目　錄
Consent

2012 年度台灣再生醫學學會學術研討會暨會員大會

2012 Annual Meeting of Formosa Association of Regenerative Medicine

Scientific Program

Time	Topic	Speakers & Authors	Institute	Moderator
08:30	**Registration 報 到**			
09:00~10:00 Oral Presentation Competition (Ⅰ)				
S-01 09:00~09:10	Intramuscular Injection of Amniotic Fluid Stem Cells Improve Cardiac Functions after Infarction	曾哲揚[1] 彭劭于[2] 吳信志[12]	國立台灣大學動物科技研究所[1] 國立台灣大學生物科技研究所[2]	張恆雄教授 張至宏主任
S-02 09:10~09:20	Spontaneous Osteogenesis of MSCs Cultured on 3D Microcarriers Through Alteration of Cytoskeletal Tension	王稚翔[1] 曾佩淇[2,3] 楊台鴻[4] 王廷明[5] 彭筱雯[2] 侯勝茂[3] 嚴孟祿[2,6]	清華大學生物科技研究所[1] 台大醫學院一般醫學科[2] 新光吳火師紀念醫院骨科部[3] 台灣大學醫學工學研究所[4] 台大醫院骨科部[5] 台大醫院婦產部[6]	
S-03 09:20~09:30	Functional Assessment of Cross-Linked Porous Gelatin Hydrogels for Bioengineered Cell Sheet Carriers	李雅婷 賴瑞陽*	長庚大學生化與生醫工程研究所	
S-04 09:30~09:40	Influence of Molecular Weight of Hyaluronic Acid on the Adhesion, Phenotypic Expression, and Biosynthetic Capacity of Cultivated Corneal Keratocytes	俞亭君 涂益豪 賴瑞陽*	長庚大學生化與生醫工程研究所	
S-05 09:40~09:50	Carbodiimide Cross-Linked Amniotic Membranes for Cultivation of Limbal Epithelial Cells	張仁杰[1] 馬惠康[2*] 賴瑞陽[1*]	長庚大學生化與生醫工程研究所[1] 長庚醫院眼科[2]	
S-06 09:50~10:00	Development of Photopolymerized Poly(2-Hydroxyethyl Methacrylate)-*co*-Poly(Acrylic Acid) for Keratoprosthetic Biomaterial	張育誠 王祖濱 賴瑞陽*	長庚大學生化與生醫工程研究所	
10:00-10:30 Coffee Break				

List of Oral Presentation

Time	Topic	Speakers & Authors	Institute	Moderator
10:30~12:00 Invited Lectures				
I-01 10:30~11:15	How and Why Do Cells Migrate Into Damage Regions: Experimental and Computational Models	Amit Gefen, Ph.D.	Department of Biomedical Engineering, Faculty of Engineering, Tel Aviv University, Israel	林峰輝教授 徐善慧教授
I-02 11:15~12:00	In Vitro and In Situ Tissue Engineering of Blood Vessels	Song Li, Ph.D.	Department of Bioengineering University of California, Berkeley	
12:00 會 員 大 會				
12:00-13:30 Lunch Break				
13:30~15:00 Invited Lectures				
I-03 13:30~14:15	Cell Surface Modification with Polymers for Cell Therapy	Hiroo Iwata, Ph.D.	Department of Reparative Materials, Institute for Frontier Medical Sciences, Kyoto University	侯勝茂教授 楊台鴻教授
I-04 14:15~15:00	Generation of Transplantable Muscular Satellite-like Cells from Mouse Embryonic and Murine Induced Stem Cells	Hsi Chang, Ph.D	Department of Pediatrics, Taipei Medical University Hospital	
15:00-15:30 Coffee Break				
15:30~16:40 Oral Presentation Competition (Ⅱ)				
S-07 15:30~15:40	Porous Gelatin Scaffolds Modified with Chondroitin Sulfate for Corneal Stromal Tissue Engineering Applications	賴孟恆 賴瑞陽*	長庚大學生化與生醫工程研究所	侯連圍教授 蔡清霖教授
S-08 15:40~15:50	Hyaluronan-enriched Fibrin Gel Enhances Chondrogenesis of Human Adipose-derived Stem Cells for Chondro-Defect Repair in Explant Cultures	黃珮詒[12] 林怡珊[2] 林松彥[2] 王昭仁[12] 何美泠[12]	高雄醫學大學醫學系生理學科[1] 高雄醫學大學骨科學研究中心[2]	
S-09 15:50~16:00	Delayed Cell Morphology Responses to Mechanical Stimulation	溫新民 趙本秀	國立台灣大學醫學工程研究所	
S-10 16:00~16:10	Spheroid Formation of Human Adipose-derived Stem Cells on Chitosan Films Enhances Stemness and Differentiation Capabilities	鄭乃禎[1,2] 王 珊[2] 楊台鴻[1]	國立台灣大學醫工所[1] 國立台灣大學醫學院附設醫院 外科部 整形外科[2]	

Time	Topic	Speakers & Authors	Institute	Moderator
S-11 16:10~16:20	Rho A signaling Contributes to Statin-Induced Osteogenesis in Bone Marrow Mesenchymal Stem Cells	戴宜均 [1,2] 王耀賢 [2] 張瑞根 [2,3] 何美泠 [+1,2]	高學醫學大學醫學研究所 [1] 高雄醫學大學骨科學研究中心 [2] 高雄醫學大學附設醫院骨科 [3]	侯連團教授 蔡清霖教授
S-12 16:20~16:30	Spheroid Formation and Neuronal Induction in Adipose Derived Stem Cells by Chitosan Surface Coating	薛元毓 [1,3] 吳佳慶 [2] 林聖哲 [1]	國立成功大學醫學院附設醫院 外科部 整形外科 [1] 國立成功大學醫學院 細胞生物暨解剖學研究所 [2] 國立成功大學臨床醫學研究所 [3]	
S-13 16:30~16:40	Effects of Low Intensity Ultrasound on Neural Stem Cells	羅子琳 [1] 李亦宸 [2] 李亦淇 [1*]	長庚大學生化與生醫工程研究所 [1] 國立台灣大學醫學工程研究所 [2]	
頒 獎 閉 幕				

Invited Lectures

10:30-11:15

I-1

How and why do cells migrate into damage regions: experimental and computational models

Amit Gefen, Ph.D.

Department of Biomedical Engineering, Faculty of Engineering, Tel Aviv University, Israel
E-mail: gefen@eng.tau.ac.il

The kinematics of cell migration is frequently being studied in the context of wound healing. Scratch wound assays *in vitro* are particularly popular, being a cost-effective method for characterizing the kinematic parameters of cultures. However, objective and standardized measures of the kinematic parameters are often missing in these studies. We addressed the issue by developing an automatic and quantitative method for determining time-dependent damage areas in "wound healing" monolayer culture experiments by means of image processing. "Wound" area over time data are then fitted to a Richards function (non-symmetric sigmoid) from which we determine, in a process which can be fully automated, some fundamental characteristics of the migration process such as the migration rate, time for onset of mass cell migration and time for end of mass cell migration. In the present talk, the utility of our above method will be demonstrated by employing time-lapse microscopy to monitor migration in fibroblast, pre-adipocyte and myoblast cultures post infliction of controlled, geometrically-defined local mechanical damage. Applications in medical research are numerous, and include tests of environmental effects, biochemical effects and effects of medications and even food supplements on the migration kinematics in cell cultures. We further use mathematical modeling, based on the theory of mechanotransduction, to describe cell migration and obtain further insights regarding factors that drive this process. Specifically, we consider the movement and viability of individual cells in cell colonies by formulating a cell-level model of the migration process. Cell motility is assumed to take place as a result of sensing strain energy density signals which are transmitted through the culture substrate and serve as localized mechanical stimuli for cell movement. The displacement and viability of each individual cell are tracked, and the intercellular signaling gradually shapes the migration pattern in the simulated colony. The modeling is qualitatively tested against experimental data, and simulations behave similarly to real-world observations. This simulation tool can be used alongside with the experiments, e.g. to test effects of the presence of pharmaceutical agents on migration performances of individual cells and masses of cells in colonies, particularly in the context of wound healing and regenerative medicine.

References:
Topman G, Sharabani-Yosef O, Gefen A. A Method for Quick, Low-Cost Automated Confluency Measurements. Microsc Microanal. 2011 Oct 28:1-8.
Topman G, Sharabani-Yosef O, Gefen A. A standardized objective method for continuously measuring the kinematics of cultures covering a mechanically damaged site. Med Eng

Phys. 2011, in press (available online), DOI: 10.1016/j.medengphy.2011.07.014
Vermolen FJ, Gefen A. A semi-stochastic cell-based formalism to model the dynamics of migration of cells in colonies. Biomech Model Mechanobiol. 2011, in press (available online), DOI: 10.1007/s10237-011-0302-6

11:15-12:00

I-2

In Vitro and In Situ Tissue Engineering of Blood Vessels

Song Li, Ph.D.
Professor

Department of Bioengineering
University of California, Berkeley
UC Berkeley-UCSF Bioengineering Graduate Program
Director, Master Program in Bioengineering
Co-Director, UC Berkeley-UCSF Master Program in Translational Medicine

Nanostructured biomaterials and stem cells have tremendous potential for tissue engineering. A challenge in vascular tissue engineering is to develop optimal scaffolds and establish expandable cell sources for the construction of tissue-engineered vascular grafts (TEVGs) that are non-thrombogenic and have long-term patency. Here we demonstrate the potential of engineering nanofibrous scaffolds and stem cells for vascular tissue engineering. Nanofibrous scaffolds with organized nanofibers are fabricated to possess the similar mechanical property to native arteries. The scaffolds are implanted in the carotid arteries with or without the seeding of bone marrow mesenchymal stem cells (MSCs). Acellular grafts result in significant intimal thickening, while MSC-seeded grafts have excellent long-term patency and exhibit well-organized layers of endothelial cells (ECs) and smooth muscle cells (SMCs), as in native arteries. In vitro experiments show that MSCs, as ECs, resisted platelet adhesion in a manner dependent on cell surface heparan sulfate proteoglycans. To develop the next generation of vascular grafts, we take an in situ tissue engineering approach that recruits two types of endogenous progenitor cells for the regeneration of blood vessels. Heparin is conjugated to microfibrous vascular grafts to suppress thrombogenic responses, and stromal cell-derived factor-1α (SDF-1α) is immobilized onto heparin to recruit endogenous progenitor cells. Heparin coating improved the short-term patency for the first 2 weeks, and SDF-1α enhanced long-term patency at 4 weeks. Within 1 week, SDF-1α effectively recruited CD34+/CD133+ endothelial progenitor cells (EPCs) to the luminal surface of the grafts. EPCs differentiated into CD31+ ECs, resulting in a complete coverage of luminal surface within 4 weeks. In contrast, the grafts without SDF-1α showed much slower endothelialization, especially in the middle portion. In addition, SDF-1α increased the recruitment of smooth muscle progenitor cells (SMPCs) to the outer surface of the grafts at 1 week, and SMPCs differentiated into smooth muscle cells (SMCs) in vivo and in vitro. Furthermore, SDF-1α-immobilized grafts had significantly higher elastic modulus. This work demonstrates the feasibility of simultaneously recruiting progenitor cells of ECs and SMCs for in situ blood vessel regeneration. The approach of recruiting endogenous stem cells and progenitor cells will have wide applications in regenerative medicine.

13:30-14:15

I-3

Cell Surface Modification with Polymers For Cell Therapy

Hiroo Iwata*

Department of Reparative Materials, Institute for Frontier Medical Sciences, Kyoto University, 53 Kawara-cho, Shogoin, Sakyo-ku, Kyoto, 606-8507, Japan.

Summary

We overview new methodologies for using amphiphilic polymers to modify the surfaces of cells and tissues. Coating the cell surface with amphiphilic polymers that can capture and immobilize bioactive substances or cells represents a promising approach for clinical applications, particularly cellular therapies.

1. Cell surface modifications with amphiphilic polymers

Amphiphilic polymers are typically derived by conjugating polyethylene glycol (PEG) to a phospholipid (PEG-lipid). When a PEG-lipid solution is added to a cell suspension, the hydrophobic alkyl chains of the PEG-lipid spontaneously form hydrophobic interactions with the lipid bilayer of the cell membrane (Fig. 1a). This spontaneous anchoring was demonstrated with a human cell line derived from T cell leukemia cells (CCRF-CEM). A solution of fluorescein isothiocyanate (FITC)-conjugated PEG-lipid was added to a suspension of CCRF-CEM. Under a confocal laser scanning microscope, the bright fluorescence from FITC was observed at the periphery of all cells (Fig. 1b). This indicated that PEG-lipids had lodged on the cell surface. The retention time of PEG-lipids on cell membranes can be controlled by adjusting the length of the lipid alkyl chain. The dissociation rate of PEG-lipid was much slower with long than with short hydrophobic domains.

Proteins can be immobilized on the cell surface with the use of a short, single stranded DNA (ssDNA) attached to the end of a PEG chain (ssDNA-PEG-lipid) (Fig. 2). First, an ssDNA-PEG-lipid is prepared by conjugating maleimide-PEG-lipid with an ssDNA that carries a thiol group. While, a protein is modified with a hetero-bifunctional cross-linker, sulfo-EMCS (*N*-(6-maleimidocaproyloxy)sulfosuccinimide); next, it is treated with a ssDNA' that is complementary to the ssDNA on the PEG-lipid. Figure 2 shows a schematic of the procedure, where the ssDNA and ssDNA' are oligo(deoxythymidine) (oligo$(dT)_{20}$) and oligo(deoxyadenine) (oligo$(dA)_{20}$), respectively. The cells with oligo$(dT)_{20}$ attached are exposed to the protein with the oligo$(dA)_{20}$ attached. The protein is immobilized on the cell through hybridization between oligo$(dT)_{20}$ and oligo$(dA)_{20}$.

3. Immobilization of bioactive substances on an islet surface

Cell transplantation has shown promise as a method for treating serious diseases. Various kinds of pluripotent stem cells have been developed or identified, including embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, and mesenchymal stem cells. Moreover, the differentiation of stem cells to functional cells has been extensively studied.

Previous studies have demonstrated that the transplantation of insulin secreting cells, that

is islet of Langerhans cells (islets), could successfully treat type 1 diabetes. Islets are insulin secreting cells found in the pancreas. Over two hundred patients with type 1 diabetes have been clinically treated with islet transplantation. To cure the disease, a single patient typically requires islets from several donors, due to the destruction of islets just after transplantation. In the clinical setting, islets are infused into the liver through the portal vein. Exposure of islets to the blood activates blood coagulation and complement systems, which induce non-specific inflammatory reactions or instant blood-mediated inflammatory reactions (IBMIR). These host defense mechanisms destroy donor islets because they are considered foreign bodies. Anticoagulants, including aspirin, heparin, and dextran sulfate, are typically administered to inhibit blood coagulation. However, systemic infusion of these drugs increases bleeding. The optimal approach would be to prevent blood coagulation at the islet. Recent studies have been able to immobilize various bioactive substances, like heparin, urokinase, thrombomodulin, and the soluble domain of human complement receptor 1 (sCR1), on islets in attempts to control local activation of the blood coagulation and complement systems.

As an example, we will describe immobilization of the fibrinolytic enzyme, urokinase (UK), on the islet surface (Fig. 3). UK could be immobilized on islets through ssDNA hybridization of oligo$(dT)_{20}$-PEG-lipid and oligo$(dA)_{20}$-UK. When the oligo$(dT)_{20}$-PEG-lipid was added to a suspension of islets, the lipid moiety spontaneously anchored to the lipid bilayer of the cell membrane through hydrophobic interactions. The oligo$(dT)_{20}$ segment was exposed on the cell surface, which made it accessible for conjugation with the oligo$(dA)_{20}$ on UK.

UK is a serine protease that activates plasminogen to plasmin. Plasmin dissolves the fibrin in blood clots. The attachment of UK to the islet surface was expected to dissolve blood clots that surrounded the islets in the liver; thus, IBMIR could be inhibited in the initial stages. A fibrin plate–based assay was performed to assess the function of the UK attached to the islets. Fifty islets with/without immobilization of UK were spotted onto a fibrin gel plate. After incubation, transparent areas around the spots indicated UK dissolution of the fibrin. Figure 4 shows the fibrin plate at 14 h after spotting the islets. Larger transparent areas were observed around the UK-islets compared to those around the unmodified islets (Fig. 4). These indicated that the immobilized UK retained its activity on the islets. UK-islets were also tested after 2 days of culture in the presence of serum (Fig. 4). UK activity rapidly decreased with 2 days in culture. The morphology of all islets after modification with UK was well maintained after 7 days of culture. Islets with UK maintained the ability to regulate insulin release in response to changes in glucose concentration (data not shown). We also performed transplantation of UK-islets by transfusion to the liver through the portal vein. The transplantation results indicated that donor islets were rescued from host defenses by attaching UK to their surfaces. It remains to be determined how long UK-islets can maintain the inhibition of IBMIR; however, these data suggested that UK immobilization on islets is a promising approach for islet transplantation.

4.3 Encapsulation of islets with living cells

The histocompatibility and blood compatibility of donor islets can be significantly improved by enclosing them inside a capsule made of the patient's vascular endothelial cells. The ssDNA-PEG-lipid method was utilized to enclose islets with living cells. The method is schematically shown in Fi. 5. Oligo$(dT)_{20}$ was introduced onto the surface of HEK293 cells with an oligo$(dT)_{20}$-PEG-lipid, and oligo$(dA)_{20}$ was introduced onto the surface of islets with

an oligo$(dA)_{20}$-PEG-lipid. Then, the oligo$(dA)_{20}$-islets were mixed with the oligo$(dT)_{20}$-HEK293 cells. The HEK293 cells were immobilized on the islet surface through DNA hybridization, as shown in Fig. 5b. Although the HEK293 cells existed as single cells on the islet just after immobilization, the surface of islets were completely covered with a cell layer after 3 days in culture (Fig. 5). No central necrosis of the islet cells was observed. Immunostaining showed that insulin remained inside the islets after culturing for 3 days. Furthermore, after cell encapsulation, insulin secretion in response to glucose stimulation was well maintained (data not shown). This technique will greatly facilitate islet transplantation for treating type 1 diabetes.

List of publications

1. Miura S, Teramura Y, Iwata H (2006) Encapsulation of islets with ultra-thin polyion complex membrane through poly(ethylene glycol)-phospholipids anchored to cell membrane. Biomaterials 27:5828-5835
2. Teramura Y, Kaneda Y, Iwata H (2007) Islet-encapsulation in ultra-thin layer-by-layer membranes of poly(vinyl alcohol) anchored to poly(ethylene glycol)-lipids in the cell membrane. Biomaterials 28:4818-4825
3. Teramura Y, Iwata H (2008) Islets surface modification prevents blood-mediated inflammatory responses. Bioconjug Chem 19:1389-1395
4. Teramura Y, Kaneda Y, Totani T et al. (2008) Behavior of synthetic polymers immobilized on a cell membrane. Biomaterials 29:1345-1355
5. Totani T, Teramura Y, Iwata H (2008) Immobilization of urokinase on the islet surface by amphiphilic poly(vinyl alcohol) that carries alkyl side chains. Biomaterials 29:2878-2883
6. Teramura Y, Iwata H (2009) Islet encapsulation with living cells for improvement of biocompatibility. Biomaterials 30:2270-2275
7. Teramura Y, Iwata H (2009) Surface modification of islets with PEG-lipid for improvement of graft survival in intraportal transplantation. Transplantation 88:624-630
8. Teramura Y, Minh Ln, Kawamoto T et al. (2010) Microencapsulation of islets with living cells using polyDNA-PEG-lipid conjugate. Bioconjug Chem 21:792-796
9. Inui O, Teramura Y, Iwata H (2010) Retention dynamics of amphiphilic polymers PEG-lipids and PVA-Alkyl on the cell surface. ACS Appl Mater Interfaces 2:1514-1520
10. Teramura Y, Chen H, Kawamoto T et al. (2010) Control of cell attachment through polyDNA hybridization. Biomaterials 31:2229-2235
11. Teramura Y, Iwata H (2010) Bioartificial pancreas microencapsulation and conformal coating of islet of Langerhans. Adv Drug Deliv Rev 62:827-840
12. Teramura Y, Iwata H (2010) Cell surface modification with polymers for biomedical studies. Soft Matter 6:1081-1091
13. Chen H, Teramura Y, Iwata H (2011) Co-immobilization of urokinase and thrombomodulin on islet surfaces by poly(ethylene glycol)-conjugated phospholipid. J Control Release 150:229-234
14. Luan NM, Teramura Y, Iwata H (2011) Immobilization of soluble complement receptor 1 on islets. Biomaterials 32:4539-4545
15. Sakurai K, Teramura Y, Iwata H (2011) Cells immobilized on patterns printed in DNA by an inkjet printer. Biomaterials 32:3596-3602
16. Takemoto N, Teramura Y, Iwata H (2011) Islet surface modification with urokinase through DNA hybridization. Bioconjug Chem 22:673-678
17. Teramura Y, Iwata H (2011) Improvement of graft survival by surface modification with

poly(ethylene glycol)-lipid and urokinase in intraportal islet transplantation. Transplantation 91:271-278.

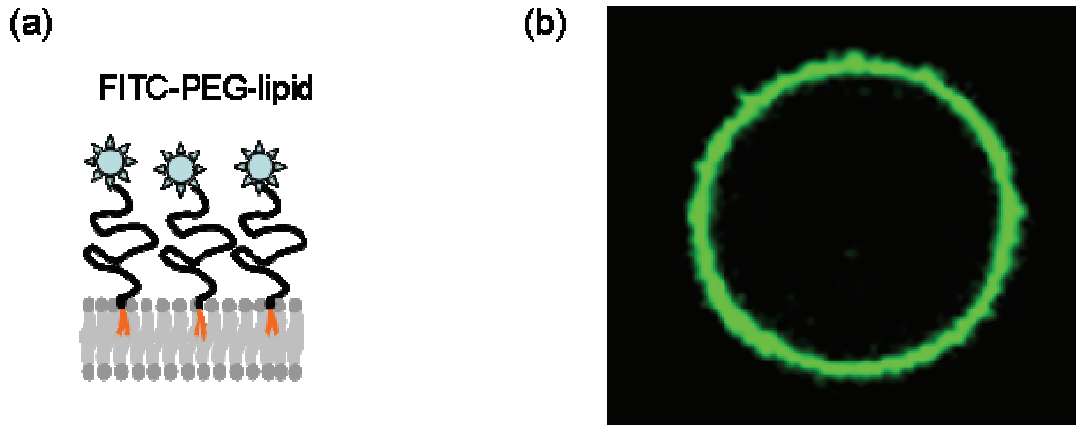

Fig. 1. Surface modification of cells with PEG-lipid. (a) Schematic representation of surface modification of a CCRF-CEM cell with FITC-PEG-lipid, (b) Confocal laser scanning microscopic image of an modified CCRF-CEM cell.

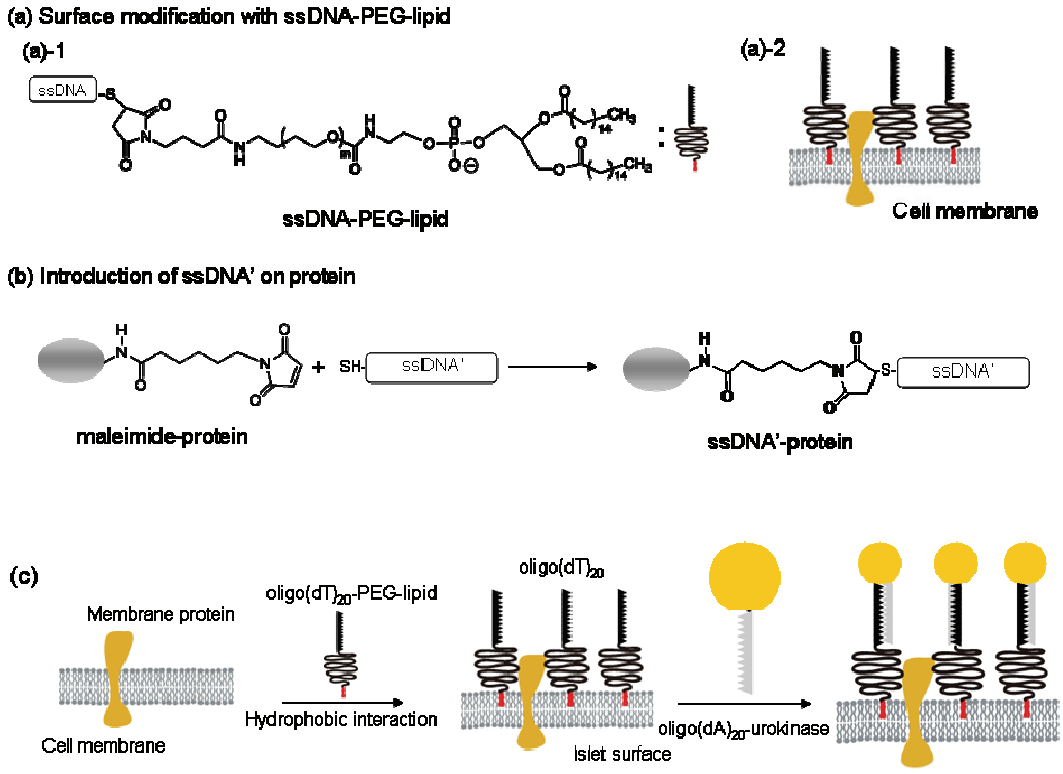

Fig. 2. Immobilization of urokinase protein on the surfaces of islet cells. (a-1) Chemical structure of DNA-conjugated PEG-phospholipid (DNA-PEG-lipid); (a-2) ssDNA-PEG-lipid anchoring to the cell membrane. (b) Introduction of a complementary ssDNA onto urokinase protein, which was first modified with a madeimide group by EMCS. (c) Schematic representation of urokinase-immobilization through DNA hybridization.

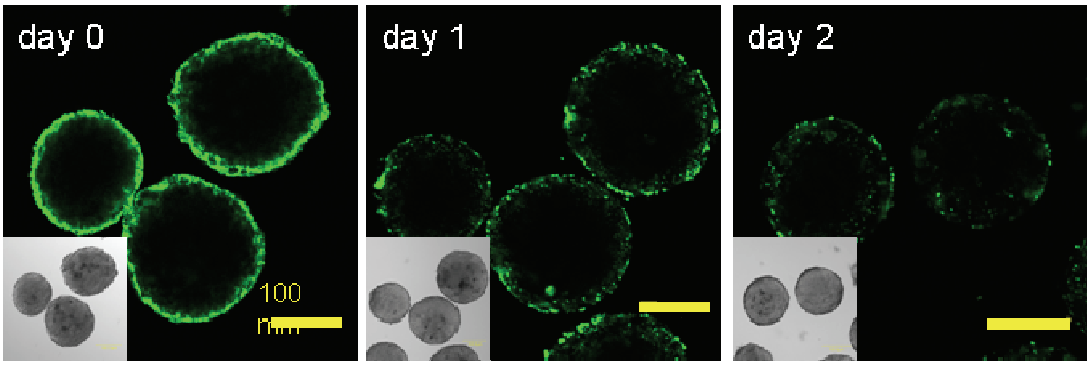

Fig. 3. Confocal laser scanning microscope images of islets with urokinase (UK) immobilized on the membrane. The green signal indicates positive immunostaining for UK. (a) Islets were modified with oligo(dT)$_{20}$–PEG-lipid (C16) then, oligo(dA)$_{20}$-UK was added to the media. Scale bar: 100 μm.

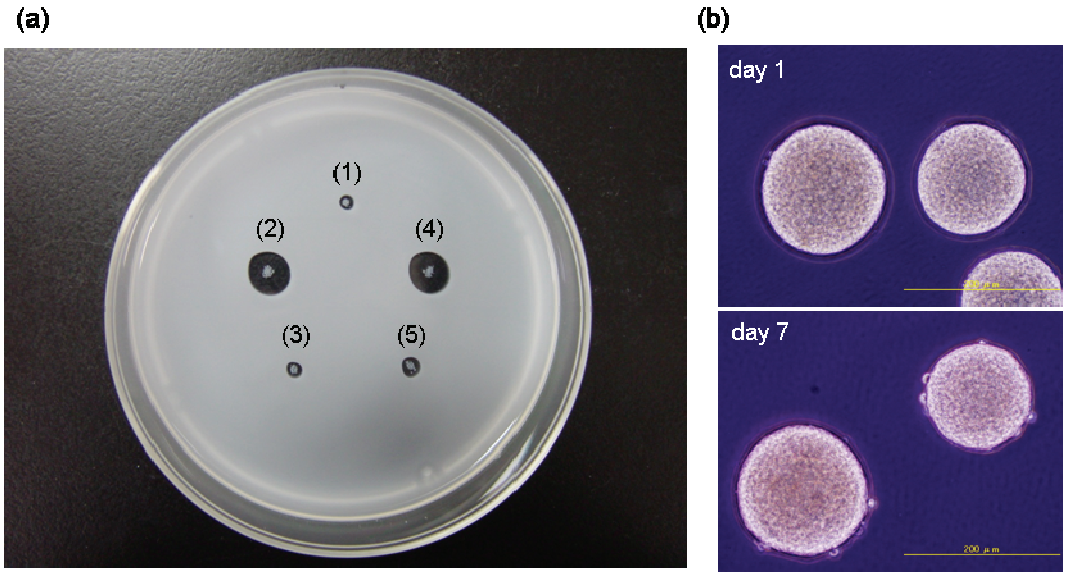

Fig. 4. Islets with immobilized urokinase (UK-islets) were tested for the ability to dissolve fibrin. (a) Fibrin in the plate gel medium was dissolved by UK-islets (clear areas). Fifty islets were applied to each spot, and the plate was observed after incubation at 37 ºC for 14 h. (1) untreated islets; (2) UK-islets (with oligo(dT)$_{20}$–PEG-lipid (C16)), just after preparation; (3) UK-islets (with oligo(dT)$_{20}$–PEG-lipid (C16)) lost activity after 2 days in culture; (4) UK-islets (with oligo(dT)$_{20}$–PEG-lipid (C18)), just after preparation; and (5) UK-islets (with oligo(dT)$_{20}$–PEG-lipid (C16)) lost activity after 2 days in culture. (b) Morphology of UK-islets after 1day and 7 days of culture.

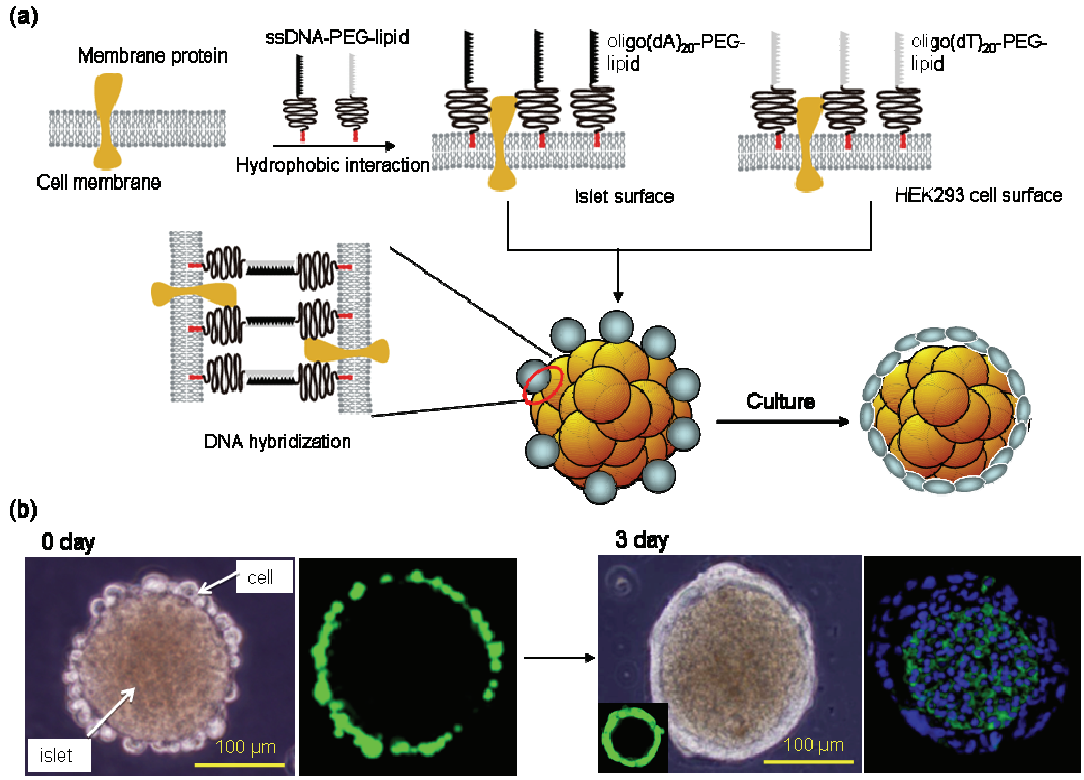

Fig. 5. Islet encapsulation within living HEK293 cells that express green fluorescent protein (GFP). (a) Schematic illustration. Islets are enclosed within a capsule of HEK293 cells (that express GFP) by introducing surface modifications of complementary single-stranded DNAs. Islets modified with oligo(dT)$_{20}$-PEG-lipid are combined with HEK293 cells that have oligo(dA)$_{20}$-PEG-lipid immobilized on the surface. DNA hybridization immobilizes the HEK293 cells to the surface of the islets. After 3 days in culture, islets are completely encapsulated within HEK293 cells. (b) Phase contrast (left panels) and fluorescence images (right panels) of islets with attached HEK293 cells. (0 days) GFP-HEK cells immobilized to islets observed with a confocal laser-scanning microscope; (3 days) Frozen sections of islets with attached GFP-HEK cells were stained with Alexa 488-labeled anti-insulin antibody (green) and Hoechst 33342 dye (blue) for nuclear staining.

11:15-12:00

I-4

Generation of transplantable muscular satellite-like cells from mouse embryonic and murine induced stem cells

Hsi Chang

Department of Pediatrics, School of Medicine, College of Medicine, Taipei Medical University,
Department of Pediatrics, Taipei Medical University Hospital

It was known that satellite cells, the muscle-specific stem cells play a key role in muscle regeneration. Satellite cells differentiate into myoblasts and form myotubes to replace the myofibers damaged by exercise and daily activities. The muscular dystrophies are inherited myogenic disorders of variable distribution and severity that are characterized by progressive muscle wasting and weakness. In many forms of muscular dystrophy, the common molecular defect of the encoded proteins, which are involved in muscular structural integrity, is observed in both immature satellite cells and mature myofibers. Duchene muscular dystrophy (DMD), which is the best-described and most serious form of muscular dystrophy, results from mutations in the X-linked dystrophin gene. Dystrophin and its associated proteins are commonly known to be indispensable for the functioning of the intracellular actin cytoskeleton, as are laminins in the extracellular matrix of muscle fibers, which protect myofibers from contraction-induced damage. Loss of dystrophin causes the rapid and continuous damage of muscles, which leads to the exhaustion of both skeletal muscles and satellite cells, even though muscular regeneration occurs at a higher frequency in DMD patients than in non-affected individuals. Despite extensive efforts to establish pharmacological agents that halt the clinical course of DMD, the disease still results in high mortality in patients during late adolescence.

Skeletal muscle stem/progenitor cell transplantation is considered to be one of the most promising therapies for the muscular dystrophies. Here we report the successful *in vitro* induction of Pax7-positive satellite-like cells from mouse embryonic stem (mES) cells and murine induced pluripotent stem (miPS) cells. Embryoid bodies were generated from both mES and miPS cells and cultured on Matrigel-coated dishes with Dulbecco's modified Eagle medium containing fetal bovine serum and horse serum. Pax7-positive satellite-like cells were enriched by fluorescence-activated cell sorting using a novel anti-satellite cell antibody, SM/C-2.6. SM/C-2.6-positive cells efficiently differentiate into skeletal muscle fibers both *in vitro* and *in vivo.* Furthermore, the cells demonstrate satellite cell characteristics such as extensive self-renewal capacity in subsequent muscle injury model, long-term engraftment up to 24 wk, and the ability to be secondarily transplanted with remarkably high engraftment efficiency compared to myoblast transplantation. This is the first report of transplantable, functional satellite-like
cells derived from mES and miPS cells. We hope these novel findings will provide a foundation for new therapies for degenerative muscle disorders.

Oral Presentations

09:00-09:10

S-1

肌肉注射羊水幹細胞於心肌梗塞治療之效能評估
Intramuscular Injection of Amniotic Fluid Stem Cells Improve Cardiac Functions after Infarction

曾哲揚[1] 彭劭于[2] 吳信志[12]
國立台灣大學動物科技研究所[1] 國立台灣大學生物科技研究所[2]

[Introduction]
Amniotic fluid stem cells (AFSCs) can be obtained with low-invasively procedure, and possess immunosuppressive properties, and shows cardiomyoplasticity in vivo and in vitro. Previous studies showed transplantation of AFSCs has beneficial effects for myocardial infarction (MI) mice, but studies focused on its secretion abilities still lacks. Hence, the aims of this study are to investigate their therapeutic potential on MI mice by using the manner of AFSCs intramuscular injection.
[Materials and Methods]
Porcine AFSCs were isolated from amniotic fluid of E70 porcine fetus of Ds-red-transgenic pig. MI was induced by ligation of the left descending coronary on 8-week-old male C57/B6 mice. MI model mice were divided into 3 groups: medium cell dose ($1x10^6$ cells per leg) group A, high cell dose ($5x10^6$ cells per leg) group B and PBS group C. Cells or PBS were directly injected into hamstring muscle 20 minutes after ligation. Echocardiographs were taken before MI, 2 weeks after MI, and 4 weeks after MI. Then the hearts were processed for histology.
[Results]
AFSCs isolated from Ds-red-transgenic pig express surface antigen CD44 and slight express CD90, do not express CD4a, CD31, and can stably express red fluorescence protein (RFP) after serial passage. Four weeks after MI surgery, injected AFSCs were resided in hamstring muscle. A and B groups preserved significantly better LV ejection fraction when compared with C group ($p<0.05$). And the ejection fraction in A and B groups four weeks after MI persevered in same level when compared to two weeks after MI. Infarct scar sizes in both A and B groups were smaller than in C group ($p<0.05$). Wall thickness in scar region also preserved thicker in A and B group ($p<0.05$).
[Discussion]
Four weeks after MI, we found that AFSCs injected were resided in hamstring muscle but not found in heart. This result suggested that intramuscular injected AFSCs possibly assist recovery of heart morphology and function through their secreting factors. Implied besides AFSC's differential potential, its factor-releasing abilities also have beneficial effects in heart repair.
[Conclusions]
Intramuscular injection of porcine AFSCs can reduce scar size, reduce dilation of ventricle, and preserve better heart function after MI.

09:10-09:20

S-2

間質幹細胞培養於三維微載體時會改變細胞骨架張力導致自發性骨化作用
Spontaneous osteogenesis of MSCs cultured on 3D microcarriers through alteration of cytoskeletal tension

王稚翔[1] 曾佩媜[2,3] 楊台鴻[4] 王廷明[5] 彭筱雯[2] 侯勝茂[3] 嚴孟祿[2,6]
清華大學生物科技研究所[1] 台大醫學院一般醫學科[2]
新光吳火師紀念醫院骨科部[3] 台灣大學醫學工學研究所[4]
台大醫院骨科部[5] 台大醫院婦產部[6]

[Introduction]

In recent years, mesenchymal stem cells have been broadly used as a therapeutic cell source for tissue engineering and autologous transplantation. For clinical applications, 3-dimentsional microcarrier (3D-MC) cell culture systems are often used for expansion of MSC cells. However, compare to 2-dimensional (2D) cell culture, effects of 3D-MC culture systems on MSC differentiations have not been well studied.

[Materials and Methods]

In this study, various sources of MSCs were cultured on 3D collagen I-coated –MCs (COL-MC). For comparison, MSC monolayer 2D culture on uncoated dish or collagen I-coated dished were used as 2D culture controls. Effects of 3D-MC culture on lineage commitment were determined by tri-lineage gene expression patterns and functional assay.

[Results]

Our data showed that proliferation of all MSCs culture on 3D COL-MC was much decreased compared to 2D culture. Unexpectedly, COL-MC cultured MSCs underwent spontaneous osteogenesis, as evidenced by increased osteogenic genes expression, ALP activity, calcium deposition and collagen I secretion. Furthermore, this spontaneous lineage commitment could be prevented by treatment with inhibitors of cytoskeletal tension and actomyosin extraction.

[Discussion]

In our study, we found that 3D-MC culture alters the cytoskeletal tension of MSCs, thereby leads to osteogenic-specific lineage commitment. Our findings are robust as evidence by the consistence of diverse source of MSCs and suggest that the combination of bioengineered MC and MSCs alone are sufficient for lineage specification.

[Conclusions]

Taken together, we demonstrated that altering the culture conditions of MSCs from 2D to 3D-MC is sufficient to induce osteogenesis without requiring exogenous addition of biochemical factors. These data have strong implications in simplifying tissue engineering strategies for therapeutic applications.

[Results]
We are success of making 3D shape (aggregate animal) from mainly TGP polymer (NIPAAm) and live cell.

[Discussion]
This technology is just making 3D shape from MRI data. And we need to make junction structure in space of separate cell. This process will need many case study by target cell and 3D shape.

[Conclusions]
In short, we proposed a comprehensive solution by merging the two existing technologies i.e. tissue engineering technology and the rapid prototyping technology using optical light stereolithography of BPS Technology Sdn Bhd to bring about a paradigm shift in the fabrication of living tissue substitute for therapeutic purposes.

09:20-09:30

S-3

交聯多孔明膠作為生醫工程細胞層片傳輸載體之功能性評估
Functional Assessment of Cross-Linked Porous Gelatin Hydrogels for Bioengineered Cell Sheet Carriers

李雅婷　賴瑞陽*
長庚大學生化與生醫工程研究所

[Introduction]
An efficient carrier for corneal endothelial cell therapy should deliver and retain the cell sheet transplants at the site of injury without causing adverse effects. In this study, we introduced a simple stirring process combined with freeze-drying (SFD) method for the development of gelatin hydrogels with enlarged pore structure that can improve the aqueous humor circulation.
[Materials and Methods]
Samples fabricated by air-drying and freeze-drying methods were used for comparison. After cross-linking with 1 mM 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide, the discs were investigated to assess their functionality.
[Results]
Among all the samples studied, the discs of SFD groups showed the best in vitro and in vivo compatibility and the most appropriate swelling characteristics without squeezing effect on the anterior segment tissues of the eye.
[Discussion]
The simultaneous presence of ice crystals and gas bubbles resulted in large pore size and high porosity of carriers. The enlarged pore structure allowed the materials to contain the highest fraction of mobile water and exhibit the lowest resistance to the glucose permeation. As a temporary supporter, the biodegradable gelatin hydrogel could facilitate cell sheet transfer and avoid long-term residence of foreign carriers in the body.
[Conclusions]
Our findings suggest that the gelatin discs prepared by a simple stirring process combined with the freeze-drying method may have potential as cell sheet carriers for intraocular delivery and corneal tissue engineering.

09:30-09:40

S-4

透明質酸分子量對體外培養之角膜基質細胞貼附、基因表現及生物合成能力影響
Influence of Molecular Weight of Hyaluronic Acid on the Adhesion, Phenotypic Expression, and Biosynthetic Capacity of Cultivated Corneal Keratocytes

俞亭君[1] 涂益豪[1] 賴瑞陽[1*]
長庚大學生化與生醫工程研究所[1]

[Introduction]
In ophthalmology, hyaluronic acid (HA) is an important extracellular matrix (ECM) component and is appropriate for use in generating a microenvironment for cell cultivation. The aim of this work was to evaluate the rabbit corneal keratocyte (RCK) growth in response to different HA coatings under serum-free conditions.
[Materials and Methods]
After modification with HA of varying molecular weights (MWs: 35-1500 kDa), the surfaces were characterized by atomic force microscopy and contact angle measurements, and were used for various cell culture studies.
[Results]
When grown on the surfaces modified with HA of higher MW (i.e., ≥ 360 kDa), the viable RCKs spontaneously aggregate to form multicellular spheroids, which are almost mitotically quiescent with enhanced phenotypes and found to exhibit greater amounts of collagen and GAG production.
[Discussion]
The polysaccharide MW-related surface properties (i.e., charge, topography, and hydrophilicity) of culture substratum are crucial for regulating the cell behaviors such as cell adhesion, spreading, and proliferation. In addition, the keratocyte configuration (i.e., 2D monolayer or 3D spheroid) affects the biosynthetic capacity and gene expression of the cells.
[Conclusions]
It is concluded that the cultured RCKs on surfaces coated with HA of different MWs can sense ECM cues, and the multicellular spheroids may potentially be used for corneal stromal tissue engineering applications.

09:40-09:50

S-5

以碳二亞胺交聯羊膜作為培養角膜輪部幹細胞之生物基質

Carbodiimide Cross-Linked Amniotic Membranes for Cultivation of Limbal Epithelial Cells

張仁杰[1] 馬惠康[2*] 賴瑞陽[1*]
長庚大學生化與生醫工程研究所[1] 長庚醫院眼科[2]

[Introduction]

In ophthalmic tissue engineering, amniotic membrane (AM) is one of the most prevalent natural matrices used for limbal epithelial cell (LEC) cultivation and transplantation. However, the application of AM scaffold is limited by its low biomechanical strength and rapid biodegradation. The present study reports the development of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide (NHS) cross-linked AM as an LEC carrier.

[Materials and Methods]

The collagenous tissue materials were modified with varying cross-linker concentrations (0-0.25 mmol EDC/mg AM) and were characterized by attenuated total reflection-Fourier transform infrared spectroscopy, differential scanning calorimetry, ninhydrin assays, electron microscopy, light transmission measurements, mechanical and in vitro degradation tests, as well as diffusion permeability and cell culture studies.

[Results]

With the optimum concentration of 0.05 mmol EDC/mg AM, chemical cross-linker could significantly enhance the mechanical and thermal stability, optical transparency, and resistance to collagenase digestion. Additionally, the EDC cross-linked samples were able to support LEC proliferation and preserve epithelial progenitor cells in vitro and in vivo.

[Discussion]

Our results showed that chemical cross-linking approaches saturation at concentrations of 0.05 mmol EDC/mg AM. The formation of cross-links (i.e., amide bonds) in these samples may cause significant aggregation of tropocollagen molecules and collagen microfibrils without affecting cell morphology of biological tissues, thereby modulating cell behavior.

[Conclusions]

It is concluded that the AM cross-linked with 0.05 mmol EDC/mg AM may be a potential biomaterial for corneal epithelial regenerative medicine.

09:50-10:00

S-6

以功能性甲基丙烯酸-2-羥基乙酯與丙烯酸共聚物作為人工角膜材料
Development of Photopolymerized Poly(2-Hydroxyethyl Methacrylate)-*co*-Poly(Acrylic Acid) for Keratoprosthetic Biomaterial

張育誠　王祖濱　賴瑞陽*
長庚大學生化與生醫工程研究所

[Introduction]
Development of functional hydrogels for use in anterior lamellar keratoplasty can potentially eliminate the risk of corneal endothelial rejection associated with penetrating keratoplasty. This study aims to copolymerize 2-hydroxyethyl methacrylate (HEMA) with acrylic acid (AAc) to fabricate a transparent, bioinert keratoprosthesis base material that evokes minimal ocular cell/tissue response and suppresses corneal epithelial cell attachment and growth.

[Materials and Methods]
In the presence of varying amounts of monomers (i.e., 72-92% HEMA and 0-20% AAc), initiators (i.e., 2-6% 2,2-diethoxyacetophenone (DEAP)), and cross-linkers (i.e., 2-6% trimethylolpropane trimethacrylate (TMPTMA)), the photopolymerized samples were synthesized successfully by varying reaction time (i.e., 10-50 min), and were characterized by various tests.

[Results]
While the introduction of PAAc into the structure of PHEMA can enhance the water content and oxygen and nutrient permeability, it simultaneously reduces mechanical and suture strength as well as resists protein adsorption and cell adhesion. The in vitro and in vivo studies showed that the membranes obtained from optimized photopolymerization conditions had good compatibility with the corneal epithelial cell cultures and the anterior segment of the eye.

[Discussion]
For an incomplete cross-linked hydrogel prepared with lowest DEAP and TMPTMA content or least UV irradiation time, significant cytotoxicity and inflammation were noted, probably due to the presence of more unreacted C=C bonds.

[Conclusions]
The properties of poly(HEMA-co-AAc) materials obtained from optimized photopolymerization conditions are comparable to those of native corneas, suggesting its promise as a keratoprosthesis.

15:30-15:40

S-7

以硫酸軟骨素修飾多孔明膠作為角膜基質組織工程支架
Porous Gelatin Scaffolds Modified with Chondroitin Sulfate for Corneal Stromal Tissue Engineering Applications

賴孟恆　賴瑞陽*
長庚大學生化與生醫工程研究所

[Introduction]
In this study, we developed corneal keratocyte scaffolds by the modification of porous gelatin materials with chondroitin sulfate (CS) using carbodiimide chemistry.
[Materials and Methods]
The water content and light transmission measurements, mechanical and in vitro degradation tests, and glucose permeation and in vitro biocompatibility studies were performed to examine the relationship between CS content and scaffold functionality.
[Results]
Scanning electron microscopy/energy dispersive x-ray spectroscopy and Fourier transform infrared spectroscopy showed that the amount of covalently incorporated polysaccharide was significantly increased with increasing CS feed concentration. The hydrophilic and mechanical properties of scaffolds modified with 0.25% CS were comparable to those of native corneas.
[Discussion]
Given that the generation of amide bonds between gelatin and CS is affected by the collision frequency of these biomacromolecules, the higher feed concentration may lead to the covalent incorporation of larger amount of CS into the porous gelatin scaffolds. Our findings also suggest that the modification gives influence on the characteristics and cell-material interactions of CS containing gelatin hydrogels.
[Conclusions]
In summary, the porous membranes with the optimal CS content may hold potential for use in corneal stromal tissue engineering.

15:40-15:50

S-8

玻尿酸/纖維蛋白膠促進人類脂肪幹細胞軟骨化於體外培養之軟骨缺損模式之研究

Hyaluronan-enriched fibrin gel enhances chondrogenesis of human adipose-derived stem cells for chondro-defect repair in explant cultures

黃珮詒[12] 林怡珊[2] 林松彥[2] 王昭仁[12] 何美泠[12]
高雄醫學大學醫學系生理學科[1] 高雄醫學大學骨科學研究中心[2]

[Introduction]
The self-repaired capability of articular cartilage is limited; moreover, cartilage injuries may result in osteoarthritis. Nowadays, tissue engineering is an alternative way to restore cartilage defects. Our previous in vitro study indicates that Hyaluronic acid (HA)/fibrin gel increased chondrogenic gene expressions and sulfated glycosaminoglycans (sGAG) formation in human adipose-derived stem cells (hADSCs), indicating HA microenvironment is critical for chondrogenic differentiation of ADSCs. In this study, we hypothesize that HA-enriched fibrin gel may induce hADSCs chondrogenesis and thus enhance the repair of porcine full-thickness chondral defects.
[Materials and Methods]
In the ex vivo study, we created a chondral defect (diameter: 2mm) at the center of isolated osteochondral disc from porcine femoral condyle. The HA/fibrin/hADSCs, fibrin/hADSCs, HA/fibrin or fibrin gel was implanted in the defects and then cultured for 4 weeks. To evaluate the effect of HA/fibrin/hADSCs in the repair porcine chondral defect using histological analysis.
[Results]
We found that HA/fibrin/hADSCs group showed more sGAG formation and have better integration with host cartilage than other groups.
[Discussion]
To evaluate the effect of HA/fibrin/hADSDs in vivo in a porcine model to confirm the results of explants culture is needed in future study..
[Conclusions]
The results demonstrated that HA/fibrin may help the regeneration of articular cartilage defect in the ADSCs-based tissue engineering.

15:50-16:00

S-9

細胞型態對機械刺激之延遲反應
Delayed Cell Morphology Responses to Mechanical Stimulation

溫新民　趙本秀
國立台灣大學醫學工程研究所

[Introduction]
Cytoskeleton is one of the proposed mechanotransducers to convert mechanical loads into intracellular chemical signals during mechanotransduction, as well as a response element to the stimulation. As cytoskeleton organizations are different for different cell types, we directly compared the effects of mechanical loading between the differentiated and undifferentiated cells. Furthermore, most studies examining cell morphology change after mechanical loading focus on the immediate effects after loading. However, there may be long term consequences and the recovery period after loading may have additional effects on the cell. We therefore examined cell morphology changes immediately after loading, as well as after 24 hours of incubation.

[Materials and Methods]
Primary anterior cruciate ligament fibroblasts (LFs) were harvested from porcine knee and human bone marrow-derived mesenchymal stem cells (MSCs) were obtained from Dr. Shih-Chieh Hung (YMU). Cells were seeded on PDMS membranes coated with type I collagen for 12 hours and subjected to 3 hours of deformational loading (10% linear tension or compression) at statically or at 0.5 Hz (dynamic). After stimulation, the cells were observed immediately or after 24 hours in the seeding configuration. Cells were stained for f-actin and visualized by confocal microscopy.

[Results]
When subjected to dynamic tensile loading, LFs exhibited increased elongation immediately after stimulation (based on aspect ratio calculations) when compared with the control group. However, LFs subjected to dynamic compressive loading or static loading of any kind, did not exhibit any morphology changes. Interestingly, after 24 hours, LFs from all of the stimulated groups exhibited elongation and alignment (perpendicular to the direction of deformation), indicating a delayed response. When MSCs were subjected to the same mechanical loads, only the static groups exhibited elongation at 3 hours. No elongation was observed for the dynamically loaded groups, or for any group after 24 hours. Further examination of the actin cytoskeleton revealed that LFs exhibited more stress fibers, which was not found in MSCs.

[Discussion]
Cells are subjected to a number of physical stimulation in vivo. For example, LFs are in constant tensile and compressive stimulation. Bone marrow-derived MSCs, on the other hand, reside in a ‘niche’ environment with minimal deformation. MSCs have also been reported to be more compliant, with a less structured cytoskeleton. Results from our study demonstrate the different response of LFs and MSCs to the same mechanical stimulation,

possibly reflecting their organization and native physical microenvironment. Moreover, while some of the MSC groups respond immediately to mechanical loads, no effect was observed after 24 hours. LFs, interestingly, exhibited a delayed response to stimulation, suggesting a long-term effect.

[Conclusions]

Differentiated LFs exhibited delayed morphological responses to both static and dynamic deformational loading, with more actin stress fibers. The undifferentiated MSCs responded immediately to static loadings, recover completely after 24 hours, and did not display stress fibers. These results may arise from the different phenotypic cytoskeleton structure as well as the native mechanical environment in situ.

16:00-16:10

S-10

脂肪組織幹細胞在甲殼素薄膜表面形成球狀體可提升其幹細胞特性及分化能力

Spheroid Formation of Human Adipose-derived Stem Cells on Chitosan Films Enhances Stemness and Differentiation Capabilities

鄭乃禎[1,2] 王珊[2] 楊台鴻[1]

國立台灣大學醫工所[1] 國立台灣大學醫學院附設醫院 外科部 整形外科[2]

[Introduction]

Because of their abilities of rapid proliferation and differentiation into multiple cell types, mesenchymal stem cells are regarded to have great potentials for application in regenerative medicine. The adipose-derived stem cells (ASCs) have been shown to share similar characteristics of mesenchymal stem cells isolated from bone marrow. Previous reports suggested that culture as 3D spheroids can increase therapeutic potentials of mesenchymal stem cells. Therefore, we aimed to manipulate the spheroid formation of human ASCs by culturing them on chitosan films.

[Materials and Methods]

Chitosan-coated tissue culture plates were prepared for human ASC culture. The ASCs were passaged 3 times and plated onto chitosan films or tissue-culture polystyrene with the culture medium consisting of DMEM-high glucose, 10% FBS, 1% penicillin-streptomycin. Human ASCs were seeded at different densities onto chitosan films, and the number and diameter of spheroids per well in each group were measured. Culture media was refreshed every 2–3 days. Cell viability was assessed by the alamar blue assay and the live/dead analysis. Cell apoptosis was analyzed by flow cytometry measuring propidium iodide uptake and annexin V labeling. Expression of stemness genes were analyzed by real-time PCR. Histology and immunohistochemistry of the ASC spheroids were also obtained. Differentiation and animal injection assays were also performed.

[Results]

Human ASCs spontaneously formed three-dimensional spheroids on chitosan films. The ASC spheroids aggregated faster and with a larger diameter as the seeding density increased. Most ASCs within the spheroid were viable, and the cells produced more extracellular molecules, like laminin and fibronectin. Comparing to monolayer culture, ASC spheroids also exhibited enhanced cell survival in serum starvation condition. Although cell proliferation was inhibited in spheroids, ASCs readily migrated out and proliferated upon transferring spheroids to another adherent growth surface. Moreover, spheroid-derived ASCs exhibited higher expansion efficiency and colony -forming activity. Importantly, we demonstrated that spheroid formation of human ASCs on chitosan films induced significant upregulation of pluripotency marker genes (*Sox-2*, *Oct-4* and *Nanog*). The protein expression of these stemness markers were confirmed by western blot analysis and immunofluorescence. By culturing the ASC spheroids in proper induction media, we found that ASC differentiation capabilities were significantly enhanced after spheroid formation,

including increased transdifferentiation efficiency into neuron and hepatocyte. In a nude mice model, we further showed a significantly higher cellular retention ratio of ASC spheroids after intramuscular injection of spheroids and dissociated ASCs.

[Discussion]

We found that ASC spheroid formation on chitosan films enhanced the stemness and differentiation capabilities of ASCs by upregulation of stemness-related genes. Different cell behaviors of ASCs derived from spheroid and monolayer cell culture were demonstrated. Cells in spheroids are in close association with each other and probably signal cues to each other much easier than in monolayer cultures. The changes in the ASCs as they form spheroids are probably the result of the nonadherent culture conditions provided by the chitosan films.

[Conclusions]

The results presented here indicated that ASCs can be activated nonchemically on chitosan films to form spheroids and express pluripotent marker genes. When cultured in appropriate madia, ASC spheroids demonstrated enhanced transdifferentiation capabilities into neuron and hepatocyte. Therefore, spheroid ASCs formed on chitosan films may have potential advantages for many clinical applications.

16:10-16:20

S-11

探討 Rho signaling 參予骨隨幹細胞進行的骨分化
Rho A signaling contributes to statin-induced osteogenesis in bone marrow mesenchymal stem cells

戴宜均.[1,2]; 王耀賢.[2]; 張瑞根.[2,3]; 何美泠 [+1,2]
高學醫學大學醫學研究所 [1] 高雄醫學大學骨科學研究中心 [2]
高雄醫學大學附設醫院骨科 [3]

[Introduction]
Statins, 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, reduce cholesterol synthesis and prevent cardiovascular disease. Previous studies showed that statin stimulated bone formation *in vitro* and *in vivo*. They have been found to inhibit proteins prenylation in recent decade. This effect decreased the active form of Rho A and further induced apoptosis in osteoclasts. However, other study showed that statins inhibited protein prenylation but increased the active form of Rho A in human erythroleukemia cells. Therefore, the role of statins in Rho A activity regulation remains unclear. Rho GTPases act as molecular switches to regulate mesenchymal stem cell differentiation. Transfection of constitutively active-form Rho A into human mensenchymal stem cells (hMSCs) 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 Rho A signaling contributes to statins-induced osteogenesis in BMSCs.

[Materials and Methods]
Pluripotent mesenchymal cells, D1, which were cloned from Balb/c mouse bone marrow cells and purchased from ATCC. For all experiments, cells were seeded at a density of 70% confluence and treated with or without simvastatin. The mRNA expression of Rho A was detected and quantified by real time PCR. The activity of Rho A was detected by pull down assay. And the mineralization effect on D1 cells was tested by Alizarin Red S Staining. The Alizrin red S staining assay showed that the simvastatin were potentially enhanced the cell mineralization on BMSCs.

[Results]
The Alizrin red S staining assay showed that the simvastatin were potentially enhanced the cell mineralization on D1 cells. The mRNA expression of Rho A showed that simvastatin significantly increased Rho A gene expression dose-dependently at first day. However, there were no significantly different between control and treatment group at third and fifth days. Simvastatin dose-dependently increased the activity of Rho A at second day.

[Discussion]
Simvastatin induces deprenylation of proteins, however deprenylation seems not to affect the activation of Rho A. We still need to clarify the status of active form Rho A. How simvastatin activate Rho A and its downstream signaling for acceleration of osteogenesis requires further investigations.

[Conclusions]
From these results, we suggest that simvastatin enhances osteogenesis rBMSCs might through activate Rho A signaling.

16:20-16:30

S-12

甲殼素表面塗佈對脂肪幹細胞形成球狀體及神經分化潛力之探討
Spheroid formation and neuronal induction in adipose derived stem cells by chitosan surface coating

薛元毓 [1,3] 吳佳慶 [2] 林聖哲 [1]
國立成功大學醫學院附設醫院 外科部 整形外科 [1]
國立成功大學醫學院 細胞生物暨解剖學研究所 [2]
國立成功大學臨床醫學研究所 [3]

[Background]
The adipose derived stem cells (ASCs) have been proven a similar potential of multipotency comparing to bone marrow stem cells and can be differentiated into adipose, cartilage and bone as well. Moreover, the ability of trans-differentiation from ASCs into neural lineage can provide a scope of post-natal stem cell therapy over the neurodegenerative disease or spinal cord injury. Chitosan, a naturally derived polysaccharide from chitin, is widely studied to facilitate nerve regeneration using chitosan neural tube to guide the direction of nerve regeneration and to improve survival of neural stem cells. The purpose of this study is aimed at the effect of transdifferentiation on spheroid formation and neuronal induction of ASCs by chitosan surface coating without any aid of neurotrophic factors.
[Materials and Methods]
Aim1: In order to obtain the detail about roughness of the chitosan coating plate, we use the atomic force microscope to test the surface roughness of chitosan coating surface. Besides, we use live and death assay to determine the optimal cell coating density and timing in order to gain the best survival rate within the spheroid. **Aim2:** After determination of optimal cell density and timing, we try to prove the transdifferentiation of ASCs from mesodermal lineage to neural lineage, by using immunocytochemisty stain and western blot. Besides, we investigate the difference of surface marker expression within the 3-D structure of spheroid in the aid of confocal microscopy. **Aim3:** We follow the neurosphere culture assay to manipulate the primary, secondary and tertiary spheroid formation. The same methods described in aim2 are adopted to investigate the neuronal lineage potential.
[Results]
The optimal condition of spheroid formation from ASCs is $2x10^4$ at 48 hours, which is determined by live and death assay using trypan blue stain. The neural lineage surface marker of nestin, neurofilament heavy chain and glial fibrillary acidic protein are expressed

by immunofluorescent stain and the western blot shows also similar protein expression. Replating of the spheroid to form secondary and tertiary sphere also shows enhanced expression of neural marker.

[Conclusions]

Spheroid formation can be induced by treating ASCs with chitosan surface coating. Besides, neural-lineage potential is induced under the influence of such microenvironment, instead of aiding various neurotrophic factors. This can provide a scope of post-natal stem cell therapy over the neurodegenerative disease or spinal cord injury in the future.

16:30-16:40

S-13

低強度超音波在神經幹細胞上的效應
Effects of Low Intensity Ultrasound on Neural Stem Cells

羅子琳[1] 李亦宸[2] 李亦淇[1*]
長庚大學生化與生醫工程研究所[1] 國立台灣大學醫學工程研究所[2]

[Introduction]
The capacity of neural stem cells (NSCs) to regenerate functional neural cells has raised hopes for the treatment of harsh neural diseases and injuries. In vitro, these NSCs demonstrate the ability to self-renew and the potential to differentiate into various cell types. Previous studies showed that the physical and chemical signals, coming from cell living microenvironment and cell-cell interaction strongly affect the stem cell behaviors. For example, low intensity ultrasound (LIU) was applied on many different research fields. Our preliminary data indicate to explore the effect of LIU on NSCs proliferation and differentiation that might provide a feasible method on the regulation of NSCs.

[Materials and Methods]
Cells were prepared from pregnant Wistar rat embryos. These cells were resuspended in a serum-free medium. LIU was operated at indicative period and intensity. The cell viability was determined by using LDH assay. Phase-contrast images were used to estimate morphology and to quantify cell process length. Immunocytochemical staining was performed to analyze differentiated cell phenotypes. We also analyze the extent of cells differentiation extent via a software.

[Results]
In our observation, except for the glass only group, neurites length on the other three culture conditions with LIU stimulation increased with the culture time. Compared to the control group, differentiated neurospheres seemed to have some thicker and longer neurites in LIU stimulation group. It suggests that the LIU stimulation will induce the NSCs differentiation and neurite outgrowth. Results of immunocytostaining revealed that LIU stimulation could promote the attachment of neurospheres and to up-regulate the percentage of neuron and astrocytes differentiation. Moreover, the neural network connected by process between neurospheres with LIU stimulation better than that on the control group .

[Discussion]
According to the literature, there are many specific bioeffects via LIU stimulation have been reported. The mechanism of cavitation and acoustic streaming probably opens some channel on NSC membrane, that might directly promote the cell metabolism.

[Conclusions]
Effects of applied LIU seem to promote NSCs differentiation and help NSCs to build up a well signaling network by the extension of neurite outgrowth. Cell response from LIU stimulation is due to combined complex physical, chemical and biological parameters. This study could help us to develop a regulated system to optimize NSCs microenvironments and their behavior.

第五屆第一次會員大會

台灣再生醫學學會第四屆理、監事名單
(照筆劃順序排列)

理 事 長：侯勝茂

常務理事：江清泉、徐善慧、陳耀昌、蔡清霖

理　　事：林峰輝、侯連圍、洪士杰、張至宏、張恒雄、
陳文哲、黃玲惠、楊俊佑、楊榮森、嚴孟祿

候補理事：林高田、陳志華、陳英和、蘇正堯

常務監事：黃義侑

監　　事：林文澧、林泰元、孫瑞昇、蔡文基

候補監事：譚傳明

第五屆第一次會員大會議程

時間：民國101年2月25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：侯勝茂 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、選舉第五屆理監事

七、散會

100年度工作報告

理事會報告

一、召開理監事會議計四次。

二、會員人數增加至一百八十一人。

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。

101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 100 年 1 月 1 日至 100 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**	**225,132**	**700,000**			
	1		會費收入	69,000	170,000		101000	含入會費常年會費
	2		捐款收入	130,000	500,000		370000	廣告攤位收入及贊助會員捐款等
	3		利息收入	26,132	30,000		3868	郵局, 銀行利息
			本會支出	**384,032**	**1,035,000**			
2	1		人事費	145,000	150,000		5,000	員工及加班費
	2	1	文具	3,770	30,000		26,230	
		2	印刷費	46,914	40,000	6,914		
		3	郵電費	2,859	20,000		17,141	
		4	雜項	2,281	60,000		57,719	
	3	1	業務費	45,000	100,000		55,000	召開理監事會及辦理相關研討會所需之費用
		2	會議費	40,937	200,000		159,063	
		3	交通費	3,500	100,000		96,500	
			其他業務費	93,771	300,000		206,229	召開2月份年會
			本期結餘	(158,900)				

理事長：侯勝茂　秘書長：楊台鴻　常務監事：黃侑義　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 101 年 1 月 1 日至 101 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	700,000	700,000			
	1		會費收入	170,000	170,000			含入會、費常年會費
	2		捐款收入	520,000	500,000	20,000		廣告攤位收入及贊助會員捐款等
	3		利息收入	10,000	30,000		20,000	郵局銀行利息
			本會支出	700,000	1,035,000			
2	1		人事費	150,000	150,000			員工薪資及加班費
	2	1	文具	15,000	30,000		15,000	
		2	印刷費	50,000	40,000	10,000		
		3	郵電費	10,000	20,000		10,000	
		4	雜項	25,000	60,000		35,000	
	3	1	業務費	100,000	100,000			召開理監事會及辦理相關研討會所需之費用
		2	會議費	100,000	200,000		100,000	
		3	交通費	20,000	100,000		80,000	
			其他業務費	200,000	300,000		100,000	
			提撥基金	30,000	35,000			依收入總額提列5%作為準備基金

理事長：侯勝茂　秘書長：楊台鴻　常務監事：黃侑義印　會計：楊惠昭　製表：楊惠昭

台灣再生醫學學會

工作人員待遇表

職稱	姓名	性別	到職日年月日	月支薪餉	備註
秘書	楊惠晶	女	97.12.01	NT$10,000	現任

理事長：侯勝茂　秘書長：楊台鴻　常務監事：黃侑義　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 100 年 1 月 1 日至 100 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	$2,080,120	本期支出	$384,032
本期收入	$225,132	本期結餘	$1,921,220
合計	$2,305,252	合計	$2,305,252

理事長：侯勝茂　秘書長：楊台鴻　常務監事：黃侑義　會計：楊惠晶　製表：楊惠晶

台灣再生醫學學會
資產負債表
中華民國 100 年 1 月 1 日至 100 年 12 月 31 日止

資　產		負　債	
科　　目	金額	科　　目	金　　額
庫存現金	$1, 921,220	累計基金	$96,000
--	--	本期結餘	$1, 825,220
合計	$1, 921,220	合計	$1, 921,220

理事長：侯勝茂　秘書長：楊台鴻　常務監事：黃侑義印　會計：楊惠昌　製表：楊惠昌

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	090	吳錫銘
002	侯勝茂	030	徐郭堯	062	范揚峰	091	李宣書
003	陳耀昌	032	吳輝傑	063	戴浩志	092	楊長彬
004	楊台鴻	033	蕭逸民	064	洪士杰	093	王貞棣
005	楊榮森	034	李建和	065	王世南	095	楊曙華
006	楊俊佑	036	黃振勳	066	劉有漢	096	邱錦輝
007	林峰輝	037	施庭芳	067	許致榮	097	郭兆塋
008	林文澧	038	侯連圍	068	黃國淵	098	陳學明
009	王清貞	039	陳志華	069	李裕滄	099	林柳池
010	黃義侑	040	李炫昇	070	陳沛裕	100	潘如瑜
011	王兆麟	041	張瑞根	072	林頌然	101	楊維宏
012	江清泉	044	李敏旭	073	游敬倫	102	劉明偉
013	石朝康	045	江鴻生	075	陳吳坤	103	王文志
014	蔡清霖	046	陳昭宇	076	鄧文炳	104	方旭偉
015	張恆雄	047	張宗訓	077	鄭耀山	105	陳敏慧
017	蘇芳慶	048	釋高上	079	簡松雄	106	張明熙
018	陳瑞明	050	張至宏	080	郭繼陽	107	陳興源
019	陳全木	051	蔡慶豐	081	王世杰	108	蔡文龍
020	童瑞年	054	楊治雄	082	蔡友士	109	郭宗甫
021	殷金儉	055	蔡文基	083	王至弘	110	王禎麒
022	何始生	056	林高田	084	張志豪	111	湯月碧
023	孫瑞昇	057	古鳴洲	085	趙建銘	112	黃玲惠
026	陳文哲	058	宋信文	087	曾鵬文	113	王佩華
027	周正義	059	姚俊旭	088	徐明洸	114	郭源松
028	陳英和	060	呂紹睿	089	詹益勝	115	翁文能

編號	姓　名	編號	姓　名	編號	姓　名	編號	姓　名
116	徐善慧	136	高國慶	156	傅再生	176	侯添財
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		
123	曾育弘	143	胡育誠	163	李一麟		
124	林佐文	144	黃維超	164	何美泠		
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		

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：