台灣再生醫學學會

2013年度台灣再生醫學學會學術研討會暨會員大會
2013 Annual Meeting of Formosa Association of Regenerative Medicine

論文摘要 & 大會手冊

2013 年 03 月 02 日

台大醫學院 **103** 講堂

台北市仁愛路一段一號

目　錄
Consent

2013 年度台灣再生醫學學會學術研討會暨會員大會
2013 Annual Meeting of Formosa Association of Regenerative Medicine
Scientific Program

Oral

Time	Topic	Speakers & Authors	Institute	Moderator
08:00	**Registration 報 到**			
08:30	**開幕致詞**			
08:40~09:40 Oral Presentation Competition (I)				
S-1 08:40~08:50	Multicellular Spheroids of Human Placenta-Derived Multipotent Cells (PDMC) Grown in Solid Freeform (SFF) Scaffolds for Cartilage Tissue Engineering	黃國祥[1] 曾清秀[2] 戴念國[3] 顏伶汝[4] 徐善慧[1,5*]	國立台灣大學高分子研究所[1] 國立中央大學機械工程研究所[2] 光田綜合醫院骨科[3] 國家衛生研究院細胞與系統醫學研究所[4] 國立台灣大學發育生物學與再生醫學研究中心[5]	方旭偉 張至宏
S-2 08:50~09:00	The Effect of Substrate-Derived MSC Spheroids on Peripheral Nerve Regeneration	曾庭箴 謝百善 徐善慧*	國立臺灣大學高分子科學與工程學研究所	
S-3 09:00~09:10	Replicative Senescence Alters Both Differentiation & Proliferation Potential Through Oxidative Stress in Human Adipose Tissue-Derived Stem Cells (hADSCs)	彭凱彥[1,2,3,4] 陳佩旻[1,2] 李柟葶[1,2] 黃令宜[1,2] 顏伶汝[4] 嚴孟祿[1,2]	台大醫學院一般醫學科[1] 台大醫院婦產部[2] 中央大學生命科學研究所[3] 國家衛生研究院[4]	
S-4 09:10~09:20	Scalable Production of Controllable Dermal Papilla Spheroids on PVA Surfaces and the Effects of Spheroid Size on Hair Follicle Regeneration	黃怡晴[1] 詹智傑[1,2] 林韋廷[1] 邱顯鎰[2,3] 蔡仁雨[4] 蔡宗樺[5] 詹融怡[2,6] 林頌然[1,2,7]	國立臺灣大學醫學工程研究所[1] 國立臺灣大學醫學院皮膚科暨附設醫院皮膚部[2] 臺大醫院新竹分院皮膚部[3] 台北醫學院皮膚部[4] 亞東紀念醫院皮膚部[5] 國泰醫院皮膚部[6] 國立台灣大學發育生物與再生醫學研究中心[7]	
S-5 09:20~09:30	The Bio-effect of Polyelectrolyte Multilayer Films on Neural Stem/Progenitor Cells	吳俐潔 李亦淇	長庚大學生化與生醫工程研究所	
S-6 09:30~09:40	Regeneration and Anti-inflammation Potentials were Synergistically Enhanced by the Combination of Platelet-rich Plasma and Hyaluronic Acid for Osteoarthritis Therapy	許維哲[1] 陳韋弘[1,2] 鄧文炳[1,2*]	臺北醫學大學生醫材料暨組織工程研究所[1] 臺北醫學大學 幹細胞研究中心[2]	

Time	Topic	Speakers & Authors	Institute	Moderator
09:40~10:00 Coffee Break				
10:00~12:00 Invited Lectures				
I-1 10:00~10:30	Integration Platform for Isolation of Adipose-Derived Stem Cells and Applications	方旭偉	國立台北科技大學化學工程與生物科技系	何美泠 嚴孟祿
I-2 10:30~11:00	Adipose-Derived Stem Cells Exhibit Anti-Oxidative and Anti-Apoptotic Properties to Rescue Ischemic Acute Kidney Injury in Rats	馬　旭	台北榮民總醫院外科部整形外科	
I-3 11:00~11:30	Biomaterial-Derived MSC Spheroids for Tissue Regeneration	徐善慧	台灣大學高分子科學與工程研究所	
I-4 11:30~12:00	The Development of an Automatic Processing Device for Adipose Tissue-Derived Stem Cell	王玲美	台灣生技整合育成中心	
12:00 會 員 大 會				
12:00-13:30 Lunch Break				
13:30~15:00 Invited Lectures				
I-5 13:30~14:00	The Bio-mechanisms of Immunomodulatory and Regenerative Effects in Adipose-derived Stem Cells	郭耀仁	高雄長庚紀念醫院整形外科	黃玲惠 徐善慧
I-6 14:00~14:30	Application of Adipose Derived Stem Cells on Articular Cartilage Regeneration	何美泠	高雄醫學大學骨科學研究中心	
I-7 14:30~15:00	Long Term Results of Engineering Predefined Shape Adipose Tissue from Uncultured Human Adipose Stromal Vascular Fraction on Collagen Matrix In Vivo	林幸道	高雄醫學大學附設中和紀念醫院整形外科	
15:00-15:20 Coffee Break				

Time	Topic	Speakers & Authors	Institute	Moderator
15:20~16:20 Oral Presentation Competition (Ⅱ)				
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S-8 15:30~15:40	Selection and Enrichment of Liver Cancer Stem Cells by Using Polyelectrolyte Multilayer Films	張仁輔 李亦淇*	長庚大學生化與生醫工程研究所	
S-9 15:40~15:50	Bioengineered Dermal Fibroblast Spheroids for Corneal Stromal Tissue Reconstruction	張仁杰 薛聖潔* 賴瑞陽*	長庚大學生化與生醫工程研究所	
S-10 15:50~16:00	Electrospun Gelatin Nanofibrous Membranes for Corneal Keratocyte Scaffolds: Effect of Multi-Stage Carbodiimide Cross-Linking	張育誠 賴瑞陽*	長庚大學生化與生醫工程研究所	
S-11 16:00~16:10	Rhoa Downstream Signaling and Regulated Cytoskeleton Tension was Involved In Simvastatin Induced Osteogenesis in Mouse Bone Marrow Mesenchymal Stem Cells	戴宜均[1,2,3] 王耀賢[3] 張瑞根[2,3,4] 何美泠[1,2,3]	高雄醫學大學醫學研究所[1] 高雄醫學大學生理學科[2] 高雄醫學大學骨科學研究中心[3] 高雄大同醫院[4]	
S-12 16:10~16:20	Preclinical Safety Evaluation of Adipose-Derived Stem Cells Engineered by Baculovirus	李奎璋 胡育誠	國立清華大學化學工程系	
頒 獎 閉 幕				

Poster

No. 編號	Topic 題目	Speaker 講者	Institute 所屬單位
P-1	A Novel Approach for Cartilage Tissue Engineering: Using Cartilage Fragments, Synovial Membranes and Mesenchymal Stem Cells	陳佳君[1] 胡家僖[1] 方旭偉[1*] 張至宏[2*]	國立台北科技大學化學工程與生物科技系[1] 亞東紀念醫院骨科[2]
P-2	PLGA-Alendronate Enhances Bone Repair in Femoral Bone Defect in Rats	王耀賢[1] 路卡曼[1] 王志光[1,3] 蔡紫琳[1] 劉瓊月[1] 張瑞根[1,4,5] 何美泠[1,2]	高雄醫學大學骨科學研究中心[1] 高雄醫學大學生理學科[2] 高雄醫學大學醫用化學系[3] 高雄醫學大學附設醫院骨科部[4] 高雄市大同醫院骨科部[5]
P-3	Porcine Mesenchymal Stem Cells of hTERT-gene Transfection combined with *in-situ* gelatin hydrogel for Cartilage Regeneration	許元銘[1,2] 蕭莉馨[1] 陳瑾霈[1] 張至宏[1,2*]	亞東紀念醫院骨科[1] 元智大學生技所[2]
P-4	Simvastatin-stimulated Osteogenic Effects is Related to Estrogen Receptor in Osteogenic Lineage Cells	莊淑君[1] 張力夫[1,2] 何美泠[1,2] 張瑞根[1,3,4]	高雄醫學大學骨科學研究中心[1] 高雄醫學大學生理學科[2] 高雄醫學大學附設中和紀念醫院骨科[3] 高雄市立大同醫院骨科[4]
P-5	Cycloxygenase-2 Contributes to Osteogenic-differentiation via $p27^{kip1}$ in Murine Bone Marrow Mesenchymal Stem Cells	莊淑君[1] 戴宜均[1,2] 張瑞根[1,3,4] 何美泠[1,2]	高雄醫學大學骨科學研究中心[1] 高雄醫學大學生理學科[2] 高雄醫學大學附設中和紀念醫院骨科[3] 高雄市立大同醫院骨科[4]
P-6	Combined Use of Hyaluronic Acid and Non-Proteinous Drugs as Bio-Factors to Enhance Chondrogenesis of Stem Cells for Tissue Engineering	張智翔[1] 吳順成[1] 張瑞根[2,3] 陳崇桓[2] 何美泠[1,4]	高雄醫學大學骨研中心[1] 高雄醫學大學附設中和紀念醫院骨科[2] 高雄市立大同醫院骨科[3] 高雄醫學大學醫學系生理學科[4]

Invited Lectures

10:00-10:30

I-1

脂肪幹細胞分離整合平台與應用
Integration Platform for Isolation of Adipose-Derived Stem Cells and Applications

方旭偉教授 Prof. Hsu-Wei Fang
國立台北科技大學 化工系

Adipose-derived stem cells (ADSCs) are population of multipotent progenitor cells that reside in adipose tissue. Due to adipose-derived stem cells are easily accessible by liposuction、 abundant、safety, their became a promising adult stem cell source use in clinical cell therapy、autologous fat implantation for cosmetic or plastic surgery.

As the adipose-derived stem cells needs increased, the effective cell isolation technology for clinical use required. However, current isolation methods are tedious、time-consuming that increased the cost of medical and affected the quality of patient and not suitable for clinical application.

One of the next challenges is required to develop the effective cell isolation technology and machine to efficient production of adipose-derived stem cells. The key strategy in our technology is to establish a semiautomatic platform with effective standard procedure for isolate of adipose-derived stem cells.

First we modified the influence factors in methods and optimized the isolation procedure that can obtain high purity and activity of ADSCs. Based on the critical parameter of instrument setting provide from experimental processing, we then constructed multi-function operating platform that contained isolation procedures necessary equipment. Furthermore, programmed standard procedure imported into multi-function operating platform to drive machine.

The semiautomatic integration platform not only provided a complete environment for isolation of ADSCs, but also offered a platform for preparation other cellular products such as growth factor, platelet-rich plasma (PRP) and autologous filling biomaterials like condensed fat tissue. In future, our integration platform could be applied in regenerative medicine and tissue engineering.

10:30-11:00

I-2

Adipose-Derived Stem Cells Exhibit Anti-Oxidative and Anti-Apoptotic Properties to Rescue Ischemic Acute Kidney Injury in Rats

Hsu Ma[1,4], Yu-Chung Shih[1*], Pei-Ying Lee[5*], Henrich Cheng[3,4,7], Chi-Han Tsai[1], and Der-Cherng Tarng[2,4,5,6]
Division of Plastic Surgery, Department of Surgery[1],
Division of Nephrology, Department of Medicine[2], and Department of Neurosurgery, Neurological Institute, Taipei Veterans General Hospital, Taipei[3]; School of Medicine[4],Department and Institute of Physiology[5] and nstitute of Clinical Medicinel[6], Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan[7]

Background: Acute kidney injury (AKI) is a major challenge in critical medicine with high rates of in-hospital morbidity and mortality. However, stem cell therapy has emerged as an evolving technology which could have a substantial impact on AKI outcomes in the critical care environment. Therefore, we investigated the therapeutic effects of adipose-derived stem cells (ASCs) in ischemic AKI of rats.
Methods: In our study, an ischemia-reperfusion (I/R)-induced AKI rat model was used. The effects of rescuing AKI were assessed in regards to different ASC numbers, and various routes of administration as compared with sham-operated and PBS-treated groups.
Results: Both intra-renal arterial (ia) and intravenous (iv) administration of ASCs reduced the elevation of BUN and creatinine, and also decreased the tubular injury score 48 hours after I/R-induced AKI in a dose-dependent manner, as compared to the PBS-treated group. In our study, it was determined that the optimal cell number was 5×10^5. Furthermore, ASC transplantation exhibited the anti-oxidative and anti-inflammatory properties to reduce apoptosis and promote proliferation of renal tubular cells. Transplantation of ASCs in any number $> 1x10^7$ generated reduced renoprotective effects in the ia route, and caused lethal pulmonary embolism when utilizing the iv route.
Conclusion: An optimal number of ASCs administered via the ia or the iv route effectively rescued I/R-induced AKI in rats. Anti-oxidative and anti-apoptotic properties of ASCs to reduce tubular cell injury also merit recognition and further study.

Keywords: adipose-derived stem cells, acute kidney injury, critical care

11:00-11:30

I-3

Biomaterial-derived MSC Spheroids for Tissue Regeneration

Shan-hui Hsu
Professor, Institute of Polymer Science and Engineering
National Taiwan University

Our previous study has demonstrated that generation of three-dimensional cellular spheroids from adipose-derived adult stem cells or other mesenchymal stem cells (MSCs) grown on substrates can help maintain the self-renewal of MSCs and promote their chondrogenic differentiation potential as well as cardiomyogenic differentiation potential in vitro. The increased endocytosis of nanoparticles during spheroid formation can also enhance the efficiency of MSC labeling. Here, the effects of the substrate-derived MSC spheroids on tissue regeneration in vivo will be briefly presented. MSC single cells or substrate-derived MSC spheroids will be combined with different biomaterial scaffolds for studying their in vivo regeneration effects. Preliminary data suggested that substrate-derived MSC spheroids may promote tissue regeneration through various effects in different tissues.

11:30-12:00

I-4

The Development of an Automatic Processing Device for Adipose Tissue-derived Stem Cell

Ching-Huai Ko[1], Dian-Kun Li[2], Ling-Mei Wang[3], Yio-Wha Shau[1]
Biomedical Technology and Device Research Labs, Industrial Technology Research Institute[1]
Buddhist Tzu Chi General Hospital[2]
Supra Integration and Incubation Center[3]

Tissue engineering and regenerative medicine for treating failing organs is an emerging field. Human adipose tissue contains multipotent stem cells and represents an alternative stem cell source to bone marrow-derived mesenchymal stem cells (MSCs). Adipose tissue-derived stem cells (ADSCs) are abundant, easier to obtain, more convenient to harvest, and are with much lower surgical risk. The clinical trials of ADSCs have launched recently in cardiovascular diseases in Europe and liver regeneration in Japan. It is believed that ADSCs will be one of the leading fields in regenerative medicine in the near future. Industrial Technology Research Institute (ITRI) has established an R&D program in ADSCs since 2007. The initial study was an exploratory project of the differentiation regulation and clinical application of ADSC in muscular injury disease. In the recent years, we have been able to isolate sufficient amount of stem cells for tissue repair within a reasonable time frame without the need of cell expansion in a good tissue practice lab. To fulfill the unmet need of applying ADSCs in a “point of care” scenario, we have initiated the development of an automatic and computerized control processing machine for the fresh ADSCs. The automatic processing machine will contain a closed disposable cell processing system which can prevent the exposure to environmental pathogens. We have now refined our manual isolation protocol and have broken down into stepwise processes which can be easily translated into an engineering design. We believe that the isolation device will become a very useful tool in stem cell therapy.

13:30-14:00

I-5

The Bio-mechanisms of Immunomodulatory and Regenerative Effects in Adipose-derived Stem Cells

郭耀仁 Yur-Ren Kuo, MD, PhD, FACS
Chairman and Professor, Department of Surgery, Kaohsiung Chang Gung Memorial Hospital, and Chang Gung University College of Medicine, Taiwan

Mesenchymal stem cells (MSCs) are attractive cell sources in regenerative medicine. MSCs are multi-potential non-hematopoietic progenitor cells of differentiating into various lineages of the mesenchyme. MSCs derived from the stroma of bone marrow, adipose tissue, umbilical cord Wharton's jelly, etc. Studies indicated that bone marrow-derived MSCs (BM-MSCs) and adipose-derived stem cells (ASCs) are equally capable of differentiating into various lineages of the mesenchyme and rendering immunosuppressive and immune modulatory effect *in vitro* and *in vivo.* In this presentation, we will present our work and focus on the bio-mechanisms of immune modulation and regeneration effect of ASCs.

According the regenerative effect of ASCs, studies indicated ASCs enhanced wound healing might through promotion of tissue repair included increasing multiple growth factors, cytokines, collagens, and promotion of keratinocyte migration. Our animal study revealed treatment with multiple rounds of ASCs significantly enhances the process of wound healing in diabetes. This improvement is characterized by associated with augmented local epithelialization, increased neovascularization and cellular recruitment. Analytical results revealed that the wound area was significantly reduced in the ASCs-treated rats as compared to the control ($P < 0.001$). Histological findings revealed significant increase in neo-vessels in ASC group—as compared to the control. In immunohistochemical stain, significant increases in EGF, VEGF, eNOS, and vWF expressions were noted in the treatment group as compared to the control. The GFP^+-ASCs were injected in wound edge and the results revealed significant expression in the subdermal layer of wound margin by fluorescence microscope.

In contrast, the immune regulatory effects of ASCs have been studied. Our study indicated ASCs exert immunomodulatory effects including suppressing T cell proliferation and increasing the expressions of CD4+/CD25+/Foxp3+ regulatory T cells *in vitro.* Matured dendritic cells (DC) are strong antigen presenting cells (APC) and highly potent immuno-stimulatory cells to induce allograft rejection. Our study revealed ASC suppressed the effect of DC induction of $CD4^+$ T-cell proliferation. DC co-cultured with ASCs showed significant inhibition of DC differentiation by the decreasing MFI of MHC class II and CD86 expressions. Recent study revealed that ASCs combined with short-term immunosuppressant have a positive effect in prolongation of rodent and miniature swine hind-limb allograft survival *in vivo*. ASC suppressed DC maturation is correlated with indoleamine 2, 3-dioxygenase (IDO) expression in mature DC *in vitro.*

Results obtained from this review provided important information of ASCs regarding the bio-mechanisms of immune-modulation and regeneration medicine.

14:00-14:30

I-6

應用脂肪幹細胞於關節軟骨再生醫學
Application of Adipose Derived Stem Cells on Articular Cartilage Regeneration

何美泠 Mei-Ling Ho, Ph.D.
Professor/Chair, Department of Physiology; Director, Orthopaedic Research Center, Kaohsiung Medical University, Kaohsiung, Taiwan.

Abstract:
Damaged articular cartilage has a limited capacity for self-repair. Cell-based tissue engineering provides a novel strategy to treat cartilage defects. Due to the poor proliferation capability and de-differentiation of chondrocytes caused by in vitro expansion, mesenchymal stem cells (MSCs) have attracted interest for possible clinical use. Among the MSCs, adipose tissue derived stem cells (ADSCs) are comparably better cell source for tissue engineering, because of their high proliferative capacity, ease to isolate from donor site, and less aging-related proliferation and differentiation limitations. Chondro-induction for ADSC-based cartilage tissue engineering plays a crucial role in the tissue regeneration. Growth factors, ie, TGFb and IGF, are the well-known induction factors; however, these factors are high cost, easily denatured, and difficult to deliver the appropriate concentration at right timing in the repaired tissue. Recently, we used the niche factor of cartilage (hyaluranan, HA) as the induction factor instead of growth factors, and found that a 2D- or 3D-HA enriched microenvironment initiated and enhanced chondrogenesis in human ADSCs (Biomaterials 2010). Several HA-enriched biomaterials were further developed to prove the concept for HA-induced ADSCs chondrogenesis and cartilaginous matrix formation in joint cavity and in a chondral defect model of porcine ariticular cartilages. Our results showed that HA-enriched biomaterials enhanced cartilaginous matrix formation of ADSCs, suggesting HA, the non-protein niche factor, may be a potent induction factor for ADSC-based cartilage tissue engineering.

14:30-15:00

I-7

Long Term Results of Engineering Predefined Shape Adipose Tissue from Uncultured Human Adipose Stromal Vascular Fraction on Collagen Matrix in Vivo

林幸道 Lin* S D., Ph.D.
Division of Plastic Surgery, Department of Surgery, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan.

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Introduction：The purpose of the current animal study was to evaluate whether freshly isolated uncultured human SVF can be engineered to generate adipose tissue using 3D porous collagen matrix as the scaffold.
Materials and Methods：SVF was isolated from freshly lipectomized fat. Adipogenic potential of freshly isolated uncultured SVF was evaluated in vitro. Following in vitro evaluation, animal study was performed to demonstrated the long-term results of newly formed adipose tissue. After isolation, SVF were seeded onto the predefined shape of collagen matrix and implanted immediately into subcutaneous pocket in nude mouse. In the same mouse, the control constructs without SVF was implanted at the other side. The implanted constructs were retrieved 2, 4 and 6 months after implantation. Macroscopically, the changes of the shape of constructs and reaction of surrounding tissue were recorded. The generation of adipose tissue was studied by H&E and Oil Red O stains. Immunostain of antihuman and antimouse vimentin antibody were used to assay the origin of adipocytes within the constructs. The presence of neovascularization was studied by H&E stain and confocal immunostain of CD31 and CD34.
Results：Adipose tissues of different cell density were demonstrated in all experimental constructs. The majority of adipocytes in the constructs of 2 and 4 months samples were human origin. However, the majority of adipocytes in the constructs of 6 months sample were murine origin. Neovascularization presented in clusters of viable adipocytes. Freshly isolated SVF seeded onto collagen matrix could generate new predefined shape of adipose tissue and remained viable as long as 6 months.
Conclusion：The human SVF generated new adipose tissue in the implanted constructs and also promoted host murine adipocytes migration into the constructs. In the constructs of 6 months samples, the majority of viable adipocytes originated from host tissue.

Oral Presentations

08:40-08:50

S-1

人體胎盤幹細胞結合實體自由成型支架於軟骨組織工程之應用
Multicellular Spheroids of Human Placenta-Derived Multipotent Cells (PDMC) Grown in Solid Freeform (SFF) Scaffolds for Cartilage Tissue Engineering

黃國祥[1] 曾清秀[2] 戴念國[3] 顏伶汝[4] 徐善慧[1,5*]
國立台灣大學高分子研究所[1] 國立中央大學機械工程研究所[2] 光田綜合醫院骨科[3] 國家衛生研究院細胞與系統醫學研究所[4] 國立台灣大學發育生物學與再生醫學研究中心[5]

Introduction：Materials serving as tissue engineering scaffolds are usually designed to have a porous structure to accommodate the suspended cells. Three-dimensional (3D) cellular spheroids have recently emerged as a new platform for cell-based therapy because of their greater regeneration capacities. The traditional microporous polymer matrices may not be suitable for carrying these relatively large spheroids. This study reveals a novel approach to create a robust scaffold for delivery of 3D cellular spheroids.
Materials and Methods：PLGA precision scaffolds were fabricated by one of the solid freeform fabrication (SFF) techniques, liquid frozen deposition manufacturing (LFDM). MSC spheroids were generated from cell self-assembly on chitosan membranes. Cells (in the form of single cells or spheroids) were seeded to the precision scaffolds. After chondrogenesis induction for 4 weeks, the constructs were analyzed for gene expression (Sox9, Aggr, and Col II) or subjected for histological examinations or biochemical analyses (cell number/GAG contents) as described later.
Results：Solid freeform fabricated (SFF) scaffolds with macroporosity are made from poly(D,L-lactide-co-glycolide) (PLGA) to accommodate these spheroids. Human mesenchymal stem cell (MSC) spheroids derived on chitosan-hyaluronan (CS-HA) membranes can be loaded in SFF scaffolds efficiently and produce a significant amount of extracellular matrix (ECM) in vitro and in NOD/SCID mice. The combination also helps recruit host cells for promoting neotissue formation in vivo.
Discussion：We have successfully designed and created a delivery system for keeping MSC spheroids functional in long term for tissue engineering and cell therapy applications. The maintenance of spheroid state is highly associated with the functions in vitro and in vivo. The well-kept spheroids express more adhesion molecules, cytokines, and greater differentiation capacities in vitro.
Conclusions：It is concluded that SFF scaffolds can be designed as robust carriers for growing and delivering 3D cellular spheroids.

08:50-09:00

S-2

以生醫材料誘導形成之間葉幹細胞球體對周邊神經再生的影響
The effect of substrate-derived MSC spheroids on peripheral nerve regeneration

曾庭箴　謝百善　徐善慧*
國立臺灣大學高分子科學與工程學研究所

Introduction：Peripheral nerve injuries are often caused by trauma and may result in functional deficits. Current treatments for peripheral nerve repair include autografts, artificial nerve guides and transplantation of mesenchymal stem cells (MSCs). Three-dimensional MSC spheroids may enhance therapeutic potential of MSCs. Our previous study has also shown that nanoparticles (NPs) or genes can be delivered to substrate-derived MSC spheroids. However, the repairing capacities of these spheroids and the advantages of enhanced NP/gene delivery have not been verified in vivo. In this study, we investigated the effect of the substrate-derived MSC spheroids vs. single cells on the regeneration of transected rat sciatic nerve.

Materials and Methods：MSCs were labeled with Fe_3O_4 NPs or transfected with brain-derived neurotrophic factor (BDNF) gene by increased endocytosis during spheroid formation. Rat sciatic nerve was transected to create 10 mm gap, where a polylactide conduit was implanted to bridge the nerve. The Fe_3O_4 NPs-labeled MSC single cells or spheroids were injected in the conduit and tracked by magnetic resonance imaging (MRI) in vivo. The regeneration capacity of MSC single cells, spheroids, or BNDF-transfected spheroids was evaluated by histological analysis and electrophysiology after 30 days of implantation.

Results：Fe_3O_4 NPs-labeled MSC single cells or spheroids in the conduits were successfully tracked by MRI, particularly for MSC spheroids. Animals receiving the conduits and BDNF-transfected spheroids had the shortest nerve connection time in ~21 days. MSC spheroids were superior to single cells in regeneration of transected peripheral nerve. Fe_3O_4-labeled cells were observed in the regenerated axons. Animals receiving MSC spheroids showed better functional recovery than those receiving single cells, based on electrophysiology.

Discussion：Migration of Fe_3O_4 NPs-labeled MSCs toward the two nerve stumps was visualized by MRI. In MSC spheroids, the gene expression level of BDNF as well as chemokine receptor Cxcr4 was increased in vitro, which might account for the enhanced cell migration and nerve regeneration in animals receiving MSC spheroids.

Conclusions：Substrate-derived MSC spheroids upregulated Cxcr4 and BDNF genes in vitro and promoted peripheral nerve regeneration in vivo. The Fe_3O_4 NPs-labeled MSCs could be monitored by MRI.

09:00-09:10

S-3

人類脂肪幹細胞(hADSCs)繼代培養的老化會透過氧化壓力來改變其增生及分化 能力
Replicative Senescence Alters Both Differentiation & Proliferation Potential Through Oxidative Stress in Human Adipose Tissue-Derived Stem Cells (hADSCs)

彭凱彥 [1,2,3,4] 陳佩旻 [1,2] 李柟葶 [1,2] 黃令宜 [1,2] 顏伶汝 [4] 嚴孟祿 [1, 2]
台大醫學院一般醫學科 [1] 台大醫院婦產部 [2] 中央大學生命科學研究所 [3] 國家衛生研究院 [4]

Introduction：Human adipose tissue-derived stem cells (hADSCs) have emerged as a particularly attractive and abundunt source of mesenchymal stem cells (MSCs) which have now been isolated from number of organs and tissues. However, since clinical stem cell therapy requires high volumes of stem cells using *ex vivo* expansion, we studied the effects of replicative senescence on hADSCs.

Materials and Methods：We assessed the functional changes on hADSCs brought upon due to replicative senescence. Cell proliferation-associated studies, tri-lineage mesodermal differentiation assays and associated studies—including gene expression profiling—as well as senescence-related assays such as -galactosidase staining (-gal) and reactive oxygen species (ROS) detection were performed. We then eludicated the mechanisms involved in mediating these functional changes brought about by replicative senescence.

Results：We found that hADSCs undergo decreased cell proliferation and alteration of differentiation capacity with prolonged *ex vivo/in vitro* cell culture. Cell proliferation ceased around passages 7 to 10, with morphological changes seen and -gal(+). While p53 expression was unchanged with passaging, expression of the cyclin-dependent kinase inhibitor $p16^{INK4a}$ correlated with the number of passages. In terms of differentiation capacity, hADSCs readily differentiated into the 3 mesodermal lineages of osteogenesis, adipogenesis, and chondrogensis. With senescence, however, an increased propensity for adipogenesis was seen while osteogenesis was decreased. Replicative senescence also increased the amount of ROS within the hADSCs, and exogenous application of ROS in terms of H_2O_2 to non-senescent hADSCs induced a decrease the proliferation potential and differentiation bias towards adipogenesis. Mechanistically, the effects of ROS on proliferative decline of senescent hADSCs are mediated through $p16^{INK4a}$, which also affect while the alterations to differentiation capacity through altering the balance between Runx2 and PPAR , the master lineage transcription factors for osteogenesis and adipogenesis, respectively.

Discussion：Replicative senescence affects the cell proliferation and differentiation capacity of hADSCs, and these changes appear to involve ROS/oxidative stress. Our data shows the detrimental effect of prolonged *ex vivo/in vitro* manipulation of human

MSCs, which remains an obstacle towards therapeutic use of these versatile stem cells. Our findings implicate the important role of oxidative stress and shed light on reversing these detrimental effects on hADSC function.

Conclusions：The effects of senescence on hADSCs—decreased proliferation and differentiation capacity changes—are mediated in large part by oxidative stress. Further research in this area should bring insights to further the use of stem cells for clinical use.

09:10-09:20

S-4

利用聚乙烯醇調控真皮乳突微組織之大小-探討對毛囊再生的影響

Scalable production of controllable dermal papilla spheroids on PVA surfaces and the effects of spheroid size on hair follicle regeneration

黃怡晴[1] 詹智傑[1,2] 林韋廷[1] 邱顯錳[2,3] 蔡仁雨[4] 蔡宗樺[5] 詹融怡[2,6] 林頌然[1,2,7]
國立臺灣大學醫學工程研究所[1] 國立臺灣大學醫學院皮膚科暨附設醫院皮膚部[2]
臺大醫院新竹分院皮膚部[3] 台北醫學院皮膚部[4] 亞東紀念醫院皮膚部[5] 國泰醫院皮膚部[6]
國立台灣大學發育生物與再生醫學研究中心[7]

Introduction：Organ size and numbers are vital issues in bioengineering for hair follicle (HF) regeneration. Dermal papilla (DP) cells are able to induce HF neogenesis when transplanted as aggregates. However, how the preparation of DP aggregates affects HF inductivity and the size of regenerated HF is yet to be determined.

Materials and Methods：Biomaterials for surface coating: including poly(vinyl alcohol) (PVA), Poly (ethylene-co-vinyl alcohol) (ethylene: vinyl alcohol=27:63) (EVAL27), Poly (ethylene-co-vinyl alcohol) (ethylene: vinyl alcohol=44:56) (EVAL44), and poly ethylene (PE) are prepared for culture plate or PCR tube.

Results：Hydrophilic PVA is a non-adhesive substrate for human and rat DP cells. In the principle experiment, we aim to examine the size effect on the efficiency and efficacy of HF regeneration. Our recent results demonstrated that rapid consistent spheroidal DP microtissue formation on PVA-coated PCR tube arrays remaining intact structure and signature gene expression after injection. We also found that both human and rat DP spheroids are able to induce HF neogenesis and larger DP spheroids exhibit higher HF inductivity.

Discussion：Our strategy can't dissolve the problem of controllable regenerated HF by tissue engineering. However, from the cell seeding to spheroid collection, this method can be automated for mass production of controllable microtissues for either transplantation or pharmacological testing. The plateaued induction efficiency of rat DP cells at higher cell numbers from 2^{10^4} to 3^{10^4} per spheroid suggests that such paracrine or surface effect may have reached a maximum in transdifferentiating keratinocytes from an interfollicular epidermal fate to a follicular fate.

Conclusions：The average diameter of regenerated hair fiber did not significantly change with the increasing size of transplanted DP spheroids. The result suggests that an appropriate size of DP spheroid is essential for HF inductivity, but its size cannot be directly translated to a thicker regenerated hair. Our results also have implications on the efficiency and efficacy in the regeneration of other epithelial organs.

09:20-09:30

S-5

聚電解質多層膜在神經幹細胞上的生物效應
The Bio-effect of Polyelectrolyte Multilayer Films on Neural Stem/Progenitor Cells

吳俐潔　李亦淇
長庚大學生化與生醫工程研究所

Introduction：So far, the related central nerve system diseases are hard to handle. Lately, many researchers have devoted to create a proper device or microenvironment for the growing of NSPCs or neural lineage cells. However, most of researches could not promote NSPCs to differentiate into large amount of neural related cells during in short time and build a good neural network. Therefore, we hope to modulate the microenvironment by series of PEM films and find a proper condition for the growing of NSPCs.

Materials and Methods：Polyelectrolyte multilayer (PEM) films were composed of positive charge and negative charge, which is poly-L-lysine and poly-L-glutamic acid sequentially. POPC and NGPE lipid solution were chosen to assemble supported lipid bilayer (SLB). NSPCs were prepared from pregnant Wistar rat embryos on day15. NSPCs were cultured on series of PEM films in serum free medium containing DMEM/F12 and N_2 supplement.

Results：NSPCs were found to have a good attachment on series of PEM films no matter on PEM only or SLB-PEM system, and also extend longer and straighter processes on PLL terminating layers than on PLGA terminating layers so that to form good neural networks. In cell differentiation, NSPCs could differentiate into large amount of neurons with increased layer of PEM and SLB-PEM films. In SLB-PEM system, neurons could grow very well with only a few of astrocytes supported.

Discussion：Few studies have shown that the good attachment of NSPCs on PEM films might due to amine group on PLL terminating layer. The stiffness property of PEM films may affect the differentiation of NSPCs, which stiffer substrate is proper for astrocytes while softer substrate is proper for neurons, and in higher number layer of PEM films could produce large amount of neurons. The lipid effect on SLB-PEM films might make NSPCs feel like growing with other cell membranes, so NSPCs could differentiate neurons with only a few astrocytes.

Conclusions：A proper microenvironment from series of SLB-PEM films were successfully created for the growing and differentiation of NSPCs. This good result could be very helpful on the application of neural tissue engineering and for the spontaneous regeneration of neural concerned diseases in the CNS.

09:30-09:40

S-6

Preferential Therapy of Cord Blood Mesenchymal Stem Cells for Osteoarthritis through Regulation of Chondrogenic Cytokines

許維哲[1] 陳韋弘[1,2] 鄧文炳[1,2*]
臺北醫學大學生醫材料暨組織工程研究所 [1] 臺北醫學大學 幹細胞研究中心 [2]

Introduction： Osteoarthritis (OA), a common rheumatic disease associated with imbalanced cartilage homeostasis, could be corrected by mesenchymal stem cells (MSCs) therapy. However, MSCs from different origins might exhibit distinct differentiation capacities. This study was undertaken to compare the therapeutic efficacies between MSCs from cord blood (CB-MSCs) and bone marrow (BM-MSCs) on OA treatment.

Materials and Methods： The surface phenotypes and multipotent capacities of CB-MSCs and BM-MSCs were first characterized. The commitment system was subsequently ultilized for comparing the patterned molecules in stage-specific chondrogenesis. For examining the therapeutic efficacies, committed CB-MSCs and BM-MSCs were encapsulated in neo-cartilage and subjected into pro-inflammatory cytokine environment. Finally, chondrogenic and inflammatory cytokine profiles in committed MSCs were evaluated.

Results： The CB-MSCs showed a markedly higher chondrogenic potential and relatively lower osteogenic and adipogenic capacities than BM-MSCs. During chondrogenesis, the committed CB-MSCs also showed significant increases in cell proliferation, adhesion molecules, signaling molecules, and chondrogenic-specific gene expressions in an coculture system. For the therapeutic efficacies, the committed CB-MSCs could strongly revocer the pro-inflammatory cytokines diminished-Col II and proteoglycan expressions in an 3D arthritic model. The IL-10, ICAM-1 and TGF- 1 were also up-regulated in committed CB-MSCs analyzed by using cytokine profiling. Our data demonstrate that CB-MSCs possess specific advantages in cartilage regeneration over BM-MSCs..

Discussions： By comparing MSCs-based therapeutic indications in arthritis, we found that CB-MSCs as OA therapy is superior to BM-MSCs by induction of chondrogenic cytokines. Our results indicated that CB-MSCs were committed rapidly to the mesenchymal condensation and chondroprogenitor stage of chondrogenesis than BM-MSCs. Prior to the cooperation of activated developmental molecules, cell fate of both committed MSCs were subsequently examined. The chondrogenic genes were highly enhanced in committed CB-MSCs. In addition, chondrogenic matrix is essential to determine the completion of chondrogenesis process. The increased levels of Col II synthesis and proteoglycan accumulation were also observed in committed CB-MSCs.

Conclusions： The CB-MSCs showed a better therapeutic potential that can contribute to advanced cell-based transplantation for clinical OA therapy could potentially be used as a therapeutic drug for clinical arthritis therapies.

15:20-15:30

S-7

使用源自脾臟之間質幹細胞治療小鼠急性胰臟炎
The Usage of Spleen-derived Mesenchymal Stem Cell for the Treatment of Acute Pancreatitis in Mice

黃千芳[1] 黃效民[2] 沈延盛[1]
成功大學醫學院臨床醫學研究所附設醫院外科部[1] 食品工業發展研究所[2]

Introduction：Acute pancreatitis is a common inflammatory disease of the pancreas by different etiologies. The mortality rate in severe acute pancreatitis ranges from 10% to 30%. Mesenchymal stem cells (MSCs) are considered to play a role in regulation of inflammation, tissue repair and regeneration, so, MSCs therapies raise the possibility of improving pancreatitis treatment. However, the origin of MSCs may influence the efficacy of MSC therapy. The spleen is a reservoir of islet stem cells supported by close interrelationships between the spleen and pancreas during development. In this study, we plan to use spleen-derived MSCs to treat acute pancreatitis occurred by the way of differentiating into pancreatic cells or suppressing the inflammation.
Materials and methods：Acute pancreatitis was in duced in C57BL/6 mice by five hourly intraperitoneal injections of 50μg/kg of cerulean. In the other hands, the MSCs derived from C57BL/6 spleen will be labeled with Qtracker in order to be recognized during in vivo experiments. The severity of pancreatitis was determined by the serum levels of amylase, lipase, and Myeloperoxidase (MPO) and the extent of pancreas cell edema, necrosis and inflammation by IHC staining. Inflammatory cytokines were also detected by RT-PCR. Those indicators were compared before and after the MSCs treatment by tail vein injection.
Results：The spleen-derived MSCs present several MSCs specific surface markers, such as CD44 (+), CD90 (+), Sca-1(+), CD73 (+), CD4 (-). In the acute pancreatitis mouse model, the improvement of lipase and MPO activity level in the serum after the MSCs treatment was demonstrated and the labeled MSCs were also recognized in pancreas. The extent of pancreatic edema, necrosis level and amylase expression in acute pancreatitis model were improved after the MSCs treatment. The level of cytokine, TNF-α was also significantly reduced.
Discussion：In our study, we can observe that the MSCs have the homing effect on reducing inflammatory cytokines to repair damaged pancreatic tissue as expected. In future, the MSCs therapy may be applied to treat the chronic pancreatitis-induced diabetes and further to be determined their function and mechanism.
Conclusions：The spleen-derived MSCs therapy can treat acute pancreatitis successfully by suppressing the inflammation and repairing the damaged tissue in acute pancreatitis model.

15:30-15:40

S-8

利用聚電解質多層膜篩選與擴增肝臟癌症幹細胞
Selection and Enrichment of Liver Cancer Stem Cells by Using Polyelectrolyte Multilayer Films

張仁輔　李亦淇*
長庚大學生化與生醫工程研究所

Introduction：癌症幹細胞是許多實質腫瘤的來源，其中包含肝癌。大部分早期肝癌患者進行化學治療與輻射線治療時，療效有限。由於缺乏高專一性和高敏感性的標記，肝癌在早期診斷與處理仍有困難。在之前研究裡，聚電解質多層膜逐層塗佈技術可有效進行細胞篩選。本研究利用一系列聚電解質多層膜創造系列微環境的變化以篩選及分離癌症幹細胞，並探討聚電解質多層膜對微環境及肝癌幹細胞篩選之影響。

Materials and Methods：本研究使用的聚電解質多層膜以帶負電的 polysodium 4-styrenesulfonate (PSS)與正電性的 polyallylamine hydrochloride (PAH)之高分子材料逐層塗佈於玻璃片上，可使材料藉由不同電性進行吸附。觀察肝癌細胞株(Huh7)於系列材料的生物效應。使用 MTT 及 LDH 進行細胞活性試驗。並以流式細胞儀(flow cytometry)分析肝癌幹細胞特徵螢光標記並分析篩選比例，評估聚電解質多層膜所建構之微環境對肝癌細胞株篩選的影響。

Results：此系統培養到 120 小時，以 PAH 為最上層之聚電解質層膜 4.5 及 6.5 層，具有單顆球狀細胞呈現或球狀細胞之間相互聚集成類球狀的形態。在第 3 天及第 7 天的 MTT 與 LDH 細胞活性試驗中，可知無明顯材料毒性，且可發現以 PAH 為最上層之聚電解質層膜培養下，其讀值相較於 PSS 為最上層之數值明顯偏低。接著標記肝臟癌症幹細胞之特定螢光染劑(CD133、CD44)並以流式細胞儀檢測篩選之純度比例，發現在 6.5 層肝臟癌症幹細胞的比例含量較高。

Discussion：根據文獻，肝臟癌症幹細胞在 5 至 8 天，這些類球狀細胞尺寸約為 70 到 100 μm。MTT 與 LDH 細胞活性試驗中，以 PAH 為最上層之聚電解質多層膜數值偏低，可能是因癌症幹細胞數量稀少或篩選的環境適合癌症幹細胞生存，而導致其他細胞死亡所致。此外，聚電解質多層膜 4.5 與 6.5 層經流式細胞儀篩選 CD133 及 CD44 螢光雙染比例高於相關文獻所篩之癌症幹細胞比例，可能是因這些條件下具有純化或擴增癌症幹細胞之特性。

Conclusions：由結果中可發現在聚電解質多層膜 4.5 與 6.5 層具有類似癌症幹細胞形態，且經由流式細胞儀測定 CD133 及 CD44 肝臟癌症幹細胞表面螢光染劑，發現在聚電解質多層膜 4.5 與 6.5 層具有明顯篩選及擴增比例。

15:40-15:50

S-9

以生物工程皮膚纖維母細胞球體進行角膜基質組織重建
Bioengineered Dermal Fibroblast Spheroids for Corneal Stromal Tissue Reconstruction

張仁杰　薛聖潔*　賴瑞陽*
長庚大學生化與生醫工程研究所

Introduction：To overcome the drawbacks associated with shortage of allograft, this study aims to develop bioengineered dermal fibroblast spheroids for corneal stromal tissue reconstruction.
Materials and Methods：Rabbit dermal fibroblast (RDF) spheroids were fabricated on hyaluronic acid (HA) coatings and were evaluated by determinations of cell proliferation and extracellular matrix production capacity. The multicellular spheroid aggregates were implanted in a rabbit model of bacterial keratitis and the postoperative outcomes were evaluated by means of clinical observations including slit-lamp biomicroscopy and pachymetry. Animals receiving dissociated cell suspensions harvested from tissue culture polystyrene (TCPS) plates were included for comparison.
Results：Our results showed that the RDFs on HA coatings exhibited poor adhesion and tended to aggregate to form spheres. When compared to the TCPS plates, the HA-coated substrates significantly hindered cell spreading and subsequent cell proliferation. The RDF spheroids possessed better biosynthetic capacity and tissue repair than their counterparts in the form of dissociated cell suspensions. The injection of multicellular spheroid aggregates into the stromal defect could improve corneal clarity and edema.
Discussion：Corneal stromal cells are not readily accessible, and it is difficult to obtain sufficient number of cells from patients. Therefore, an alternative strategy for corneal stromal reconstruction should be established to meet the increasing medical service needs. Here, we demonstrate the feasibility of cultivating dermal fibroblast spheroids on biomaterial coatings and transplanting these cell grafts to treat corneal wound.
Conclusions：Bioengineered RDF spheroids may have potential for use in corneal stromal regenerative medicine.

15:50-16:00

S-10

電紡明膠奈米纖維薄膜作為角膜基質細胞支架：碳二亞胺多階段交聯效應
Electrospun Gelatin Nanofibrous Membranes for Corneal Keratocyte Scaffolds: Effect of Multi-Stage Carbodiimide Cross-Linking

張育誠　賴瑞陽*
長庚大學生化與生醫工程研究所

Introduction：The application of gelatin as a cell scaffold is limited by its poor biostability. Here, the electrospun gelatin nanofibrous membranes (GNMs) were treated by carbodiimide cross-linking, and the effects of multi-stage cross-linking on the characteristics of scaffold materials were investigated.
Materials and Methods：The test samples were obtained from multi-stage carbodiimide cross-linking of electrospun GNMs (CG1-CG4) by varying the solvent composition (an ethanol-water mixture).
Results：The increase in the cross-links formed between gelatin molecules at higher cross-linking stage number significantly enhanced the cross-linking degree, mechanical strength, and dissolution resistance of GNMs. By means of live/dead assays, all the test samples displayed good biocompatibility toward corneal stromal cells. Interestingly, while the cells well spread on their counterparts from CG1 and CG2 groups, the cultures on the CG3 and CG4 samples aggregated to form spheroids. A better biosynthetic capacity associated with a higher prevalence of elevated extracellular matrix production was found for the cells in a spherical configuration.
Discussion：Development of nanofibrous membranes for corneal stromal cell cultivation and tissue replacement is considered to be promising given that the major structural components of stroma are collagen nanofibers. Gelatin is a denatured collagen and has been used for corneal cell sheet delivery in our laboratory. Here, we demonstrated the corneal stromal cell growth in response to chemically modified nanostructured gelatin matrices.
Conclusions：Cross-linking condition of GNMs may be crucial to determine the scaffold characteristics and corneal stromal cell-material interaction.

16:00-16:10

S-11

RhoA及其調控之細胞骨架張力在simvastatin引導骨隨幹細胞成骨的角色
RhoA Downstream Signaling and Regulated Cytoskeleton Tension was Involved in Simvastatin Induced Osteogenesis in Mouse Bone Marrow Mesenchymal Stem Cells

戴宜均[1,2,3] 王耀賢[3] 張瑞根[2,3,4] 何美泠[1,2,3]
高雄醫學大學醫學研究所[1] 高雄醫學大學生理學科[2]
高雄醫學大學骨科學研究中心[3] 高雄大同醫院[4]

Introduction：Statins, 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, reduce cholesterol synthesis and prevent cardiovascular disease. They also have been found to inhibit prenylation of Rho proteins in recent decade. Previous reports showed that statins inhibited protein prenylation and decreased the active form of RhoA in osteoclasts. Others reports indicated that statins inhibited protein prenylation but increased the active form of RhoA in human erythroleukemia cells. Therefore, the role of statins regulates RhoA activity remains unclear. Rho GTPases act as molecular switches to regulate mesenchymal stem cell differentiation. Previous study showed that transfected constitutively active-form of RhoA into human mensenchymal stem cells (hMSCs) which leaded differentiation of hMSCs into osteoblasts. On the other hand, dominant negative RhoA leaded differentiation of hMSCs into adipocytes. According to the description above, we want to investigate whether RhoA signaling contributes to simvastatin-induced osteogenesis in BMSCs.

Materials and Methods：Mouse mesenchymal stem cells, D1, were purchased from ATCC. For all experiments, cells were treated with or without simvastatin (SIM) in osteo-induction medium. The mRNA expression of osteogenic marker genes were detected and quantified by real time PCR. The mineralization effect on D1 cells was tested by Alizarin Red S Staining. Actin rearrangement was observed by phalloidin alexa 488. The active form of RhoA was detected by pull down assay. Cells were transfected with constitutive-activated RhoA and dominate negative RhoA plasmid using lipofectamine 2000.

Results：The SIM 0.5 and 1uM appeared to alter cytoskeletal organization at day 1. And SIM 1uM enhanced the osteogenic gene expressions of Runt-related transcription factor 2 (Runx-2), bone morphogenetic protein 2 (BMP-2), Alkaline phosphatase (ALP) and dose-dependently increased the mineralization at day 5 in D1cells culture. We further found that SIM 1uM sustainably increased active-form RhoA level at 0.5 and 1 hrs in D1cells culture. We preliminary found that constitutive-active form of RhoA induced increased the effect of mineralization rather than dominate negative form RhoA in D1cell culture. RhoA downstream downstream inhibitor partially inhibited simvastatin

induced mineralization and osteogenic gene expression.

Discussion： SIM enhanced mineralization through activated osteogenic gene expression in mBMSCs. SIM induced active form RhoA level in mBMSCs. Constitutive-active form of RhoA induced osteogenic genes expression and further enhaced mineralization in mBMSCs.

Conclusion： We suggest Rho A signaling may contribute to SIM-enhanced osteogenesis in mBMSCs.

16:10-16:20

S-12

以桿狀病毒基因改質脂肪幹細胞之臨床前安全性評估
Preclinical Safety Evaluation of Adipose-derived Stem Cells Engineered by Baculovirus

李奎璋　胡育誠
國立清華大學化學工程系

Introduction：Previous studies showed that mesenchymal stem cells derived from adipose tissues (ASCs) can be genetically engineered by Baculovirus (BV) to express bone morphogenetic protein 2 (BMP2) and vascular endothelial growth factor (VEGF). Implantation of the transduced cells helps to repair bone defects in rabbits. However, the effect of BV transduction on cell characteristics and in vivo immune responses are not clear. In order to advance this technology into the clinical applications, this study aims to assess the safety of BV transduced human ASCs.

Materials and Methods：Stemness, proliferation and immunosuppressive ability of BV transduced human ASCs were analyzed by flow cytometry and BrdU cell proliferation assay. Tumorigenicity and chromosome stability were detected by qRT-PCR and CGH array. A critical-size defect at the femora of mini pig was established to evaluate the in vivo immune responses. The peripheral blood mononuclear cell (PBMC) was analyzed by flow cytometry and cytokines in serum were analyzed by ELISA.

Results： BV transduction did not impair the ASCs' viability, proliferation, immunophenotyping and immunesuppressive ability. Furthermore, neither tumor suppressor gene nor oncogene gene expression was disturbed and the chromosome was stable. As to the in vivo immune responses, $CD4^+$, $CD8^+$ and macrophage percentage of PBMC demonstrated similar responses from 1 to 28 days post-transplantation (dpt) in mock and BV-engineered group. The Th1/Th2 cell cytokine decreased from 1 to 28 dpt while TNF-α increased significantly from 14 to 28 dpt in BV-engineered group.

Discussion：The Th1/Th2 cell cytokine decreased is due to secretion of BMP2 by transduced cells. The literature reports that BMP2 inhibit B- and T-cell lymphopoiesis and lead to decrease of cytokine secretion. The TNF-α increased significantly from 14 to 28 dpt, suggesting that BMP2 accelerated bone healing and remodeling.

Conclusions：This study demonstrated BV vector was a safe gene therapy carrier and validated safety of BV-engineered ASCs in tissue engineering. The results are crucial for future applications of the technique in the clinical setting.

Poster Presentations

P-1

一種新穎之軟骨組織工程：利用軟骨碎片、滑液膜碎片以及間葉幹細胞
A Novel Approach for Cartilage Tissue Engineering: Using Cartilage Fragments, Synovial Membranes and Mesenchymal Stem Cells

陳佳君[1] 胡家僖[1] 方旭偉[1*] 張至宏[2*]
國立台北科技大學化學工程與生物科技系[1] 亞東紀念醫院骨科[2]

Introduction：A key for successful cartilage regeneration from MSCs is to correctly modulate cells to differentiate into the chondrogenic lineage and express normal cartilage extracellular matrix (ECM). Therefore, the stem cell microenvironment is critical for cell differentiation along the right lineage. In this study, we applied cartilage fragments to drive bone marrow-derived MSCs toward chondrogenesis. Besides, the MSCs resided in synovial membranes increased the total cell numbers and might result in better outcomes. This would be a novel strategy of using cartilage fragments, synovial membranes and MSCs to mimic the knee joint environment for cartilage tissue engineering.

Materials and Methods：The cell suspension containing MSCs, cartilage fragments and synovial membranes were mixed together. After centrifugation, the supernatant was discarded and the mixture was embedded into type I collagen. Cells, cartilage fragments and synovial membranes were dispersed in the collagen-medium mixed solution and allowed to gelatinization. Finally, the construct containing MSCs, cartilage fragments and synovial membranes was acquired.

Results：SEM images showed that MSCs attached and grew well in the constructs and exhibited polygonal cell morphologies. Histological analyses revealed that the constructs showed round cell appearances with positive Alcian blue staining. After 28 days of in-vitro culture, the gene expressions of type II collagen were significantly increased. Therefore, we suggested that the constructs containing cartilage fragments, synovial membranes and MSCs resulted in better outcomes for chondrogenesis.

Discussion：Histological results revealed that synovial membrances could fill the gaps between cartilage fragments and MSCs and encouraged the production of GAGs. This suggested that the constructs resulted in better integration and chondrogenesis. We considered that the cartilage synovium-fragment-MSC constructs resulted in better outcomes for chondrogenesis.

Conclusions：In conclusion, the constructs used in this study included cartilage fragments, synovial membranes and bone marrow-derived MSCs to mimic a knee joint environment. This approach achieved better outcomes of chondrogenesis and might benefit the clinical cartilage repair.

P-2

大鼠股骨缺損模式探討聚乳酸–甘醇酸接合 Alendronate 促骨修復
PLGA-Alendronate Enhances Bone Repair in Femoral Bone Defect in Rats

王耀賢[1] 路卡曼[1] 王志光[1,3] 蔡紫琳[1] 劉瓊月[1] 張瑞根[1,4,5] 何美泠[1,2]
高雄醫學大學骨科學研究中心[1] 高雄醫學大學生理學科[2] 高雄醫學大學醫用化學系[3]
高雄醫學大學附設醫院骨科部[4] 高雄市大同醫院骨科部[5]

Introduction：Alendronate (Aln) is known as an anti-resorptive drugs for treatment of osteoporosis. It is able to inhibit the mevalonate pathway and induces apoptosis in osteoclasts. In our previous study indicates that Aln combined human adipose stem cells enhances bone repair in carvarial bone defect model1. In this study, we modified PLGA conjugated with Aln as a short-term controlled-release drug for local use in bone repair.
Materials and Methods：The fabrication of PLGA-Aln is the two stage process, first the activation of the carboxylic acid end group of PLGA by EDC/NHS method and second is the cross linking reaction. The morphology of the PLGA-Aln was observed by the scanning electron microscopy (SEM). We created a bone defect site with 4x1 mm^2 on femoral bone in rats and treated with PLGA-Aln for 6 weeks. After micro-CT evaluation, we employed 3 points bending test to evaluate bone quality. H&E or Masson staining were employed to indicate the morphology and archeology of new forming bone. Immuno- histochemistry staining was used to indicate the expression of osteogenesis factors. All values are expressed as the mean ± standard error of the mean (SEM) of at least three independent experiments. A one-way ANOVA (analysis of variance) was used to test for statistical differences, and multiple comparisons were performed using Scheffe's method. Statistical significance was set at $p < 0.05$.
Results：PLGA-ALN enhances biomechanical property of repaired bone and increases bone formation (trabecular and cortical bones), BMP-2 and osteocalcin level at the new formed trabecular bones at the repair site. Short-term released alendronate from PLGA scaffold with concentration of 10^{-7}M enhances bone formation in bone defect model.
Discussion：We demonstrated that controlled-release PLGA-Aln enhances bone repair and increased new bone formation. Aln might enhance bone growth rather than inhibit bone resorption in bone repair.
Conclusions：Short-term and controlled-release PLGA-Aln for local-use of bone defect could avoid the side-effects of systemic administration of Alendronate. The PLGA-Aln may be applied to treat non-union bone fracture and/or bone defect.

P-3

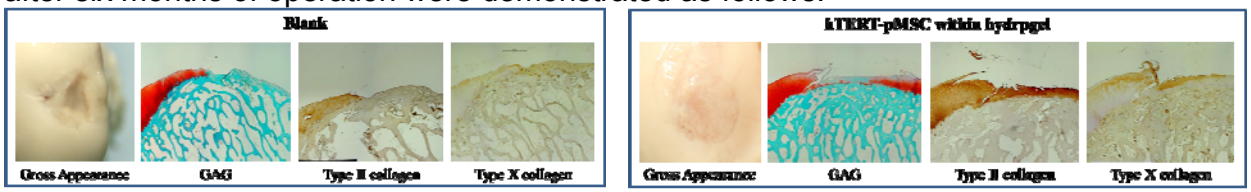
以原位成膠之水膠包覆經人類端粒酶反轉錄酵素基因轉殖之幹細胞作為軟骨再生之療法
Porcine Mesenchymal Stem Cells of hTERT-gene Transfection Combined with *in-situ* Gelation Hydrogel for Cartilage Regeneration

許元銘[1,2] 蕭莉馨[1] 陳瑾霈[1] 張至宏[1,2*]
亞東紀念醫院骨科[1] 元智大學生技所[2]

Introduction：Mesenchymal stem cells (MSCs) are an essential source for tissue engineering and gene therapy, since they possess the ability to differentiate into variety of cell types. Due to limited proliferation of MSCs, introduction of human telomerase reverse transcriptase (hTERT) into the MSCs has been carried out in our study. Result demonstrated that introduction of hTERT into MSCs will not alter the characteristics of the cells and able to prolonged their life span and differentiation ability. A hydrogel of methyl cellulose and chitosan was developed in our previous study. An *in vivo* study with mini-pig was carried out to provide a pre-clinical test results for evaluating the hTERT-pMSCs and hydrogel as a treatment in further application.

Materials and Methods：Porcine MSCs with hTERT gene transfected were analyzed by PCR, telomerase activity, flow cytometry, and Telomere length assays. For *in vivo* study, three sexually mature miniature pigs were used. All operations and interventions were performed under general anesthesia. A full-thickness chondral defect with diameter of 8 mm was created in medial condyles of both knee joints; one received hTERT-transduced MSCs entrapped in hydrogel and covered by periostium while another remained defect as control group. The pigs were sacrificed after six months. The specimens from the knee joints underwent fixation, decalcification and eventually embedded in paraffin and cut into slices for further staining. Then histological analysis (GAG) and immunohistochemical analysis (Type II and X collagen) were performed.

Results：The PCR results showed the hTERT gene was successful transfected into pMSCs. The telomere length, telomerase activity, and differentiation activity of pMSCs were increased after hTERT gene transfected. The histological and immunohistochemical results of hTERT-PMSC in hydrogel and Blank (control group) after six months of operation were demonstrated as follows.



Discussion：According to the results, regeneration of cartilage can be seen in defected site after implantation of hTERT-pMSCs entrapped in hydrogel. Moreover, results showed increased expression of GAGs and Type II collagen and average expression of X collagen in treatment group.

Conclusions：Porcine MSCs of hTERT-transfection entrapped in hydrogel can be a potential treatment in repairing cartilage defect.

P-4

雌激與 simvastatina 在骨源細胞中促進骨分化的關係之研究
Simvastatin-Stimulated Osteogenic Effects is Related to Estrogen Receptor in Osteogenic Lineage Cells

莊淑君 [1] 張力夫 [1,2] 何美泠 [1,2] 張瑞根 [1,3,4]
高雄醫學大學骨科學研究中心 [1] 高雄醫學大學生理學科 [2]
高雄醫學大學附設中和紀念醫院骨科 [3] 高雄市立大同醫院骨科 [4]

Introduction：Simvastatin is known to inhibit cholesterol biosynthesis and recent studies have reported that simvastatin stimulated bone formation in vivo and vitro. However, the mechanism of osteogenic cell differentiation regulated by simvastatin is not investigated in detail. In our previous study, we clarified the simvastatin-stimulated osteogenesis was related to estrogen receptor by using antagonist ICI 182.780 (ICI). However, the ICI is the antagonist of ERα and agonist of GPR30. Therefore, in this study, we want to clarify the roles of osteogenesis on ERα and GPR30. Our purposes of this study were to know if simvastatin can act through ERα or GPR30 and to know how simvastatin effects on osteogenic gene expression and osteogenesis.

Materials and Methods：Murine bone marrow mesenchymal stem cells (D1 cell) were used in this study. Simvastatin was used to stimulate D1 osteogenesis for 5 days and then changed into osteo-induction medium for 5 days. The specific antagonist MPP was used to block ERα function. The specific specific antagonist, G15, was used to block GPR30. The siRNA ERα was used to knockdown the expression of ERα. The gene expressions were measures by real-time PCR. Mineralization was tested by Alizarin red S staining. In lucificase reporter assay, human estrogen receptor vector (pHE0) and estrogen response element reporter vector (pERE-Luc) were co-transfected to MG63 and C2C12 to detect if the simvastatin can react on ERα directly. Significant differences were tested by using ANOVA. The mean of different treatment groups was tested using Duncan's new multiple-range test. A p value < 0.05 was taken as significant.

Results：After treated with simvastatin, the mineralization in D1 cell was increased and had dosage dependent effect. MPP inhibited the mineralization, but G15 didn't. It meant simvastatin stimulated mineralization is related to ERα, but not GPR30. The osteogenic gene expressions were increased by simvastatin treatment, but attenuated by MPP. The ERα gene expression was knockdown by siRNA ERα and the mineralization and osteogenic gene expression was attenuated by siRNA ERα. In lucificase reporter gene assay, pERE-Luc vector express was increased after treated simvastatin for 24 hr in MG63 and C2C12 cells, but the ICI inhibited the pERE-Luc vector expression.

Discussion：According to our previous study, we clarified the simvastatin-stimulated osteogenesis was related to estrogen receptor by using ICI. However, the ICI is the antagonist of ERα and agonist of GPR30. Therefore, it is necessary to clarify the role of ERα and GPR30 on osteogenesis. According to our data, when treated with MPP, the mineralization and osteogenic gene expressions were decreased the simvastatin-stimulated osteogenesis. But the mineralization of D1 cells was not

significantly changed when treated with G15 combined with simvastatin. Besides, the mineralization and osteogenic gene expression was decreased by treated with siRNA ERα. It meant ERα contributed to simvastatin-stimulated osteogenesis, but not GPR30. Simvastatin can activate lucificase gene in C2C12 and MG63 when co-transfect pHE0 and pERE-Luc vector, but the reaction was reversed by using ICI. It meant that simvastatin can bind to ERα directly or be a co-activator in ERα signaling pathway.

Conclusions：The results showed the physiological roles of estrogen receptor, ERα and GPR30, on murine bone marrow mesenchymal stem cell osteo-differentiation. It provides new information and basic medicine science in bone physiology.

P-5

環氧氫酶透過 p27^{kip1} 調控小鼠骨隨間葉幹細胞骨分化
Cycloxygenase-2 Contributes To Osteogenic-differentiation Via p27^{kip1} in Murine Bone Marrow Mesenchymal Stem Cells

莊淑君 [1] 戴宜均 [1,2] 張瑞根 [1,3,4] 何美泠 [1,2]
高雄醫學大學骨科學研究中心 [1] 高雄醫學大學生理學科 [2]
高雄醫學大學附設中和紀念醫院骨科 [3] 高雄市立大同醫院骨科 [4]

Introduction：Cyclooxygenase (COX), including isoenzymes such as COX-1, COX-2 and COX-3, is a central enzyme in converting arachidonic acid into prostaglandins (PGs).Our previous study indicated that COX-2 not only contributes to osteogenic cell proliferation via PTEN/Akt/p27^{Kip1} signaling, but also plays as a negative regulator to promote osteogenic differentiation. However, the mechanism of osteogenic cell differentiation regulated by COX-2 is not well investigated. A recent report indicated that p27 ^{Kip1} and Akt contribute to promoting osteogenic cell differentiation. Accordingly, we hypothesize that COX-2 may be a negative regulator to control osteogenic differentiation via Akt and p27 ^{Kip1} signaling. This study is to test the hypothesis, and murine bone marrow mesenchymal stem cells are used to examine the function of COX-2 on osteogenic differentiation.

Materials and Methods：Murine bone marrow mesenchymal stem cells (D1 cell) were used in this study. D1 cells were cultured to sub-confluence and changed into osteo-induction medium to promote differentiation. COX-2 inhibitor, NS398 (10 or 20 uM), was used to block the COX-2 function. The siRNA p27^{kip1} was used to block the gene expression of p27^{kip1}. The osteogenic marker genes such as Runx-2, BMP-2, osteocalcin (OC) and alkaline phosphatase (ALP) were measured by real-time PCR. Additionally, the changes of COX-2 and p27 gene expressions were also evaluated by real-time PCR. Mineralization was tested by Alizarin red S staining. Significant differences were analyzed by using one-way ANOVA. The mean of different treatment groups was tested using Duncan's new multiple-range test. A p value < 0.05 was taken as significant.

Results：The COX-2 gene expression was decreased when the D1 cells were cultured from sub-confluence (day 0) into confluence (day 2). After NS398 treatment, the mineralization in D1 cells were increased in a time and dose dependent manner (**, p<0.05 compared to the control group; # compare to the day 4 group). The gene expression of Runx-2, osteocalcin and ALP were increased after NS398 treatment (**, p<0.05 compared to the control group; # compare to the day 1 group). The p27^{Kip1} gene expressions in NS398 treated group were higher than that in the control group. The gene expression of p27kip1 was decreased after treated with siRNA p27kip1, and the

COX-2 inhibition caused mineralization increase was attenuated by $p27^{kip}$ silence.

Discussion：According to our previous study, COX-2 contributes to proliferation via PTEN/Akt/$p27^{Kip1}$ signaling in osteogenic cells. In this study, we found that COX-2 plays a negative regulator to promote osteo-differentiation via $p27^{kip1}$. COX-2 expression has a dynamic change when D1 cells from proliferation stage (sub-confluence) to osteo-differentiation stage (confluence). When treated with COX-2 inhibitor, the gene expressions of Runx-2 and osteocalcin significantly increase, but not in BMP-2. Besides, the inhibition of COX-2 function increasing mineralization was attenuated by $p27^{kip}$ silence. It implies that the COX-2 may be a negative regulator to promote the D1 cell osteo-differentiation via $p27^{Kip1}$.

Conclusions：The results clarify the physiological roles and molecular mechanisms of the constitutively expressed COX-2 on osteo- differentiation. The finding provides new information for the basic medical science in bone physiology and new drug development that act via COX-2 function.

P-6

結合玻尿酸及非蛋白質藥物作為生物因子促進幹細胞軟骨分化之組織工程研究
Combined Use of Hyaluronic Acid and Non-Proteinous Drugs as Bio-Factors to Enhance Chondrogenesis of Stem Cells for Tissue Engineering

張智翔[1] 吳順成[1] 張瑞根[2,3] 陳崇桓[2] 何美泠[1,4]
高雄醫學大學骨研中心[1] 高雄醫學大學附設中和紀念醫院骨科[2] 高雄市立大同醫院骨科[3]
高雄醫學大學醫學系生理學科[4]

Introduction：The recent stem cell based tissue engineering has offers many advantages over current techniques in the repair of damaged cartilage. Adipose tissue derived stem cells (ADSCs) are comparably better cell source for tissue engineering, because of their high proliferative capacity and multipotent differentiation capacity. Our previous results demonstrated that hyaluronic acid (HA)-microenvironment can both initiate and promote chondrogenic differentiation of ADSCs. On the other hand, previous reports also indicated both statin and bisphosphonate induced chondrogenic maker genes expression, type II collagen and aggrecan, and concomitant with increasing BMP-2 expression in ADSCs. The aim of this study is to fabricate a novel scaffold that not only possess a good chondrogenic microenvironment but also can release chondrogenic induction factors for promoting chondrogenic differentiation of ADSCs for articular cartilage tissue engineering.
Materials and Methods：In examination of statin or bisphosphonate enhancing ADSCs chondrogenesis in a HA-enriched micro-environment, isolated hADSCs were cultured in HA-coated plate or in a 3-D culture in HA/Fibrin hydrogel with of Simvastain (Sim) or Alendronate (Aln). Cells were analyzed sGAG formation by DMMB assay and the chondrogenic gene expression by real-time RT-PCR.
Results：After incubated hADSCs in HA coated or non-coated plates for 7 days, cells were harvested and analyzed their sGAG formation. The results showed hADSCs cultured in HA-coated plate with Sim or Aln formed more extracellular matrix than non-coated group with a dose-dependent manner.
We further found that hADSCs seeded in HA/Fibrin hydrogel with 10-6 M Sim supplement also presented higher sGAG formation after 7 days.
Discussion：Construction of a 3D biomaterial with autologous adult stem cells to regenerate defected articular cartilage may become a viable clinical option. In this study, we first examined HA can be a enhancing factor for ADSCs to differentiate during chondrogenesis by using HA-coated wells, and demonstrated that the Sim supplied in a HA-enriched microenvironment further enhanced cell aggregations and sGAG formation. The 3-D culture of hADSCs in the HA/Fibrin hydrogel with Sim supplement was further confirmed.
Conclusions：In this proposed project we demonstrated the effect of statin and bisphosphonate for ADSCs chondrogenesis *in vitro* in a 2-D and 3-D culture with HA-based hydrogel. Our results emphasize that these non-protein drugs enhaced hADSCs chondrogenesis capacity a HA-enriched microenvironment. It may be applied for more effective articular cartilage tissue engineering.

第五屆第二次會員大會

台灣再生醫學學會第五屆理、監事名單
(照筆劃順序排列)

理 事 長　楊台鴻

秘 書 長　洪士杰

常務理事　林峰輝、徐善慧、陳耀昌、蔡清霖

理　　事　王至弘、王兆麟、方旭偉、江清泉、侯連圍、張至宏、黃玲惠、楊俊佑、楊榮森、嚴孟祿

候補理事　林高田、張恒雄、陳文哲、陳志華、陳英和

常務監事　黃義侑

監　　事　林文澧、林泰元、孫瑞昇、陳敏慧

候補監事　顏伶汝

第五屆第二次會員大會議程

時間：民國102年3月2日(星期六) 12:00

地點：臺大醫學院103講堂(台北市仁愛路一段一號)

主席：楊台鴻 理事長

一、大會開始

二、主席致詞

三、理、監事會工作報告

四、討論事項

1. 通過年度工作計劃
2. 通過年度經費收支、決算表

五、臨時動議

六、散會

101年度工作報告

理事會報告

一、召開理監事會議計四次。

二、會員實際人數一百六十八人。

監事會報告

一、理事會處理會務均係依據大會章程辦理，遇有重大事項召開理監事聯席會議商討決定。

二、理事會所編財務報告，業經本監事會審核無誤。

三、本屆理事會竭盡全力推展會務。

102年工作計劃

一、招收會員

二、舉行四次理監事會議。

三、舉辦學術交流研討會。

四、隨時通知會員學會相關訊息。

台灣再生醫學學會
收支決算表
中華民國 101 年 1 月 1 日至 101 年 12 月 31 日止

科目				決算數	預算數	決算與預算比較數		說明
款	項	目	科目			增加	減少	
1			**本會收入**	**334,889**	**700,000**			
	1		會費收入	93,500	170,000		76,500	含入會費、常年會費
	2		捐款收入	240,000	520,000		280,000	廣告攤位收入及贊助會員捐款等
	3		利息收入	1,389	10,000		8,611	郵局、銀行利息
			本會支出	**562,212**	**700,000**			
2	1		人事費	164,000	150,000	14,000		員工及加班費
	2	1	文具	892	15,000		14,108	
		2	印刷費	70,652	50,000	20,652		
		3	郵電費	3,549	10,000		6,451	
		4	雜項	3,613	25,000		21,387	
	3	1	業務費	54,179	100,000		45,821	召開理監事會及辦理相關研討會所需之費用
		2	會議費	61,000	100,000		39,000	
		3	交通費	10,500	20,000		9,500	
			其他業務費	193,827	200,000		6,173	召開2月份年會
			提撥基金	0	35,000		35,000	
			本期結餘	(227,323)				

理事長：　秘書長：　常務監事：　會計：楊惠昌　製表：楊惠昌

台灣再生醫學學會
收支預算表
中華民國 102 年 1 月 1 日至 102 年 12 月 31 日止

科目				預算數	上年度預算數	本年度與上年度預算比較數		說明
款	項	目	科目			增加	減少	
1			**本會收入**	**630,000**	**700,000**			
	1		會費收入	170,000	170,000			含入會、費常年會費
	2		捐款收入	450,000	520,000		70,000	廣告攤位收入及贊助會員捐款等
	3		利息收入	10,000	10,000			郵局銀行利息
			本會支出	**630,000**	**700,000**			
2	1		人事費	170,000	150,000	20,000		員工薪資及加班費
	2	1	文具	5,000	15,000		10,000	
		2	印刷費	70,000	50,000	20,000		
		3	郵電費	5,000	10,000		5,000	
		4	雜項	10,000	25,000		15,000	
	3	1	業務費	70,000	100,000		30,000	召開理監事會及辦理相關研討會所需之費用
		2	會議費	80,000	100,000		20,000	
		3	交通費	20,000	20,000			
			其他業務費	200,000	200,000			
			提撥基金	31,500	35,000		3,500	依收入總額提列5%作為準備基金

理事長：　秘書長：　常務監事：　會計：楊惠晶　製表：楊惠晶

台灣再生醫學學會
現金出納表
中華民國 101 年 1 月 1 日至 101 年 12 月 31 日止

收入		支出	
科目名稱	金額	科目名稱	金額
上期結餘	$1,921,220	本期支出	$562,212
本期收入	$334,889	本期結餘	$1,693,897
合計	$2,256,109	合計	$2,256,109

理事長：　秘書長：　常務監事：　會計：楊惠昭　製表：楊惠昭

台灣再生醫學學會
資產負債表
中華民國 101 年 1 月 1 日至 101 年 12 月 31 日止

資產		負債	
科目	金額	科目	金額
庫存現金	$1,693,897	累計基金	$96,000
--	--	本期結餘	$1, 597,897
合計	$1,693,897	合計	$1,693,897

理事長：　秘書長：　常務監事：　會計：楊惠昭　製表：楊惠昭

台灣再生醫學學會章程

第一章 總 則

第 一 條 本會名稱為 **台灣再生醫學學會**(以下簡稱本會)。

第 二 條 本會以國內外人士共同發揚再生醫學之研究、教學及應用為宗旨。

第 三 條 本會以全國行政區域為組織區域。

第 四 條 本會會址設於主管機關所在地區。本會得視會員人數及分配與會務進行之需要設分會與各種委員會，其組織簡則由理事會擬訂，報請主管機關核准後實施，變更時亦同。

第 五 條 本會之任務如左：

一、提倡再生醫學之研究並發揚醫學倫理道德。

二、調查國內外再生醫學之發展，徵集有關圖書資訊以供各學術團體之參考及交流。

三、舉辦學術演講及討論會。

四、出版會誌及有關書刊。

五、獎助再生醫學及組織工程學人才及舉辦其他有關事宜。

六、與有關公司、廠商及機構合作，共求再生醫學及組織工程學之發展與應用。

第 六 條 本會之主管機關為內政部。

本會之目的事業應受各該事業主管機關之指導、監督。

第二章 會 員

第 七 條 本會會員申請資格如下：

一、個人會員：凡贊同本會宗旨、取得中華民國醫師執照者或取得與再生醫學、組織工程學相關博士學位者並經理事會通過後得申請為本會個人會員。

二、贊助會員：贊助本會工作之團體或個人。 申請時應填具入會申請書，經理事會通過，並繳納會費後，始得為本會贊助會員。

三、準 會 員：凡贊同本會宗旨的碩、博士班學生、博士後研究員、 住院醫師、研究助理或等同資格者，由會員二人推薦，經理監事會審查通過，得為本會準會員。

第 八 條 會員(會員代表)有表決權、選舉權、被選舉權與罷免權。每一會員(會員代表)為一權。 贊助會員、準會員無前項權利。

個人會員另享有 1. 參加本會年會及本會所舉辦之其他集會之權利。
2. 參加本會所舉辦各種活動或事業之權利。
3. 本會各種書刊訂閱優待之權利。

贊助會員享有 1. 參加本會年會及本會所舉辦之其他集會之權利。
2. 本會出版之資訊及刊物贈閱之權利。

準會員享有　　1. 參加本會年會及本會所舉辦之其他集會之權利。
2. 本會出版之資訊及刊物贈閱之權利。

第　九　條　會員有遵守本會章程、決議及繳納會費之義務。
會員每年年初需繳納會費，以利本會之運作。未繳納會費者，不得享有會員權利；連續二年未繳納會費者，視為自動退會。會員經出會、退會或停權處分，如欲申請復會或復權時，除有正當理由經理事會審核通過者外，應繳清前所積欠之會費。

第　十　條　會員(會員代表)有違反法令，章程或不遵守會員大會決議時，得經理事會決議，予以警告或停權處分，其危害團體情節重大者，得經會員(會員代表)大會決議予以除名。

第 十一 條　會員喪失會員資格或經會員大會決議除名者，即為出會。

第 十二 條　會員得以書面敘明理由向本會聲明退會。

第三章　組織及職權

第 十三 條　本會以會員大會為最高權力機構。
會員人數超過三百人以上時得分區比例選出會員代表，再召開會員代表大會，行使會員大會職權。會員代表任期二年，其名額及選舉辦法由理事會擬訂，報請主管機關核備後行之。

第 十四 條　會員大會之職權如左：
一、訂定與變更章程。
二、選舉及罷免理事、監事。
三、議決入會費、常年會費、事業費及會員捐款之數額及方式。
四、議決年度工作計畫、報告及預算、決算。
五、議決會員(會員代表)之除名處分。
六、議決財產之處分。
七、議決本會之解散。
八、議決與會員權利義務有關之其他重大事項。前項第八款重大事項之範圍由理事會定之。

第 十五 條　本會置理事十五人、監事五人，由會員(會員代表)選舉之，分別成立理事會、監事會。 選舉前項理事、監事時，依計票情形得同時選出候補理事五人，候補監事一人，遇理事、監事出缺時，分別依序遞補之。本屆理事會得提出下屆理事、監事候選人參考名單。
理事、監事得採用通訊選舉，但不得連續辦理。通訊選舉辦法由理事會通過，報請主管機關核備後行之。

第 十六 條　理事會之職權如左：

一、審定會員(會員代表)之資格。

二、選舉及罷免常務理事、理事長。

三、議決理事、常務理事及理事長之辭職。

四、聘免工作人員。

五、擬訂年度工作計畫、報告及預算、決算。

六、其他應執行事項。

第 十七 條　理事會置常務理事五人，由理事互選之，並由理事就常務理事中選舉一人為理事長。理事長對內綜理督導會務，對外代表本會，並擔任會員大會、理事會主席。理事長因事不能執行職務時，應指定常務理事一人代理之，未指定或不能指定時，由常務理事互推一人代理之。理事長、常務理事出缺時，應於一個月內補選之。

第 十八 條　監事會之職權如左：

一、監察理事會工作之執行。

二、審核年度決算。

三、選舉及罷免常務監事。

四、議決監事及常務監事之辭職。

五、其他應監察事項。

第 十九 條　監事會置常務監事一人，由監事互選之，監察日常會務，並擔任監事會主席。常務監事因事不能執行職務時，應指定監事一人代理之，未指定或不能指定時，由監事互推一人代理之。

監事會主席(常務監事)出缺時，應於一個月內補選之。

第 二十 條　理事、監事均為無給職，任期二年，連選得連任。理事長之連任，以一次為限。

第二十一條　理事、監事有左列情事之一者，應即解任：

一、喪失會員(會員代表)資格者。

二、因故辭職經理事會或監事會決議通過者。

三、被罷免或撤免者。

四、受停權處分期間逾任期二分之一者。

第二十二條　本會置秘書長一人，承理事長之命處理本會事務，其他工作人員若干人，由理事長提名經理事會通過聘免之，並報主管機關備查。但秘書長之解聘應先報主管機關核備。前項工作人員不得由選任之職員擔任。工作人員權責及分層負責事項由理事會另定之。

第二十三條　本會得設各種委員會、小組或其他內部作業組織，其組織簡則經理事會通過後施行，變更時亦同。

第二十四條　本會得由理事會聘請名譽理事長一人，名譽理事、顧問各若干人，其聘期與理事、監事之任期同。

第四章　會　　議

第二十五條　會員大會分定期會議與臨時會議二種，由理事長召集，召集時除緊急事故之臨時會議外應於十五日前以書面通知之。定期會議每年召開一次，臨時會議於理事會認為必要，或經會員(會員代表)五分之一以上之請求，或監事會函請召集時召開之。本會辦理法人登記後，臨時會議經會員(會員代表)十分之一以上之請求召開之。

第二十六條　會員(會員代表)不能親自出席會員大會時，得以書面委託其他會員(會員代表)代理，每一會員(會員代表)以代理一人為限。

第二十七條　會員(會員代表)大會之決議，以會員(會員代表)過半數之出席，出席人數較多數之同意行之。但章程之訂定與變更、會員(會員代表)之除名、理事及監事之罷免、財產之處分、本會之解散及其他與會員權利義務有關之重大事項應有出席人數三分之二以上同意。

本會辦理法人登記後，章程之變更以出席人數四分之三以上之同意或全體會員三分之二以上書面之同意行之。本會之解散，得隨時以全體會員三分之二以上之可決解散之。

第二十八條　理事會、監事會至少每六個月各舉行會議一次，必要時得召開聯席會議或臨時會議。前項會議召集時除臨時會議外，應於七日前以書面通知，會議之決議，各以理事、監事過半數之出席，出席人數較多數之同意行之。

第二十九條　理事應出席理事會議，監事應出席監事會議，不得委託出席。 理事、監事連續二次無故缺席理事會、監事會者，視同辭職。

第五章　經費及會計

第 三十 條　本會經費來源如左：

一、入會費：個人會員新台幣壹仟元，於會員入會時繳納。
　　贊助會員新台幣壹仟元，於會員入會時繳納。
　　準會員新台幣五百元，於會員入會時繳納。

二、常年會費：個人會員新台幣壹仟元。
　　贊助會員新台幣貳仟元。
　　準會員新台幣五百元。

三、事業費。
四、會員捐款。
五、委託收益。
六、基金及其孳息。
七、其他收入。

第三十一條　本會會計年度以曆年為準，自每年一月一日起至十二月三十一日止。

第三十二條　本會每年於會計年度開始前二個月由理事會編造年度工作計畫、收支預算表、員工待遇表，提會員大會通過(會員大會因故未能如期召開者，先提理監事聯席會議通過)，於會計年度開始前報主管機關核備。並於會計年度終了後二個月內由理事會編造年度工作報告、收支決算表、現金出納表、資產負債表、財產目錄及基金收支表，送監事會審核後，造具審核意見書送還理事會，提會員大會通過，於三月底前報主管機關核備(會員大會未能如期召開者，先報主管機關。)

第三十三條　本會解散後，剩餘財產歸屬所在地之地方自治團體或主管機關指定之機關團體所有。

第六章　附　　則

第三十四條　本章程未規定事項，悉依有關法令規定辦理。

第三十五條　本章程經會員(會員代表)大會通過，報經主管機關核備後施行，變更時亦同。

第三十六條　本章程經本會93年2月7日第一屆第一次會員大會通過。
報經內政部93年5月14日台內社字第0930018951號函准予備查。

台灣再生醫學學會會員名單：

編號	姓　名	編號	姓　名	編號	姓　名	編號	姓　名
001	劉華昌	029	林瑞模	061	鍾瑞嶂	091	李宣書
002	侯勝茂	030	徐郭堯	062	范揚峰	092	楊長彬
003	陳耀昌	032	吳輝傑	063	戴浩志	093	王貞棣
004	楊台鴻	033	蕭逸民	064	洪士杰	095	楊曙華
005	楊榮森	034	李建和	066	劉有漢	096	邱錦輝
006	楊俊佑	036	黃振勳	067	許致榮	097	郭兆塋
007	林峰輝	037	施庭芳	068	黃國淵	098	陳學明
008	林文澧	038	侯連圍	069	李裕滄	099	林柳池
009	王清貞	039	陳志華	070	陳沛裕	100	潘如瑜
010	黃義侑	040	李炫昇	072	林頌然	101	楊維宏
011	王兆麟	041	張瑞根	073	游敬倫	102	劉明偉
012	江清泉	044	李敏旭	075	陳吳坤	103	王文志
013	石朝康	045	江鴻生	076	鄧文炳	104	方旭偉
014	蔡清霖	046	陳昭宇	077	鄭耀山	105	陳敏慧
015	張恆雄	047	張宗訓	079	簡松雄	106	張明熙
017	蘇芳慶	048	釋高上	080	郭繼陽	107	陳興源
018	陳瑞明	050	張至宏	081	王世杰	108	蔡文龍
019	陳全木	051	蔡慶豐	082	蔡友士	109	郭宗甫
020	童瑞年	054	楊治雄	083	王至弘	110	王禎麒
021	殷金儉	055	蔡文基	084	張志豪	111	湯月碧
022	何始生	056	林高田	085	趙建銘	112	黃玲惠
023	孫瑞昇	057	古鳴洲	087	曾鵬文	113	王佩華
026	陳文哲	058	宋信文	088	徐明洸	114	郭源松
027	周正義	059	姚俊旭	089	詹益聖	115	翁文能
028	陳英和	060	呂紹睿	090	吳錫銘	116	徐善慧

編號	姓　名	編號	姓　名	編號	姓　名	編號	姓　名
117	蘇正堯	137	徐新生	157	蔡宗廷	177	賴文福
118	楊世偉	138	許文明	158	羅文政	178	施子弼
119	林偉彭	139	黃鶴翔	159	王德原	179	黃鼎鈞
120	謝豐舟	140	陳偉勵	160	賴志毅	180	陳宣佑
121	方紀宇	141	劉席瑋	161	吳佳慶	181	邵宏仁
122	蘇慶華	142	李冠瑢	162	沈延盛	182	徐永康
123	曾育弘	143	胡育誠	163	李一麟	183	賴瑞陽
124	林佐文	144	黃維超	164	何美泠	184	薛元毓
125	史　中	145	陳安泰	165	楊宗霖		
126	鄭乃禎	146	謝清河	166	吳坤佶		
127	謝式洲	147	彭慶安	167	趙本秀		
128	蘇鴻麟	148	劉滄梧	168	鄭明德		
129	曾清秀	149	薛敬和	169	李源芳		
130	劉百栓	150	林毅誠	170	嚴孟祿		
131	唐逸文	151	顏君哲	171	顏伶汝		
132	王清正	152	陳江山	172	林泰元		
133	王盈錦	153	侯君翰	173	陳尹愷		
134	吳信志	154	吳俊昇	174	許元銘		
135	簡雄飛	155	廖振焜	175	鄭有仁		
136	高國慶	156	傅再生	176	侯添財		

台灣再生醫學學會　個人會員入會申請書

姓　　名		性別		出　生 年月日		出生地		身分證 號　　碼	
學　　歷	民國　　年　　月畢業於								
戶籍住址									
現任職務	醫院或單位：			科部：		職稱：			
服務單位 地　　址						專科醫師證書字號： (無者免填)			
電　　話	(公)			(宅)		傳真：			
其他連絡 方　　式	電子信箱(e-mail)：					行動電話：			
審查結果 (由學會填寫)		會員類別 (由學會填寫)			會員證號碼 (由學會填寫)				

本人贊同貴會宗旨，擬加入為會員，嗣後並願意遵守會章，共圖發展

此致　　台灣再生醫學學會　　申請人：　　(簽章)

中　華　民　國　　年　　月　　日

會員資料異動申請書

本單填妥後請回傳至台灣再生醫學學會

Fax: 02-8921-3969

會員姓名：

變 更 為：

郵遞區號：

通訊地址：

服務單位：

聯絡電話：

傳　　真：

e-mail：

COLLAMATRIX

An expert in biopolymer technology

Our Company

Collamatrix Co., Ltd., established since 2003, is located in Taipei, Taiwan that specializes in the extraction, purification and transfiguration of natural biopolymers. Its R&D team, comprises professional of the fields of biochemistry, biomedical and tissue engineering, is dedicated to developing innovative biopolymer-based medical devices.

The proprietary technology and sophisticated manufacturing plant enable **Collamatrix** to produce superior biopolymers featured by its structural integrity, safety, biodegradability, biocompatibility and are ideal for various medical applications, such as tissue repair, regeneration and replacement.

R&D, Production & Marketing

Collamatrix has established a research and development laboratory, a quality control laboratory and a manufacturing plant. The manufacturing quality and controlled environments strictly adhere to Good Manufacturing Practice, in compliance with ISO 13485 and FDA Quality System regulations.

Collamatrix has received regulatory clearance for its **CollaWound** and **CollaDental** product lines indicated for the repair, the regeneration and the replacement of soft and hard tissues. We strive to continually expand the versatility of our products to satisfy global customers' requirements.

CollaWound

Collagen Sponge

Collagen Granules

CollaDental

Matrix

Graft

Barrier

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COLLAMATRIX

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