions that impede access to most therapies for progressively abraded corneas. An advanced therapeutic hydrogel sheet functionalized with a ternary drug-carrier system can potentially overcome the drawbacks associated with treatment modality for corneal wound healing.

Methods: In this work, the smart hydrogels were constructed from acrylate and chitosan. This biomaterial system was further assembled with the ternary drug carriers comprising liposomes containing nanoceria and polysaccharide nanoparticles carrying insulin-like growth factor. In vitro and in vivo studies were performed to evaluate the performance of hydrogels.

Results: Due to the tailored degradability of the ternary system, the smart hydrogels exhibited multistage drug release, consequently allowing successive drug administration onto an abraded cornea for the suppression of inflammatory response and oxidative stress by the fast release of nanoceria, followed by promotion of wound healing via the slow release of insulin-like growth factor. In an animal model, the smart hydrogels could effectively repair injured cornea tissues and demonstrated successful tissue recovery.

Discussion and Conclusion: The smart hydrogel materials may open up a new avenue for the treatment of corneal wound and other complex ocular diseases.

Acknowledgement: This work was supported by National Science and Technology Council of Taiwan #MOST110-2221-E-182-001-MY3.
Introduction: Pancreatic islet transplantation is promising and has potential in the treatment of type 1 diabetes (T1D) patients whereas ensuring successful engraftment and functioning of the transplanted islets has consistently posed a significant obstacle in the field. Native pancreatic islets have a dense network of blood vessels that facilitate glucose sensing and insulin secretion. However, during islet transplantation, the harvested islets lose their vascular connections which leads to hypoxia and reduced function. The pre-vascularization strategy aims to establish a functional microvasculature system in transplanted islets prior to transplantation, improving their long-term survival and performance.

Subjects and Methods: In this work, we fabricated the implantable, pre-vascularized, islet-encapsulated, fibrin-based hydrogel system with the co-culture of endothelial cells and fibroblasts. We examined the microvasculature formation surrounding the intact islets and analyzed the in vitro insulin release profile of the hydrogel construct. Moreover, after the induction of T1D mice model using streptozotocin, we transplanted the pre-vascularized islet construct to the subcutaneous site of the T1D mice model and monitored non-fasting blood glucose levels and body weight periodically for 30 days.

Results: The co-culture hydrogel system exhibited improved viability and functionality of both islets and vasculature. In vitro studies showed the successful formation of the hydrogel constructs with vasculature surrounding the encapsulated islets. Insulin secretion was observed from the islets encapsulated in the pre-vascularized constructs, in both in vitro and in vivo. Compared to the non-vascularized islet model, our pre-vascularized islet construct showed accelerated engraftment, resulting in faster attainment of normoglycemia.

Discussion and Conclusion: Our study demonstrated the effectiveness of the pre-vascularized islet construct in promoting swift vasculature regeneration and insulin secretion, offering a potential treatment for T1D. Given the essential role of vascular regeneration in engineered cells or tissue implants, this system holds promise for the application of other endocrine organs as well.

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Sodium containing magnesium alloys enhance bone regeneration by angiogenesis aided osteogenesis and regulated biological apatite formation

Prof. Huafang LI1,2, Prof. Jiankun XU2, Prof. Yang LIU3, Prof. Cuie WEN4, Prof. Yufeng ZHENG3, Prof. Ling QIN2

1School of Materials Science and Engineering, University of Science and Technology Beijing, Beijing, China, 2Musculoskeletal Research Laboratory of Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, 3School of Materials Science and Engineering, Peking University, Beijing, China, 4School of Engineering, RMIT University, Melbourne, Australia

Title: Sodium containing magnesium alloys enhance bone regeneration by angiogenesis aided osteogenesis and regulated biological apatite formation

Authors: Huafang Lia, Jiankun Xub, Yang Liuc, Ling Qinb, Cuie Wend, Yufeng Zhengc.

Affiliations: a School of Materials Science and Engineering, University of Science and Technology Beijing, Beijing 100083, China. b Musculoskeletal Research Laboratory of Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China. c School of Materials Science and Engineering, Peking University, Beijing 100871, China. d School of Engineering, RMIT University, Melbourne, Victoria 3001, Australia. (*Corresponding author: Prof. Huafang Li huafangli@ustb.edu.cn)

Category: Design and Application of Biomaterials

Background: Biodegradable magnesium (Mg) alloys have attracted great attention in research and development (R&D) of medical implants in the past decades because of their biodegradability and biocompatibility. Significant milestones include but are not limited to mechanism explanatory of calcitonin gene-related peptide (CGRP) involved in Mg2+-induced osteogenesis, commercial products in terms of bone screws and coronary stents, through translational progress and clinical trials, as well as better understanding of the corrosion behavior of Mg-based biodegradable metals.

Methods: In the present study, in order to increase the mechanical property and corrosion resistance with essential element Na, by using Sn-Na master alloy, Na was micro-alloyed to fabricate MgSnZnNa alloy, and for the first time to investigate its potential as biodegradable Mg scaffold, with comparison to high purity Mg and Na-free MgSnZn alloy both in vitro and in vivo. Their mechanical properties, corrosion resistance, cell viability and ability for calvarial defect regeneration have been compared.

Results: Micro-CT imaging along with derived bone volume density (BV/TV) after 6 and 12 weeks were presented (Fig.a and b). No significant bone mineralization was found in the blank/sham group over time, while MgSnZn scaffold showed limited bone mineralization after 6 weeks and 12 weeks. In comparison, Na incorporated Mg scaffold showed a significant higher bone mineralization in the defects which accounted for 37.17 ± 4.13% after 6 weeks and 42.84 ± 3.92% after 12 weeks. In consistence, pixel-based bone coverage of the defect showed that the new bone coverage for MgSnZnNa is 88.48% after 12 weeks implantation, which was much higher than the MgSnZn (43.02%) or blank (7.49%). Besides, most new bone was found formed at the edge of the defects in Mg6Sn5Zn group. According to the scoring system described in the protocol, bony bridging over partial length of defect (scoring 3) and bony bridging entire span of defect at longest point (diameter) (Scoring 4) were found in Na-incorporated Mg scaffold 6 weeks and 12 weeks after implantation, respectively. In contrary, no bony bridging over length was found in the blank or Mg scaffold groups. The scoring for different group after 6 weeks and 12 weeks was shown in Fig. c.

Discussion and conclusion: we demonstrate for the first time a Na micro-alloyed biodegradable Mg-based scaffold that achieves a unique co-release of Mg and Na for enhancing bone regeneration.
Acknowledgement: This work was supported by the Fundamental Research Funds for the Central Universities and the Youth Teacher International Exchange & Growth Program (No. QNXM20220020).
Phosphorylated focal adhesion kinase by mild reduction of cell surface proteins inhibits integrin α5β1-dependent patient-derived cancer cell migration and invasion

Dr. Joo Hyun Kim¹,², Laurensia D. Anggradita¹,³, Jae Hong Park², Myung Jin Ban², Yongsung Hwang¹,³

¹Soonchunhyang Institute of Medi-bio Science (SIMS), Soonchunhyang University, Cheonan-si, South Korea, ²Department of Otolaryngology, Soonchunhyang University Cheonan Hospital, Cheonan-si, South Korea, ³Department of Integrated Biomedical Science, Soonchunhyang University, Asan-si, South Korea

Authors: Joo Hyun Kim¹,², Laurensia D. Anggradita¹,³, Jae Hong Park², Myung Jin Ban²,*, and Yongsung Hwang¹,³,*

Affiliations: ¹Soonchunhyang Institute of Medi-bio Science (SIMS), Soonchunhyang University, Cheonan-si 31151, ²Department of Otolaryngology, Soonchunhyang University Cheonan Hospital, Cheonan-si 31151, ³Department of Integrated Biomedical Science, Soonchunhyang University, Asan-si 31538, Republic of Korea. (*Correspondence)

Background: Although head and neck squamous cell carcinomas (HNSCCs), including tongue, vocal cord, and buccal cancers, are one of the most common cancers worldwide with a mortality rate of more than 40%, there have been no solid standard biomarkers for effectively diagnosing HNSCCs at early stages and no effective protocols available to support in vitro expansion of HNSCC cells.

Methods: To establish a cell culture method to support in vitro self-renewal of HNSCC cells, patient-derived tumor was enzymatically digested, and the tongue cancer epithelial cells were seeded onto feeder cells with the Rho kinase inhibitor (Y-27632). Additionally, to test our hypothesis that free thiol groups in the cells, generated by a mild reduction of cell surface proteins using the reducing agent tris(2-carboxyethyl) phosphine hydrochloride (TCEP), would modulate cancer cell adhesion and migration, we assessed the expression levels of integrins, focal adhesion kinase (FAK), and phosphorylated FAK (pFAK) of tongue cancer epithelial cells cultured on both soft and rigid polyacrylamide (PAAm) hydrogel-based cell culture substrates.

Results: Patient-derived tongue cancer epithelial cells exhibited self-renewal ability and maintained cancerous phenotypes, with high expressions of P53, Ki67, E-cadherin, CD44, and AHDH1A1. Treatment with the reducing agent (TCEP) increased the degree of cell adhesion through the significantly upregulated integrin α5β1-mediated phosphorylation of FAK, resulting in inhibited single-cell and collective cell migration as well as invasion. Additionally, when these cells were cultured on stiff PAAm hydrogels mimicking the tumor microenvironment, the cells exhibited accelerated cell migration behaviors, compared to cells on soft hydrogels.

Conclusion: MEFs and Rho kinase inhibitors can promote the self-renewal of patient-derived HNSC cells. Using this cancer cells and CAFs can be used to study cancer development and progression and test new therapies. It can also be used to study the effects of environmental factors on cancer development.
“Decellularized Extracellular Matrix-Based Models for Breast Cancer Research: Organoid Culture and Pharmacological Screening”

Mrs. Julia López de Andrés¹, Laura de Lara-Peña¹, Carmen Griñán-Lisón², Carmen Granados², Juan Antonio Marchal¹, Gema Jiménez¹

¹Department of Human Anatomy and Embryology, Faculty of Medicine, Granada, España, ²Instituto de Investigación Biosanitaria ibs.GRANADA, University Hospitals of Granada - University of Granada, Granada, España, ³BioFab i3D Lab- Biofabrication and 3D (bio)printing Singular Laboratory, University of Granada, Granada, España, ⁴Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Granada, España

Category: Design and Application of Biomaterials and SYIS

Background: Breast cancer (BC) is the second most prevalent tumor type worldwide and the leading cancer in women. The tumor microenvironment (TME) plays a crucial role in influencing the behavior of BC cells. The TME consists of stromal cells (fibroblasts, mesenchymal stem cells, immune cells, and vascular structures) embedded within an extracellular matrix (ECM), creating a favorable and heterogeneous environment for tumor development. Recent research has highlighted the importance of non-cellular components, such as ECM protein composition and biomechanical properties, in BC progression and metastasis. Recent advances have made it possible to obtain in vitro reconstituted ECM, known as decellularized ECM (dECM), which provides a robust platform for recapitulating key tumor characteristics and model the TME more realistically.

Objectives and Methods: This study focuses on the development of a bioink using dECM derived from different molecular subtypes of BC cell lines, enabling the advancement of 3D model development. To achieve this, MCF-7, MDA-MB-231, SKBR3, and BT474 cell lines were cultured, and an optimized decellularization protocol was implemented to obtain the highest quantity and quality of the matrix. The structural components, growth factors, and ECM properties of the dECMs from the different cell lines were characterized and quantified. The resulting bioinks were then tested for their suitability in culturing stromal and epithelial cells from the TME, as well as organoids derived from patient-derived xenografts (PDXs).

Results: The matrices from different sources exhibited distinct structures, compositions, and properties, which correlated with the clinicopathological characteristics of the molecular subtypes of BC. Additionally, they induced noticeable differences in the cellular morphology of stromal cells cultured within the hydrogels, and variations were observed in how stromal cells interact with their environment in the different models. Furthermore, these models enabled the cultivation of BC organoids derived from (PDX).

Discussion and Conclusion: The diversity and heterogeneity observed in the composition of healthy, tumoral, and premetastatic ECM highlight the importance of recapitulating the entire ECM component set of BC for studying tumor behavior, rather than focusing on individual ECM proteins. The dECM derived from tumor culture demonstrates its efficacy and emerges as a promising starting point for the development of bioinks that can encompass the BC heterogeneity, including various molecular types and subtypes of cancer.

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Decellularised Extracellular Matrix-Based Bioinks for Osteochondral Tissue Regenerative Medicine

Ms. Ana VOLTES MARTÍNEZ1,2,3,4,5,6, Julia LÓPEZ DE ANDRÉS1,2,3,4,5, Daniel MARTÍNEZ MORENO1,2,3,4,5, Carmen GRÍÑÁN LISÓN2,7,8, Gema JIMÉNEZ1,2,3,4,5, Juan Antonio MARCHAL1,2,3,4,5, Elena LÓPEZ RUIZ1,2,3,5,9

1Biopathology and Regenerative Medicine Institute (IBIMER), Centre for Biomedical Research (CIBM), University of Granada, Granada, Spain, 2Instituto de Investigación Biosanitaria ibs.GRANADA, University Hospitals of Granada, University of Granada, Granada, Spain, 3BioFab i3D—Biofabrication and 3D (Bio)Printing Laboratory, University of Granada, Granada, Spain, 4Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Granada, Spain, 5Excellence Research Unit “Modeling Nature” (MNat), University of Granada, Granada, Spain, 6Bio-Health Research Foundation of Eastern Andalusia - Alejandro Otero (FIBAO), Granada, Spain, 7GENYO, Centre for Genomics and Oncological Research, Pfizer/University of Granada/Andalusian Regional Government, Granada, Spain, 8UGC de Oncología Médica, Hospital Universitario de Jaén, Jaén, Spain, 9Department of Health Sciences, University of Jaén, Jaén, Spain

Authors: Ana Voltes-Martínez 1,2,3,4,5,6, Julia López-de Andrés 1,2,3,4,5, Daniel Martínez-Moreno 1,2,3,4,5, Carmen Gríñán-Lisón 2,7,8, Gema Jiménez 1,2,3,4,5, Juan Antonio Marchal 1,2,3,4,5*, Elena López-Ruiz 1,2,3,5,9*

Affiliations: 1Biopathology and Regenerative Medicine Institute (IBIMER), Centre for Biomedical Research (CIBM), University of Granada, Granada, Spain. 2Instituto de Investigación Biosanitaria ibs.GRANADA, University Hospitals of Granada, University of Granada, Granada, Spain. 3BioFab i3D—Biofabrication and 3D (Bio)Printing Laboratory, University of Granada, Granada, Spain. 4Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Granada, Spain. 5Excellence Research Unit “Modeling Nature” (MNat), University of Granada, Granada, Spain. 6Bio-Health Research Foundation of Eastern Andalusia - Alejandro Otero (FIBAO), Granada, Spain. 7GENYO, Centre for Genomics and Oncological Research, Pfizer/University of Granada/Andalusian Regional Government, Granada, Spain. 8UGC de Oncología Médica, Hospital Universitario de Jaén, Jaén, Spain. 9Department of Health Sciences, University of Jaén, Campus de las Lagunillas SN, Jaén, Spain. (*Correspondence)

Introduction: Osteochondral tissue regeneration is a significant challenge in regenerative medicine. Despite the many existing techniques using different types of biomaterials and biomolecules, a fully functional cure has not yet been found due to the complexity of fully mimicking the multiphasic properties of this tissue. Decellularised extracellular matrix (dECM)-based bioinks as part of new osteochondral graft designs aims to fill these gaps by preserving and maintaining the structural and biochemical interactions of the native environment.

Methods: Mesenchymal Stromal Cells (MSCs) from various healthy donors had been isolated and grown under good laboratory practices (GLP) conditions. To generate cartilage-dECM and bone-dECMs, MSCs were grown under a combination of differentiation factors. Cartilage-dECMs and bone-dECMs were characterised and evaluated in vitro by immunofluorescence and histological techniques assays. The addition of the dECMs to a combination of fluid biomaterials allowed the generation of novel 3D bioprintable bioinks that were subsequently evaluated by assessing biomechanical properties, cell viability, cell differentiation, and biocompatibility assays by immunofluorescence staining assays.

Results: After obtaining the cell-derived extracellular matrices, their cell differentiation and correct decellularisation were confirmed. Cartilage-dECM and bone-dECM demonstrated remarkable biochemical properties for osteochondral regenerative medicine. The results showed that our dECM-based bioinks possess mechanical viscoelastic properties suitable for use as an injectable hydrogel. Hydrogels loaded with MSCs generated using dECM-based bioinks maintained cell viability and
proliferation over time. Pre-differentiation of MSCs was observed after 21 days in standard cell culture conditions.

Discussion and Conclusion: This work demonstrates that MSCs from lipoaspirate, thanks to their capacity for self-renewal and differentiation into other cell types, are a suitable source for generating differentiated extracellular matrices. Furthermore, cartilage-dECMs and bone-dECMs show good biocompatibility and biochemical properties like those of native tissues. Our results indicate that dECM is a promising, easily adaptable, low-cost biomaterial for bone regenerative medicine use. All this suggests that dECM-based bioinks could be successfully applied as an implant to promote regeneration in osteochondral damaged tissue.

Acknowledgement: This work was supported by Fundación Mutua Madrileña (FMM AP17196 2019).
Recreating the Pancreatic Cancer Microenvironment: Breakthroughs in Organoid-Based Tumor Models for Advanced Therapeutics Research

Laura de Lara-Peña1,2,3,4, Mrs. Julia López de Andrés1,2,3,4, Carmen Griñán-Lisón1,2,3,4, Mari Paz Zafra1,2,3,4, Juan Roberto Rodriguez-Madoz6,7, Felipe Prósper6,7,8,9, Juan José Lasarte10, Francisco Martín5,11, Gema Jimenez1,2,3,4, Juan Antonio Marchal1,2,3,4

1Biopathology and Regenerative Medicine Institute (IBIMER), Centre for Biomedical Research (CIBM), Granada, Spain, 2Instituto de Investigación Biosanitaria ibs.GRANADA, University Hospitals of Granada- University of Granada, Granada, Spain, 3Excellence Research Unit “Modeling Nature” (MNat), Granada, Spain, 4Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Granada, Spain, 5Genomic Medicine Department, Centre for Genomics and Oncological Research, Pfizer-University of Granada (Andalusian Regional Government) (GENYO), Granada, Spain, 6Hemato-Oncology Program, Cima Universidad de Navarra, IdiSNA, Pamplona, Spain, 7Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain, 8Hematology and Cell Therapy Department, Clínica Universidad de Navarra (CUN), Pamplona, Spain, 9Cancer Center Universidad de Navarra (CCUN), Pamplona, Spain, 10Immunology and Immunotherapy Program, Cima Universidad de Navarra, IdiSNA, Pamplona, Spain, 11Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, University of Granada, Granada, Spain

Background: Pancreatic cancer (PC) represents the seventh leading cause of cancer death worldwide and its incidence is increasing every year. Survival in this type of tumor is less than 5% due to lack of early diagnosis, rapid disease progression, high rate of metastasis and treatment failure. In PC, the stroma constitutes more than 90% of the tumor mass and is composed of many different elements. This highlights the need to create new 3D in vitro models incorporating the tumoral microenvironment (TME), as tumor progression and response to treatments depend to a large extent on it. These models may help us to understand and overcome the resistance of PC to several therapeutic strategies. Thus, the incorporation of dECM-based bioinks provides 3D models of a more physiological TME, giving them their characteristic complexity and allowing interactions between cells and with the ECM.

Methodology: For the generation of extracellular matrix-based bioinks, isolation of human fibroblasts and mesenchymal stem cells was performed, followed by their malignancy induction through different strategies to obtain a cancer-associated fibroblast (CAFs) phenotype specific to the TME. Upon differentiation, the secretion of extracellular matrix (ECM) by CAFs was induced using a vitamin and growth factor cocktail, and then characterized and combined with other biomaterials to create a biomimetic tumor context for the cultivation of patient-derived tumor organoids.

Results: The different malignancy induction strategies resulted in cell cultures with a specific molecular profile of CAFs, exhibiting differences in the expression of various proteins among the cell cultures. The matrices generated from these cultures, and subsequently decellularized, proved to be rich in collagens, glycosaminoglycans, and tumor-related growth factors, and therefore, a potential platform for patient-derived organoids culture.

Discussion and Conclusion: The incorporation of dECM in in vitro PC models makes it possible to mimic characteristics of native tumors: development and metastasis, and treatment outcomes. In addition, organoids are good personalized medicine platforms, useful in pharmacotyping studies because they show parallel sensitivity and resistances in patients. Some treatments strategies, as CAR-T therapy, find many challenges to be effective in solid tumors. Because PC organoids can mimic the native tissue, they can facilitate the identification of novel antigens which could serve as targets for CAR-T therapy and also can help us to better understand resistance to it.
Use of autologous patient-derived cell culture dECM as biomimetic material for tissue engineering

Ms. Ana Voltes Martínez1,2,3, Julia López de Andrés1,2,3, Laura de Lara-Peña1,2,3, Ana Fernández González4, Antonio Manuel Lizana5, Elena López Ruiz1,2,3,4,5, Gema Jiménez1,2,3,6,7, Carmen Griñán Lisón1,2,3,6, Salvador Arias4, Juan Antonio Marchal1,2,3,6,7
1Biopathology and Regenerative Medicine Institute (IBIMER), Centre for Biomedical Research (CIBM), University of Granada, Granada, Spain, 2Excellence Research Unit “Modeling Nature” (MNat), University of Granada, , Spain, 3BioFab i3D- Biofabrication and 3D (bio)printing Laboratory, University of Granada, , Spain, 4Unidad de Producción Celular e Ingeniería Tisular. Instituto de Investigación Biosanitaria ibs.GRANADA. Complejo Hospitalario Universitario de Granada, Granada, Spain, 5Department of Health Sciences, University of Jaen, Jaen, Spain, 6Instituto de Investigación Biosanitaria ibs.GRANADA, Hospitales Universitarios de Granada-Universidad de Granada, Granada, Spain

Introduction: The high incidence of patients with chronic skin injuries and major burns requires the development of new strategies based on tissue engineering to produce functional skin grafts, either to replace tissue lost in skin injuries or to generate human skin models for research. However, currently available skin substitutes present different limitations, such as high costs, abnormal skin microstructure and graft failure or, in the case of autografts, the availability of healthy donor tissue in patients suffering a major injury is restricted. Among all the possible biomaterials used in tissue engineering, the use of decellularized extracellular matrix (dECM) derived from primary cultures obtained from patients has emerged as a source of ECM that allows recapitulating all the components of the native tissue, including growth factors and other communication molecules essential for proper tissue homeostasis.

Methods: We have developed different bioinks based on ECMs obtained from mesenchymal stem cells (MSCs), fibroblasts (FBs), and MSCs differentiated to cutaneous cell lineages, isolated from patient samples, in combination with biomaterials of different origins to generate biomimetic models that closely resemble native contexts. The ECMs obtained were decellularized by physicochemical and enzymatic treatments and solubilized so that they could be combined with the other biomaterials. Finally, dECMs were characterized by histology techniques, immunofluorescence techniques and by quantitative analysis of structural proteins such as collagen and GAGs.

Results: Histological and immunofluorescence characterization of MSCs, FBs and MSCs differentiated to cutaneous cell lineages dECMs before and after the decellularization process, compared with native dermal tissue and decellularized native dermal tissue showed correct decellularization, and a
matrix rich in native tissue components. Quantitative analyses of DNA, collagen and glycosaminoglycan of the matrices content confirmed the previous results.

Discussion and Conclusion: The dECMs generated retain the main components of the native tissues, being especially rich in collagens. The matrices mimicking the hypodermal tissue retain more GAGs, while the dermal ones have a more fibrillar profile. All of them can crosslink again after solubilization, which makes them suitable for the culture of cells embedded in the matrix for the generation of artificial skin tissues.
Bionic microenvironmental modulated scaffold based on DLP 3D printing with vesicle self-assembly modification for large segment bone regeneration

Dr. Wenbin Jiang

Huazhong University of Science and Technology, Wuhan, China

• Introduction

Large segmental bone defects caused by trauma, infection and bone tumor resection have limited regenerative capacity and the associated loss of function severely reduces the quality of life of patients, resulting in extensive demand for the repair of large segmental bone defects. Currently, in situ bionic scaffold implantation for large segmental bone repair has gained immense popularity in the field of regenerative medicine. However, no major clinical breakthroughs have been achieved in the repair of large segmental bone defects with bone in situ bionic scaffolds due to the lack of suitable early microenvironmental modulation capabilities.

Inspired by the natural microchannels and cortical meshwork of bone, and combining active ingredients to modulate the local angiogenesis and osteogenic microenvironment, we designed highly bionic scaffolds loaded with engineered vesicles. In our study, we used DLP 3D printing technology to fabricate bionic bone repair scaffolds with bone cortex, cancellous bone containing complex reticular structures, Haversian canals, and transversely penetrating Volkmann canals, and loaded engineering adipose stem cell vesicles (ADSC-ENs) stably onto the scaffold surface via a biotin-streptavidin system. The scaffold provided a bionic structural support with suitable active ingredients for the bone defect site, artificially constructing the local vascularization and osteogenic microenvironment of the defect.

• Subjects and Methods

We first synthesized a polycaprolactone material with light-curing properties (PCLMA) and used DLP 3D printing technology to enable fine control of the bionic bone repair scaffold in terms of structure and size, and then engineered human adipose-derived stem cell vesicles were uniformly and densely loaded onto the scaffold via a perfusion device to construct an in situ bone repair scaffold with local microenvironmental modulation. In addition, the loading stability of ADSC-ENs on the scaffolds was investigated in vitro and in vivo, respectively, and their application for repairing large bone defects was verified by a rabbit radius model.

• Results

Scanning electron microscopy images of different parts of the scaffold in the perfusion grafting group showed that ENs achieved uniform and efficient loading on all parts of the surface of the scaffold.

Animal experiments showed that in vivo CT imaging at months 1, 2, and 3 showed significant differences in the status of bone defect repair in the three groups of rabbits, with most of the bone defects in the experimental group having been repaired by month 3, with more complete and continuous osseointegration on both sides of the defect area, and normal motor and activity functions having been restored.

Consistent with the above examination, histological staining at 12 weeks showed relatively complete and continuous bone formation throughout the bone defect area in the experimental group, with much immature collagen visible. Positive staining for CD31 confirmed that the bionic bone scaffold loaded with ENs promoted angiogenic differentiation in vivo, and that endothelial cells developed well and were continuous at 12 weeks postoperatively. These results confirmed that the bionic bone scaffold system loaded with vesicles promoted the growth of host vessels and accelerated the regeneration of radial bone. In addition, immunohistochemical staining (BMP-2, Runx2, and OCN) was performed at 12 weeks post-implantation to investigate possible osteogenesis-related expression during bone regeneration. Quantitative analysis showed enhanced bone-related expression of BMP-2, Runx2 and OCN in the experimental group.
Bioactive components play a crucial role in the construction of the local microenvironment to regulate the repair of bone defects, and in traditional bone tissue engineering techniques, seed cells inoculated on the scaffold surface provide the bioactive components for the construction of the local microenvironment. Extracellular vesicles, which are cell-derived but not true cells, have attracted the attention of researchers in recent years for their low immunogenicity, good targeting ability and rich biological functions. However, the high cost and low yield greatly limit the clinical application of cellular vesicles. Here, we obtained engineered vesicles from ADSC by stepwise mechanical extrusion, and clinically accessible ADSC have been shown to have significant osteogenic differentiation and pro-vascularization capacity. Compared to conventional methods for obtaining extracellular vesicles, this simple method reduces the cost and increases the yield, requiring only a certain number of cells without the need for prolonged ultracentrifugation and a tedious supernatant collection process. More importantly, the engineered vesicles we extracted had similar surface membrane proteins and contents as the naturally secreted vesicles. The above results could indicate that ENs could be a good alternative to conventional extracellular vesicles.

In addition to the active ingredient, the construction of the fine bionic structure of the bone repair scaffold is also indispensable. The microstructure and porosity inside the scaffold not only affect the cell attachment and growth, but also the local biological environment. Whang et al. have experimentally demonstrated that the optimal pore size range of implants for bone regeneration is 100-350 μm. However, most of the bone bionic scaffold fabrication techniques used in current studies have shortcomings such as insufficient precision and insufficient uniformity of porosity, which affects the construction of scaffold microchannels and limits their clinical application. In contrast, thanks to DLP 3D printing technology, we can improve the printing accuracy of scaffolds up to 100 μm, enabling the construction of complex vascular systems and interlaced meshwork of cancellous bone within the scaffold. In addition, the structural parameters of the scaffold can be precisely adjusted in 3D modeling software to accommodate different sizes of bone defects. The integrated fine molding of the complex combined structure in this study is challenging for other scaffold construction approaches.

Overall, this study demonstrates a method to load engineered vesicles on a high-precision scaffold to achieve a regulated local osteogenic microenvironment, which can effectively achieve stable connection of vesicles to the scaffold and thus improve the efficiency of uniform loading of vesicles on the scaffold. Compared with conventional methods, our method has higher attachment efficiency and longer vesicle stability, while also providing a more highly accurate bionic microenvironment. In vivo experiments, we found that using our scaffold construction method can significantly promote the osteogenesis process at the site of bone defects and improve the repair outcome.
Fabrication and mechanical enhancement of a cell-laden aligned porous construct for tendon tissue regeneration.

Mr. Youngwon Koo¹, Dr. Jaeyoon Lee¹, Dr. Dogeon Yoon⁴, Prof. Geun Hyung Kim¹,2,3
¹Department of Precision Medicine, Sungkyunkwan University School of Medicine (SKKU-SOM), Suwon, Republic of Korea, ²Institute of Quantum Biophysics, Department of Biophysics, Sungkyunkwan University, Suwon, Republic of Korea, ³Biomedical Institute for Convergence at SKKU (BICS), Sungkyunkwan University, Republic of Korea, ⁴Burn Institute, Hangang Sacred Heart Hospital, College of Medicine, Hallym University, Seoul, Republic of Korea

Introduction: Tendon is one of the anisotropic tissues of human body that connects bone and muscle. Since it has low capability of self-repair, treatments in tissue engineering and regenerative medicine have been widely attempted to regenerate the tendon tissue. Relevantly, there are two main factors to consider; aligned structure and sufficient mechanical properties. Conventionally, synthetic polymers were mostly utilized to fabricate an aligned and mechanically firm fibrous structure, usually using electrospinning. The materials or methods are, however, hardly compatible with cells, requiring additional process to remove the cytotoxicity or to insert a cell-friendly elements before seeding/attaching cells. Natural polymers, which are cyto-compatible, were also used for tendon tissue regeneration. However, low mechanical properties have been one of the main drawbacks.

Subjects and Methods: In this work, we use biocompatible materials, collagen and silk fibroin (SF) to fabricate a mechanically enhanced cell-laden aligned structure. Collagen and SF were mixed with our unique method in various composition to enhance the mechanical properties. Then the cell-laden composite bioink were printed and collected using various printing/collecting conditions to fabricate an aligned construct. The cell-laden constructs were then crosslinked and cultured for further in vitro experiments to be compared with normally printed (non-aligned) cell-laden constructs; cell viability, proliferation, alignment, and tenogenic differentiation were examined.

Results: Collagen/SF composite in particular mixing condition showed highly increased mechanical properties compared to collagen only or SF only groups. The composite bioink was successfully utilized to fabricate cell-laden aligned constructs using an optimized printing/collecting condition. In the cellular experiments, the aligned constructs have shown more capability to promote tendon regeneration compared to non-aligned constructs.

Discussion and Conclusion: We demonstrated that the collagen/SF aligned cellular constructs can support tenogenic differentiation by the aligned structure and enhanced mechanical properties. The interpenetrating connections between collagen and SF are assumed to cause the increase of mechanical properties. The currently developed biomaterials and methods can be a promising fabrication system for biomimetic cell-laden constructs capable of regenerating not only tendon but also other anisotropic tissues.

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Using stem cell-derived nanovesicles (scNVs) as a scalable, cell reprogramming therapeutic strategy for cardiac repair

Mr. Jonathan LOZANO¹ ² ³, Dr Alin RAI¹ ² ³ ⁴, Dr Jarmon G LEES⁵ ⁶, A/Prof Shiang Y LIM⁵ ⁶ ⁷, A/Prof David W GREENING¹ ² ³ ⁴

¹Baker Heart and Diabetes Institute, Australia, ²Baker Department of Cardiovascular Research Translation and Implementation, La Trobe University, Australia, ³School of Agriculture, Biomedicine and Environment, La Trobe University, Australia, ⁴Baker Department of Cardiometabolic Health, University of Melbourne, Australia, ⁵Cardiac Regeneration Laboratory, O'Brien Institute Department, St Vincent's Institute of Medical Research, Australia, ⁶Department of Surgery and Medicine, University of Melbourne, Australia, ⁷National Heart Research Institute Singapore, National Heart Centre, Singapore.

Title: Using stem cell-derived nanovesicles (scNVs) as a scalable, cell reprogramming therapeutic strategy for cardiac repair

Authors: Jonathan LOZANO¹ ² ³, Jarmon G LEES⁵ ⁶, Alin RAI¹ ² ³ ⁴, Shiang Y LIM⁵ ⁶ ⁷, David W GREENING¹ ² ³ ⁴

Affiliations: ¹Baker Heart and Diabetes Institute, Australia. ²Baker Department of Cardiovascular Research Translation and Implementation, La Trobe University, Australia. ³School of Agriculture, Biomedicine and Environment, La Trobe University, Australia. ⁴Baker Department of Cardiometabolic Health, University of Melbourne, Australia. ⁵Cardiac Regeneration Laboratory, O'Brien Institute Department, St Vincent's Institute of Medical Research, Australia. ⁶Department of Surgery and Medicine, University of Melbourne, Australia. ⁷National Heart Research Institute Singapore, National Heart Centre, Singapore.

Category: SYIS (Student and Young Investigator Section) + Tissue Engineering and Regeneration.

Background: Accumulating reports indicate that stem cell-originating extracellular vesicles (EVs, including exosomes) provide a potential strategy for cardiac tissue repair, including for ischaemia-reperfusion injury. However, the disadvantages of natural EVs, such as fast decrease of biological activity, scalable generation for therapeutic purpose, and unknown biological mechanisms, limit their clinical application. Stem cell-derived nanovesicles (scNVs) offer a promising pathway towards reproducible, rapid, and scalable deliverable therapy for cardiac repair.

Methods: Herein, NVs were rapidly generated from different human-induced pluripotent stem cells through serial membrane-based extrusion strategy in large quantities (yield 900× natural EVs). The regulatory mechanism of NV function in cardiac cell repair was explored using quantitative proteomic profiling in cell-based and cardiac organoid systems representing models of ischaemia-reperfusion injury.

Results: NVs isolated using density-gradient separation (1.13 g/mL) are spherical in shape and morphologically intact (~100 nm) and readily internalised by human cardiomyocytes, primary cardiac fibroblasts, and endothelial cells. NVs captured the dynamic proteome of parental cells and include pluripotency markers (LIN28A, OCT4) and regulators of cardiac repair processes. Functionally, single-dose NVs significantly promoted tubule formation of endothelial cells (angiogenesis) (p<0.05) and survival of cardiomyocytes exposed to low oxygen conditions (hypoxia) (p<0.0001), as well as attenuate activation of cardiac fibroblasts (p<0.0001). In human cardiac organoids we demonstrate NVs preserve overall contractility function; total contraction duration, time to peak, relaxation time and ratio of relaxation to contraction velocities (p<0.05). Quantitative proteome profiling of target cell and organoid proteomes following NV treatment revealed upregulation of pro-survival network (MDH2, LRPPRC, NIPSNAP1), tissue repair (HSP70, CYFIP1), and cardiac function (XIRP1, SLMAP, MYH6, CTNNA1, NDUFS2, GPD2).
Discussion and conclusion: In summary, this study showcases a scalable approach to generating functional NVs, highlights their multimodal therapeutic potential, and identifies key regulatory players involved in cell reprogramming for cardiac repair.

Acknowledgement: This work was supported by the National Heart Foundation of Australia (DG: Vanguard), NHMRC project/ideas grants (DG), MRFF (DG), Pankind (DG), Stafford Fox Medical Research Foundation (SYL), and the Victorian Government’s Operational Infrastructure Support Program. JL is supported by an Australian Government Training Program (RTP), La Trobe University-Baker Heart and Diabetes Institute joint scholarship.
Binary colloidal crystals (BCC): A platform for investigating mechanotransduction and cell fate specification during reprogramming

Mr. Javad Harati¹, Dr. Ping Du¹, Dr. Peng-Yuan Wang², Dr. Pan Haobo¹
¹Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China,
²Oujiang Laboratory; Key Laboratory of Alzheimer’s Disease of Zhejiang Province, Institute of Aging, Wenzhou Medical University, Wenzhou, China

Background: Non-soluble signals of the microenvironment influence cell fate via the generation of relevant biophysical cues. Engineering cell culture substrate properties such as topography and chemistry not only allow for the use of biophysical signals to improve reprogramming efficiency and direct cell fate, but it also aids in the understanding of mechanotransduction mechanisms during cell fate specification. Biophysical signals are transmitted through the cytoskeleton and signalling pathways, changing the expression of several genes. However, the detailed mechanism, which includes epigenetic alteration involved with cell fate specification, is not well known.

Methods: In this work, we employed a nano-microstructured pattern, BCC, as the cell culture substrate during reprogramming, which was self-assembled from two distinct particles, BCC1: Si 5µm and Ps 0.4 µm and BCC2: Si 2 µm and Ps 0.065 µm. Human fibroblasts were directly reprogrammed into induced neurons (iNs) using a cocktail of small molecules.

Results: The BCC substrate drove the fibroblasts to modify their states by altering their shape and focal adhesion to a smaller size, migration to faster speed, and proliferation to a slower rate. Furthermore, we identified and validated a link between cell surface receptors, integrins αVβ3 and Syndecan 1, and the expression of markers associated with neuronal subtypes such as NR4A2 and ISL1. The generation of iNs on the BCC substrate demonstrated that the physicochemical features of the substrate determine the iNs subtypes, i.e., monoaminergic on the BCC1 and dopaminergic on the BCC2. Further mechanistic investigation indicated that signalling pathways like Hippo and Wnt may be involved in neuronal subtype determination. Histone epigenetic modification as the tipping point was detected on the BCC substrate, which led to a condensed chromatin state.

Discussion and Conclusion: Our findings demonstrate that specialised biophysical signals on a BCC artificial matrix effectively alter cell fate during direct cell reprogramming via epigenetic state modulation and distinct mechanotransduction pathways that regulate neuronal subtype-specific gene expression in a surface-dependent manner. In addition, we introduced BCC as a powerful cell culture platform with a wealth of combination choices for cellular engineering applications.
3D-cultured cancer spheroid behavior within decellularized extracellular matrix (dECM) powder based bioink.

**Dr. Mako Kobayashi**\(^1\), Lucas COLLET\(^1\), Keito TSUTAHARA\(^1\), Dr Yoshihide HASHIMOTO\(^2\), Dr. Tsuyoshi KIMURA\(^2\), Dr. Akio KISHIDA\(^2\), Dr. Masaya YAMAMOTO\(^1\)

\(^1\)Department of Materials Processing, Graduate School of Engineering, Sendai, Japan, \(^2\)Institute of Biomaterials and Bioengineering, Tokyo, Japan

**Background:** The mechanisms of cancer invasion and metastasis are still largely unknown. One reason for this is the heterogeneity and complexity of cancer tissue. Cancer cells interact with various surrounding normal cells and extracellular matrix components to form a cancer microenvironment. In this study, we focused on the relationship between cancer cells and the extracellular matrix, and aimed to evaluate the behavior of cancer cells arranged three-dimensionally in the extracellular matrix and to explore the factors that contribute to the construction of the in vivo-like cancer microenvironment.

**Methods:** Decellularized tissue powder was mixed with sodium alginate and/or collagen hydrogel to prepare bioink. Spheroids of cancer cells were embedded in bioink, and 3D structures were printed. 3D constructs were removed from the supporting bath by incubation at 37°C for 1 hour. After immersion in culture medium and incubation for certain days, cell behavior, cell morphology, and invasive metastatic ability were evaluated by fluorescent immunostaining.

**Results:** The 3D constructs were immersed in culture medium, and after 1, 3, 5, and 14 days of incubation, calcein-AM staining was performed and observed with a laser confocal microscope. In both sodium alginate hydrogel alone and sodium alginate hydrogel mixed with dECM powder, HepG2 cells were observed to be viable, but cells were well proliferated in the hydrogel with dECM powder added. After 14 days, cell aggregates were observed in the sodium alginate-only 3D constructs, and the number of cell viability decreased, while in the 3D construct prepared with the dECM powder-mixed hydrogel, cells were observed to be well growing and progressing.

**Discussion and Conclusion:** It was found that various dECM powders affect the survival and proliferation of cancer cells in 3D environments, indicating the importance of the choice of the extracellular matrix in establishing the cancer microenvironment. In addition, the findings obtained through the investigation of the 3D bioprinting method of dECM powder-mixed hydrogel using a supporting bath are expected to lead to the realization of the design of 3D cancer tissue models.

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Fabricating a biphasic scaffold mimicking the osteotendinous and tendomuscular region to study stem cell progression

**Akshay Bhatt**, Robert Kapsa, Peter Kingshott, Subha Narayan Rath, Simon Moulton

1 Regenerative Medicine and Stem Cell Laboratory, Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, Hyderabad, India.
2 School of Science, Computing and Engineering Technologies, Swinburne University of Technology, Hawthorn, Melbourne, Australia.
3 School of Engineering, RMIT University, Melbourne, Australia.

**Title**: Fabricating a biphasic scaffold mimicking the osteotendinous and tendomuscular region to study stem cell progression

**Authors**: Akshay Bhatt, Robert Kapsa, Peter Kingshott, Subha Narayan Rath, Simon Moulton

**Affiliation**: 1 Regenerative Medicine and Stem Cell Laboratory, Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, NH-65, Kandi, Sangareddy 502285, Telangana, India.
2 School of Science, Computing and Engineering Technologies, Swinburne University of Technology, Hawthorn 3122, Victoria, Australia.
3 School of Engineering, RMIT University, Melbourne, Victoria 3000, Australia. († Presenting Author # Corresponding Author)

**Category**: Design and Application of Biomaterials

**Background**: Interphase tissue regeneration has always been a challenge in medicine. These tissues are commonly located in the osteotendinous and tendomuscular regions. More than 90% of graft rejections have been reported in these regions due to mechanical failure (Calejo, I et al. 2019). Hence, there is a need for an in vitro model which could mimic the complexity of such tissues that can be used to study stem cell progression under trauma conditions.

**Method**: The osteogenic phase was first fabricated using the electrospinning technique, in which polycaprolactone was used incorporating nanohydroxyapatite to mimic the bone tissue matrix. The physicochemical and mechanical properties of electrospun scaffolds were analyzed using scanning electron microscopy (SEM), optical profilometry, Fourier transform infrared spectroscopy (FTIR), and dynamic mechanical analysis (DMA). For the tendon and muscle phase, a composite hydrogel system of methacrylate gelatine (GelMA) and tendon-derived extracellular matrix (tdECM) was used. The change in the physicochemical and viscoelastic properties of the composite hydrogel was confirmed using FTIR and rheology. The cellular biocompatibility of electrospun scaffolds was evaluated using MG63 osteoblast-like cells, and for the hydrogel system, adipose-derived stem cells (ADSCs) were used. Finally, both phases were combined by 3D printing of the GelMA-tdECM hydrogel on top of the electrospun scaffold.

**Results**: The SEM images showed randomly oriented hydroxyapatite-containing microfibers. The same was confirmed from elemental analysis. The functional group analysis using FTIR confirmed the presence of PCL and hydroxyapatite, and the optical profilometry reported a roughness increase. The DMA analysis showed increased scaffold stiffness with nanohydroxyapatite addition. Furthermore, the GelMA-tdECM composite hydrogel demonstrated increased peak intensity, viscosity, storage, and loss modulus compared to pure GelMA hydrogel. The compressive modulus measurement also showed an increase in the modulus of the GelMA-tdECM composite hydrogel compared to pure GelMA hydrogel. The MG63 osteoblast cells showed over 70% proliferation across 28 days of culture on the electrospun scaffolds, while a similar trend was observed for adipose-derived stem cells (ADSCs) when encapsulated in the GelMA-tdECM hydrogel system.
Discussion and Conclusion: We have developed a novel in vitro model for interphase tissue by combining two phases that mimic osteotendinous and tendomuscular regions using different fabrication techniques. Our in vitro model is a promising method aimed at overcoming the limitations of existing models that can be used to further study cellular progression under both healthy and traumatic conditions.


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Methylcellulose hydrogel-based compressible strain sensor fabricated by combination of projection lithography and direct ink writing methods

Dr. Sorour Sadeghzade, Jinre Cao, Hongyan Yuan
1Shenzhen Key Laboratory of Soft Mechanics & Smart Manufacturing, Department of Mechanics and Aerospace Engineering, Southern University of Science and Technology, Shenzhen, China

Title: Methylcellulose hydrogel-based compressible strain sensor fabricated by combination of projection lithography and direct ink writing method

Authors: Sorour Sadeghzade, Jinre Cao, Hongyan Yuan*
Affiliations: Shenzhen Key Laboratory of Soft Mechanics & Smart Manufacturing, Department of Mechanics and Aerospace Engineering, Southern University of Science and Technology, Shenzhen, 518055, China
*Corresponding author: yuanhy3@sustech.edu.cn

Category: Design and Application of Biomaterials

Background: Hydrogel-based sensors have several advantages, including their sensitivity, biocompatibility, and ability to protect sensitive components. However, they also have limitations related to swelling, dehydration, and degradation in specific environments, which can negatively affect the sensors' performance and reliability. To overcome these limitations, hydrogel-elastomer-based strain sensors utilize an elastomer matrix to encapsulate the hydrogel circuit. This approach prevents dehydration, improves sensor performance, and maintains biocompatibility. Existing methods for patterning conductive features in hydrogel-elastomer sensors involve either directly encapsulating titanium wires or trapping liquid metals in pre-formed channels. However, both methods come with their own set of limitations. The use of titanium wires restricts the stretchability of hydrogel devices, while liquid metal circuits require leads that can potentially lead to leakage issues. Moreover, these methods heavily rely on manual operations, which limits precision and scalability. Consequently, there is a demand for advanced techniques that can enable precise and scalable patterning of conductive features in hydrogels while ensuring biocompatibility, mechanical stability, and electrical stability are maintained.

Methods: In this work, we assembled a methylcellulose hydrogel-based strain sensor by combination of projection lithography and direct ink writing method with capability of printing, which are conductive with capability of detecting a wide range of strain variations and using in smart prosthetics.

Results: The methylcellulose-based hydrogel, which is typically not known for its flexibility and shape recovery properties, demonstrated an unexpected ability to exhibit these characteristics when subjected to compressive loading. This unique behavior defies the inherent nature of such hydrogels. The process of creating compressible and conductive hydrogel and elastomer precursors was described, and the mechanical and electrical properties of these materials were evaluated. Furthermore, an application scenario was presented wherein a manually produced compressible strain sensor was affixed to a 3D-printed scaffold. This setup allowed for the measurement of strain and bending angles during actuation, providing a practical example of the sensor's capabilities.
Discussion and Conclusion: We demonstrated that the methylcellulose hydrogel-elastomer-based can be introduced as a novel compressible sensor with photopolymerization capability under UV. A potential application of conductive compressible hydrogel and elastomer can be an effective monitoring of bone-implant interfaces during the daily living of patients.

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Stepwise Dual-release Microparticles of BMP-4 and SCF in Induced Pluripotent Stem Cell (iPSC) Spheroids Enhance Differentiation into Hematopoietic Progenitor Cells

Dr. Alvin Bacero Bello1, Mr. Kevin Kent Canlas2, Mr. Nityanand Prakash1, Prof. Hansoo Park2, Prof. Soo-Hong Lee1

1Department of Biomedical Engineering, Dongguk University, Goyang-si, Ilsandong-gu, Dongguk-ro, South Korea, 2School of Integrative Engineering, Chung-Ang University, Seoul-si, Dongjak-gu, Heukseok-ro, Seoul, South Korea

Stepwise dual-release microparticles of BMP-4 and SCF in induced pluripotent stem cell (iPSC) spheroids enhance differentiation into hematopoietic progenitor cells

Alvin Bacero Bello1, Nityanand Prakash1, Kevin Canlas2, Hansoo Park2, Soo-Hong Lee1*

1Department of Biomedical Engineering, Dongguk University, Seoul 04620, Korea
2School of Integrative Engineering, Chung-Ang University, Seoul 06911, Korea
*Correspondence: soohong@dongguk.edu (Tel.: +82-031-961-5153, Fax: +82-31-961-5108)

Category: Stem Cells and Cell-Based Therapies

Background: Allogeneic bone marrow transplantation (BMT) is the intravenous infusion and re-engraftment of pre-conditioned hematopoietic stem cells (HSC) from a healthy donor into a patient to replace defective cells and re-establish the function of the BM. This is an essential procedure in treating patients suffering from various hematologic conditions such as leukemia, myeloma, and lymphoma. The growing demand for BMT however is impeded by the critically low population (0.01% of all the bone marrow cells) of HSCs in the BM which limits their potential medical applicability. Recently, the formation of three-dimensional (3D) cell aggregates known as embryoid bodies (EBs) grown in media supplemented with HSC-specific morphogens has been used to investigate the directed differentiation of pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), into clinically relevant HSCs. However, delivering growth factors and transferring nutrients have become ineffective in inducing synchronous differentiation of cells owing to their 3D conformation. Moreover, irregularly sized EBs often lead to the formation of necrotic cores in larger EBs, impairing differentiation.

Methods: In this study, we developed two gelatin microparticles (GMPs) with different release patterns and two HSC-related growth factors conjugated to them. Slow and fast-releasing GMPs were conjugated with bone morphogenic factor-4 (BMP-4) and stem cell factor (SCF), respectively.

Results: The sequential presentation of BMP-4 and SCF resulted in varying degrees of hematopoietic differentiation, as indicated by the gene and protein expression of several mesoderm (Brachyury, NCAD, VEGF) and HSC-related markers (CD34, cKit, CXCR4, and SCL). Moreover, cytokine secretion analysis revealed that the GMP-conjugated EBs produced greater concentrations of HSC-related cytokines (IL-6, IL-10, SDF-1, FGF, G-CSF). Interestingly, our technique was able to generate CD133+ (13.74%), CD34+ (4.21%), and FLT3+ (19.48%) cells with similar cellular and molecular morphology as the naïve HSCs that can produce colony units of different blood cells.

Discussion and Conclusion: This study demonstrates the ability to generate HSCs from iPSC via a single-step 3D composite spheroid culture with the stepwise presentation of HSC growth factors.

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Graphical Abstract: Dual microparticle system for the sequential differentiation of embryonic stem cells into mesoderm and hematopoietic progenitor cells.
Fibro-adipogenic progenitors-derived TGF-β1 is a key molecule in regulating muscle regeneration and ectopic mineralization

**Dr. La Li**, Dr. Dai Li, Dr. Jingxian Zhu, Dr. Yiqun Wang, Dr. Feng Zhao, Dr. Jin Cheng, Dr. Rocky Tuan, Dr. Xiaqing Hu, Dr. Yingfang Ao

1Peking University Third Hospital, Beijing, China, 2The Chinese University of Hong Kong, Hong Kong, China

Introduction: Previously we have demonstrated that stress response-induced high glucocorticoid levels could be the underlying cause of failed muscle regeneration and traumatic heterotopic ossification (HO) formation. However, it is not known how glucocorticoids initiate muscle ectopic mineralization (EM) at a cellular and molecular level. It is well known that HO formation is associated with inflammation. But it is counterintuitive to learn that glucocorticoids, usually regarded as immune suppressants, can cause a hyperinflammatory state leading to muscle EM formation. Therefore, glucocorticoids must target other cell types whose regulatory roles can override those of inflammatory cells during muscle regeneration. This study thus aims to explore the regulatory role of fibro-adipogenic progenitors (FAPs) in glucocorticoid-induced EM.

Methods: Glucocorticoid-induced HO model was created by systemic administration of a high dose of dexamethasone (DEX) to mice with muscle injury induced by cardiotoxin (CTX) injection. A PDGFRα-creERT: tdTomato reporter mouse line was used to trace FAPs during muscle regeneration and enable sorting out FAPs for gene expression analysis. A PDGFRα-creERT: TGF-β1flox/+ transgenic mouse model was utilized to specifically knock out the TGF-β1 gene in PDGFRα-positive FAPs.

Results: We showed that DEX treatment inhibited inflammatory cell infiltration into CTX-injured muscle, but inflammatory cytokine production in the muscle was significantly increased. Accompanying this phenotype, TGF-β1 expression in FAPs was greatly downregulated, while a number of categories related to the inflammatory response and inflammatory cell chemotaxis were enriched in FAPs after DEX treatment (Figure 1A). Transgenic mice that specifically knocked out the TGF-β1 gene in FAPs exhibited a significant decrease in circulating and muscle TGF-β1 levels, which resulted in a hyperinflammatory state demonstrated by increased inflammatory cell infiltration into the muscle (Figure 1B). These FAPs TGF-β1 gene knock-out mice also spontaneously developed EM following muscle injury, suggesting that downregulation of TGF-β1 in FAPs is the cause of failed muscle regeneration and subsequent EM formation after DEX treatment (Figure 1B).

Discussion and conclusion: The regulatory role of FAPs in muscle regeneration and maintaining muscle homeostasis is gaining increasing attention. In this study, by using a transgenic mouse model to specifically knock out the TGF-β1 gene in FAPs, we have demonstrated that FAP-derived TGF-β1 is a key molecule in regulating muscle inflammatory response and subsequent EM, and that glucocorticoids exert their effect via downregulating TGF-β1 in FAPs. Our results thus provide a mechanistic explanation for how glucocorticoids induce EM, and shed light on the therapeutic strategies for promoting muscle regeneration, i.e., enhancing TGF-β1 signaling in FAPs to inhibit inflammatory response and prevent muscle EM.
Multiplexed PLGA scaffolds with nitric oxide-releasing zinc oxide and melatonin-modulated extracellular vesicles for chronic kidney disease

Dr. Dong Keun HAN1, Dr. Won-Kyu RHIM1, Mr. Jun Yong KIM1, Dr. Eun Hye LEE2, Dr. Bum Soo KIM2, Dr. Tae Gyun GWON2

1CHA University, Seongnam, Korea, 2Kyungpook National University, Daegu, Korea

Background: Chronic kidney disease (CKD), a major public health issue with progressive and irreversible structural and functional abnormalities of the kidney, has only a few treatment options as dialysis and kidney transplantation. Due to the lack of effective treatments for CKD, although researchers concentrate on tissue engineering and regenerative medicine methods for replacing and restoring kidney function, kidney tissue regeneration is difficult due to the relatively low reproducibility of renal basal cells and the limited bioactivities of implanted biomaterials.

Methods: The structural construction of PMEZ scaffolds was supported by numerous analyses, and the inclusion of mEV was also investigated by comparing surface properties and a fluorescence staining approach. The bioactivities of PMEZ/mEV were proven in vitro utilizing a variety of biological tests, and they were later confirmed in 5/6 nephrectomy mouse models, which closely resemble severe human CKD.

Results: The continuous NO-releasing property of ZO-ALA and augmented regeneration ability of mEV, accelerated angiogenesis, and anti-inflammation activities, resulting in the recovery from renal fibrosis and apoptotic damages. The morphology of the glomerulus was also regenerated by the functionality of the multifunctional scaffold system.

Discussion and Conclusion: The optimal microenvironments for the morphogenetic formations of renal tissues and functional restorations have successfully enabled the synergistic bioactivities of noteworthy components for PMEZ/mEV.
Generation of universal hiPSCs and their differentiation into RPE

Ms. Kailibinuer Maitiruze1, Prof. Akon Higuchi1,3, Tzu-Cheng Sung1, Guoping Fan2

1State Key Laboratory of Ophthalmology, Optometry and Visual Science, Eye Hospital, Wenzhou Medical University, Wenzhou, China, 2Department of Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, United States, 3Department of Chemical and Materials Engineering, National Central University, Jhongli, Taiwan

Kailibinuer Maitiruze1, Tzu-Cheng Sung1, Guoping Fan2 and Akon Higuchi1, 3
1 State Key Laboratory of Ophthalmology, Optometry and Visual Science, Eye Hospital, Wenzhou Medical University, Wenzhou, Zhejiang, 325027, China
2 Department of Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA 90095, USA
3 Department of Chemical and Materials Engineering, National Central University, Jhongli, Taoyuan, 32001 Taiwan, China

Introduction
Age-related macular degeneration (AMD) is an aging disease, which refers to the degeneration of the choroid and pigment epithelium in the macula of the eye caused by abnormal metabolism in the elderly. Besides, AMD has progressive damage to retinal pigment epithelium, which shows irreversible blinding. Human pluripotent stem cells (hPSCs) including human induced pluripotent stem cells (hiPSCs), human embryonic stem cells (hESCs) have been used to differentiate into retinal pigment epithelium (RPE) cells to treat AMD recently. However, hPSCs with various types of human leukocyte antigen (HLA) is major barrier of allograft transplantation of hPSC-derived RPE cells. Banking hPSCs having different HLA types cost huge money and need a lot of stuffs to maintain. The patients still need to administrate life-long immune suppressive drugs. It is necessary to generate an hypoimmunogenic (universal) hPSC-derived RPE cells to apply on cell treatment of AMD patients in future clinical trial in order to avoid to use immunosuppressive drugs after treatment and to reduce the cost of stem cell therapy.

Experiments
Universal hiPSCs were generated from the reprogramming of human amniotic fluid stem cells (hAFSCs), which were derived from multi-donors of amniotic fluid (AF) where the mixing process of multi-donors of AFs is a key technology to generate universal hiPSCs. We chose one protocol (NIC84 protocol) to induce differentiation of universal hiPSCs and conventional hiPSCs into RPE cells. After the successful differentiation of hiPSCs into RPE cells, we evaluated the human leukocyte antigen (HLA) class Ia and class II and RPE markers (ZO-1, PAX-6, MITF, RPE 65) expression. We injected conventional and universal hiPSCs (HPS0077, mix2) cells and RPE (HPS0077-derived RPE and mix2-derived RPE) cells into the subretinal of Royal College of Surgeons (RCS) rats of 21 days, and then evaluated whether the vision of the rats improved after four weeks and eight weeks. We compared hypoimmunogenic effect between universal and conventional hiPSCs and RPE cells derived from those cells by using humanized mice.

Results and Discussion
The immunostaining of RPE cells, which were differentiated from universal hiPSCs and conventional hiPSCs showed the RPE markers (ZO-1 and RPE 65). We are evaluating the HLA Ia and HLA II expression of RPE cells derived from conventional hiPSCs and universal hiPSCs. We will use humanize mice to investigate the hypoimmunogenic effect of universal hiPSCs. We expect the universal hiPSCs-derived RPE can escape from immune rejection and will be used for the treatment of AMD patients in the future.

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References
Continuous 3D Printing of Gelatin/Oxidized Alginate Scaffolds with Gradient Porosity for Bone Tissue Regeneration Applications

Ms. Fariza Mukasheva¹, Dr Alexander TRIFONOV¹, Dr Dana AKILBEKOVA¹

¹Department of Chemical Engineering, School of Engineering and Digital Sciences, Nazarbayev University, Astana, Kazakhstan

Title: Continuous 3D printing of Gelatin/Oxidized Alginate scaffolds with gradient porosity for bone tissue regeneration applications

Authors: F. Mukasheva¹, A. Trifonov¹, D. Akilbekova¹

Affiliations: ¹Department of Chemical Engineering, School of Engineering and Digital Sciences, Nazarbayev University, 010000, Astana, Kazakhstan

Presenting Author: Fariza Mukasheva, fariza.mukasheva@alumni.nu.edu.kz

Category: SYIS (Student and Young Investigator Section) + Design and Application of Biomaterials

Introduction: The transition between cortical and cancellous bone layers is characterized by gradient porosity and pore sizes [1]. Investigation of extracellular matrix formation on the transition border between the layers is of high interest for bone tissue engineering [2]. Here, we describe the method of fabricating gradient-porosity Gelatin/Oxidized Alginate cryogel scaffolds using continuous extrusion 3D printing for in-vitro and in-vivo applications in bone tissue regeneration.

Methods: Three biopolymer inks, varying in gelatin and oxidized alginate concentrations, were printed sequentially in a contact mode, layer-by-layer, in a rectangular spiral (1 cm on each side), followed by crosslinking with a 0.1% glutaraldehyde solution and freeze-drying. Printing accuracy was evaluated for each ink. Scaffold morphology was visualized with a scanning electron microscope. Mechanical properties, swelling capacity, and degradation rate were investigated. The scaffolds were seeded with rat-derived mesenchymal stem cells and incubated in osteogenic cell media for four weeks. The cells’ viability and migration were observed under a confocal microscope, and differentiation into osteoblasts was assessed by defining the amount of alkaline phosphatase (ALP) and dentin matrix phosphoprotein (DMP1). The tissue formation was verified by histological analysis with H&E and Alizarin red.

Results: The inner gradient structure starts with the layer of small diameter pores of 20-50 µm, ascends to 70-120 µm, and ends with the macroporous layer of pores > 200 µm. The layers have a graded porosity of 11 to 90%. The scaffold showed high elasticity and an optimal degradation rate. The cells’ attachment and viability were above 80% and 87%, respectively. The osteoblasts grew better at the bottom layer, where the highest deposition of calcium was found. ALP and DMP activity slightly fluctuated but overall remained constant throughout the incubation period (Figure 1).

Discussion and Conclusion: It was shown that the porous gradient structure supports the attachment of cells to the walls of small pores, osteogenic differentiation in medium-sized pores, and mineralization and collagen production in the macroporous layer. The method is advantageous because of its simplicity and ability to precisely control the pores’ size and gradient thickness.

Acknowledgement: This project was supported by AP13067719 “Towards the human bone organotypic model: From bioink to biocompatible 3-D printed model” and AP14869460 “Lactate-triggered shape adaptive scaffold for advanced bone tissue regeneration: Injectable applications” by the MES RK.


Keywords: gradient porosity, bone tissue
LDH-doped Gelatin-chitosan Scaffold With Aligned Microchannels Improves Anti-inflammation And Neuronal Regeneration With Guided Axon Growth For Effectively Recovering Spinal Cord Injury

Ms. Zhaoqi Wang¹, Tongling Zhang¹, Rongrong Zhu¹
¹Key Laboratory of Spine and Spinal Cord Injury Repair and Regeneration of Ministry of Education, Department of Orthopedics, Tongji Hospital affiliated to Tongji University, School of Life Science and Technology, Tongji University, Shanghai, China

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Authors: Zhaoqi Wang, Tongling Zhang, Rongrong Zhu

Affiliations: a Key Laboratory of Spine and Spinal Cord Injury Repair and Regeneration of Ministry of Education, Department of Orthopedics, Tongji Hospital affiliated to Tongji University, School of Life Science and Technology, Tongji University, Shanghai, P.R, China. (*Correspondence)

Category: Design and Application of Biomaterials

Background: Spinal cord injury (SCI) is a central nervous system injury and always accompanied by axonotmesis, neuronal death, inflammation resulting in severe motor and sensory dysfunctions and even permanent limb paralysis. Recovery from traumatic spinal cord injury is challenging due to difficulties in the neural circuit reconstruction impeded by cavity formation, glial scarring and harsh microenvironment. Therefore, development of multifunctional strategy that can simultaneously suppress the inhibitory and inflammatory microenvironment and promote the neuronal regeneration with guided axon growth at SCI site has attracted much attention.

Methods: In this study, we designed a type of scaffold with aligned channels by mixing gelatin/chitosan/Mg-Fe layered double hydroxides (GC-LDH) solutions followed by anisotropic freeze then lyophilization, then evaluated its repair efficiency through building mouse 2 mm complete spinal cord transected injury model, further investigated the potential repair mechanisms caused by the aligned microchannels structure using transcriptome sequencing.

Results: The results of in vitro experiments proved that adding Mg-Fe LDH could improve the differentiation efficiency of neurons from neural stem cells (NSCs). Implantation of the anisotropic scaffold (AS) into mouse 2 mm complete spinal cord transected injury model activated anti-inflammatory polarization of macrophages and microglia, promoted neurogenesis and guided axon growth from the rostral to caudal of the lesion site. BMS score and MEP result showed a better recovery of motor function for mice was achieved in AS group compared with the isotropic scaffold (IS) with chaotic channels. Transcriptome sequencing uncovered the significant regulating effect of focal adhesion signaling pathway in spinal cord injury repair. The inhibition of Myosin II by the aligned channels enhanced axonal extension and promoted connection of neurons, resulting nerve conduction restoration.

Discussion and Conclusion: A Mg-Fe LDH doped gelatin-chitosan scaffold with aligned microchannels combined chemical and structural repair effects was designed and developed for repairing SCI. The scaffold activated anti-inflammatory polarization of macrophages and microglia, reversing inhibitory and inflammatory microenvironment, it also promoted neuronal regeneration and guided the directional growth of axons. Additionally, Myosin II as the key mediator of negatively regulating axon growth was suppressed by the aligned microchannels structure, accelerating reconstruction of neuronal circuit. The LDH-doped gelatin-chitosan scaffold with multifunctional repair effect provides a fundamental design basis and molecular mechanism for developing scaffold for repairing SCI, and
the multifunctional repair effect in vivo indicates its enormous potential of clinical application in nerve regeneration.
3D Printing of Gelatin/Oxidized Carboxymethyl Cellulose Scaffolds with Gradient Porosity for Bone Tissue Regeneration Applications

Mr. Aibek DYUSSENBINOV\(^1\), Dr. Dana AKILBEKOVA\(^1\)

\(^1\)Department of Chemical Engineering, School of Engineering and Digital Sciences, Nazarbayev University, Astana, Kazakhstan

Introduction: Bone tissue regeneration is a critical area of research in tissue engineering, aiming to create scaffolds that promote the restoration, repair, and regeneration of damaged or diseased bone. 3D printing technology has gained significant attention for fabricating complex structures with precise control over pore size and distribution. In this work, we employed cryogelation and 3D printing methods to create gelatin/oxidized carboxymethyl cellulose (OCMC) scaffolds, attempting to simulate the hierarchical structure of real bone by altering the gelatin to OCMC ratio to create gradient porosity.

Subjects and Methods: Gelatin/OCMC scaffolds were produced using 3D printing. The gelatin to OCMC ratio was systematically adjusted to produce scaffolds with different porosity gradients. Scaffold crosslinking was achieved by treatment with a 0.3% glutaraldehyde solution, followed by freeze-drying to enhance stability and mechanical properties. Scaffold characterization included scanning electron microscopy (SEM) analysis to confirm interconnected pore presence throughout the structure. The scaffolds' suitability for bone tissue regeneration applications was tested by evaluating their elasticity, degradation rates, and cytocompatibility by assessing cell attachment and viability using rat-derived mesenchymal stem cells. Histological studies, including staining with hematoxylin and eosin (H&E), alizarin red, and Masson's trichrome, were used to confirm tissue development within the scaffolds.

Results: The fabricated gelatin/OCMC scaffolds exhibited gradient porosity, with distinct layers possessing varying pore sizes, with averages of 70-90 µm, 100-140 µm, and above 200 µm. SEM analysis confirmed interconnected pore presence, facilitating nutrient diffusion, cell infiltration, and tissue regeneration. Scaffolds demonstrated excellent cytocompatibility, indicated by cell attachment and high cell viability observed through confocal microscopy. Differentiation of osteoblast-like cells into osteoblasts was evident through increased alkaline phosphatase (ALP) activity and dentin matrix phosphoprotein (DMP1) expression. Histological analysis using H&E, alizarin red, and Masson's trichrome staining confirmed tissue formation within the scaffolds.

Discussion and Conclusion: The construction of gelatin/OCMC scaffolds with gradient porosity, closely matching the hierarchical structure of natural bone, was achieved using cryogelation and 3D printing technologies. The systematic adjustment of the gelatin-to-OCMC ratio resulted in distinct layers within the scaffolds, each characterized by a specific range of pore sizes. SEM analysis confirmed interconnected pore presence throughout the scaffold structure, essential for promoting nutrient diffusion, cell infiltration, and tissue formation. Moreover, the scaffolds exhibited optimal elasticity and degradation rates and supported cell attachment and viability, crucial for successful tissue regeneration. The histological analysis provided further confirmation of tissue formation within the scaffolds.

In conclusion, our study successfully produced gelatin/OCMC scaffolds with gradient porosity using cryogelation and 3D printing techniques. The ability to control the scaffold's porosity and pore size distribution, achieve optimal elasticity and degradation rates, promote cell attachment and viability, and confirm tissue formation through histological analysis, opens possibilities for customized scaffold designs to meet specific tissue engineering requirements. Future investigations should evaluate these scaffolds' in vivo performance and long-term efficacy in promoting bone regeneration, considering their mechanical properties, degradation behavior, and ability to support cell functions.

Acknowledgement:
This work was supported by AP14869460 “Lactate-triggered shape adaptive scaffold for advanced bone tissue regeneration: Injectable applications” by the MES RK and FDRCG SEDS2020020 from Nazarbayev University.
Development of a 3D osteogenic niche (3DON) to support the survival of acute myeloid leukemia (AML) cells in cultures for drug screening

Ms. Hoi Lam CHEUNG1,2, Ms. Jessica Evangeline Tan KABIGTING1, Dr. Yu Hin WONG1,2, Dr. Yuk Yin LI1, Mr. Lok Him KO1,2, Mr. Koon Chuen CHAN4, Prof. Anskar Yu Hung LEUNG4, Prof. Barbara CHAN1,2,3
1 Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China, 2 Advanced Biomedical Instrumentation Centre, Hong Kong, China, 3 School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China, 4 Department of Medicine, School of Clinical Medicine, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, China

Authors: Hoi Lam Cheung1,2, Jessica Evangeline Tan Kabigting1, Yu Hin Wong1,2, Yuk Yin Li1, Lok Him Ko1,2, Koon Chuen Chan4, Anskar Yu Hung Leung4, Barbara Pui Chan1,2,3

Affiliations:
1 Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong, China
2 Advanced Biomedical Instrumentation Centre, Hong Kong, China
3 School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China
4 Department of Medicine, School of Clinical Medicine, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, China

* Email: bpchan@cuhk.edu.hk

Category: SYIS, Enabling Technologies

Background: Acute myeloid leukemia (AML) is the most lethal hematopoietic cancer, only half of the patients achieve overall survival [1]. The poor therapeutic outcome may be caused by the highly heterogeneous nature of AML. Personalized cancer drug screening could be a solution, but primary AML cells undergo spontaneous apoptosis in ex vivo cultures [2], causing difficulty to carry out personalized drug screening. Studies have shown that stromal cells and extracellular matrix (ECM) provide a pro-tumoral environment to AML cells. We hypothesized that the bone marrow microenvironment mimicked by osteolineage cells encapsulated in ECM called 3D osteogenic niche (3DON) would support the survival of primary AML cells, and the presence of a biomimetic 3D cancer microenvironment would provide more accurate screening results.

Methods: Our laboratory has been developing a 3D microencapsulation platform using naturally occurring ECM to fabricate physiologically relevant and ECM-based 3D microtissues. We aimed to develop a drug screening platform consisting of osteogenic differentiated human mesenchymal stem cells (hMSCs) encapsulated in collagen microspheres co-cultured with primary AML cells. The 3DON was produced by encapsulating AML-derived or healthy donor-derived hMSCs in collagen microspheres and differentiated osteogenically for 21 days. 3DMN was prepared by encapsulating AML or healthy hMSCs in collagen without differentiation, and 2DMN was prepared by seeding AML or healthy hMSCs as a 2D feeder layer. Primary AML cells were cultured under different experimental conditions for 3 days and collected for viability test by trypan blue exclusion test, apoptosis test by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, and CD33, CD34 phenotype test by flow cytometry. The medium from different groups was also collected for cytokine secretion analysis. Drug tests were performed under different culture conditions.

Results: Our results showed that the viability of primary AML cells was around 40-49% when being cultured alone but it was significantly higher when being co-cultured with AML 3DMN (74%) and AML 3DON (74%). The percentage of apoptotic cells was reduced from 12% to 6% in AML 3DON when compared with PC. This could be explained by the high levels of secretion of pro-tumoral cytokines such as IL-8, CXCL-1, and OPG. Stage M1 and M5 AML phenotypes of CD33+ and CD34- were
maintained in 88% of the AML cells in the AML 3DON co-culture system, in comparison to NC1 and NC2 with less than 70% of the population. Moreover, the IC50s of daunorubicin on AML cells when co-cultured with 3DON (2.05µM) were significantly lower than the PC (34.32µM).

Discussion and Conclusion: The AML 3DON maintained the viability and phenotypes of primary AML cells, suggesting that the AML 3DON environment may be more biomimetic and hence may achieve more reliable drug screening results. The high drug sensitivity of AML cells in healthy 3DON suggested the future application of healthy 3DON as an adjuvant to chemotherapy in AML. Future investigation in using AML 3DON for drug screening will be carried out.

References:

Acknowledgement: This work was supported by the General Research Fund (17164116) from the Research Grant Council, the Mid-Stream Research Program (MRP/047/21) and the Health@InnoHK program from the Innovation and Technology Commission, of the Hong Kong Special Administrative Region government.
Generation of universal hiPSCs and their differentiation into RPE on matrigel-coated surface

Ms. Kailibinuer Maitiruze¹, Prof. Akon Higuchi¹
¹State Key Laboratory of Ophthalmology, Optometry and Visual Science, Eye Hospital, Wenzhou Medical University, Wenzhou, China, ²Department of Chemical and Materials Engineering, National Central University, Jhongli, Taiwan

Introduction
Age-related macular degeneration (AMD) is an aging disease, which refers to the degeneration of the choroid and pigment epithelium in the macula of the eye caused by abnormal metabolism in the elderly. Besides, AMD has progressive damage to retinal pigment epithelium, which shows irreversible blinding. Human pluripotent stem cells (hPSCs) including human induced pluripotent stem cells (hiPSCs), human embryonic stem cells (hESCs) have been used to differentiate into retinal pigment epithelium (RPE) cells to treat AMD recently. However, hPSCs with various types of human leukocyte antigen (HLA) is major barrier of allograft transplantation of hPSC-derived RPE cells. Banking hPSCs having different HLA types cost huge money and need a lot of stuffs to maintain. The patients still need to administrate life-long immune suppressive drugs. It is necessary to generate an hypoimmunogenic (universal) hPSC-derived RPE cells to apply on cell treatment of AMD patients in future clinical trial in order to avoid to use immunosuppressive drugs after treatment and to reduce the cost of stem cell therapy.

Experiments
Universal hiPSCs were generated from the reprogramming of human amniotic fluid stem cells (hAFSCs), which were derived from multi-donors of amniotic fluid (AF) where the mixing process of multi-donors of AFs is a key technology to generate universal hiPSCs. We chose one protocol (NIC84 protocol) to induce differentiation of universal hiPSCs and conventional hiPSCs into RPE cells. After the successful differentiation of hiPSCs into RPE cells, we evaluated the human leukocyte antigen (HLA) class Ia and class II and RPE markers (ZO-1, PAX-6, MITF, RPE 65) expression. We injected conventional and universal hiPSCs (HPS0077, mix2) cells and RPE (HPS0077-derived RPE and mix2-derived RPE) cells into the subretinal of Royal College of Surgeons (RCS) rats of 21 days, and then evaluated whether the vision of the rats improved after four weeks and eight weeks. We compared hypoimmunogenic effect between universal and conventional hiPSCs and RPE cells derived from those cells by using humanized mice.

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The immunostaining of RPE cells, which were differentiated from universal hiPSCs and conventional hiPSCs showed the RPE markers (ZO-1 and RPE 65). We are evaluating the HLA Ia and HLA II expression of RPE cells derived from conventional hiPSCs and universal hiPSCs. We will use humanize mice to investigate the hypoimmunogenic effect of universal hiPSCs. We expect the universal hiPSCs-derived RPE can escape from immune rejection and will be used for the treatment of AMD patients in the future.

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References
Enhancing Bone Tissue Regeneration: An Enzyme-Triggered Cross-Linking Approach For 3D Printing Of Cell-Laden Scaffolds

Ms. Bota YERNAIMANOVA¹, Dr. Alexander TRIFONOV¹, Dr. Dana AKILBEKOVA¹
¹Department of Chemical and Materials Engineering, School of Engineering and Digital Sciences, Nazarbayev University, Astana, Kazakhstan

Introduction: Previous studies have demonstrated that clinically relevant bone grafts for bone fracture treatments should incorporate mesenchymal stem cells (MSCs) and structurally supportive grafts with an extracellular matrix (ECM)-like structure to enhance cell proliferation and vascularization [1]. In this project, we propose a fundamentally innovative approach to cell-laden scaffold engineering for bone regenerative medicine - employing enzyme-triggered cross-linking aimed at advancing bone tissue regeneration. This method allows for precise control over the shape and structure of the printed scaffolds, facilitating the inclusion of cells within the scaffold matrix [2]. The enzyme-triggered cross-linking mechanism assures the development of stable, biocompatible scaffolds, promoting the growth and regeneration of bone tissue.

Methods: For bioink preparation, we combined gelatin, alginate, lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) photoinitiator, and methacrylated gel (GelMA) with different concentrations of catalase (Cat) and glucose oxidase (GOx) enzymes. We achieved desired pore sizes by manipulating Gel/GelMA and enzyme concentrations. The kinetics of the cascading reactions in the gelatin medium was examined using electrochemical methods. A lattice-rod model was extruded during 3D Printing (Cellink BIOx) at a pressure of 80-100 kPa, maintaining the system temperature between 15-20 °C. The scaffolds were photo-crosslinked with UV light and a 0.25 M CaCl₂ solution, and subsequently soaked in a high glucose Dulbecco’s Modified Eagle Medium (DMEM) for one week. After producing the enzyme-triggered hydrogel, we introduced osteoblasts to the most promising formulations and assessed the material’s biocompatibility in vitro. Viability (live/dead staining), swelling, and degradation tests were conducted. We analyzed tissue formation using H&E, Sirius Red, and Alizarin Red staining. The morphology was studied through scanning electron microscopy (SEM), and mechanical properties were measured using a texture analyzer.

Results: We incorporated cells into the prepared solutions and 3D printed scaffolds with varying levels of porosity, featuring a range of pore sizes from 50 to 400 µm (Figure 1). The scaffold with an increased concentration of Cat demonstrated higher swelling capacity, but it also degraded significantly faster than the GOx-rich scaffold, which exhibited superior tensile modulus and mechanical properties. Fluorescence images revealed enhanced proliferation of osteoblasts in the scaffold with the highest GOx concentration and largest pore sizes compared to the control and Cat-rich constructs. Histological images indicated a more homogeneous distribution of osteoblasts within the Cat-rich scaffold. However, the largest amount of calcium nodules and collagen fibrils were observed within the GOx scaffold. Compared to both, the control scaffold had a meager number of nuclei, and the intensity of Alizarin and Sirius red was weak.

Discussion and Conclusion: Our objective was to develop a gel-like Gel/GelMA-based material capable of generating a mechanically stable, yet biodegradable construct through intermolecular crosslinking upon exposure to GOx. To achieve this, we employed a tandem of two enzymes, Cat and GOx. Given its excellent cytocompatibility and proliferation rates, this hydrogel construct can be employed in the development of in vitro bone organoids.

References:
Acknowledgment: This research project was supported by AP14869460 “Lactate-triggered shape adaptive scaffold for advanced bone tissue regeneration: Injectable applications” by the MES RK.

Keywords: In vitro bone organoids, enzyme-triggered cross-linking
Modified exosome carrier for targeted gene delivery of glioblastoma

Ms. Minji Kang1, Minhyung Lee

1Department of Bioengineering, College of Engineering, Hanyang University, Seoul, South Korea

Title : Modified exosome carrier for targeted gene delivery of glioblastoma

Authors : Subin Kang1, Minhyung Lee*

Affiliations : Department of Bioengineering, College of Engineering, Hanyang University, Republic of Korea.

Category : Design and Application of Biomaterials

Background : Glioblastoma (GBM) is an aggressive type of cancer that can occur in the brain or spinal cord. Many treatments are being studied, but there is a limitation that the recurrence rate after surgery is high and the drug's BBB permeability is low. Therefore, there is a need for better treatments to overcome challenges.

Methods : In this study, we modified the exosome using peptides that was able to target the receptors in the BBB and GBM. The conjugation of Chlorotoxin-Exosome/antagomir21c (CTX-Exo/AMO21c) has been tested for its effect on mRNA inhibition and targeting efficiency.

Results : TEM showed that Exo/AMO21c formed a compact complex. Dynamic light scattering confirmed the temperature-dependent stability of the carrier. MTT assay were conducted to verify minimum cytotoxicity of CTX-Exo/AMO21c compared to Lipofectamine/AMO21c. In addition, flow cytometry and confocal laser microscopy indicated that CTX-Exo/AMO21c had higher cellular uptake than control.

Discussion and Conclusions : We demonstrated that modified exosomes increased delivery efficiency to glioblastoma. This result suggest that CTX-Exo may be useful for gene therapy of glioblastoma.
Enhanced Bone and Tissue Regeneration Potential with multi-Substituted Nanoparticles Synthesis, Characterization, and In Vitro Evaluation

Dr. Fahad Alhamoudi¹, Aqif A. Chaudhry², Gwendolen C. Reilly³, Ihtesham U. Rehman⁴

¹Department of Dental Technology, College of Applied Medical Science, King Khalid University, Abha, Kingdom of Saudi Arabia, ²Interdisciplinary Research Centre in Biomedical Materials (IRCBM), COMSATS University Islamabad, Punjab, Pakistan, ³INSIGNEO institute for in silico Medicine, University of Sheffield, United Kingdom, ⁴Head of Translational Research, University of Central Lancashire, Preston, United Kingdom

Title: Enhanced Bone and Tissue Regeneration Potential with multi-Substituted Nanoparticles Synthesis, Characterization, and In Vitro Evaluation

Authors: Fahad H. Alhamoudi¹, Aqif A. Chaudhry², Gwendolen C. Reilly³ and Ihtesham U. Rehman⁴

Affiliations: ¹Dental Technology Department, applied medical Science, King Khalid University, Abha 62529, Kingdom of Saudi Arabia. ²Interdisciplinary Research Centre in Biomedical Materials (IRCBM), COMSATS University Islamabad, Lahore Campus, Punjab, Pakistan. ³INSIGNEO institute for in silico Medicine, University of Sheffield, United Kingdom. ⁴Head of Translational Research, University of Central Lancashire, Preston, England, United Kingdom

Category: Tissue Engineering and Regeneration / Design and Application of Biomaterials

Introduction: Bone and tissue regeneration plays a crucial role in regenerative medicine, addressing bone loss due to trauma or disease. Hydroxyapatite (HA), a bioceramic material resembling the mineral component of bone, has demonstrated significant potential in bone repair and tissue engineering due to its osteoconductive properties and biocompatibility. The substitution and co-substitution of carbonate, fluoride, and citrate in HA can enhance its properties for bone tissue engineering applications. This study aims to synthesize, characterize, and evaluate in vitro the potential of these substituted HAs for bone and tissue regeneration.

Methods: Various HA formulations were synthesized using hydrothermal methods and characterized using Fourier-transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). The synthesized HAs were assessed for cell attachment and viability using osteoblasts (MG63) and mesenchymal stem cells (hTERT-BMSCs) at two concentrations over time.

Results: Innovative hydroxyapatite (HA) formulations were successfully synthesized for bone and tissue regeneration applications, focusing on variations in substitutions and co-substitutions of carbonate, fluoride, and citrate. FTIR spectra revealed successful HA synthesis with differing substitution patterns, while TEM analysis showcased a shift from rod-shaped to needle-shaped particles in citrate-substituted HA. The presence of citrate also resulted in reduced particle sizes, enhancing bioactivity and biocompatibility. Cell attachment and viability tests demonstrated an overall improvement with fluoride and citrate co-substituted HA (F-Cit-HA), attributable to the increased surface area of nanoparticles. Carbonate substitution improved HA solubility and bioactivity, while fluoride substitution influenced cell attachment and subsequent activities within an appropriate concentration range. Citrate substitution played a vital role in biomineralization and particle size control, impacting the biological outcome, particularly as a co-substituted element.
Discussion & Conclusion: Co-substituted hydroxyapatite, especially F-Cit-HA, displayed promising properties for bone tissue engineering applications, including enhanced cell attachment, improved solubility, and controlled particle size. These findings support the potential use of these materials in scaffold fabrication, coating materials, and injectable gels for bone and tissue regeneration. Future research should further explore these innovative biomaterials to optimize their potential in regenerative medicine.

Acknowledgments
Financial support was provided by King Khalid University; Ministry of Education; Kingdom of Saudi Arabia. Cell viability tests were conducted at the Kroto Research Institute; Sheffield University.

References
Glucose As A Stimulus For Dynamic Crosslinking Of Hydrogel Scaffolds: Injectable And Shape-Adaptive Hydrogel Scaffolds For Bone Tissue Regeneration Applications.

Ms. Ainur Zhanbassynova1, Ahmer SHEHZAD, Birzhan ABDIKHAN, Dr. Alexander TRIFONOV, Dr. Dana AKILBEKOVA

1Department of Chemical Engineering, School of Engineering and Digital Sciences, Nazarbayev University, Astana, Kazakhstan

Introduction: Hydrogel scaffolds are valuable tools in regenerative medicine and tissue engineering due to their hydrophilic nature and tunable morphology and physical properties, which provide a conducive environment for new tissue formation. The ability to control the scaffold’s degradation rate and porosity is essential to support multiple processes associated with tissue formation, such as cell proliferation and ECM deposition. Control over the scaffold’s properties includes consideration of several variables, e.g., precursor materials, their concentration, nature, and concentration of crosslinker. To endow the scaffold with greater biocompatibility, natural substances such as gelatin, alginate, and hyaluronic acid, as well as several synthetically derived materials, like polyethylene glycol, polyvinyl alcohol, are frequently used due to their low cytotoxicity. The robustness of hydrogel constructs is controlled via crosslinking process: namely, the crosslinker used, reaction duration, and conditions. For example, photoinitiated radical polymerization, utilization of bifunctional crosslinking agents, such as glutaraldehyde or BS3, direct enzymatic crosslinking (e.g., transglutaminase), or cryogelation are frequently used in scaffold fabrication. However, the use of classical crosslinking approaches is not always suitable due to the excessive cytotoxicity of some materials or certain technical challenges of providing conditions for gelation. For example, in surgery-free bone regeneration, the hydrogel is injected with a syringe directly into the bone fracture to facilitate the healing process. This treatment method imposes certain conditions: the hydrogel has to be injectable and form a robust scaffold when in contact with bone tissue to fill the voids and maximize contact with the surface.

Here we demonstrate the development of shape-adaptive hydrogel biomaterial for bone grafting and tissue regeneration applications with minimal invasion. The project utilizes an enzymatic cascaded reaction based on Glucose Oxidase (GOx) and Catalase (CAT) for glucose-triggered radical polymerization through enzymatic Fenton’s reaction. This approach not only allows control of gelation time, but also eliminates the need for cytotoxic chemical crosslinkers, and creates a highly porous hydrogel matrix and favorable conditions for tissue growth due to the increase of local oxygen concentration as a result of enzymatic activity.

Subjects and Methods: In this work, we proposed a novel injectable, biocompatible, and stimuli-responsive hydrogel-based biomaterial for improved osteogenesis and directed bone regeneration. The proposed precursor material contains a solution of Gel/GelMA and a combination of enzymes forming an amorphous gel-like substance, suitable for convenient application of the material via injection. Enzymatic cascaded reactions are used for the initiation of radical polymerization by Fenton’s mechanism and the dynamic formation of macroporous morphology of the scaffold due to enzymatic oxygen evolution. Control over the kinetics of the process can be achieved via pH, and/or enzyme ratio and concentration.

Results: The study focused on analyzing a range of compositions to identify the optimal conditions for achieving both sufficient radical polymerization and the formation of a macroporous structure in the hydrogel. Electrochemical studies revealed that GOx and CAT remain active during the hydrogelation, despite a significant pH drop resulting from the conversion of glucose to gluconic acid by GOx. It was also observed that smooth moderation of acidity enhanced the polymerization rate, Consequently, stable irreversibly crosslinked hydrogels based on GelMA were successfully produced, featuring interconnected macropores capable of filling bone defects.

Discussion and Conclusion: Our findings demonstrated the possibility of crosslinking GelMA-based hydrogels through a non-conventional method, eliminating the need for photoinitiators and instead utilizing enzymatically produced ·OH radicals as initiators. This novel approach allows for a dynamic
and controlled formation of a biodegradable macroporous matrix, enabling injectable in-vivo applications for improved bone regeneration. Additionally, the oxygen-rich macropore structure created in the hydrogels can mitigate anoxic conditions, thereby positively influencing bone cell proliferation, vascularization, and therefore osteoconductivity.
Stem cell therapy using cell-assembling collagen microgel for critical limb ischemia repair

**Prof. Sangheon Kim**, Ms. Haeun Jung, Mr. Changgi Hong, Dr. Jungkyun Choi, Mr. Jongwan Kim

1Sbiomedics, Seoul, Korea, 2Seoul National University, Seoul, Korea, 3Korea Institute of Science and Technology, Seoul, Korea

Background: Critical limb ischemia is a devastating disease characterized by the progressive blockade of blood vessels. Stem cell therapy has emerged as an angiogenic therapy for CLI, as it showed promising therapeutic potential via the paracrine effect of growth factors. An approach to deliver stem cells that can enhance cell survival and engraftment is a key for successful therapeutic outcome. Collagen microgel (CMG), 30~40 mm, was fabricated from polyionic complex of collagen and hyaluronic acid as a novel scaffold to deliver cells for improving transplantation efficiency.

Methods: In this study, a collagen microgel (CMG) was fabricated from polyionic complex of high-concentrated collagen (10%) and HA (5%) by mechanical fragmentation. Size distribution of CMG particles were analyzed using an automated Mophologi G3 optical microscope (Malvern Panalytical Ltd). We characterized CMG assembly as carrier for cell delivery and investigated the microstructure, mass transfer, cell viability, and angiogenic functions of CMG assembly as therapeutics for CLI treatment. Finally, therapeutic efficacy of CMG assembly was demonstrated in mouse CLI model.

Results: Physiological interaction of hASCs with CMG resulted in injectable 3-dimensional (3D) cell construct (CMG-hASCs) which was inhibited by integrin beta1 blocking antibody and Rho kinase inhibitor. CMG-hASCs assembly was an architecture with increased microporosity in a manner dependent on the increase in the dose of the CMG. Glucose uptake and hypoxic state in CMG-hASCs assembly increased and decreased, respectively, depending on the amount of CMG. We verified had CMG-hASCs assembly improved survival of hASCs compared to hASCs 3D construct. Further analysis with heatmap revealed that genes related to actin contraction, mass transfer, anti-apoptosis, and angiogenesis were upregulated in CMG-hASCs assembly (4:1 in the ratio of CMG:hASCs), compared to hASCs 3D construct and 2D cultured hASCs. CMG-hASCs was verified to have high angiogenic potential in vitro and ex vivo angiogenesis assay. Immunofluorescent images of thigh muscles collected from CMG-hASCs injected mice CLI model showed muscle regeneration and angiogenesis with enhanced survival of transplanted hASCs.

Discussion and Conclusion: Taken together, we propose that CMG-hASCs is a potential therapeutics to treat CLI and CMG is a novel scaffold platform to fabricate 3D cell construct for regenerative medicine. Future research is required to dissect the molecular mechanisms underlying the upregulation of key angiogenic factors. The identification of the possible signaling pathways involved in this enhanced angiogenic potential will pave the way for breakthroughs in enhancing treatments for CLI.

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Integrative in-situ 3D bioprinting platform for programmable delivery to regenerate knee cartilage

Dr. Kai Xie¹, Dr. Chunliang WANG², Prof. Molly STEVENS¹,²,³
¹Department of Bioengineering, Imperial College London, London, United Kingdom, ²Department of Materials, Imperial College London, London, United Kingdom, ³Institute of Biomedical Engineering, Imperial College London, London, United Kingdom

Title: Integrative in-situ 3D bioprinting platform for programmable delivery to regenerate knee cartilage

Background: Currently, clinical delivery of cartilage implants is still a highly manual process that requires surgeon to either inject pre-formulated polymers or fix pre-fabricated implants to fill the defect regions. While these methods are simple and straightforward, both provide little control of final implantation structure, homogeneity, and composition, hindering its therapeutic efficacy to regenerate cartilage tissues.

Methods: To enable a fully programmable therapeutic delivery, an Integrative Printing on Tissue System (iPoTS) has been built to incorporate surface sensing, geometrical reconstruction, and bioprinting on non-planar tissue surface. The robot mimics an arthroscopic surgery procedure to access cartilage in a keyhole surgery manner.

Results: Inside the highly confined knee cavity, iPoTS can sense the defected region to digitally reconstruct the printing substrate. Upon mapping the substrate, it calculates the regenerative model to fill the defect, and optimises printing trajectory to comply with the non-planar substrate. The printing can then be started to delivery therapeutic polymers, which offers programmable control of implant structure and composition.

Discussion and Conclusion: The development of iPoTS is a clinically-oriented project to achieve programmable delivery of therapeutic biomaterials, and is compatible with current clinical practices. The controllable implant patterning and composition can promote the scaffold integration by improving mass transportation efficiency and cell migration, which has the potential to shorten the post-surgical recovery time and promote long-term therapeutic efficacy.

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A Therapeutic Nanovaccine that Generates Anti-Amyloid Antibodies and Amyloid-Specific Regulatory T Cells for Alzheimer’s Disease

Dr. Mungyo JUNG1, Ms. Songmin LEE2, Prof. YoungSoo KIM2, Prof. Byung-Soo KIM1
1School of Chemical and Biological Engineering, Seoul National University, Seoul, Republic of Korea, 2Department of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, College of Pharmacy, Yonsei University, Incheon, Republic of Korea

Background: Various immunotherapy approaches for Alzheimer’s disease (AD), such as anti-Aβ monoclonal antibody (mAb) therapy, Aβ vaccines, and adoptive regulatory T (Treg) cell transfer have exhibited unsatisfactory therapeutic efficacy. Because targeting a single pathophysiological mechanism doesn’t overcome a complex condition characterized by multiple pathophysiological mechanisms including amyloid-β (Aβ) plaque accumulation and neuroinflammation in the brain. Moreover, Aβ vaccines could worsen therapeutic efficacy by inducing T helper 1 (Th1) cell-mediated inflammatory responses.

Methods: In this work, we developed a nanovaccine (LNP-R/Aβ) targeting multiple pathophysiological mechanisms. LNP-R/Aβ effectively delivers Aβ peptides and rapamycin to dendritic cells, and Aβ presenting tolerogenic dendritic cells (tDCs) induce both anti-Aβ antibodies and Aβ-specific Treg cells.

Results: LNP-R/Aβ was taken up by dermal DCs and induced tDCs, and the tDCs then activated Aβ-specific Treg cells in vitro and in vivo. Moreover, LNP-R/Aβ-administered 5XFAD mice showed a high level of anti-Aβ antibodies in the plasma and brain lysates. As a result, LNP-R/Aβ exhibited therapeutic efficacies against AD such as reduced Aβ plaque, attenuated neuroinflammation, and suppressed cognitive decline in 5XFAD mice.

Discussion and Conclusion: LNP-R/Aβ shows great therapeutic effect in removing Aβ plaques in the brain, alleviating neuroinflammation, preventing Th1 cell-mediated excessive immune responses, and inhibiting cognitive impairment in 5XFAD mice. Furthermore, LNP-R/Aβ does not require complicated and costly manufacturing processes compared to current immunotherapies. The nanovaccine can represent a novel treatment option for AD.
The in vivo efficacy evaluation of MSC-XenoMEM-graft and MSC-sheets in diabetic wound healing

Dr. SHANSHAN DU
Dr. CLARA NOGUES
Dr. XIZHE CHEN
CAOMHAN LYONS
ALAN KEANE
SANDRA CALCAT
STEPHEN ELLIMAN
DIMITRIOS ZEUGOLIS
Prof TIMOTHY O’BRIEN

1Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), University of Galway, Galway, Ireland, 2Regenerative Medicine Institute (REMEDi), University of Galway, Galway, Ireland, 3Orbsen Therapeutics Ltd, Galway, Ireland, 4Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), Charles Institute of Dermatology, Conway Institute of Biomolecular & Biomedical Research and School of Mechanical & Materials Engineering, University College Dublin, Dublin, Ireland

Introduction: Decellularized grafts emerged as a promising cell delivery platform, as they retain the native extracellular matrix (ECM) composition and structure of the tissues from which they have derived, providing a favourable microenvironment for cell migration and proliferation. Macromolecular crowding is a biophysical phenomenon and has been shown to enhance and accelerate the native ECM deposition of cells in vitro. Culturing the MSCs in a thermo-responsive polymer-coated dish under macromolecular crowding conditions could generate an MSC sheet with good cell localization and its own ECM and secretome. Herein, we ventured to investigate the therapeutic effect of a decellularized graft (XenoMEM™) mediated MSC delivery method (MSC-XenoMEM-graft) and a scaffold-free approach (MSC-sheets) in a pre-clinical diabetic wound model.

Subjects and Methods: MSCs were cultured either on XenoMEM™ membrane or thermo-responsive polymer-coated dishes for three days under the MMC conditions. Animals were induced to hyperglycaemia using streptozotocin (65 mg/Kg) and wounds were treated as per groups: Control, XenoMEM, XenoMEM+MSC, XenoMEM+MSC+MMC, MSC sheet and MSC+MMC sheet. Wound closure, morphometric parameters and angiogenesis were assessed.

Results: Gross view of wounds on day 7 showed no significant difference in wound closure between the control and treatment groups. Histology analysis revealed no significant difference in re-epithelialization between all groups. However, the granulation tissue area was significantly increased in the MSC+MMC sheet group than in the control group. RT-PCR analysis further confirmed the RNA expression of collagen type I and III was significantly increased in the wound bed of the MSC+MMC sheet group than the control group. Further angiogenesis analysis is ongoing.

Discussion and Conclusion: Current data indicate that XenoMEM-graft mediated MSC delivery method may not be effective for improving diabetic wound healing at an early stage. Scaffold-free approach (MSC+MMC sheet) may be an effective treatment for accelerating wound healing as it improved the regeneration of granulation tissue in the wound bed via increasing the RNA expression of collagen type I and III. Further analysis is needed to determine the angiogenesis in the wound bed and the underlying mechanism of action. This study provided a promising scaffold-free approach for MSC therapy in the treatment of diabetic wounds.

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Natural nanoparticles for the treatment of osteoporosis by synergistically enhancing bone formation and reducing bone resorption

Prof. Xi Chen, Weihui Wu, Changsheng Liu

1 Key Laboratory for Ultrafine Materials of Ministry of Education, Frontiers Science Center for Materiobiology and Dynamic Chemistry, Engineering Research Center for Biomaterials of Ministry of Education, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai, China

Title: Natural nanoparticles for the treatment of osteoporosis by synergistically enhancing bone formation and reducing bone resorption

Authors: Xi Chen*, Weihui Wu, Changsheng Liu*

Affiliations: Key Laboratory for Ultrafine Materials of Ministry of Education, Frontiers Science Center for Materiobiology and Dynamic Chemistry, Engineering Research Center for Biomaterials of Ministry of Education, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai, China (* Correspondence)

Category: Biomaterial-based delivery technologies

Background: Osteoporosis (OP) is a systemic disease of bone metabolism characterized by microstructural degradation of bone tissue due to greater bone resorption than bone formation. Nanomaterials are recently considered for systemic treatment of osteoporosis. Particularly, the nanotechnology of metal-polyphenol network (MPN) structure has attracted great extensive attention, which provide valuable inspiration for the design of MPN-based biomaterial scaffolds to regulate osteoporosis

Methods: In this work, we select EGCG, natural catechin that affects bone tissue metabolism, as phenol component and Sr, a key trace metal in human bone, as metal element to construct metal-polyphenol network nanoparticles, and its effects on bone formation and bone resorption in vitro and in vivo.

Results: The MPN nanoparticles exhibited expedited release of the constructed EGCG and Sr in response to acidic OP microenvironment, which can reach the targeted bone matrix. The in vitro studies showed that the MPN nanoparticles inhibit mature osteoclastic differentiation of macrophage-like RAW264.7 cells, while the MPN nanoparticles can enhance differentiation and mineralization of MC3T3-E1 preosteoblast cells via the released two components. Furthermore, the in vivo evaluation in ovariectomized mouse model showed that the MPN nanoparticles can both inhibit bone resorption and enhance the in-situ regeneration of bone.

Discussion and Conclusion: Based on the multifunction metal-phenol network structure, we demonstrated that MPN nanoparticles could inhibit bone resorption and promote bone formation through the systemic circulation of nanovesicles, the targeting of bone matrix, and the degradation in the OP microenvironment. This research provides a new strategy for the preparation of novel nano-drug delivery systems for the treatment of osteoporosis.

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Modulating the modulus: how lattices can be used to prevent periprosthetic osteopenia following endoprosthesis reconstruction surgery

Dr. Reza Sanaei¹, Dr. Charles Neil Pagel¹, Dr. Babatunde A. Ayodele¹, Dr. Bill Lozanovski², Dr. Thierry Beths¹, Prof. Martin Leary², Dr. Darpan Shidid², Dr Endri Kastrati², Dr Joe Elambasseril², Mr. Ulrich Bühner⁴, Dr. Tom Williamson³, Dr. Stewart Ryan¹, Prof. Milan Brandt²

¹Melbourne Veterinary School, The University of Melbourne, Melbourne, Australia, ²RMIT Centre for Additive Manufacturing, RMIT University, Melbourne, Australia, ³Stryker Australia Pty. Ltd., Australia, ⁴Stryker Leibinger GmbH & Co. KG, 79111 Freiburg, Germany

Introduction: Megaendoprosthetic reconstruction is the treatment of choice when extensive surgical resection of bone is required. A common complication that is associated with such surgeries is periprosthetic osteopenia, which is attributed to stress shielding of the host bone by the large rigid implant. As such, it has been observed that implants made of less stiff materials are less likely to result in this complication. We demonstrate here that inclusion of an open lattice structure into additively manufactured Ti6Al4V ELI prostheses, in order to reduce the implant modulus, protects the periprosthetic bone against the effects of stress shielding in a sheep model.

Methods: The control sheep (solid implant group) received solid-core protheses that were custom designed to fit a large distal femoral defect based on an initial CT scan of their hindlimbs. The test group (lattice implant group) received similar implants that had a gradient face-centred cubic with z-strut lattice structure forming their cores. Both implant types had a 4 mm thick interfacial lattice to facilitate rapid osteointegration and load sharing with the host bone. Implants and surrounding tissue were harvested at 12 weeks and subjected to back-scattered SEM (BSEM) as well as fluorescent and light microscopy to complete a range of static and dynamic morphological evaluations.

Results: Based on BSEM evaluations, incorporation of the lattice resulted in less porosity in the more proximal region of interest (ROI) and improved bone density in the more distal ROI (Figure 1).

Discussion and Conclusion: Our findings support the hypothesis that reduced implant modulus, through incorporation of an open lattice, protects the host bone from the effects of stress shielding. This has significant implications for orthopaedic implant design, suggesting that strategically incorporating open-lattice structures into megaendoprostheses could improve surgical outcome and long-term implant survivorship by optimising bone-implant interactions.

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Tissue inhibitor matrix metalloproteinase 1 overexpression improves cell viability and therapeutic efficacy of cell spheroids

Prof. Sangheon Kim1,2, Dr. Jungkyun Choi1,2, Dr. Wonyeong Jang1, Mr. Jongwan Kim, Haeun Chung1,2

1Center for Biomaterials, Biomedical Research Institute, Korea Institute of Science and Technology (KIST), Seoul, South Korea, 2Division of Bio-Medical Science & Technology, KIST School, University of Science and Technology, Seoul, South Korea

Title: Tissue inhibitor matrix metalloproteinase 1 overexpression improves cell viability and therapeutic efficacy of cell spheroids

Authors: Jung-Kyun Choi1,2, Won Young Jang1, Haeun Chung1,2, Sang-Heon Kim1,2*

Affiliations: 1 Center for Biomaterials, Biomedical Research Institute, Korea Institute of Science and Technology (KIST), Seoul 02792, Republic of Korea. 2 Division of Bio-Medical Science & Technology, KIST School, University of Science and Technology, Seoul 02792, Republic of Korea (* Correspondence)

Category: Stem Cells and Cell-Based Therapies

Background: Extending the lifespan of transplanted cells and improving therapeutic effect is an important issue for stem cell therapy [1]. In our previous work, we have demonstrated that TIMP1 acts as an anti-apoptotic factor thereby increasing cell survival of transplanted cells [2]. In this study, we aimed to increase the efficacy of human adipose-derived mesenchymal stem cell (hASC) therapy for critical limb ischemia (CLI) repair by overexpressing TIMP1 in the cell spheroid using a recombinant adenovirus.

Methods: We transduced hASCs with recombinant adenovirus (rAd)-TIMP1. The transduced hASCs were trypsinized and seeded in each well of MBP-FGF2-coated 384-microwell (1×104 cells/well) and cultured for 24 h in a 37 °C incubator with 5% CO2 to prepare cell spheroids. TIMP1 expression level was analyzed by RT-PCR, ELISA, IF staining. rAd-TIMP1-FECS-Ad were injected into CLI mouse model and its therapeutic effects were evaluated by a laser doppler system and histological analyses.

Results: The Optimized dose of hASCs transduction for TIMP1 overexpression was determined to be multiplicity of infection (MOI) of 100 and TIMP1 protein was overexpressed in hASCs for up to 35 days. Overexpression of TIMP1 in hASCs spheroids activated FAK signaling pathway compared to non-transduced hASCs spheroids, ultimately improving cell survival and therapeutic efficacy in mouse CLI model.

Discussion and Conclusion: We demonstrated that the anti-apoptotic effect of TIMP1 can be further enhanced by overexpressing TIMP1 via rAd infected transduction. In this study, we propose overexpression of TIMP1 as a strategy to improve therapeutic efficacy of stem cell therapy.

Acknowledgement: This work supported by a grant funded by the Ministry of Health & Welfare (HI16C2234) in Republic of Korea.
Comparing 3D culture platforms: Microwell-type plate vs. Novel cell culture plate fabricated using particle array film technology

Dr. MinKyu KIM, Dr. HyunWoo JOO, Dr. SoonSun BAK, Prof. YoungKwan SUNG, Dr. HyoSop KIM, Dr. Huyen T. M. PHAM, Prof. JaeHo KIM

1Department of Biomedical Science, School of Medicine, Kyungpook National University, Daegu, Republic of Korea, 2BK21 FOUR KNU Convergence Educational Program of Biomedical Sciences for Creative Future Talents, Department of Biomedical Sciences, School of Medicine, Kyungpook National University, Daegu, Republic of Korea, 3Department of Molecular Science and Technology, Graduate School & Department of applied Chemistry and Biological Engineering, College of Engineering AjouUniversity, Suwon, Republic of Korea

Introduction:

Advancements in three-dimensional culture technology have played a pivotal role in rectifying the limitations of traditional two-dimensional cell cultures, which often exhibited physiologically inaccurate behaviors. The evolution of cell culture techniques has ushered in the era of organoids, demanding a substantial upswing in the utilization of three-dimensional culture methods. Introducing PAMCELLTM, our innovative large-scale 3D culture platform designed to seamlessly align with the demands of the organoid era, boasting both convenience and exceptional performance.

Methods:

Initiate the process by arranging the surface-modified particles compatible with proteins on the cell membrane within the polymer mold, simultaneously securing PEG and the adhesive film. Following UV light irradiation for PEG curing, proceed to eliminate the mold. This step enables modification of particle surface qualities, dimensions, and arrangement patterns to suit individual preferences.

Results:

We first compared the morphology of spheroids formed within the confined reverse-pyramid-shaped microwell platform and PAMCELLTM. Notably, no discernible distinctions were observed in morphology. A subsequent LIVE/DEAD Cell Viability Assay was performed, revealing a significantly higher proportion of viable cells cultured on the PAMCELLTM platform compared to the alternative. Afterward, we performed immunofluorescence staining for specific cell functional markers. The expression of these markers through fluorescent staining was equally robust or even superior in the case of PAMCELLTM spheroids.

Discussion and Conclusion:

In addition to its remarkable cell nurturing capabilities, the PAMCELLTM 3D cell culture plate offers significantly greater convenience compared to other platforms when performing Immunocytochemistry (ICC) staining procedures. The spheroids adhering to the substrate surface, coupled with their inherent transparency and minimal fluorescence absorption, allow for capturing fluorescence images of hundreds of spheroids under the same culture conditions. This confers a substantial advantage in high-throughput screening data. Moreover, the PAMCELLTM plate offers a versatile attribute enabling customization of surface characteristics to align with cell type characteristics. Leveraging the advantages of the PAMCELLTM plate, the enhanced 3D spheroids will offer a comprehensive cytophysiological perspective for advancing organoid development.
Preconditioning with Substance-P can enhance the efficacy of aged human adipose-derived stem cells under inflammatory conditions by restoring angiocrine and repopulation potential

Dr Jiyuan Piao¹, Mr Hyunchan Cho¹, Prof. Hyun Sook Hong²,³,⁴
¹Department of Genetic Engineering, Graduate School of Biotechnology, Kyung Hee University, Yong In, South Korea, ²Department of Biomedical Science and Technology, Graduate School, Kyung Hee University, Seoul, South Korea, ³East-West Medical Research Institute, Kyung Hee University, , South Korea, ⁴Kyung Hee Institute of Regenerative Medicine (KIRM), Medical Science Research Institute, Kyung Hee University Medical Center, , South Korea
Jiyuan Piao1, Hyunchan Cho1, Hyun Sook Hong* 2,3,4

Introduction: Age affects ADSCs’ activity to cause low efficacy after transplantation. Considering stem cell therapy is mostly applied to the aged, the strategies to restore paracrine disorders and low cellular activity due to aging should be established during ex vivo culture. TNF-α can exert its inflammatory action via Erk by binding TNFR1 and survival action via Akt by binding TNFR2. Aged cells are reported to be an insufficient expression of TNFR-2, indicating that aged ADSC is susceptible to inflammation stress. Substance-P (SP) is an endogenous neuropeptide involving cell viability and proliferation and promoting stem cells’ paracrine ability.

Subjects and Methods: This study aimed to evaluate the effect of preconditioning with SP on the activity of ADSCs derived from elderly donors (ADSCs-E) under inflammatory conditions, compared to young donors (ADSCs-Y). ADSC from young and elderly was cultured to compare morphology, viability and cytokine secretion. SP was added to ADSC-E, followed by treatment of high-dose TNF-α. 24 or 48h later, the activity of ADSC was determined by determining viability, survival-related signaling, and cytokine secretion

Results: This discovered that ADSCs-Y has expression of TNFR2 higher than ADSCs-E in normal conditions, which difference was bigger under inflammatory stress. Under the stimulus of TNF-α, ADSC-E had a low survival rate and lacked VEGF/HGF production and PCNA expression, compared to ADSC-Y. This phenomenon might be due to the lack of TNFR2 in ADSC-E. However, preconditioning with SP clearly elevated cell viability and PCNA/HGF level in ADSC-E with TNF-α. Pretreatment of SP could maintain active Akt signaling in ADSC-E. From this, SP treatment is inferred to restore the cellular activity of ADSC-E that lacks TNFR-2.

Discussion and Conclusion: These results suggested that preconditioning with SP prior to transplantation can enhance the therapeutic effect of ADSCs-E, possibly by restoring survival and paracrine potential.

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Mimicking in vivo dermal papilla of hair follicle

Dr. Hyun Woo Joo\textsuperscript{1}, Dr. Min Kyu Kim, Dr. Soon Sun Bak, Ms. Thanh Hang Ngo, Mr. Hyo Sop Kim, Prof. Young Kwan Sung
\textsuperscript{1}Kyungpook National University, , South Korea

\textbullet introduction

The dermal papilla, which is located in the hair bulb of the hair follicle, is connected to the capillaries during the hair follicle growth phase and plays a key role in hair follicle growth and signal transmission within the hair follicle. It will be possible to derive effective results through the formation of a spheroid structure similar to the dermal papilla in vivo, which can be applied to hair follicle bioengineering as well as developing screening platforms for hair growing compounds.

\textbullet method

In this study, we formed spheroids of human dermal papilla cells (hDPC) in the presence or absence of human umbilical vein endothelial cells (HUVEC). In addition, keratinocytes were added to the obtained spheroids to form a hair follicle-like structure (HFLS) and elongation of the keratinocyte layer was compared.

\textbullet result

Instead of random mixing and aggregation of the hDPC and HUVEC, a specific zone is formed within the spheroid similar to the arrangement of blood vessels in the dermal papilla structure in the body. In addition, we observed that the keratinocyte layer of the HFLS was more elongated in the spheroids containing HUVEC.

\textbullet Discussion& conclusion

Through 3D co-culture with HUVEC, vascularized spheroids and vascularized HFLS similar to the in vivo dermal papilla and hair follicle, respectively, were constructed. Vascularized HFLS with enhanced keratinocyte layer strongly suggest that vascularization provides the positive effect to hair growth and regeneration. Furthermore, the possibility of a new hair loss treatment method can be inferred by promoting angiogenesis in the hair follicle.
A 3D Patterned Polymeric Bone Mimicking Scaffold as an Effective Bone Substitute

**Prof. Jeong Ok (Grace) Lim** 1, Professor Chang-Wug Oh2, Yu Ri Hong1

1Biomedical Research Institute, Joint Institute for Regenerative Medicine, Kyungpook National University Hospital, Daegu, South Korea, 2Dept. of Orthopedic Surgery, Kyungpook National University Hospital, Daegu, South Korea

Title: A 3D Patterned Polymeric Bone Mimicking Scaffold as an Effective Bone Substitute

Authors: Yu Ri Hong1, Chang-Wug Oh 2, Jeong Ok Lim 1*

Affiliations: 1 Biomedical Research Institute, Joint Institute for Regenerative Medicine, Kyungpook National University Hospital, Kyungpook National University School of Medicine, Republic of Korea 2 Dept. of Orthopedic Surgery, Kyungpook National University Hospital, Kyungpook National University School of Medicine, Republic of Korea (* Correspondence)

Category: Design and Application of Biomaterials

Introduction: Despite the extensive development of bone substitutes, much remains to investigate for application to clinical settings. In order for promotion of bone regeneration, various bone growth factors, particularly bone morphogenic protein-2 (BMP-2), are required for effective treatment of significant bone loss. However, the currently available bone substitutes cannot sustain prolonged BMP-2 release and are inconvenient to use. In this study, we developed a bone mimicking, easy handling and highly functional bone substitute by sequential conjugation of BMP to a three-dimensional (3D) biodegradable synthetic polymer scaffold in a block shape using novel molecular adhesive materials that will enable reduction of operation time and prolongation of BMP release.

Subjects and Methods: Using a bioprinter, 3D Poly (L-lactide) (PLLA) scaffold was printed and BMP-2 conjugated with alginate-catechol and collagen. For dose dependent study, different concentrations of BMP-2 were tested and evaluated for bone regeneration in vitro and in vivo using a mouse calvarial model. ELISA was used to measure BMP-2 release kinetics, and histological analysis and micro-CT image analysis were performed to evaluate new bone formation.

Results: Hierarchically patterned PLLA scaffold exhibited a pore size of 400 micrometer and grid thickness of 187–230 micrometer. Release kinetics of BMP-2 demonstrated an initial burst, followed by a sustained release for 14 days. Released BMP-2 maintained osteoinductivity in vitro and in vivo. Micro-computed tomography and histological findings demonstrate that the PLLA scaffold conjugated with 2 microgram/ml of BMP-2 induced optimal bone regeneration in a mouse calvarial model.

Conclusion: The bone mimic 3D-printed polymeric scaffold conjugated with BMP-2 enhanced bone regeneration, demonstrating its potential as a novel bone substitute.


Acknowledgements: The Korean Fund for Regenerative Medicine funded by the Ministry of Science and ICT and by the Ministry of Health and Welfare (21C0705L1-11) supported this research.

Figure: Micro-CT analysis of volume of the newly regenerated bone at each time point. Data are expressed as mean ± SD (n = 4). Significant difference: *p>0.05; **p >0.01; ***p> 0.001. Sham, sham control; PLLA, PLLA only; PLLA+Collagen, collagen-coated PLLA; BMP-2 L, PLLA containing 1 µg BMP-2; BMP-2 H, PLLA containing 2 µg rhBMP-2
A Novel Ultra-Thin Deposition of Graphene Oxide on the Plastic Culture Substrate and Its Biomedical Application Potential

Prof. Jeung Soo Huh¹, Jae-Bum Park¹, Danbi Park¹, Professor Jeong Ok Lim²

¹ School of Convergence & Fusion System Engineering, Institute of Global Climate Change and Energy, Kyungpook National University, San Kyuk-dong, South Korea, ²Biomedical Research Institute, Joint Institute for Regenerative Medicine, Kyungpook National University Hospital, Kyungpook National University School of Medicine, Dongdeok-ro, South Korea, ³ Kyungpook National University, Daegu, South Korea, ⁴ Kyungpook National University, Daegu, South Korea

Title: A Novel Ultra-Thin Deposition of Graphene Oxide on the Plastic Culture Substrate and Its Biomedical Application Potential

Authors: Jae-bum Park 1, Danbi Park 1, Jeong Ok Lim 2, Jeung Soo Huh 1*

Affiliations: 1 School of Convergence & Fusion System Engineering, Institute of Global Climate Change and Energy, Kyungpook National University, San Kyuk-dong 386, Daegu 41566, Republic of Korea 2 Biomedical Research Institute, Joint Institute for Regenerative Medicine, Kyungpook National University Hospital, Kyungpook National University School of Medicine, Dongdeok-ro 135, Jung-gu, Daegu 41944, Republic of Korea (* Correspondence)

Category: Design and Application of Biomaterials

Introduction: For cell engineering in regenerative medicine, it is very important to produce high-quality cells in large quantities in a short time period. Recently, many studies have shown big potential of graphene oxide as a biocompatible substance to enhance cell growth. We investigated if graphene oxide-coated culture plate can promote production efficiency of stem cells.

Subjects and Methods: Three types of graphene oxide were used for this study. They are highly concentrated graphene oxide solution, single-layer graphene oxide solution, and ultra-highly concentrated single-layer graphene oxide solution with different single-layer ratios, and coated on cell culture plates using a spray coating method. Physiochemical and biological properties of graphene oxide-coated surface were analyzed by atomic force microscope (AFM), scanning electron microscope (SEM), cell counting kit, a live/dead assay kit, and confocal imaging.

Results: Graphene oxide was evenly coated on cell culture plates with a roughness of 6.4 * 38.2 nm, as measured by SEM and AFM. Young’s Modulus value was up to 115.1 GPa, confirming that graphene oxide was strongly glued to the surface. The ex vivo stem cell expansion efficiency was enhanced as bone marrow-derived stem cell doubling time on the graphene oxide decreased compared to the control (no graphene oxide coating), from 64 to 58 h, and the growth rate increased up to 145%. We also observed faster attachment and higher affinity of stem cells to the graphene oxide compared to control by confocal microscope.

Discussion and Conclusion: This study demonstrated that graphene oxide dramatically enhanced the ex vivo expansion efficiency of stem cells. Spray coating enabled an ultra-thin coating of graphene oxide on cell culture plates. The results supported that utilization of graphene oxide on culture plates can be a promising mean for mass production of stem cells for commercial applications.

Acknowledgement: This work was supported by the Central Research Center, Corestem Inc.& Energy Convergence Graduate Program.

Figure. Surface image and roughness parameters of a GO deposited substrate (left) & force curve analysis (right)
Polymethylpentene-based new culture plates can overcome the diffusion limitation of oxygen and enable aerobic respiration toward better physiological in vitro liver tissue models

Prof. Yasuyuki Sakai¹, Dr. Ya Gong¹, Dr. Tomoaki Matsugi², Dr. Jingjing Yang², Dr. Katsuhiro Esashika², Dr. Jun Takahashi², Prof. Hiroshi Arakawa³, Prof. Yukio Kato³, Dr. Masaki Nishikawa¹

¹University of Tokyo, Bunkyo-ku, Japan, ²Mitsui Chemicals, Inc., Minato-ku, Japan, ³Kanazawa University, Kanazawa, Japan

Introduction: Oxygenation of cells in vitro is a fundamental issue, because sufficient oxygen supply enables aerobic respiration for in vivo-like efficient ATP production, leading to better reorganization of tissue structure and physiological functionality/responses. However, in vitro oxygenation, particularly in static culture using conventional tissue culture-treated polystyrene (TCPS), has been a kind of a blind spot in the modern cell culture format but not completely been solved yet, although simple diffusion limitation by the layer of the culture medium was pointed out almost 50 years ago in liver cell culture [1]. We have been demonstrating the advantages of direct oxygenation of hepatocytes using highly gas-permeable polydimethylsiloxane (PDMS) as a cell adhering bottom membrane. Such a PDMS plate remarkably enhances the oxygen supply flux to hepatocytes, allowing their aerobic respiration even while keeping physiological low oxygen concentrations, leading to better expressions of hepatic functions and/or stable pseudo-3D multilayer [2]. However, a remaining problem is the sorption of drugs/chemicals in PDMS membranes, causing incorrect evaluation of the their metabolic responses, hindering its wide uses in cell culture.

Materials and Methods: We developed a new oxygen-permeable plates, polymethylpentene (PMP), “InnoCell®” and evaluated its efficacy in various liver cell cultures. When prepared in a thin membrane (50 μm), the oxygen permeation flux becomes almost equal to that of conventional polydimethylsiloxane (PDMS) membrane (300-500 μm). The cells can be oxygenated directly and satisfactorily, by avoiding the oxygen diffusion limitation. In addition, the surface modification of PMP, such as hydrophilization and ECM protein coating etc., is much easier than PDMS.

Results and Discussion: When rat hepatocytes were cultured on PMP plates, the oxygen consumption rate was as large as that of PDMS plates and it was fully met at a level enough for the cellular aerobic respiration. Versatile functions of the hepatocytes were almost the same as those cultured in PDMS plates, exceeding those in conventional TCPS plates. Specifically, cytochrome P450-dependent metabolite productions of drugs on the PMP plates was much larger than those in TCPS. This is both because the high metabolic functions of the hepatocytes and because reduced sorptions of both the substrates and their metabolites in the plates [3]. In addition, the PMP plates enabled simple organization of advanced liver tissues composed of both parenchymal and non-parenchymal liver cells stacked in a in vivo-mimicking hierarchical manner. This can be called as “open organoid” as opposed to 3D spherical organoid. Its open structure is better compatible with perfused MPS to address possible interactions with other organs.

Conclusions: The new oxygen-permeable PMP plate, “InnoCell®”, has a high potential in hepatocyte-based drug/chemical screenings. Furthermore, simple realization of cellular aerobic respiration in vitro is an important scientific advantage of the plates. Therefore, it is highly expected to become the new cell culture format instead of conventional TCPS plates toward better physiological culture of various organ-derived cells.

References
Role of podoplanin for cellular senescence-related changes in mesenchymal stem cells

**Dr. Ha Yeong Kim**, 1, Professor **Han Su Kim**

1Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, Ewha Womans University, Yangcheon-gu, South Korea

Title: Role of podoplanin for cellular senescence-related changes in mesenchymal stem cells

Authors: Ha Yeong Kim1 and Han Su Kim1, *

Affiliations: 1 Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, Ewha Womans University, 1071 Anyangcheon-ro, Yangcheon-gu, Seoul 07985, Republic of Korea (*Correspondence)

Category: Stem Cells and Cell-Based Therapies

Background: Although mesenchymal stem cells (MSCs), which are multipotent cells with the ability to differentiate into various cell types, have shown potential as important resources for developing regenerative medicine, they have limitations in maintaining their quality during in vitro expansion resulting in cellular senescence. Here, we investigated promising cellular senescence-related markers to conserve cellular stemness and minimize senescence.

Methods: Tonsil-derived MSCs (TMSCs) were obtained from patients under 10 years old undergoing tonsillectomy and isolated through an enzymatic procedure. TMSCs were cultured for over 15 passages to measure doubling time. To determine whether the cells were senescent, we conducted several analyses, including measurements of cell size, telomere length, the activity of senescence-associated β-galactosidase (SA-β-gal), MSC-specific characteristics, and flow cytometry.

Results: TMSCs passed over 15 passages (later passaged TMSCs) significantly increased doubling time, cell width, and expression of SA-β-gal. On the other hand, late-passaged TMSCs showed lower expression of telomere length, pluripotent markers, and multilineage differentiation capacities such as adipogenesis, chondrogenesis, and osteogenesis. Interestingly, the expression of podoplanin (PDPN), a transmembrane glycoprotein involved in various cellular functions, was significantly reduced in a passage-dependent manner. PDPN-negative cells identified by fluorescence-activated cell sorting (PDPN−) had significantly lower multi-lineage differentiation potential and senescence-associated preliminary characteristics compared to PDPN-positive cells (PDPN+). Absence of PDPN by knockdown on early passaged TMSCs also negatively regulated proliferation rate, cell cycle progression, and migration ability.

Discussion and Conclusion: Altogether, these findings suggest the importance of noting the potential roles of PDPN in cellular senescence-related changes in MSCs. PDPN can be used as a promising biomarker for modulating stemness or senescence and ensuring high-quality MSC-based therapeutic applications in regenerative medicine.

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An Adhesive Drug-loaded Photo-crosslinked Hydrogel To Alleviate Ulcerative Colitis By Endoscopic Delivery Strategy

Dr. Wen WU1, Dr. Ting CHEN2
1School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China, 2Department of Colorectal Surgery, Fudan University Shanghai Cancer Center, Shanghai, China

Introduction: Ulcerative colitis (UC) is a chronic inflammatory bowel disease, which has evolved into a global burden because of its high and substantial increasing incidence. Some nonspecific systematic drug administration (oral or intravenous) nowadays, such as use of 5-aminosalicylic acid (5-ASA) and glucocorticoids, however, caused undesired efficacy and multiple side effects. Local therapies can reduce systematic exposure but are difficult to retain or have limited retrograde distance. Thus, we conducted a drug-loaded hydrogel endoscopic treatment platform to alleviate UC.

Subjects and Methods: To address above issue, firstly, we designed and synthesized an adhesive drug-loaded biodegradable photo-crosslinking hydrogel and characterized its mechanical strength, tissue adhesion, retention time, drug release profile, stability, and biodegradation. Then, we compared the therapeutic effect of drug-loaded hydrogel in a rat model of DSS-induced chronic colitis with conventional systematic drug administration.

Results: The designed hydrogel possesses moderate swelling profile, ultrafast gelation (which can fast gel in situ within 4 s under 395-nm LED light irradiation, 50 mW/cm²), tunable mechanical strength, biodegradation and long-term tissue adhesion properties. Simultaneously, the drug-loaded hydrogel realized sustained release of 5-ASA and budesonide respectively, achieving spatiotemporal specificity for lesion site. Compared with systematic drug administration, the novel drug-loaded hydrogel showed excellent therapeutic effect (i.e., superior pharmacokinetics, longer retention and higher drug concentration of inflamed sites) by local sustained drug release, and protection of inflamed wound by long-term adhesive hydrogel barrier, thus boosting colitis repair, and alleviating fibrosis in chronic colitis.

Discussion and Conclusion: Our study presents a strategy for wet adhesive, sustained therapy and precision treatment of ulcerative colitis, which will pave the way for smart all-in-one drug-loaded hydrogel with minimally invasive medical interventions.
Phenotypic Maturation of hiPSC-derived Notochordal Cells for Intervertebral Disc Regeneration

Ms. Julie WARIN, Dr. Nicolas VEDRENNE, Mr. Nathan LAGNEAU, Ms. Garance SAINT-PE, Ms. Claire CHEDEvILLE, Dr. Jerome GUICHEUX, Dr. Vianney DELPLACE, Dr. Anne CAMUS
1INSERM UMR 1229, RMeS, Nantes University, Nantes, FRANCE, 2INSERM UMR 1248, Université de Limoges, Limoges, FRANCE

Phenotypic Maturation of hiPSC-derived Notochordal Cells for Intervertebral Disc Regeneration
Julie Warin, Nicolas Vedrenne, Nathan Lagneau, Garance Saint-Pé, Claire Chedeville, Jérôme Guicheux, Vianney Delplace, Anne Camus
1Nantes Université, Oniris, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-4400 Nantes, FR, 2 Current address, Inserm, Université de Limoges, UMR 1248
Pharmacologie et transplantation P&T, Limoges, FR,

Low back pain is a worldwide health problem. Its onset is often associated with the degeneration of the nucleus pulposus (NP), the central part of the intervertebral disc (IVD). The founder cells of the NP originate from the embryonic structure called the notochord. After birth, the matured notochordal cells (NC) have been shown to exert rejuvenating effects upon degenerated IVD cells [1]. Human induced pluripotent stem cells (iPSC) differentiation toward notochordal-like cells (NLC) has been reported for cell-based therapeutic interventions to mediate regeneration. However, these NLC harbor a transcriptomic signature of immature embryonic NC [2]. Phenotypic maturation should result in functional NLC producing specialized matrix that exert anabolic effects on degenerated disc cells. Therefore, there is a need to create an optimized microenvironment suitable to sustain NLC survival and to support their maturation toward a secretory phenotype.

In this context, we investigated the effect of different 3D culture models on NLC maturation and differentiation. Based on previously described protocol, iPSC were first differentiated into mesendoderm progenitors (MEPC) by WNT activation, and then, NLC differentiation was triggered by NOTO mRNA transfection [2]. The MEPC transfected or not with NOTO were either aggregated into micromass or encapsulated in HA-based hydrogels grafted with specific peptides. These 3D constructs were kept in culture in NLCs differentiation medium or in nucleopulpogenic medium to study their maturation toward NP-like cell phenotype [3]. Cells’ identity, proliferation and differentiation were assessed at different time points by qPCR, immunohistochemistry, or immunofluorescence for confocal imaging.

Our results demonstrated that the tested 3D culture promotes the commitment of the MEPC toward notochordal lineage. Indeed, MEPC aggregation significantly induced NOTO endogenous expression even without transfection compared to monolayer culture. Interestingly, when MEPC were encapsulated in hydrogels, we observed clusters of cells with notochordal identity and cytoplasmic vacuoles suggesting that cells began their maturation. Following 28 days differentiation in nucleopulpogenic medium, aggregated MEPC micromass showed a cell-dense part with positive staining for known NP markers including CA12, SOX9, CD166 and CK8 associated with a matrix-rich part composed of glycosaminoglycan proteins such as aggrecan.

This work demonstrates that 3D microenvironment is favourable for phenotypic maturation of the NLC. Quantitative phenotypic evaluations are ongoing to determine cells responses to different viscoelasticity properties and investigate cell-matrix interactions.

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Gelatin methacryloyl nanofibers with crimping structures control morphological attributes of cultured cells

Mr. Chien Wei Wu, Ms. Tsu-Yin Huang, Mr. Liang-Jie Huang, Mr. Yi-Wei Kuo, Ms. Chia-Pei Wang, Professor Po-Ling Kuo

1Graduate Institute of Biomedical Electronics and Bioinformatics, College of Electrical Engineering and Computer Science, National Taiwan University, Taipei, Taiwan, 2Department of Physical Medicine and Rehabilitation, National Taiwan University Hospital, Taipei, Taiwan

Introduction: The collagenous fibrous network is an omnipresent architecture within the human body and bears a distinctive wavy or "crimped" manifestation across a myriad of tissues. Though the crimping structure is found to be more prominent within nascent tissues and intact tendons, a comprehensive understanding of the intricate mechanisms and interactions between the crimped architecture and the residing cells still eludes our grasp. Establishing in vitro platforms simulating the natural network with tunable crimping fiber angles is paramount as a pivotal conduit for delving into the aforementioned enigmas. However, while accomplishments have been attained in fashioning nanoscale, controllable crimped architectures via diverse synthetic polymers, the fabrication of such feats using proteinaceous materials sourced from the extracellular matrix presents a formidable conundrum. Here we reported the synthesis of a cell-laden in vitro proteinaceous nanofiber platform with controllable fiber crimping to simulate natural tissue, and the relationship between the fiber crimping and the morphology of the adhered cells.

Subjects and Methods: Matrices composed of gelatin methacryloyl (GelMA) nanofibers aligned in parallel were synthesized using electrospinning. Fiber crimping was effectuated through the immersion of the matrices in ethanol solvents of varying compositions, and the stability of the synthesized fibers within aqueous milieus was bolstered by employing photopolymerization. NIH-3T3 fibroblasts and bone marrow-derived mesenchymal stem cells (BMSCs) were cultured on the fiber matrix. Cell viability and morphology were examined through viability essays and scanning electron micrographs (SEM).

Results: GelMA electro-spun fiber matrices with modifiable crimping degrees were successfully synthesized. The diameter of the fiber was around 600 nm, and the deviation of the orientation of the fiber concerning the principal axis of alignment of the fiber was 28°. Fiber crimping was seen after the ethanol treatment. The crimping degree (quotient of the fiber contour length and the straight distance between two ends of the fiber) was about 0.88±0.11 without cells and 0.90±0.07 with cell culture, which was similar to that of natural tendon tissues (0.895-0.93) reported by Diamant et al. [1]. The viability of NIH-3T3 fibroblasts cultured on the crimped nanofibers for 72 hours was 96.43±3.65 %, corroborating the biocompatibility of synthesized fibers. Scanning electron micrographs divulged the pronounced impact of the crimped configuration upon the morphological attributes of cultured cells. When cultured on crimped fibers, adhered cells appeared less elongated. The cell aspect ratio (the ratio of the cellular major and minor axes, 1.00 for a round shape) was 3.44±2.22 and 2.01±0.80 for the cells cultured on the straight and crimped fibers, respectively.

Discussion and Conclusions: The reported fabrication of proteinaceous GelMA fibrous networks, characterized by adjustable crimped structures, engenders a faithful recapitulation of the form exhibited by natural tissue. The crimping of the nanofibers may result from the hydrogen bonding and entropic bending provided by the partially hydrated, polar environment. When compared with the matrices made of straight fiber, those composed of crimped fibers formed three-dimensional structures with larger pores. Note that the crimped structure was expected to be less stiff in the small strain regime. These factors may promote cells cultured on the crimped structure to form a rounded, rather than flattened and elongated shape. In conclusion, this engineered matrix, capable of controlling morphological attributes of residual cells, exhibits considerable potential as a versatile
substrate for in vitro platforms dedicated to unraveling the intricate dynamics underpinning cell-matrix interactions.

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Acknowledgment: This work was supported by the Taiwan National Science and Technology Council with grant number 112-2425-H-028-002 and 112-2221-E-002-232.
Fabrication of Anisotropic Polyvinyl Alcohol Hydrogels for Tissue Engineering

Mrs. Xiao Chaonan, Kai Li, Qiuning Lin, Linyong Zhu

1School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China

Title: Fabrication of Anisotropic Polyvinyl Alcohol Hydrogels for Tissue Engineering

Authors: Chaonan Xiao, Kai Li, Qiuning Lin*, Linyong Zhu*

Affiliations: 1School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China (*Correspondence)

Category: Tissue Engineering and Regeneration

Background: Hydrogels show strong promise in many fields due to their highly hydrated nature. However, the mechanical properties of hydrogels, such as flexibility, strength, and fatigue resistance, are still insufficient, which hinders their further design and application.

Methods: Based on previous research, here, we report anisotropic polyvinyl alcohol hydrogels which achieve extremely high tensile strength in the complete swelling state. It is mainly achieved through two aspects, one is to form a highly ordered arrangement structure, so as to share the stress received, and the other is to establish biomimetic soft-hard material interface microstructures, the soft end is used to improve the ductility of the material, and the hard end is used to dissipate the stress transmitted by the soft end.

Results: By constructing a hydrogel network combining soft and hard ends and neatly arranging with a certain orientation structure, it is successful to improve the mechanical properties of hydrogels. And obtain hydrogel materials with both certain strength and good toughness. Through this method, the hydrogel obtained a tensile strength of up to 35 MPa and a tensile strain of 200% in the complete swelling state while maintaining structural stability that rivals or even beats commonly used elastomer (non-hydrated) materials.

Discussion and Conclusion: Considering its high tensile strength, our hydrogel will likely foster advances in tissue engineering. It can be transformed into bioengineering soft materials with unique mechanical properties, such as sensors, actuators, bioremediation materials, artificial heart valves, blood vessel replacement, etc.

Acknowledgment: This work was supported by Shanghai Jiao Tong University.
Effects of chronic microgravity culture on contractile function and gene expression responses in 3D tissue-engineered muscle.

Ryo Nakajima¹, Ms. Yuka NAKANAGA¹, Sho Yokayama², Takashi Ishida³, Masahiko Morita⁴, Naoki Sako⁴, Toshiya Fujisato⁵, Tomohiro Nakamura⁶
¹Graduate School of Engineering, Osaka Institute of Technology, Osaka, Japan, ²Department School of Mechanical Engineering, Osaka Institute of Technology, Osaka, Japan, ³Kyowa Hakko Bio Co., Ltd., Tokyo, Japan, ⁴Kirin Central Research Institute, Kirin Holdings Company, Limited, Kanagawa, Japan, ⁵Department School of Biomedical Engineering, Osaka Institute of Technology, Osaka, Japan, ⁶Division of Human Sciences, Osaka Institute of Technology, Osaka, Japan
Title: Effects of chronic microgravity culture on contractile function and gene expression responses in 3D tissue-engineered muscle.

Authors: Yuka Nakanaga¹, Ryo Nakajima¹, Sho Yokayama², Takashi Ishida³, Masahiko Morita⁴, Naoki Sako⁴, Toshiya Fujisato⁵, Tomohiro Nakamura⁶

Affiliations: ¹Graduate School of Engineering, Osaka Institute of Technology, Osaka, Japan. ²Department School of Mechanical Engineering, Osaka Institute of Technology, Osaka, Japan. ³Kyowa Hakko Bio Co., Ltd., Tokyo, Japan. ⁴Kirin Central Research Institute, Kirin Holdings Company, Limited, Kanagawa, Japan. ⁵Department School of Biomedical Engineering, Osaka Institute of Technology, Osaka, Japan. ⁶Division of Human Sciences, Osaka Institute of Technology, Osaka, Japan.

Category: Tissue engineering and Regeneration

Background: Although previous in vitro culture models have already examined the effects of acute microgravity on skeletal muscle cells, there is no in vitro model that can examine the effects of chronic microgravity on skeletal muscle cells over time, including its contractile function. Therefore, this study examined the chronic microgravity effects on skeletal muscle cells using 3D tissue-engineered muscle, which can evaluate contractile function over time.

Methods: Mouse myoblast cell line C2C12 was embedded in collagen gel and 3D differentiated for 2 weeks; after 2 weeks of differentiation, cultured muscle was placed in custom-made containers and cultured in microgravity for 2 weeks in clinostat (Zeromo, CL-1000, Kitagawa Corporation, Hiroshima, Japan). Contractile force was assessed every week. Gene expression in muscle differentiation and ubiquitin proteasome was evaluated by ΔΔCt method after 2 weeks of microgravity culture.

Results: Compared to the control group, the microgravity culture group showed an increased variation in contractile force at 1 week and a decreasing trend after 2 weeks. In addition, some cultured muscles showed zero contractile force. Compared to the control group, Myod1, a gene involved in muscle differentiation, was significantly decreased in the microgravity culture group, and myogenin (Myog) showed a decreasing trend. MyHC-embryonic (Myh3) was also significantly decreased. Murf1 and Mafbx, genes of the ubiquitin-proteasome system, showed no change.

Discussion and Conclusion: These results indicate that chronic microgravity incubation on 3D tissue-engineered muscle suppressed muscle differentiation and showed a reducing trend in contractile force. Therefore, it is expected to be a novel in vitro model to verify the chronic microgravity effect on muscle cells.

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Discovery of novel vasculogenic multipotent stem cells from the adipose tissues and their characterization in vitro.

Mr. Do Young Kim1, Ms. Gabee PARK2, Ms. Bora LEE3, Mr. Sung-Vin YIM2, Ms. Youngsook SON2,3, Ms. Hyun Sook HONG1,4

1Department of Biomedical Science and Technology, Graduate School, Kyung Hee University, Seoul, Republic of Korea, 2Research and Development Department, Elphis Cell Therapeutics Inc, Yong In, Republic of Korea, 3Graduate School of Biotechnology and Department of Genetic Engineering, Kyung Hee University, Yong in, Republic of Korea, 4East-West Medical Research Institute, Kyung Hee University, Seoul, Republic of Korea

Affiliations: 1Department of Biomedical Science and Technology, Graduate School, Kyung Hee University, Seoul, Republic of Korea, 2Research and Development Department, Elphis Cell Therapeutics Inc, Yong In, Republic of Korea, 3Graduate School of Biotechnology and Department of Genetic Engineering, Kyung Hee University, Yong In, Republic of Korea, 4East-West Medical Research Institute, Kyung Hee University, Seoul, Republic of Korea.

Introduction: Stem cell therapies have a role in regenerative medicine for critical disease. Of diverse stem cell sources, adipose tissue has been recently used for stem cell transplantation and, notably, ex vivo cultured adipose derived stem cells (ADSCs) are reported to be able to reduce vascular damage by providing angiocrine factors. This study aims to discover novel vasculogenic stem cell as well as ADSC resides in adipose tissue.

Subjects and Methods: Adipose tissues were harvested from human donor and stromal vascular fraction (SVF) was isolated by enzyme digestion. SVF was cultured in endothelial cell growth medium (vascular multipotent stem cell; VMSC) and mesenchymal stem cell medium (ADSC), respectively. Cellular characteristics of each cell were analyzed by flow cytometry, western blot, immunofluorescence, tube formation assay, and enzyme-linked immunosorbent assay (ELISA) post ex vivo culture.

Results: VMSCs were positive to CD29, 44, 73, 90, and 105 and, negative to CD34 and CD45. Similar to ADSC, VMSC had the ability to differentiate into adipocyte, chondrocyte, and osteoblast. However, VMSCs could be distinguished from AD-MSC by high expression of CD141/c-MET, and low expression of transgelin. Analysis for angiocrine factor showed vascular endothelial growth factor (VEGF) was mainly produced in ADSC, and hepatocyte growth factor (HGF), from AD-VMSCs. Moreover, VMSC could make a tubular structure in Matrigel, whereas ADSC aggregated easily to show the pellet structure. Importantly, the combination of VMSC and ADSC could make the perfect vasculature structure, where VMSC made endothelium-like structure and ADSC encircled tubular structure with VMSC. From this, VMSC and ADSC are inferred to have distinct features as endothelium and smooth muscle cells of the vasculature.

Discussion and Conclusion: This study has confirmed the presence of vasculogenic stem cells as well as mesenchymal stem cells in adipose tissue and, the sufficient number of VMSC/ADSC was acquired by ex vivo culture. Thus, transplantation of VMSC and ADSC is anticipated to contribute to vascular regeneration in ischemic disease.

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Generating Steerable Stem-cell Based Microrobots for Neurogenesis

Dr. Ki Chan¹, Prof. Chengfei Zhang
¹The University of Hong Kong, , Hong Kong SAR

Introduction: The study aims to develop novel and advanced bio-functional microrobot which can be actuated magnetically and transport therapeutic cells such as stem cells from apical papillae (SCAPs) to a precise location, direct stem cells toward a pre-destined cell lineage, and support cells’ survivability, proliferation, and differentiation. By applying 3D bioprinting technology, nanoparticle internalization, and droplet microfluidic platform, the steerable and biodegradable SCAP-based microrobots are engineered and compared for targeted delivery of SCAPs within a controlled microenvironment for increasing survival and neurodifferentiation of SCAPs.

Subjects and Methods: The proposed study has applied bioengineering techniques to encapsulate SCAPs within 3D gelatin methacrylate (GelMA) hydrogel loaded with calcium dioxide and brain-derived neurotrophic factor for fabricating SCAP-mediated microrobots called SCAPBOT. Three types of SCAPBOT have been fabricated using (i) 3D bioprinting, (ii) nanoparticle internalization and (iii) droplet microfluidics. Proliferative ability of SCAPs is examined by cell counting kit-8; Oxygen release capacity is detected by oxygen sensor meter; Migration capability of SCAPs is observed by time-lapse microscope; Neural differentiation is detected by immunohistochemistry.

Results: Each type of SCAPBOT was actuated towards the target location by rotating magnetic fields generated by computer-controlled electromagnetic coils. SCAPs can be transported and actuated remotely by the 3D bio-printed and microfluidic droplet fabricated microrobots for directing SCAPs to undergo neural differentiation and demonstrate positive neuroprotective potential.

Discussion and Conclusion: By comparison, SCAPBOT fabricated by droplet microfluidics can be navigated efficiently by magnetic field and retain its differentiation capacities to the neurogenic lineage. As a result of the SCAPBOT providing a suitable microenvironment with oxygen and neural growth factor, the SCAPs can undergo neurogenesis. Thus, it can be viewed as a potential alternative bioengineering approach for the development of the SCAP-based microrobot with embedded functionalities in the field of targeted stem cell therapy.

Acknowledgement: This work was supported by Oral Health Research and Innovation Fund of the HKU Faculty of Dentistry.
Evaluation of novel cellular arrhythmia models in Brugada syndrome patient-specific iPSC-derived cardiomyocytes

Ms. Na Kyeong Park, Mrs. Soon-Jung Park, Mr. Yun-Gwi Park, Mr. Sung-Hwan Moon, Mr. Hun-jun Park, Mr. Sung Jun Kim, Mr. Seong Woo Choi

Seoul National University, South Korea

Introduction: Life-threatening ventricular arrhythmias, Brugada syndrome (BrS) leading to sudden cardiac death are a major cause of morbidity and mortality. BrS is often the result of genetic defects in special membrane proteins such as voltage-gated Na+ channel α-subunit 5 (NaV1.5). Understanding the pathophysiology of inherited arrhythmias can be challenging because of the complexity of the disorder and the lack of appropriate cellular and in vivo models. Recent advances in human-induced pluripotent stem cell technology have provided remarkable progress in comprehending the underlying mechanisms of ion channel disorders or channelopathies by modeling these complex arrhythmia syndromes in vitro in a dish. Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are now promising options for either patient-specific or patient-independent applications.

Subjects and methods: In this study, we characterized the hiPSC-CMs obtained in BrS patient. The arrhythmia properties of hiPSC-CMs were evaluated with electrophysiological methods using patch clamp. The arrhythmic mechanisms were also evaluated by a heterologous expression system to analyze the loss-of-function of NaV1.5 in HEK293 cells transfected with the wild-type (WT) and mutant.

Results: The iPSC was evaluated from a 38-year-old male patient with BrS, and two mutations were detected on SCN5A (guanine 1,153 to adenine and guanine 1,511 to cytosine), encoding a mutated protein with A385T and R504T. In vitro differentiation and characterization of CMs were performed for the preparation and analysis of the cell line-derived CMs. Spontaneous beating was observed around differentiation day 8 and the cells were purified in a culture lacking glucose. After 15 days of cultivation, the cells exhibited characteristics CM specific marker proteins, such as cTnT and alpha-sarcomere actinin. The differentiation efficiency of the purified CMs was measured using flow cytometry and calculated as the percentage of cTnT-positive cells. The Electrophysiological properties of BrS-CMs were recorded using the patch-clamp. The BrS-CMs showed notable differences in action potential phenotype compared to normal. In the heterologous expressing system, the density of INaV was not altered in A385T/R504T NaV1.5 alone. Furthermore, a rightward shift of the voltage-dependent inactivation and faster recovery from inactivation was observed, suggesting a gain-of-function state contrary to the BrS phenotype. Intriguingly, the co-expression of SCN1B with A385T/R504T revealed a significant reduction of INaV and slower recovery from inactivation, consistent with the BrS phenotype. The SCN1B-dependent reduction of INaV was observed in R504T while not in A385T single mutation. In contrast, the slower recovery from inactivation with SCN1B was observed in A385T while not in R504T.

Discussion and conclusion: The expression of SCN1B is indispensable for the electrophysiological phenotype of BrS with the novel double mutations; A385T and R504T contributed to the slower recovery from inactivation and reduced current density of NaV1.5, respectively.
Next-generation cell and material-based therapies for cartilage regeneration

Ms. Jacqueline Leon Ribas¹, Dr. Saki RAHEEM¹, Prof. Silvia María DÍAZ PRADO²,³,⁴,⁵, Dr. Pooja BASNETT¹, Dr. Bradley ELLIOT¹, Dr. John MURPHY¹, Prof. Brendon NOBLE¹

¹School of Life Science, University of Westminster, London, United Kingdom, ²Grupo de investigación en Terapia Celular y Medicina Regenerativa, Departamento de Fisioterapia, Medicina y Ciencias Biomédicas, CICA - Centro Interdisciplinar de Química y Biología, Facultad de Ciencias de la Salud, Universidad de Coruña (UDC, A Coruña, Spain, ³Grupo de Investigación en Terapia Celular e Medicina Regenerativa, Instituto de Investigación Biomédica de A Coruña (INIBIC), Complejo Hospitalario Universitario A Coruña (CHUAC), Universidade da Coruña (UDC), A Coruña, Spain, ⁴Centro de Investigación Biomédica en Red (CIBER) de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, Spain, ⁵Fundación Profesor Novoa Santos, A Coruña, Spain

Category: SYIS (Student and Young Investigator Section) + Design and Application of Biomaterials.

Background: Whilst the use of Mesenchymal Stem Cells (MSCs) for cell therapies has attracted great attention for cartilage regeneration in osteoarthritis, it presents two major complications. Namely, the need for cell support in an inflammatory environment, and the need for large quantities of cells for effective transplantation. Biomaterials may present the possibility to overcome both these limitations. Most studies to date supplement these biomaterials with active molecules to aid MSC adhesion, proliferation, and differentiation. There is little research on biomaterials that are not only capable of promoting cell adhesion without pre-treatment, but also can considerably increase cell proliferation for the large-scale production of MSCs for cell therapies. Additionally, previous studies have proposed that material stiffness can influence the differentiation pathways of MSCs. Therefore, being able to identify a polymer that will induce chondrogenic differentiation without the addition of costly biomolecules may lead to a significant positive impact on cell therapies. This study assessed the biocompatibility, the potential to support cell adhesion, proliferation, and to induce differentiation of two immortalised Mesenchymal Stem Cell lines.

Methods: The biocompatibility of the polymers after 72 hours and 21 days was assessed by means of cell counts and MTT assay. Cell adhesion was evaluated by DAPI-nuclear staining of the cells adhered to the polymer surfaces. Proliferation, after 72 hours, was assessed by DAPI-nuclear staining and EdU staining. The polymers tested in this study were a Polyhydroxyalkanoate, polyisoprene trans, a 50:50 blend of both, and two chemically modified versions of Hydroxypropyl methylcellulose (HPMC 1 and HPMC 2) to make them water-insoluble.

Results: The findings confirmed that none of the polymers tested induced indirect cytotoxicity after 72-hour exposure to MSCs. However, after a 21-day exposure, selected polymers exhibited limited cytotoxicity. Moreover, all polymers were able to support cell adhesion. However, HPMC 1 and HPMC 2 presented a cell adhesion and proliferation comparable to that of tissue culture plastic (TCP) without the need for pre-treatment with additional active compounds. Additionally, this study identified that one of our chemically modified polymers may potentially lead to an increased cell proliferation (with an 843% cell counts relative increase, compared to a 154% cell counts increase of the TCP) and, therefore, may be useful for bulking up MSC populations for tissue engineering. The potential for chondrogenic differentiation based on the mechanical properties of polymers is currently under investigation.

Discussion and conclusion: These findings suggest that some of the biomaterials tested may have the potential for their use in cell therapies for the large-scale production of Mesenchymal Stem Cells.
A wearable far-infrared emitting device for effectively ameliorating postoperative peritoneal adhesion

Prof. Zheng Wang

Introduction
Postoperative adhesion is the most common complications of abdominal and pelvic surgery, leading to numerous issues, including small bowel obstruction, female infertility, chronic abdominal pain, and second injuries caused by reoperation. Pharmacological treatments and physical barriers are two main approaches to ameliorate postoperative adhesion; however, the therapeutic effect is limited by rapid drug clearance and incomplete coverage of the injured tissues. It remains challenging to develop a safe, effective, and convenient approach to prevent postoperative adhesion. Following abdominal surgery, a series of cascading events, including peritoneal wound healing, inflammation reaction, coagulation activation and tissue infection, were involved in the formation of postoperative adhesion. Interestingly, far infrared (FIR) radiation with a wavelength between 3~1000 μm possessed a variety of fascinating biological effects on human bodies, such as the promoting of wound healing and inhibition of inflammatory response, indicating its potential therapeutic effect on peritoneal adhesion. However, to the best of our knowledge, there was no researcher to study the therapeutic effect of FIR on peritoneal adhesion.

Subjects and Methods
The fabrication process of a wearable far-infrared emitting device, which was mainly composed of the graphene composite film (F-GCF), included three steps: (1) mixing the graphene with the conductive graphite, strength intensifiers, and adhesives; (2) spreading the mixture on a polyethylene terephthalate membrane and rolling it into a 0.1-cm thick film using a three-high mill; (3) installing copper electrodes on both ends of the film after dying at 120 °C. The characteristics of this F-GCF were examined using the scanning electron microscopy, Fourier transform infrared spectrometer, and the FLIR One Pro system in vitro, respectively. The effect of far infrared (FIR) radiation on preventing adhesion formation was assessed in rat and porcine models. We then evaluated the degrees of adhesion between the abdominal wall and cecum 7 days after operation. Adhesion tissue samples and blood sera were obtained for further histological and molecular biological analyses. Moreover, a lipopolysaccharide (LPS) activating macrophage rat model was established to test the effect of FIR on macrophage polarization. The underlying molecular mechanism was further investigated by mRNA and ELISA assays.

Results
Herein, we developed a wearable far-infrared emitting device based on F-GCF for effectively ameliorating postoperative peritoneal adhesion (Scheme 1). This F-GCF was stitched into the inner layer of a rectangular cotton cloth, making it easy to wear, and the device’s signal could be captured by a smart telephone through near-field communication (NFC) technology. We firstly characterized the physicochemical properties of F-GCF. SEM images showed that F-GCF possessed a layer structure with uniformly dispersed graphene. The electric-thermal radiation conversion efficiency of F-GCF was 83%. The emission wavelength of F-GCF ranged from 5 to 15 μm with a peak of 8.5 μm. The FIR generated by F-GCF could penetrate tissue to a depth of nearly 5 cm. Moreover, F-GCF could be easily bent to 45° without obvious breakage, suggesting its satisfactory flexible property. Then, this device was used to treat peritoneal-adhesion rat models. Only 2 of 6 FIR-treated rats (33%) formed thin and easily separable adhesion with an average area of 12.5 mm², and the proportion was significantly lower than that of the untreated rats and chitosan-treated rats. Consistently, the similar results were also found in peritoneal-adhesion porcine models, as FIR-treated pigs had the least adhesion formation. We further examined the long-term (10 weeks) adhesion-preventing effect of FIR irradiation. The results showed that the peritoneum of rats in FIR-treated group was healed with a clearly visible mesothelial monolayer, and only two rats left a few adhesive strips. For histological
analysis, the infiltrated cell numbers of macrophages and neutrophils in the adhesion tissues of FIR-treated rats were reduced by 5.3~8 times and 7~10 times, respectively, compared with that of untreated rats. Within the FIR-treated sites, the numbers of CD206+ cells (M2 phenotypic marker) were significantly higher than that of CD86+ cells (M1 phenotypic marker), indicating that FIR drives macrophage polarization towards M2 phenotype. In addition, FIR treatment upregulated the ratios of tissue plasminogen activator (t-PA) to plasminogen activator inhibitor (PAI-1) at mRNA levels in these LPS-treated primary peritoneal macrophages. This observation was consistent with the increased serum protein ratios of t-PA/ PAI-1 in FIR-treated rats. Taken together, these results demonstrate that FIR generated by F-GCF can reduce inflammation during adhesion formation through modulating macrophage polarization.

Discussion and Conclusion
In this study, we developed a wearable far-infrared emitting device and found that FIR generated by this device could effectively prevent the formation of adhesion post-operation. The prevention rate of adhesion formation could reach almost 100% in the animal models receiving long-term treatments. Analyses of these adhesion tissues confirmed the obvious alleviation of inflammatory responses. Unlike current antiadhesion materials serving solely as physical barriers, FIR could reduce inflammation during adhesion formation through early decreasing pro-inflammatory (M1-polarized) macrophages while augmenting immunomodulatory (M2-polarized) macrophages. Collectively, this wearable device could produce the FIR, safely and effectively ameliorating peritoneal adhesion. This work presents a novel and promising approach for the treatment of postsurgical adhesion.
A Mn-MOF modified sericin nerve conduit capable of remodeling Schwann cells for effectively repairing long-gap peripheral nerve injury

**Prof. Lin Wang**

1Wuhan Union Hospital, Wuhan, China

**Introduction**

Peripheral nerve injuries (PNI) were mainly caused by fall injuries, accidents, tumor extirpation, sports injuries or other types of traumas, resulting in the loss of sensory and motor functions in the innervation zone and some may cause severe disabilities. Following long-distance peripheral nerve injury (PNI), distal Schwann cells (SCs) underwent morphological atrophy and functional degradation coupled with extensive downregulation of regeneration-associated genes (RAGs) due to lack of contact with newly regenerated axons for an extended period of time, resulting in an imbalance of nerve regeneration microenvironment. To remodel the damaged microenvironment, two strategies, including the replenishment of fresh SC sources and the release of neurotrophic factors, were commonly utilized for treating long-distance PNI. However, they were limited by the implanted SC’s low survival rate, and the burst release and bioactivity loss of the loaded factors. Therefore, it is highly desired to effectively reactivate the regeneration-promoting function of these atrophic SCs for long-distance PNI repair.

**Subjects and Methods**

To fabricate the Mn-MOF and characterize their physicochemical properties, the typical hydrothermal method was utilized to synthesize these frameworks, and these XRD, SEM and cytocompatibility assays were performed; to investigate whether Mn-MOF had an impact on SCs, the mRNA sequencing was performed on these Mn-MOF treated RSC96 cells, and qRT-PCR was conducted to verify the mRNA expression results; to test the cellular phenotype of Mn-MOF-treated SCs, the RSC96-PC12 co-culture cell model was established, and the neurites of PC12 cells were measured; to prepare the Mn-MOF/sericin composite nerve conduit and characterize its properties, the chemically crosslinked gelling and mold casting technique were jointly utilized to fabricate the composite conduit, and then the SEM, element mapping, swelling dynamics, FTIR spectra and mechanical tests were performed; to investigate whether the in vivo implantation of Mn-MOF/sericin nerve conduit could promote the microstructural regeneration of transected sciatic nerves, the Tuj1 and S100 staining assays were performed to visualize the regenerated axons and SCs; to investigate whether this composite conduit improved the electrical conduction performance and functional recovery outcomes of regenerated nerves, the electrophysiological test and footprint assay were performed, respectively.

**Results**

The Mn-MOF was successfully synthesized evidenced by the XRD results. The SEM images showed that this framework had spherical shape with the diameter of approximately 50 nm. After 24-hour treatment, no significant cytotoxicity of Mn-MOF to SCs was observed. The mRNA sequencing results showed that the Mn-MOF treatment strongly changed the mRNA expression profiles of SCs, and it’s worth noting that the regeneration-associated genes (RAGs) were upregulated and its gene signature was closely correlated with Mn-MOF treatment in SCs. In the RSC96-PC12 co-culture cell model, Mn-MOF treatment enhanced the neurite lengths of PC12 cells, indicating that this framework enables SCs to promote the neurite outgrowth. Then, we incorporated the Mn-MOF with silk sericin, aiming to develop a bioactive hybrid nerve conduit capable of reactivating the regeneration-promoting function of SCs for long-distance PNI repair. Highly porous microstructures, uniformly dispersed frameworks within this conduit, stable swelling behaviors, and appropriate mechanical strength were well proven in this Mn-MOF/sericin nerve conduit by these results of SEM, element mapping, swelling dynamics, and mechanical tests. After being implanted in vivo to treat the 13-mm sciatic nerve critical defect for 14 weeks, this hybrid conduits significantly enhanced the regeneration of axon and SCs. The results of electrophysiological tests demonstrated that Mn-MOF/sericin nerve
conduit treatment amplified the nerve conduction velocity (NCV) and compound muscle action potential (CMAP) of PNI rat model. In addition, the improved functional recovery after Mn-MOF/sericin nerve conduit implantation was evidenced by the result of footprint analysis.

Discussion and Conclusion
In this study, Mn-MOF, as a bioactive additive, was incorporated into the silk sericin scaffold to fabricate a composite nerve conduit, which termed Mn-MOF/SC (Scheme 1). Mn-MOF/SC effectively upregulated the mRNA levels of vital RAGs, and promoted the axon extension of cocultured PC12 cells through paracrine pathway, as evidenced by the results of qRT-PCR and RSC96/PC12 coculture assay. After being implanted to treat a rat model with 13-mm sciatic nerve critical defect, MOF-SS enhanced microstructural regeneration of axons and myelin sheath, improved the restoration of nerve conductivity, and significantly promoted the functional and behavioral recovery. This study suggests that the utilization of a Mn-MOF modified sericin nerve conduit is capable of remodeling Schwann cells for the effective repair of a long-gap peripheral nerve injury in a rat model.

References
Bio-Monitoring of a Reconstructed Human Skin Equivalent Using the In Vitro ‘Living’ Diffusion System

Ms. Chaewon Woo1, Mrs. Jina Byun1, Ms. Heeseon Yoo1, Ms. Chaewon Woo1
1Soongsil University, , South Korea

Introduction: For testing drug delivery through the skin, the Franz diffusion cell is widely used as an in vitro method to assess skin absorption. However, this diffusion system requires improvements due to its limited surface area, inaccurate membrane thickness, and inability to simulate in vivo conditions. In this study, we quantitatively evaluated skin permeability and the bio-effects of model molecules using an in vitro ‘Living’ diffusion system, incorporating living reconstructed human full-thickness skin equivalents.

Subjects and Methods: In the full-thickness skin equivalent, we introduced the 3D Agarose-Well to enhance structural integrity and enable increased flux during the culturing of the skin equivalent. This is because the medium can be supplied not only to the lower surface of the culture vessel but also to the side surface. Additionally, we measured the resistance value of the current passing through the skin equivalent using the TEER (Trans Epithelial Electrical Resistance) to verify the structural integrity of the skin barrier before conducting the skin permeation test. Subsequently, we utilized skin penetrating peptides (SPPs), which are widely studied as an effective non-invasive strategy for macromolecule delivery into the skin. Since we introduced a ‘Living’ diffusion system with living full-thickness skin equivalent containing the dermis and epidermis, we can evaluate the bio-effects of the skin such as procollagen synthesized in dermal layer of the skin. We evaluated procollagen synthesized using adenosine, which is actively used in anti-wrinkle cosmetics. Moreover, using COMSOL Multiphysics, skin permeation tests were simulated under the condition of using or not using the ‘Living’ diffusion system with 3D Agarose-Well.

Results: When comparing the skin equivalent in the trans-well insert to the one in the 3D Agarose-Well under the same culture process and time point, it is observed that in the trans-well insert, detachment from the culture vessel occurs due to contraction of the dermal layer caused by the interaction between collagen and fibroblasts present in the dermal layer. On the other hands, in the 3D Agarose-Well, a structurally improvement prevents detachment from the culture vessel due to the interaction between the agarose gel and the collagen in the dermal layer. To calculate the kinetic rate constant (k), the cumulative % of penetrated model molecules was fitted with the Ritger-Peppas equation. The kinetic rate constant of SPP-BP-1, SPP-BP-2, and non-skin penetrating peptide was quantified 32.9, 9.1, and 0.12, respectively. Furthermore, it was confirmed that almost all of adenosine was permeated after 6 hours of skin permeation, and procollagen synthesis was promoted after adenosine passes through the epidermis and acts on the dermis. Statistical significance was evident when comparing the control group (without adenosine permeation) and the group permeated with adenosine at 6 hours, as well as when comparing the differences at 24 hours. These differences were verified using T-test (*p<0.05).

Discussion and Conclusion: When using the trans-well insert, an existing culture vessel, the analysis of molecule permeation is only possible after it has passed through the membrane of the insert and reached the lowermost part of the dermis layer. In contrast, the use of the ‘Living’ diffusion system with the 3D-Agarose Well, which allows for lateral diffusion, enables a more accurate analysis of the permeability of model molecules. These features of system were validated through simulation results obtained using COMSOL Multiphysics. Furthermore, as this ‘Living’ diffusion system can utilize a living full-thickness skin equivalent, it offers the ability to simultaneously evaluate permeability and assess the bio-effects occurring in the skin. Overall, this diffusion system would be actively used in drug delivery, in vitro testing and tissue engineering.

Reference

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Enhanced 3D Alignment of C2C12 Myotubes by Magnetic Field

Mr. Suk Hyeon Hong¹, Mr. Sungwoo Cho¹, Mr. NamHyun Kim¹, Heeseon Yoo¹
¹Soongsil University, South Korea

Background: Contractile skeletal muscle cells which are applied in bio-robots, organoids, and organ-on-a-chips have been actively explored in various biomedical and tissue engineering studies. Alignment of myotubes is essential for better contractile properties and force generation.

Methods: In this work, we present a new strategy to enhance the 3D alignment of C2C12 skeletal muscle cells via magnetism of biomaterials-functionalized iron oxide nanoparticles (fIONPs), which are suitable for cell adhesion. To design 3D alignment of C2C12 skeletal muscle cells, we fabricate 3D muscle band that were similar to that of native muscle extracellular matrix (ECM). The formation of muscle band was controlled by unidirectional magnetic field varying strength, period, and interval. The degree of alignment was quantified using directionally plugin from ImageJ and calculated based on the order parameter.

Results: The fIONPs were sufficiently strong to spontaneously demagnetize a previously saturated assembly of particles and the phenomenon was referred as superparamagnetism, indicating that the fIONPs were facilitated to the 2D and 3D structural alignment of myotubes with controlling magnetic field. This study showed that the C2C12 myotubes were 63.27% more aligned at the direction of 600 gauss external magnetic field by magnetization of fIONPs. The alignment of myotubes in the direction of magnetic force was enhanced on higher magnetic force. Furthermore, this method is also applicable to 3D structural alignment using muscle band. Also, magnetic nanoparticles were effective as T2 contrast agents in magnetic resonance imaging. Thus, it is possible for T2-weighted MRI diagnosis of fIONPs encapsulated muscle band.

Discussion and Conclusion: We demonstrated that the 3D C2C12 myotubes are aligned unidirectionally using fIONPs at the magnetic field. Unlike previous studies, there is no necessity for patterning on the bottom surface for C2C12 alignment. Overall, this new strategy to align C2C12 myotubes using biomaterials and fIONPs would be actively utilized for cell culture system and bio-robots in the tissue engineering.

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Catalase-Mimetic Artificial Biocatalysts with Ru Catalytic Centers for ROS Elimination and Periodontitis Treatment

Dr. Yimin Sun¹
¹Sichuan University, Chengdu, China

Introduction: The accumulation of oxidative damage, especially DNA damage, often leads to stem cell senescence and impedes the repair of tissue defects. Current artificial enzymes have high metal content, low atomic utilization, and poor catalytic performance, making it difficult to achieve good therapeutic effects. Thus, it is of vital importance to improve the ROS scavenging efficiency and biocompatibility.

Subjects and Methods: In this work, we simulate the structure of natural catalase, and constructs ruthenium coordination artificial enzymes (Ru-NC) with three atomic scale catalytic centers (single atom, cluster, and nanoparticles), and studies the relationship between material structure and therapeutic effects.

Results: Artificial enzymes with ruthenium single atom (Ru@SA-NC), ruthenium cluster (Ru@NC-NC), and ruthenium nanoparticles (Ru@NP-NC) have been successfully synthesized and characterized. Among them, Ru@NC-NC possesses the highest reaction efficiency of a single catalytic center because of the three-dimensional coordination structure. All of them are safer at the working concentration. Furthermore, Ru@NC-NC can reduce the level of reactive oxygen species in periodontal tissue and reduce the absorption of alveolar bone.

Discussion and Conclusion: We demonstrate that Ru@NC-NC can reduce intracellular ROS levels, protects the survival, adhesion, and osteogenic differentiation abilities of hMSCs, and reduces tissue ROS levels in the rat periodontitis model. The current study opens up a promising avenue to develop high-performance ROS-scavenging materials in stem cell-based therapeutics and many other ROS-related diseases.

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