細胞與其衍生物應用於再生醫學暨2021年台灣再生醫學學會學術研討會

Regenerative Medicine with Cells and Cell Derivatives
2021 Annual Meeting of FARM

台灣再生醫學學會
Formosa Association Regenerative Medicine
Since 2004

摘要集

2021 年 3 月 27 日
亞東紀念醫院國際會議廳

主辦單位： 台灣再生醫學學會、亞東紀念醫院骨科部
協辦單位： 國家衛生研究院、科技部生命科學研究推動中心、
衛生福利部、台灣細胞醫療協會、臺灣幹細胞學會

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Content

亞東紀念醫院

特管辦法開放後兩年
台灣再生醫療現況與未來 揭密
再生醫學研討會 3/27 在亞東舉行

理事長的話

台灣自 107 年 9 月通過「特管法」(全稱「特定醫療技術檢查檢驗醫療儀器施行或使用管理辦法」修正條文)，開放 6 項細胞治療技術，再加上衛福部積極推動「再生醫療專法」立法；雙法齊下，使台灣的「再生醫療」從如火如荼的研發階段正式進入臨床應用。

成立於 2003 年的台灣再生醫學學會，每年都會舉辦國內/國際研討會。在秘書長鄭乃禎教授與副秘書長楊凱強教授的協助之下，今年年會將於 3 月 27 日(六)於亞東醫院盛大舉行，大會主題定名為「細胞與其衍生物應用於再生醫學」。今年雖然有新冠肺炎疫情，仍然邀請到三位國際學者參與。

近年來再生醫療的發展，為台灣的病人帶來極大的福祉。在台灣公布特管法之後，已經有許多醫院開始執行自體細胞再生醫療的實際臨床應用。這些都是歷年來台灣醫界學界的基礎研究，加上衛福部政策的導引開放，以及各位專家勇於應用於臨床治療的結果。因此本次學會年會，特別著重在政策發展，基礎研究，與臨床成果這三大面向。

首先，在政策方面，邀請到我國衛福部石崇良次長談『台灣再生醫療相關專法的展望』，財團法人醫藥品查驗中心審查員的湯伊寧醫師談『特管辦法細胞治療技術成效評估機制與風險分級』，韓國 Bryan Choi 教授談『Overview about the New Regulation on Regenerative Medicine in Korea』三位主講者分享。

其次，基礎研究方面，邀請到長庚兒童醫學中心名譽院長林奏延教授談『Prospective of Mesenchymal Stem Cell Derived Exosome』，Prof. Pburnouf Thierry 談『Applications of extracellular vesicles in cell therapy and regenerative medicine』，Prof.L. James Lee 談『Extracellular Vesicles Based Regenerative Medicine and Disease Therapy』。高雄長庚紀念醫院外科部部長謝青華教授談『Sharing of the experience in exosome research from isolation, characterization, and experiments in the cells and animals』。學者專家、臨床醫療人員，齊聚一堂進行「再生醫學治療與外泌體等幹細胞衍生物」最新研究成果的交流。

最後的壓軸主題，有鑒於特管辦法通過後，大部分的計畫都著重在癌症的免疫細胞治療。但是我們仍注意到國內許多專家，在癌症醫療以外的組織之再生與修復上，仍然提出許多成功的治療案例。因此學會特別敦聘本會創會理事長及台大榮譽教授劉華昌醫師談『Repairing cartilage defects with chondrocyte precursor』，義大醫院院長杜元坤教授談『細胞療法在軟骨重建與神經再生的最新發展』，花蓮慈濟醫院林欣榮院長談『Stem cells and combination therapy with rehabilitation for chronic stroke』，三軍總醫院戴念梓教授談『整形外科細胞治療案例分析與成果報告』，中國醫藥大學附設醫院整合幹細胞中心洪士杰教授談『Epigenetic regulation of self- renewal, differentiation and oncogenesis of mesenchymal stem cells』，林口長庚紀念醫院關節重建科主任張毓翰教授談『細胞治療在退化膝關節的應用』。至於我本人，也將分享世界上首次將膝關節脂肪墊培養出來的幹細胞，用於治療膝關節退化性關節炎之人體試驗成果，以及進一步通過特管辦法後，以此細胞治療結合矯正截骨術與關節內視鏡技術的治療成效。

本次年會敬邀台灣學界、醫界的國內再生醫學研究人員參加。3/27 當天的研討會，從早上 9:00 密集進行到下午 6:00，總計 14 個講題。並且舉辦論文競賽，各界各院校投稿壁報論文共計 25 篇，以鼓勵相關領域之研究人員。期望在新冠肺炎的疫情之下，學會仍然能持續扮演橫跨政策，研究與臨床應用之間的橋樑，持續為台灣再生醫療貢獻心力。也期待各位同好的熱心參與與支持。

張至宏 理事長

細胞與其衍生物應用於再生醫學暨 2021 年台灣再生醫學學會學術研討會
Regenerative Medicine with Cells and Cell Derivatives / 2021 Annual Meeting of FARM
Scientific Program

Time	Topic	Speaker	Institute	Moderator
08:30	**Registration 報 到**			
Session 1				
09:00~09:20	Opening Remark 邱冠明教授 亞東紀念醫院副院長 林啟禎教授 中華民國骨科醫學會理事長 / 醫策會董事長			
I-01 09:20~09:45	台灣再生醫療相關專法的展望	石崇良常務次長	行政院衛生福利部	陳耀昌教授 林峯輝教授
I-02 09:45~10:10	Prospective of Mesenchymal Stem Cell Derived Exosome	林奏延教授	長庚兒童醫學中心名譽院長 林口長庚紀念醫院兒童感染科	
10:10~10:30 **Group photo / Coffee Break**				
Session 2				
I-03 10:30~10:55	特管辦法細胞治療技術成效評估機制與風險分級	湯依寧醫師	財團法人醫藥品查驗中心	楊榮森教授 沈家寧教授
I-04 10:55~11:20	Overview about the New Regulation on Regenerative Medicine in Korea	Prof. Bryan Choi	Inha University College of Medicine	
I-05 11:20~11:45	Sharing of the Experience in Exosome Research from Isolation, Characterization, and Experiments in the Cells and Animals	謝青華教授	高雄長庚紀念醫院整形外科	陳敏慧教授 楊台鴻教授
I-06 11:45~12:10	Applications of Platelet Derivatives and Extracellular Vesicles in Cell Therapy and Regenerative Medicine	Prof. Thierry Burnouf	臺北醫學大學生物醫學工程學院生醫材料暨組織工程研究所	
12:10 **會 員 大 會** 12:10~13:30 Lunch Break				

Time	Topic	Speaker	Institute	Moderator
Session 3				
I-07 13:30~13:55	Extracellular Vesicles Based Regenerative Medicine and Disease Therapy	Prof. L. James Lee 李利教授	美國俄亥俄州立大學 化學與分子工程學系 國立陽明交通大學生物藥學研究所	何美泠教授 王至弘教授
I-08 13:55~14:20	Repairing Cartilage Defects with Chondrocyte Precursor	劉華昌教授	臺安醫院骨科部	
I-09 14:20~14:45	以膝關節脂肪墊幹細胞治療退化性關節炎的簡介與初步臨床成果	張至宏理事長	亞東紀念醫院骨科部	陳志華院長 孫瑞昇教授
I-10 14:45~15:10	細胞療法在軟骨重建與神經再生的最新發展	杜元坤教授	義大醫院院長/骨科部	
15:10~15:30 **Group photo / Coffee Break**				
Session 4				
I-11 15:30~15:55	Stem cells and Combination Therapy with Rehabilitation for Chronic Stroke	林欣榮教授	花蓮慈濟醫院院長/ 神經外科	張瑞根教授 黃玲惠教授
I-12 15:55~16:20	細胞治療案例分析與成果報告	戴念梓教授	三軍總醫院整形外科	
I-13 16:20~16:45	Epigenetic Regulation of Self-renewal, Differentiation and Oncogenesis of Mesenchymal Stem Cells	洪士杰教授	中國醫藥大學附設醫院骨科部 整合幹細胞中心	嚴孟祿教授 徐善慧教授
I-14 16:45~17:10	細胞治療在退化膝關節的應用	張毓翰教授	林口長庚紀念醫院骨科部	
17:10~17:30 Closing Remarks & Poster Competition Award				

壁報 Poster

評審委員：黃義侑教授、楊凱強教授

壁報論文解說時段：13:00~14:00。

No.	Classification	Topic	Authors	Institute
P-01	Biomaterials	Bioactive Perovskite Quantum-Dots/Gelatin Nanoparticle for Mornitoring Drug Delivery Applications in Eyes	Le Ngoc Hoang [1] 林群哲 [2,*] 曾靖孋 [1*]	臺北醫學大學生醫材料暨組織工程研究所 [1] 國立臺北科技大學分子科學與工程系暨有機高分子研究所 [2]
P-02	Biomaterials	Cell Detachment on pH-responsive Blended Surfaces: The Development of Continuous Cell Harvest of Human Adipose-Derived Stem Cell with Enhanced Regenerative Capacity	顏嘉祥 [1] 楊台鴻 [1] 鄭乃禎 [2]	國立台灣大學醫學工程研究所 [1] 國立台灣大學附設醫院外科部 [2]
P-03	Biomaterials	Use the Platelets Extracellular Vesicles to Lading the Kaempferol for Anti-angiogenic	張哲禕　白台瑞 曾靖孋	台北醫學大學生醫材料暨組織工程研究所
P-04	Bone Marrow Stem Cells	Resident vs. Non-resident Multipotent Mesenchymal Stromal Cell Interactions with B Lymphocytes Result in Disparate Outcomes	李瑋 [1,2] 王麗姿 [3] 嚴孟祿 [3] 徐珮茹 [2] 李雨薇 [2] 劉柯俊 [4] 林國儀 [5] 蘇郁文 [6] 司徒惠康 [7] 顏伶汝 [2]	國防醫學院生命科學研究所 [1] 國家衛生研究院細研所 [2] 台大醫學院婦產科 [3] 國衛院癌研所 [4] 中央研究院基因體中心 [5] 國衛院免疫所 [6] 國衛院感疫所 [7]
P-05	Bone Marrow Stem Cells	The Possibility of Using Binary Colloidal Crystals Arrayed on Surface to Modulate Stem Cells Differentiated in to Retinal-like Epithelium Cells for Retina Repair	張哲禕 [1] 陳盈汝 [1] 張睿 [1] 范育睿 [1] 王鵬元 [2, 3] 曾靖孋 [1]	台北醫學大學生醫材料暨組織工程研究所 [1] 澳洲國立旋濱科技大學 [2] 中國科學院大學 [3]
P-06	Bone Marrow Stem Cells	Ultrasound and Piezoelectric Field Stimulate Human Mesenchymal Stem Cells Migration, Proliferation and Differentiation	劉禹呈　黃文顥 林若梅　王兆麟	國立台灣大學醫學工程研究所
P-07	Neural Stem Cells	An *in vivo* Study of Inducing Neurogenesis in Mouse Brain Upon Ultrasound Stimulation	戴小芯　林若梅 朱亞成　王兆麟	國立台灣大學醫學工程研究所
P-08	Others	Intratumoral Injection of Autologous CD16^{+} Dendritic Cells and Anti-PD-L1 Antibody Combined with Radiotherapy: The Triple-Regimen Therapy in a Psoriatic Patient with Advanced Cutaneous Squamous Cell Carcinoma	楊秉恆 [1,2] 李玉鳳 [3] 王麗姿 [4] 黃文彥 [5] 劉峰誠 [6] 楊崑德 [7,8,9]	國防醫學院醫學科學研究所 [1] 三軍總醫院病理部臨床病理科 [2] 國防醫學院醫學系 [3] 台大醫院婦產部 [4] 三軍總醫院放射腫瘤部 [5] 三軍總醫院內科部風濕免疫科 [6] 國防醫學院微生物及免疫學科暨研究所 [7] 國立陽明大學臨床醫學研究所 [8] 馬偕紀念醫院兒童過敏免疫風濕科 [9]

P-09	Regenerative Medicine	3-Dimensional (3D) Sphere Formation in Stem Cells vs. Somatic Cells: Involvement of Conserved Pathways	張家齊 [1, 2, 4] 江士昇 [3] 李雨薇 [2] 徐珮茹 [2] 顏伶汝 [2] 嚴孟祿 [4]	國防醫學院生命科學研究所 [1] 國家衛生研究院細胞及系統醫學所 [2] 國家衛生研究院癌症研究所 [3] 台灣大學醫學院醫學系婦產科 [4]
P-10	Regenerative Medicine	Development of 3D Cell Spheroids of MSC-derived Schwann-like Cells for Treating Peripheral Nerve Injury	李筠蔚 [1] 林郁婕 [1] 張哲瑋 [1,2] 黃玠誠 [1*]	國立清華大學生物醫學工程研究所 [1] 國立清華大學醫學科學系 [2]
P-11	Regenerative Medicine	Injectable 3D Hybrid Cell Spheroid as a Platform for Replenishing Glomerular Podocyte	陳立騏 [1] 楊文妤 [1] 許翔皓 [2] 黃玠誠 [1*]	國立清華大學生物醫學工程研究所 [1] 林口長庚醫院腎臟科 [2]
P-12	Regenerative Medicine	Intravital Imaging of the Mammalian Tissues by Lightsheet Microscopy	吳岳峰 [1] 張煒塋 [2] 譚欣媛 [3] 潘明楷 [4] 陳壁彰 [5#] 江安世 [2#] 朱麗安 [2,6#] 林頌然 [1, 2, 7, 8#] [#]Contributed equally	台大醫學工程學系 [1] 清大腦科學研究中心 [2] 林口長庚醫院眼科 [3] 台大藥理學科暨研究所 [4] 中研院應用科學研究中心 [5] 清大生醫工程與環境科學系 [6] 台大醫院皮膚科 [7] 台大發育生物學與再生醫學研究中心 [8]
P-13	Regenerative Medicine	Mesenchymal Stem Cells Primed with Nitric Oxide-Releasing Dinitrosyl Iron Complex Exhibit Enhanced Post-engrafted Survival and Therapeutic Potential	王歆雯 謝麗虹 [1] 魯才德 [1] 黃玠誠 [1*]	國立清華大學生物醫學工程研究所
P-14	Tissue Engineering	A Versatile 3D Stem Cell Spheroid-derived Matrix Scaffold System for Promoting Tissue Regeneration	何肇庭 [1] 江承恩 [1] 方怡喬 [1] 王鈺婕 [1] 黃玠誠 [1*]	國立清華大學生物醫學工程研究所
P-15	Tissue Engineering	Adipose-derived Stem Cells Laden into HAMA/GelMA Photo-cured Based Hydrogel to Build a 3D Biomimetic Scaffold for Cartilage Tissue Reengineering	Swathi Nedunchezian[1,2] 王志光 [1,2]	高雄醫學大學醫藥暨應用化學系 [1] 再生醫學與細胞治療研究中心 [2]
P-16	Tissue Engineering	Calcium Signaling Reaction between Myoblast and Myotube by Ultrasound Stimulation	黃粵軍 朱亞成 林若梅 王兆麟	國立台灣大學醫學工程研究所
P-17	Tissue Engineering	Gelatin Scaffold with Multifunctional Curcumin-loaded Lipid-PLGA Hybrid Microparticles for Regenerating Corneal Endothelium	蔡孟妤 [1] 陳思靜 [1] 李佩蓁 [1] 沈柍君 [1] 陳宏吉 [2] 黃玠誠 [1*]	國立清華大學生物醫學工程研究所 [1] 林口長庚醫院眼科部 [2]
P-18	Tissue Engineering	Recellularization of Supercritical Carbon Dioxide Decellularized Blood Vessel: An *in vivo* Approach	宋世英 [1] 蔡建松 [1] 蔡宜廷 [1] 林怡彣 [2] 林豐彥 [3,4] 顏克中 [5] Periasamy Srinivasan[5] 謝達仁 [5]	三軍總醫院外科部心血臟血管外科 [1] 國立陽明大學口腔生物所 [2] 台北醫學大學醫學系內科 [3] 台北醫學大學附設醫院心血管研究中心及心臟內科 [4] 亞果生醫股份有限公司 [5]

P-19	Bone Marrow Stem Cells	An Intermediate Concentration of Calcium with Antioxidant Supplement in the Culture Medium Enhances the Proliferation and Decreases the Aging of Bone Marrow Mesenchymal Stem Cells	陳崇桓 [1,2,3,4,5,6,7] 莊淑君 [1,2], 鄭琮霖 [1,2,8] 林松彥 [1,2,3,4,5,6] 林怡珊 [1,2] 何美泠 [1,2,8,9,10] 張瑞根 [1,2,3,4,5]	高雄醫學大學骨科學研究中心 [1] 高雄醫學大學再生醫學及細胞治療研究中心 [2] 高雄醫學大學醫學系骨科 [3] 高雄醫學大學附設醫院骨科 [4] 高雄市立大同醫院 [5] 高雄醫學大學醫學研究所 [6] 中山大學醫學科技研究所 [7] 高雄醫學大學生理學科 [8] 高雄醫學大學臨床醫學研究部 [9] 國立中山大學海資系 [10]
P-20	Regenerative Medicine	Effects of Extra Cellular Vesicles Secreted from Adipose Derived Stem Cells on Osteoblastic Functions	歐彥廷 [1#] 陳振偉 [2] 吳順成 [2*] 邵佩琳 [3*]	高雄醫學大學生物科技學系 [1] 再生醫學與細胞治療研究中心 [2] 亞洲大學護理學系 [3]
P-21	Regenerative Medicine	Extracellular Vesicles Released from Human Adipose-derived Stem Cells Enhance Articular Chondrocyte Function	吳順成 [1,2] 張玲華 [1,2] 陳振偉 [1,2] 伍哲緯 [1,2] 陳崇桓 [1,2,3] 張瑞根 [1,2,3#] 何美泠 [1,2,3,4*#]	高雄醫學大學再生醫學與細胞治療研究中心 [1] 骨科學研究中心 [2] 骨科 [3] 生理學科 [4]
P-22	Tissue Engineering	Decellularized porcine cartilage graft with PRP attenuated OA progression and regenerated articular cartilage in ACLT-induced OA rats	Periasamy Srinivasan 葉怡君 賴意芊 謝達仁	亞果生醫股份有限公司
P-23	Tissue Engineering	Innovative 3D Semisolid Gel Engineered Using Supercritical Carbon Dioxide Decellularized Porcine Cartilage: An *ex vivo* Analysis	唐逸文 [1] 史瑞尼 [2] 葉怡君 [2] 賴意芊 [2] 謝達仁 [2]	高雄榮民總醫院骨科部 [1] 亞果生醫股份有限公司 [2]
P-24	Tissue Engineering	Supercritical Carbon Dioxide Decellularized Porcine Nasal Cartilage Graft Cultured with Chondrocyte Derived a Novel Histotypic 3D Construct for Potential Rhinoplasty	Su-Shin Lee[1,2,3,4] Yi-Chia Wu [1,2,3] Shu-Hung Huang [1,2,3] Ying-Che Chen Periasamy Srinivasan[5], 謝達仁 [5] 葉怡君 [5] 賴意芊 [5] Yun-Nan Lin[1]*	高雄醫學大學附設醫院整形外科 [1] 高雄醫學大學醫學院醫學部外科學系 [2] 高雄醫學大學再生醫學與細胞療法研究中心 [3] 高雄小港醫院外科部 [4] 亞果生醫股份有限公司 [5]
P-25	Tissue Engineering	Supercritical Carbon Dioxide Extraction Technology-enabled Tissue and Organ Scaffold Production	Periasamy Srinivasan 謝達仁	亞果生醫股份有限公司

Invited Lectures

Curriculum Vitae

石　崇　良

11558 台北市南港區忠孝東路六段 488 號 8 樓
聯絡電話：(02) 85907300
電子信箱：md01@mohw.gov.tw

現職
2020/09 迄今　行政院衛生福利部常務次長

學歷
2008/5　部定助理教授
2006/1　台灣大學公共衛生學院健康政策與管理研究所博士
1991/6　私立高雄醫學大學醫學士

經歷
2016/08~2020/08 行政院衛生福利部醫事司司長
2015/02~2016/07 行政院衛生福利部主任秘書
2013/07~2015/01 行政院衛生福利部綜合規劃司司長
2012/08~2013/07 行政院衛生署企劃處處長
2008/06~2012/07 行政院衛生署醫事處處長
2013　美國聯邦文官學院(FEI)受訓結業
2010　新加坡李光耀學院受訓結業
2008　行政院國家政務研習班第一期結業
2007/07~2008/05 行政院衛生署桃園醫院醫務秘書
2005/03~2008/03 財團法人醫院評鑑暨醫療品質策進會副執行長
2002/01~2008/05 台灣大學附設醫院品質管理中心副執行長
1998/07~2007/06 台灣大學附設醫院急診醫學部主治醫師

專長領域
急診醫學、模擬分析、病人安全與醫療品質管理、醫事法律、公共衛生

09:20-09:45

I-01

台灣再生醫療相關專法的展望

石崇良 常務次長
行政院衛生福利部

衛生福利部於 107 年 9 月發布特管辦法，開放風險較低之自體免疫細胞、自體脂肪幹細胞、自體骨髓間質幹細胞、自體纖維母細胞及自體軟骨細胞等六類細胞治療技術可於國內核准之醫療機構施行，截至 110 年 2 月 28 日止，共接獲近 218 件申請案，並已核准 70 件細胞治療計畫，遍布於全國 30 家醫院。110 年 2 月 9 日再度發布特管辦法修正案，將細胞保存庫納管、強化病人登錄及後續追蹤，同時考慮將細胞治療範圍從自體細胞擴增至異體細胞，讓細胞治療運用更廣泛。未來只要通過一、二期臨床試驗確認安全性及療效，就能依照特管法第 13 條提出專案許可，開放患者接受異體細胞治療。

本次演講，將就目前國內細胞治療技術之執行現況進行報告，並探討面臨之問題與挑戰，提出未來之策進作為與政策方向，以建構良性發展之醫療照護體系與生技產業環境，並朝台灣再生醫療相關專法的立法邁進。

Curriculum Vitae

NAME

林奏延　TZOU-YIEN LIN

林口長庚紀念醫院兒童感染科

EDUCATION

臺北醫學大學醫學系

水牛城紐約州立大學醫學院水牛城兒童醫院小兒感染科研究員

達拉斯德州大學醫學院達拉斯兒童醫院小兒感染科研究員

SPECIALIZED FIELD

兒科學、感染症、流行病學、 醫療政策

PROFESSIONAL BACKGROUND

2016/03 迄今	國家衛生研究院董事長
2015/12 迄今	長庚大學小兒科特聘教授
2015/12 迄今	林口長庚兒童醫學中心名譽院長
2017/11 迄今	辜公亮基金會董事長
2016/05 迄 2017/02	行政院衛生福利部部長
2013/07 至 2015/12	行政院衛生福利部政務次長
2011/11 至 2013/07	行政院衛生署副署長
2009/01 至 2012/01	台灣感染症醫學會理事長
2005/04 至 2008/05	台灣兒科醫學會理事長
2003/08 至 2017/07	長庚大學醫學系小兒科教授
1997/10 至 2011/11	長庚兒童醫院院長

HONORS AND AWARDS

1. 財團法人瑞信兒童醫療貢獻獎第七屆終身貢獻獎(2017年)
2. 行政院衛生福利部一等衛生福利獎章(2015年)
3. 台灣兒科醫學會醫學教育貢獻獎(2015年)
4. 台灣感染症醫學會醫療貢獻獎 (2012年)
5. 中華民國醫師公會全國聯合會台灣醫療典範獎 (2011年)
6. 桃園縣優良醫師金醫獎 (2010年)
7. 行政院H1N1防疫一等功績獎章 (2010年)
8. 台灣兒科醫學會獎 (2009年)
9. 疾病管制局2007防疫獎勵個人組疫情防治獎 (2007年)

PUBLICATION LIST

1. Tsai MS, Chen CJ, Lin TY, Huang YC. Nasal methicillin-resistant Staphylococcus aureus colonization among otherwise healthy children aged between 2 months and 5 years in northern Taiwan, 2005-2010. J Micro Immuno Infect 2018,51: 756-62.

2. Chen CJ, Lin TY, Huang YC. Letter to the editor: Occurrence of modified measles during outbreak in Taiwan in 2018. Eurosurveillance. 2018 Sep;23(37):1800485. doi: 10.2807/1560-7917.
3. Chen CH, Kuo KC, Hwang KP, Lin TY, Huang YC. Risk factors for and molecular characteristics of methicillin-resistant Staphylococcus aureus nasal colonization among healthy children in southern Taiwan, 2005-2010. J Microbiol Immunol Infect 2019;52:929-36.
4. Chien YS, Luo ST, Tsao KC, Wang YH, Lin TY, Huang YC, Lee MS. Genomic analysis of enterovirus D68, including one strain isolated from a child with Wilson's disease in Taiwan. J Formos Med Assoc. 2019;118:641-6.
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6. Pan HH, Huang YC, Chen CJ, Huang FL, Ting PJ, Huang JY, Chiu CH, Lin TY, Chen PY. Prevalence of and risk factors for nasal methicillin-resistant Staphylococcus aureus colonization among children in central Taiwan. J Microbiol Immunol Infect.2019;52:45-53.
7. Huang KA, Rijal P, Jiang H, Wang B, Schimanski L, Dong T, Liu YM, Chang P, Iqbal M, Wang MC, Chen Z, Song R, Huang CC, Yang JH, Qi J, Lin TY, Li A, Powell TJ, Jan JT, Ma C, Gao GF, Shi Y, Townsend AR. Structure-function analysis of neutralizing antibodies to H7N9 influenza from naturally infected humans. Nat Microbiol. 2019;4:306-15.
8. Hung HM, Yang SL, Chen CJ, Chiu CH, Kuo CY, Huang KA, Lin TY, Hsieh YC, Gong YN, Tsao KC, Huang YC. Molecular epidemiology and clinical features of rhinovirus infections among hospitalized patients in a medical center in Taiwan. J Microbiol Immunol Infect. 2019;52(2):233-41.
9. Huang YC, Su LH, Wu TL, Lin TY. Methicillin-resistant Staphylococcus aureus nasal carriage in international medical conference attendees. J Microbiol Immunol Infect. 2019;52(2):242-7.
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✳ 11. Lu CY, Chiang CS, Chiu CH, Wang EZ, Chen YY, Yao SM, Chang LY, Huang LM, Lin TY*, Chou JH. Successful control of Streptococcus pneumoniae 19A epidemic with a catch-up primary vaccination program in Taiwan. Clin Infect Dis2019;69(9):1581-7. **(Corresponding author)**
12. Chang LY, Lin HY, Gau SF, Lu CY, Hsia SH, Huang YC, Huang LM, Lin TY. Enterovirus A71 neurologic complications and long-term sequelae. J Biomed Sci 2019;26:57 (doi.org/10.1186/s12929-019-0552-7)
13. Lee JT, Yen TY, Shih WL, Lu CY, Liu DP, Huang YC, Chang LY, Huang LM, Lin TY. Enterovirus 71 seroepidemiology in Taiwan in 2017 and comparison of those rates in 1997, 1999 and 2007. PLOS ONE 2019;14(10):e0224110.
14. Huang KA, Huang YC, Chiu CH, Tsao KC, Lin TY. Impaired Vaccine-Induced Antibody Response Against Clade 6B H1N1 Viruses in Individuals Before Viral Emergence. Open Forum Infect Dis 2020; 11:7(1).
✳ 15. Hsia SH, Lin JJ, Chan OW, Lin TY*. Cardiopulmonary failure in children infected with enterovirus A71. J Biomed Sci 2020;27:53 **(Corresponding author)**
16. Rajendra Prasad Janapatla, Hsu Mh, Chen CL, Wei SH, Yu MJ, Su LH, Lin TY, Chiu CH. Persistence of immunity in children immunised with 13-valent pneumococcal conjugate vaccine and impact on nasopharyngeal carriage: a cross-sectional study. THORAX 2020;75:689-92.
✳ 17. Huang KA, Huang PN, Huang YC, Yang SL, Rsao KC, Chiu CH, Shih SR, Lin TY*. Emergence of genotype C1 Enterovirus A71 and its link with antigenic variation of virus in Taiwan. PLOS PATHOGENS 2020; 16(9): e1008857. **(Corresponding author)**

09:45-10:10

I-02

間質幹細胞胞外體的應用展望
Prospective of Mesenchymal Stem Cell Derived Exosomes

林奏延
長庚兒童醫學中心 名譽院長
長庚大學小兒科 客座教授

間質幹細胞近十年間為廣泛被探討領域，已知具有組織修復、免疫調節等功能，間質幹細胞分泌胞外體，其結構約 30～150 奈米大小的脂質雙分子層，攜帶蛋白質、脂質、核酸、生長因子、細胞素扮演細胞間傳遞訊息角色。近幾年間間質幹細胞胞外體已證實參與組織修復、抗發炎、抗氧化傷害等功效，胞外體不具細胞核更具安全性被認為未來替代細胞治療或攜帶藥物傳送更好選擇。

台灣每年有 2 萬多人在等待器官移植，只有不到 4000 人獲得器官移植。更有 10 萬人需要洗腎度日。這些器官衰竭的人如果能過早一點因為使用間質幹細胞及其胞外體(Exosomes)治療而阻止或減緩器官衰竭，而不至於拖累個人、家庭和社會的生活與經濟狀況。衛福部修訂特管法，鼓勵開發安全有效的細胞及其延生物治療，特別是針對老人 3 大天敵:癌症、感染和炎症退化疾病的治療。期待這個發展可以在國內和國際上，對千百萬器官失能者，提供防治或減緩器官失能的作用。

台灣隨著人口老化，大於 65 歲的老年人口已經來到 15%，30 年後上看 25-30%的趨勢。老年人的退化性疾病至少 10 種的盛行率 >1%，包括 1).退化性關節炎、2). 肌少症、3). 失智症、4). 慢性腎臟病、5). 巴金森症、6). 自體免疫病、7).中風、8). 憂鬱症、9). 慢性肺病、10). 心臟衰竭 (1%)等。這些疾病可能可以使用間質幹細胞的胞外體加以治療，特別是年輕的臍帶間質幹細胞的胞外體已經在動物實驗或初期的臨床試驗獲得安全使用和初步成效的報告。目前間質幹細胞及其胞外體的應用已經進入第 2 期臨床試驗，例如早產兒肺病、缺氧性腦病變；成人心、肺或腎臟纖維化病變；以及老年人的退化性關節炎、巴金森症或肌萎縮側索硬化症。胞外體相較於母細胞治療避免移植排斥更具安全性且方便製備成不同劑型儲存與運送，可以經由皮膚提供美容、抗發炎和抗老，或是經由鼻腔、關節或硬腦膜下腔的給藥的特性，未來對退化性疾病可以提供防治或減緩器官失能的作用，救濟成千萬甚至上億老人和早產兒的生活品質做出貢獻。

Curriculum Vitae

Name: TANG, I-Ning, M.D.,Ph.D.
Position: Deputy director, Center of consultation
Affiliation: Center for Drug Evaluation, Taiwan

Current Position
Deputy director, Center of consultation, Center for Drug Evaluation, Taiwan

Education
2020 Ph.D. National Taiwan University. Graduate Institute of Biomedical Electronics and Bioinformatics, College of Electrical Engineering and Computer Science.
2010 M.Sc. Chang Gung University. Graduate Institute of Rehabilitation Science.
2001 M.D. National Cheng Kung University. Department of Medicine, College of Medicine.

Professional Experience
2018-2020 Section chief, Center for Drug Evaluation (CDE), Taiwan.
2012-2018 Senior medical reviewer, Center for Drug Evaluation (CDE), Taiwan.
2010-2012 Senior reviewer, Division of Medical Devices, Food and Drug Administration (FDA), Taiwan.
2006-2010 Attending physician, Metropolitan and community teaching hospitals.
2005-2006 Research fellow, Taipei Medical University Hospital.
2001-2005 Resident and Chief resident, Department of Physical Medicine and Rehabilitation, National Cheng-Kung University Hospital.

Specialty
Regulatory science, Rehabilitation science, Neuroscience

Honors and Awards
2020-now Council member, Taiwan Association for Cellular Therapy (TACT).
2017-2019 Invited Speaker, International Society of Cell and Gene therapy (ISCT).
2019-now Board member, Chang Gung Medical Foundation Institutional Review Board.
2016 -now Board member, Taipei Medical University JIRB.
2016 -now International pharmaceutical regulators programme (IPRP) gene therapy working group (GTWG) member.
2014 Visiting expert, European Medicines Agency (EMA).

10:30-10:55

I-03

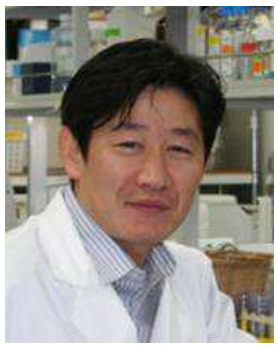
特管辦法細胞治療技術成效評估機制與風險分級

I-Ning Tang M.D.,Ph.D.
Center for Drug Evaluation (CDE), Taiwan

自民國107年9月「特定醫療技術檢查檢驗醫療儀器施行或使用管理辦法(特管辦法)」修正公告以來，細胞治療技術即在台灣蓬勃發展。特管辦法開放細胞治療的適應症包括癌症、退化性關節炎及膝關節軟骨缺損、慢性或滿六周未癒合之困難傷口等，截至110年2月1日為止，已核准70件細胞治療技術。根據該辦法規定: 醫療機構執行細胞治療技術，應於每年度終了三個月或中央主管機關要求之期限內，提出施行結果報告。前項報告之內容，應包括下列事項：一、治療案例數。二、治療效果。三、發生之不良反應或異常事件。四、其他經中央主管機關指定之事項。且中央主管機關必要時，得公開醫療機構之治療統計結果。

本次演講旨在介紹衛生主管機關對於成效追蹤的評估機制以及風險考量。

Curriculum Vitae



Byung Hyune Choi, Ph.D. (Bryan Choi)

Professor, Inha University College of Medicine

Vice-director, Strategic Center for Regenerative Medicine (SCRM)

1. CONTACT INFORMATION

Department of Biomedical Sciences, Inha University College of Medicine
Rm 322, 60th Anniversary Memorial Bldg. Inha University
100 Inha-ro, Nam-gu, Incheon 22212, Korea
Phone) 82-32-860-9881, Fax) 82-32-885-8302
Mobile) 82-10-9273-0715 E-mail) bryan@inha.ac.kr

2. CURRENT POSITIONS:

- Professor, Inha University College of Medicine, Korea
- Vice-director, Strategic Center for Regenerative Medicine (SCRM), Korea
- Secretary General, Council for Advanced Regenerative Medicine (CARM), Korea
- Director of R&D Center, ATEMs Inc., Korea
- Asia Regional Vice-President Elect, International Society for Cell and Gene Therapy (ISCT)
- Director of International Affairs, Korean Tissue Engineering and Regenerative Medicine Society (KTERMS)
- Director of Legislation, Korean Society of Cartilage and Osteoarthritis (KSCO), Korea
- Member of Korea Society for Stem Cell Research (KSSCR), Korea

3. RESEARCH INTEREST

My research covers relatively broad area of cell therapy and regenerative medicine. I am working on basic research on stem cell biology and translational research to develop regenerative medicine. The translational research on regenerative medicine is based on mesenchymal stem cells (MSCs) and cartilage progenitors from human donors, and utilized various technologies incorporating gene modification of cells, biomaterials and tissue engineering. Target indications include the cartilage defect, osteoarthritis, fibrosis and spinal cord injury. Since 2011, I have been working for Strategic Center for Regenerative Medicine (SCRM). It is a non-profit center commissioned by Ministry of Health and Welfare, Korea and plays a think-tank for the government and related stakeholders in the RM field in Korea.

10:55-11:20

I-04

Overview about the New Regulation on Regenerative Medicine in Korea

Bryan Choi, Ph.D.
Professor, Inha University College of Medicine

ABSTRACT
South Korea has rich experiences of more than 20 years in the commercialization of cell therapies particularly using stem cells. Following the recent global innovations in the field, Korea is also reforming the ecosystem of regenerative medicine including the regulatory framework. The Act on the Safety and Support for Advanced Regenerative Medicine and Advanced Biopharmaceuticals was granted by the Korea Congress in August 2019 and enacted in one year later. The act governs two different regulatory tracks for the clinical research and commercial clinical trials. The clinical research track is aiming at facilitating the application of advanced therapies to patients in desperate need early in the developmental process. The aims of the commercial trial track are to improve quality assessment and allow fast approval of advance therapies. The presentation will illustrate details about the Advanced Regenerative Bio Act in short of Korea.

Curriculum Vitae

謝青華 (Hsieh, Ching-Hua)

高雄長庚紀念醫院整形外科

Education: 台灣大學醫學系畢業(1985/09/01-1992/06/31)
Graduate : 長庚大學臨床醫學研究所博士班畢業 (2005/09/1-2008/07/31)
Postgraduate: 聖路易華盛頓大學整形外科博士後研究 (2009/09/01-2010/08/31)

高雄長庚紀念醫院現職

1. 醫師研究員(2011-)
2. 外科部副部長(2016-)
3. 手術室委員會主席(2018-)
4. 醫教會副主席(2017-)
5. 專科護理師委員會副主席(2013-)
6. 院務委員會委員(2018-)
7. 人評會委員(2018-)
8. 醫品會委員(2018-)
9. 研審會委員(2018)
10. 臨審會委員(2018-)
11. 急診管理委員會委員(2016-)

教職

1. 義守大學兼任講師(2003/08/01-2005/07/31)
2. 義守大學兼任助教授(2005/08/01-2009/07/31)
3. 長庚紀念醫院學術組講師((92)台北醫聘字第0099號, 2003/07/01-2004/06/30)
4. 長庚紀念醫院學術組助教授((93)台北醫聘字第0081號, 2004/07/01- 2008/06/30)
5. 長庚紀念醫院學術組副教授((97)台北醫聘字第0083號, 2008/07/01-2014/06/30)
6. 長庚紀念醫院學術組教授((2014)台北院聘字第0011號, 2014/07/01- present)
7. 長庚大學兼任講師((92)長庚大教聘兼字第92429號, 2003/08/01-2005/07/31)
8. 長庚大學兼任助教授((94)長庚大教聘兼字第94103號, 2005/08/01- 2011/07/31)
9. 長庚大學兼任副教授((100)長庚大教聘兼字第100274號, 2011/08/01- 2017/07/31)
10. 長庚大學兼任教授((105)長庚大教聘兼字第106280號, 2017/08/01- present)
11. 教育部部定講師(講字第078873號, 2004/08/01-2005/07/31)
12. 教育部部定助教授(助理字第016923號, 2005/08/01- 2011/09/30)
13. 教育部部定副教授(副字第042265號, 2011/10/01- 2015/09/31)
14. 教育部部定教授(教字第141966號, 2016/10/01- present)

經歷:

1. 台大醫院外科住院醫師 (1994-1996)
2. 台大醫院整形外科住院醫師, 總醫師 (1996-2000)

3. 紐約西奈山醫院(Mount Sinai Hospital) fellowship一個月 (1998)
4. 高雄長庚紀念醫院外傷科主治醫師 (2001-2017)
5. 高雄長庚紀念醫院整形外科主治醫師 (2017-present)
6. 高雄縣事故傷害防制委員會高雄長庚紀念醫院代表 (2003-)
7. 台灣品質指標計畫(TQIP)指標到院服務會議高雄長庚外傷科代表 (2004)
8. 參與高雄長庚醫院簡介編輯會議 (2004, 2005)
9. 高雄長庚紀念醫院病歷委員會外傷科代表 (2006-2010, 2013)
10. 慈濟綜合醫院院內醫療科技研究計劃審查 (2004)
11. 長庚大學課程：組織移植(英文教學) (2005, 2008)
12. 衛生署第一屆及第二屆內外科專科護理師甄審口試委員 (2007, 2008)
13. 高雄長庚醫院病歷寫作比賽外科組試題影片製作 (2008)
14. 高雄長庚紀念醫院藥事委員會委員 (2008)
15. 高雄長庚紀念醫院動物管理委員會委員 (2008-2009)
16. 長庚醫院助理教授級主治醫師職位晉升評選小組初選委員 (2009)
17. 高雄長庚紀念醫院病歷委員會委員 (2008-2010)
18. 高雄長庚醫院2011及2013年病歷記載品質優良(2011)。
19. 負責PGY核心課程之[縫合手術操作]及[蜂窩性組織炎] (2011)
20. 負責PGY核心課程之[縫合手術操作]及[燒傷及蜂窩性組織炎] (2012-2016)
21. 負責PGY核心課程之[縫合手術操作] (2017-)
22. 協辦2011年全國醫學校院聯合試辦臨床技能OSCE測驗考試(2012)
23. 長庚大學醫學院臨床醫學研究所分子生物學課程[Toll-Like Receptors] (2012)
24. 協助2012年全國醫學校院聯合試辦臨床技能OSCE測驗考試 (2013)
25. 擔任Intern OSCE考試考官(2014-2015)
26. 台灣專科護理師學會外科專科護理師甄審口試委員(2016, 2017)
27. 高雄長庚紀念醫院專科護理師醫師監督下執行醫療業務範圍教育訓練講師(2017)
28. 高雄長庚紀念醫院2017年病歷記載品質優良(2017)
29. 長庚紀念醫院人體試驗倫理委員會獨立諮詢專家(2017-)
30. 施行細胞治療技術醫師訓練課程16小時，(2018)高雄研訓字第0941號
31. 高雄長庚紀念醫院實地稽核委員教育訓練講習班，(2019)高雄研訓字第0022號

得獎及榮銜

1. 台大醫院外科八十四年度臨床病例討論會年度最佳演說獎。
2. 美國外科學院2005年國際參訪學者獎學金(International Guest Scholarship 2005 from American College of Surgeons)。簡介：美國外科學院(The American College of Surgeons, ACS)提供獎學金International Guest Scholarship每年給十位從世界各國選出來對教學研究有興趣及有發展潛力的外科醫師參訪美國各大醫學中心的機會。本獎項為頒發給美加地區以外的人士，從1968開始迄今，本人為第二位獲獎之醫師。
3. 美國外科學院會士(Fellowship of the American College of Surgeons, FACS, since 2007)
4. 國際外科醫學會2008年論文佳作
5. Biomedical Journal, 2018「論文為 SCI 收錄期刊引用獎」
6. Biomedical Journal, 2018「Most Cited Author Award」

11:20-11:45

I-05

Sharing of the Experience in Exosome Research from Isolation, Characterization, and Experiments in the Cells and Animals

Ching-Hua Hsieh, MD, PhD, FACS
Kaohsiung Chang Gung Memorial Hospital

Exosomes are a group of secreted membrane vesicles characterized by nanoscale dimensions and a complex composition made up of proteins, lipids and nucleic acids. The release of exosomes occurs by the fusion of multivesicular bodies with the plasma membrane and serves for intercellular communication. In this presentation, we will share our experience of exosome research in the past five years and describe our know-how, propose possible solution, and point out pitfall and obstacle in a practical point of view. These topics will include (1) the isolation of exosomes from a variety of extracellular vesicles such as microvesicles or apoptotic bodies and the current concept of small extracellular vesicles; (2) different kinds of isolation methods for exosomes (ultracentrifugation, size-exclusion chromatography, density gradient medium, and polymer precipitation); (3) characterization of the exosomes based on minimal information for studies of extracellular vesicles (MISEV); (4) our experience in the exploring the function of exosome in the *in vitro* experiment, regarding the different function of subpopulation of exosomes, transfection of exosomes, and the exploration of exosomal content; (5) our experience in exploring the function of exosome treatment in the mice, especially the consideration of the amount, the way, and the biodistribution of exosome treatment.

Curriculum Vitae

BURNOUF Thierry

PhD, Biochemistry
Born in France
e-mail: thierry@tmu.edu.tw thburnouf@gmail.com

Taipei Medical University, College of Biomedical Engineering: Vice-Dean ; Distinguished Professor ; **Director,** International PhD Program in Biomedical Engineering ; **Full Professor**, Graduate Institute of Biomedical Materials and Tissue Engineering ; **Adjunct Professor**, PhD Program in Cell Therapies and Regenerative Medicine, College of Medicine, Taipei Medical Univers **Adjunct Professor**, PhD Program on Brain, Mind, and Consciousness, College of Humanities and Social Sciences ; **Visiting Professor**, University of Lille, France (2020-present) ; **Visiting Professor**, Sorbonne Paris Cité University - Paris 13 University, Paris, France (2013-2017) ; **Executive Committee member and Treasurer, Working Party on "Cellular Therapies", International Society of Blood Transfusion** (2014-present) ; **Organizing Committee member and Secretary, Working Party on "Global Blood Safety", International Society of Blood Transfusion** (2013-present) **; Consultant/Expert**, **World Health Organization** : blood product safety, plasma for fractionation, contract fractionation programs, animal-derived antisera, improving access to safe blood products to developing countries through transfers of technology. **Chair (Jan 2020-present) drafting group of Guidance** on Access to plasma-derived medicinal products in low- and middle-income countries ; **Member**, **Special Advisory Board (Advanced Biopharmaceuticals)**, Ministry of Food and Drug Safety, South Korea (2017 - present) **; Editorial Board Member:** Frontiers in Medicine-Haematology (Associate Editor); Frontiers in Neuroscience (Guest Associate Editor); Biologicals - Journal of the International Association of Biologicals; Recent Patent on Nanotechnology; Current Nanoscience; Transfusion Clinique et Biologique (IF: 1.3); Annals of Blood.

RESEARCH and TEACHING AWARDS

- 2019 Award, **International Plasma and Plasma Fractionation Association (IPFA)** in "recognition of his exceptional scientific contributions to new plasma fractionation technologies and programmes, and virus inactivation and removal procedures."
- Award for **Recruitment and Retention of Special and Outstanding Talents** by **Ministry of Science and Technology (MOST)** of Taiwan in **2017** and in **2018** academic years.
- **Excellent teacher award for Research, Teaching and Administration**, **Taipei Medical University**, Taipei, Taiwan, Jan 2018
- Prize of Outstanding Research Award, **Taipei Medical University**, Taipei, Taiwan 2017, 2018, 2020
- 3-years grant from **National Science Council, Taiwan,** as foreign expert (2009-2011)
- **Special Visiting Scientist** position from the National Council for Scientific and Technological Development; CNPq), Brazil Federal Government (from Sept 2013
- Fellowship of the **International Rotary Foundation** (1982) for postdoctoral studies in the USA

FIELDS of SPECIALTY : Blood transfusion; plasma fractionation; blood protein processing; blood protein purification; virus inactivation and removal procedures; Regenerative

medicine;
Platelet lysates for stem cell expansion; Blood-cell derived extra-cellular vesicles; Platelet growth factors for cell therapy

PUBLICATIONS : > **250** SCI publications; Web of Science : h-index: **39**; total citations: >**6200** ; Research Gate score: **65.15**; h-index: **44;** >**122'000** Reads; >**7900** citations ;Google Scholar: h-index: **50**; total citations:>**9500**. Inventor, 24 international patent families.

Publications related to the presentation

1. Delila L, Wu Y-W, Nebie O, Widyaningrum R, Chou M-L, Devos D, **Burnouf T***. Extensive characterization of the composition and functional activities of five preparations of human platelet lysates for dedicated clinical uses. *Platelets* (accepted).
2. Barro L, Burnouf P-A, Chou M-L, Nebie O, Wu Y-W, Chen M-S, Radosevic M, Knutson F, **Burnouf T*.** Human platelet lysates for human cell propagation. *Platelets* (accepted).
3. Johnson J, Wu Y-W, Blyth C, Lichtfuss G, Goubran H, **Burnouf T***. Prospective Therapeutic Applications of Platelet Extracellular Vesicles. *Trends in Biotechnology,* Published:November 04, 2020DOI:https://doi.org/10.1016/j.tibtech.2020.10.004
4. Barro L, Nebie O, Chen M-S, Wu YW, Koh MBC, Knutson F., Watanabe N, Takahara M, **Burnouf T***. Nanofiltration of growth media supplemented with human platelet lysates for pathogen-safe xeno-free expansion of mesenchymal stromal cells. *Cytotherapy* 2020; 22:458-472. https://doi.org/10.1016/j.jcyt.2020.04.099
5. Nebie O, Barro L, Wu YW, Knutson F, Buée L, Devos D, Peng CW, Blum D*, **Burnouf T***. Heat-treated human platelet pellet lysate modulates microglia activation, favors wound healing and promotes neuronal differentiation *in vitro*. *Platelets,* https://doi.org/10.1080/09537104.2020.1732324.
6. Schallmoser K, Henschler R, Gabriel C, Koh MBC, Burnouf T*. Production and quality Requirements of human platelet lysate: A Position Statement from the working party on cellular therapies of the International Society of Blood Transfusion. *Trends in Biotechnology,* 2020;38:13-23. https://doi.org/10.1016/j.tibtech.2019.06.002
7. Sung TC, Li HF, Higuchi A, Kumar S, Lin QD, Wu YW, **Burnouf T**, Nasu M, Umezawa A, Lee KF, Wang HC, Chang Y, Hsu ST. Effect of cell culture biomaterials for completely xeno-free generation of human induced pluripotent stem cells. *Biomaterials*, 230, p119638. Published: 2020-Feb. https://doi.org/10.1016/j.biomaterials.2019.119638
8. Nebie O, Devos D, Vingtdeux V, Barro L, Devedjian JC, Jonneaux A, Chou ML, Bordet R, Buée L, Knutson F, Blum D*, Burnouf T*. The neuroprotective activity of heat-treated human platelet lysate biomaterials manufactured from outdated pathogen-reduced (amotosalen/UVA) platelet concentrates. *Journal of Biomedical Science*, 2019; 26, 89. doi:10.1186/s12929-019-0579-9
9. **Burnouf T***, Agrahari V*, Agrahari V*. Extracellular vesicles as nanomedicines: hopes and hurdles in clinical translation. *The International Journal of Nanomedicine* 2019:14 8847–8859.
10. Gao Y, Ku N-J, Sunb T-C, Higuchi A, Hung C-S, Lee H H-C, Ling Q-D, Cheng N-C, Umezawa A, Barro L, **Burnouf T**, Ye Q, Chen H. Effect of human platelet lysate on the differentiation ability of human adipose-derived stem cells cultured on ECM-coated surfaces**.** *Journal of Materials Chemistry B* 2019 Dec 7;45:7110-7119.

11. Gouel F, Rolland A-S, Devedjian JC, **Burnouf T**, Devos D. Past and Future of Neurotrophic Growth Factors Therapies in ALS: From Single Neurotrophic Growth Factor to Stem Cells and Human Platelet Lysates. *Frontiers in Neurology,* August 2019, 10, Article 835. doi: 10.3389/fneur.2019.00835.
12. Obeid S, Sung PS, Le Roy B, Chou ML, Shieh, SL, Elie-Caille C, **Burnouf T**, Boireau W. NanoBioAnalytical characterization of extracellular vesicles in 75-nm nanofiltered human plasma for transfusion: a tool to improve transfusion safety. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 2019;20:101977.
13. Barro, L, Su YT, Nebie O[c], Wu[c YW], Huang YH, Koh MB, Knutson F, **Burnouf T***. A double virally-inactivated (Intercept-solvent/detergent) human platelet lysate for *in vitro* expansion of human mesenchymal stromal cells. *Transfusion*, 2019; 59:2061-2073.
14. Chen MS, Wang TJ, Lin HC, **Burnouf T***. Four types of human platelet lysates, including one virally inactivated by solvent-detergent, can be used to propagate Wharton Jelly mesenchymal stromal cells. *New Biotechnology* 2019; 49: 151-160.
15. Agrahari V, Agrahari V, Burnouf PA, Chew CH, Burnouf T*. Extracellular Microvesicles as New Industrial Therapeutic Frontiers. *Trends in Biotechnology* 2019;37:707-729.
16. Henschler R, Gabriel C, Schallmoser K, **Burnouf T**, Koh M*. Human Platelet Lysate: Current Standards and Future Developments. *Transfusion,* 2019;59:1407-1413.
17. Gouel F, Do Van B, Chou ML, Jonneaux A, Moreau C, Bordet R, **Burnouf T**, Devedjian JC, Devos D* The protective effect of human platelet lysate in models of neurodegenerative disease: involvement of the Akt and MEK pathways. *J Tissue Eng Regen Med*, 2017; 11: 3236-3240
18. Chou ML, Wu JW, Gouel F, Jonneaux A, Timmerman K, Renn TY, Laloux C, Chang HM, Lin LT, Devedjian JC, Devos, D*, **Burnouf T***. Tailor-made purified human platelet lysate concentrated in neurotrophins for treatment of Parkinson's disease. *Biomaterials*, 2017; 142: 77-89
19. Tseng CL, Chen ZY, Renn TY, Hsiao SH, **Burnouf T***. Solvent/detergent virally inactivated serum eye drops restore healthy ocular epithelium in a rabbit model of dry-eye syndrome. *PLoS One* 2016, April 21, 1-14.
20. **Burnouf T**, Strunk D*, Koh M, Schallmoser K*. Human platelet lysate: replacing fetal bovine serum as a gold standard for human cell propagation? *Biomaterials* 2016; 76:371-87.

11:45-12:10

I-06

Applications of Platelet Derivatives and Extracellular Vesicles in Cell Therapy and Regenerative Medicine

Thierry Burnouf
Graduate Institute of Biological Materials & Tissue Engineering,
College of Biomedical Engineering
Taipei Medical University, Taipei, Taiwan

There is mounting experimental and clinical evidence to support that standardized human platelet derivatives biomaterials, including human platelet lysate (HPL), can be of therapeutic and clinical interest in various fields of cell therapy, tissue engineering and regenerative medicine. The clinical benefits of these platelet-derived biomaterials are based, at least in part, on their unique proteome made of a powerful range of cell growth- and repair- promoting biomolecules, including growth factors (PDGF; VEGF; BDNF; TGF-ß; EGF; HGF; etc.), cytokines (CXCL4 or PF4; CCL5 or RANTES), anti-inflammatory and anti-oxidative factors, capable to trigger cellular functions as well as protective and regenerative signaling pathways. Recent data also indicate that HPL contain a substantial concentration of extra-cellular vesicles (EVs) that may be responsible for some of the physiological activity of HPL.

One of the best-established benefits of HPL made from therapeutic-grade human platelet concentrate (PC) is as a supplement of growth media to substitute for fetal bovine serum for the xeno-free ex vivo clinical-grade isolation and propagation of human cells for transplantation. Most experimental evidence of the superiority of HPL over FBS to expand human cells was obtained so far using bone marrow- or adipose tissue- mesenchymal stromal cells (MSCs) but has recently been confirmed for MSC from other tissues sources (Wharton jelly, umbilical cord, amniotic fluid, dental pulp, periodontal ligaments, etc.). HPL xeno-free supplementation is also now proving successful for expanding human differentiated cells, such as chondrocytes, corneal endothelium and epithelium cells, and tenocytes. Most recent developments suggest the possibility to use HPL for expanding immune cells such as macrophages, dendritic cells, and chimeric antigen receptor-T cells. Therefore, strong scientific rationale supports the use of HPL as a universal growth medium supplement for isolating and propagating therapeutic human cells for transplantation and tissue engineering. Our research focuses on developing procedures for optimal standardization and pathogen safety of HPL to secure its reliability for clinical-grade cell-therapy and regenerative medicine products and tissue engineering.

Growing experimental evidence in the last few years have demonstrated the benefit of HPL and other forms of platelet biomaterials in orthopedic, maxillofacial, dermatologic, ophthalmologic fields, and in sport medicine. We will more particularly describe the pre-clinical work of our laboratory to develop tailor-made allogeneic platelet secretome preparations, rich in neurotrophins, angiogenins and EVs, for administration to the brain and treatment of neurodegenerative disorders and brain trauma, using intracranial and/or intranasal routes of delivery depending upon disease severity. Our data support the scientific rationale for a clinical translation in indications like amyotrophic lateral sclerosis, Parkinson's disease, and traumatic brain injury.

In conclusion, there is a scientific rationale for clinical translation of standardized, well-characterized, and pathogen-free platelet and EV biomaterials for cell therapy and advanced regenerative medicine fields.

Curriculum Vitae

LY JAMES LEE

A. Professional Preparation
BS in Chemical Engineering
National Taiwan University, Taipei, Taiwan, 1972
Graduate Research Associate Case Western Reserve University, Macromolecular in Polymer Science Science Department, Cleveland, OH, 1974-75
Ph.D. in Chemical Engineering
The University of Minnesota, MPLS, MN, 1979

B. Personal Statement
My research interest is to design and develop nanoscale biochips/devices for diagnostic and therapeutic biomedical applications including cancer diagnosis/therapy and regenerative medicine. I have published extensively in this field and have been serving on the NIH Study Sections. I have an established history of research collaboration with many medical researchers and clinicians in the US and Taiwan. I have advised more than 70 PhD students and more than 100 postdoc researchers/research associates/visiting scholars in the past 38 years. I have also raised more than US$120M research grants from federal and local government agencies as well as industry, and have managed several large-scale interdisciplinary, cross-college research and education programs/centers at the Ohio State University (OSU) from 2002 to 2015. I have retired to an emeritus status on September 1, 2018, but remain active in research and student/postdoc training at OSU.

C. Positions and Honors
ACADEMIC EXPERIENCE

2018-present	Jade Mountain Scholar, National Yang Ming University, Taiwan
2018-present	Emeritus Helen C. Kurtz Professor, The Ohio State University
2000-2018	Helen C. Kurtz Professor, The Ohio State University
1990-2000	Professor, Chemical Engineering, The Ohio State University
1986-90	Associate Professor, Chemical Engineering, The Ohio State University
1982-86	Assistant Professor, Chemical Engineering, The Ohio State University

INDUSTRIAL AND GOVERNMENTAL EXPERIENCE

1979-82	Senior Research Engineer, 1979-82 The General Tire & Rubber Company (GenCorp), Akron, Ohio

MAJOR AWARDS AND HONORS

2016	Lifetime Achievement Award, Society of Advanced Molding Technology
2010	International Award, Society of Plastics Engineers
2008	Malcolm E. Pruitt Award, Council of Chemical Research
2006	Fellow, American Institute for Medical and Biological Engineering Central Ohio AIChE Section, Innovation in Chemical Engineering
2002-2007	Director of NSF Integrated Graduate Education and Research Training (IGERT) Program on Molecular Engineering of Micro-Devices
2004-2015	Director of NSF Nanoscale Science and Engineering Center for Affordable Nanoengineering of Polymer Biomedical Devices (CANPBD)
2005-2012	Director of Ohio Center for Affordable Multifunctional Polymer Nanomaterials and Devices (CMPND)

D. Contribution to Science and Key Publications (>450 referred journal papers, >15,000 citations)

1. Non-viral Gene Delivery by Nanochannel Electroporation- The ability to deliver precise amounts of biomolecules and nanofabricated probes into living cells offers tremendous opportunities for biological studies and therapeutic applications. It may also play a key role in the non-viral generation of engineered stem cells and induce pluripotent stem cells with high efficiency and non-carcinogenic properties. Currently-available transfection approaches are heavily dependent upon diffusion- and endocytosis-based mechanisms, which results in highly stochastic transfection. We have overcome this problem by developing a new technology, nanochannel electroporation (NEP) allowing transfection of many small sized and delicate cells with precise control over dose and timing. Cell mortality from NEP is virtually zero. We show dose control effects on a variety of transfection agents such as oligonucleic acids, molecular beacon, quantum dots and efficient delivery of large DNA directly into the nucleus using nanoparticle "bullets." Dosage controlled delivery to multiple cells is not achievable with any existing techniques. NEP also leads to mass secretion of extracellular vesicles containing functional RNAs from transfected cells.

- P. E. Boukany, et.al. and **L.J. Lee**, "Nanochannel Electroporation Delivers Precise Amounts of Biomolecules into Living Cells", **Nature Nanotechnology**, 6, 747-754 (2011), research highlight in **Nature Methods**, 8, 996-997 (2011).
- D. Gallego-Perez, et.al **L.J. Lee** and C.K. Sen, "Topical Tissue Nano-transfection Mediates Non-viral Stroma Reprogramming and Rescue", **Nature Nanotechnology**, :10.1038/nnano.2017.134 (2017).
- Z. Yang, et.al. and **L.J. Lee**, "Large-Scale Generation of Functional mRNA Containing Exosomes via Cellular Nanoporation", **Nature Biomedical Engineering**, 4, 69-83 (2020).

2. Targeted Delivery by Lipoplex Nanoparticles- Small nucleic acids such as microRNA, siRNA and ODN regulate a network of tumor promoting and tumor suppressor genes that are critical for tumor cell survival, epithelial-to-mesenchymal and mesenchymal-to-epithelial transition (EMT/MET), escaping the host immune system, resistance to therapy, and angiogenesis. Modulating tumor cells and its microenvironment with miRNA replacement (MRT) and anti-miRNA therapy (AMT) can potentially inhibit tumor growth and sensitize tumor cells to existing therapy. A critical barrier to the clinical development of MRT/AMT is that oligonucleotides are sensitive to nucleases, subject to renal and reticuloendothelial system (RES) clearance with minimum membrane permeability. Delivery systems based on targeted lipid nanoparticles can address these problems.

- B. Yu, et.al. and **L.J. Lee**, "Novel Lipid Nanoparticle Design for Delivery of Small Interfering RNA to Liver and Liver Tumor", **Biomaterials**, 33, 5924-5934 (2012).
- B. Yu, et.al, **L.J. Lee** and N. Muthusamy, "Liposomal Targeted Delivery Overcomes Off-target Immunostimulatory Effects of RNA Oligonucleotide", **Blood**, 121, 136-147 (2013).
- X. Huang, et.al., **L.J. Lee** and G. Marcucci, "Targeted Delivery of microRNA-29b by Transferrin Conjugated Anionic Lipopolyplex Nanoparticles: A Novel Therapeutic Strategy in Acute Myeloid Leukemia", **Clinical Cancer Research**, 19(9), 2275-2292 (2013).

3. Tethered Lipoplex Nanoparticle Biochips for Extracellular Vesicles Based Circulating RNA Biomarkers- Biomolecules such as miRNAs, lncRNAs, mRNAs and protein antigens can be useful as biomarkers for cancer and other diseases. Recent studies show that they are excreted by cells in the form of extracellular vesicles (EVs) including exosomes and can be detected in the blood. However, existing methods based on next generation sequencing (NGS), hybridization microarrays, and qRT-PCR have limited sensitivity and require tedious and expensive sample preparation and detection procedures. They also need several hundred microliters of blood for EV analysis. This necessitates the sacrifice of animals in murine studies, and making it impractical for tumor monitoring in murine tumor model therapy trials. To address these issues, my lab has developed "tethered lipid nanoparticle (TLN)" technology that can be used for ultra-sensitive detection of miRNAs, lncRNA, mRNA and

protein antigen targets in EVs. ic biomarkers that can be used for early disease diagnosis and to monitor and predict disease response to therapy.

- Y. Wu, et.al. and **L.J. Lee**, "Detection of Extracellular RNAs in Cancer and Viral Infection by Tethered Cationic Lipoplex Nanoparticles", **Analytical Chemistry**, 85(23), 11265-11274 (2013).
- **L.J. Lee**, et.al. and S.P. Nana-Sinkam, "Extracellular mRNA Detected by Tethered Lipoplex Nanoparticle Biochip for Biomarker Development in Lung Cancer", **American Journal of Respiratory and Critical Care Medicine**, 193(12), 1431-1433 (2016).
- Hu, Y. Sheng, K.J. Kwak and **L.J. Lee**, "A Signal-amplifiable Biochip Quantifies Extracellular RNAs for Early Cancer Detection", **Nature Communication**, 8(1),1683 (2017).

4. Cell Based Drug Delivery- Cell-based therapeutic strategy has been proposed as a tissue engineering application for several decades. Primary or cell line with certain product secretion can be used as "seed" cell for therapeutic function. Alternative resource of cells is from gene recombination-mediated methods. As most tissue or cellular transplants, the cellular grafts are subject to immunorejection in the absence of chronic immunosuppression. For cell-based device applications *in vivo*, the device must provide proper cell immunoprotection with minimal inflammatory response. Furthermore, the device should possess controllable degradation characteristics such that the implant does not need to be removed after use via invasive second surgery. My lab has conducted considerable research in this area.

- X. Zhang, H. He and **L.J. Lee**, "A Biodegradable, Immunoprotective, Dual Nanoporous Capsule for Cell-Based Therapies", **Biomaterials,** 29, 4253-4259 (2008).
- F. Yang, et.al., **L. J. Lee**, S. Rajagopalan, and O. Ziouzenkova, "The Prolonged Survival of Fibroblasts with Forced Lipid Catabolism in Visceral Fat Following Encapsulation in Alginate-poly-L-lysine", **Biomaterials**, 33(22), 5638-5649 (2012).
- H. He, et.al. and **L.J. Lee**, "A Nanoporous Cell-Therapy Device with Controllable Biodegradation for Long-Term Drug Release", **Journal of Controlled Release**, 165(3), 226-233 (2013).

13:30-13:55

I-07

Extracellular Vesicles Based Regenerative Medicine and Disease Therapy

L. James Lee (李 利)
Department of Chemical and Biomolecular Engineering
The Ohio State University, USA
and
Institute of Biopharmaceutical Sciences
National Yang Ming University, Taiwan

ABSTRACT
Nucleic acid therapeutics including small interfering RNA (siRNA), microRNA (miRNA), microRNA antagonists (antagomiRs), antisense oligonucleotides, messenger RNA (mRNA), and DNA plasmids have great potential for disease treatment. However, a major limiting factor is the ability to deliver well-defined amounts of these relatively large and negatively charged molecules into target tissues and cells. A variety of cell transfection techniques have been developed for in vivo gene delivery, including viral vectors and chemical methods (e.g. liposomal and polymeric nanoparticles). But they suffer from severe immunogenicity, poor efficacy, and/or high cost. Recently, cell-secreted vesicles that encapsulate genetic and proteomic materials have emerged as promising therapeutic agents However, only a few cell types such as multipotent stem cells are found to secret high numbers of extracellular vesicles that exhibit immunosuppressive activity. Here we show the development of a new technology platform, nanochannel electroporation (NEP) for highly effective cell transfection and vesicle secretion. The potential of those transfected cells and their secreted vesicles is demonstrated in several frontier medical fields including non-viral generation of induced endothelial cells (iECs) for wound healing, therapeutic neutrophils for rheumatoid arthritis (RA) treatment, and therapeutic exosomes for glioblastoma multiforme (GBM) and pancreatic ductal adenocarcinoma (PDAC) treatment.

References:

- P. E. Boukany, et.al. and **L.J. Lee**, "Nanochannel electroporation delivers precise amounts of biomolecules into living cells", **Nature Nanotechnology**, 6, 747-754 (2011), research highlight in **Nature Methods**, 8, 996-997 (2011).
- D. Gallego-Perez, J.J. Otero, et. al. and **L.J. Lee**, "Deterministic transfection drives efficient nonviral reprogramming and uncovers reprogramming barriers", **Nanomedicine**, 12, 399-409 (2016).
- D. Gallego-Perez, et.al, I. Nakano, and **L.J. Lee**, "On-chip clonal analysis of oligo RNAs on glioma stem cell motility and drug resistance", **Nano Letters**, 16(9), 5326-5332 (2016).
- D. Gallego-Perez, D. Pal, S. Ghatak, V. et.al, **L.J. Lee** and C.K. Sen, "Topical tissue nano-transfection mediates non-viral stroma reprogramming and rescue", **Nature Nanotechnology**, :10.1038/nnano.2017.134 (2017).
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Curriculum Vitae

Professor Hwa-Chang Liu was graduated from school of medicine,National Taiwan University in 1969. He received his training as an orthopaedic surgeon at National Taiwan University Hospital and at Department of Orthopaedic Surgery ,University of Tokyo. After he was honored PhD degree in 1977, he started his career as a faculty member at the Department of Orthopaedic Surgery ,National Taiwan University for teaching, research and clinical services.

He has treated hundred thousands patients,severed many academic societies as president in orthopaedic ,rheumatologic and regenerative medicine.He was appointed as the inaugural director of Institute of Biomedical Engineering,National Taiwan University in 1999 and elected as one of the five distinguished member in SICOT (International Society of Orthopaedic Surgery and Traumatology) in 2011. He got award on writing teaching materials. Never the less, he continued his research with his team as Professor of Orthopaedic Surgery Emeritus since 2008,specially on repairing cartilage defect which he will present in this congress.

13:30-13:55

I-08

Repairing Cartilage Defects with Chondrocyte Precursor

Hwa-Chang Liu MD, Ph.D., FACS, FICS
Department of Orthopaedic Surgery, Taiwan Adventist Hospital; of Orthopaedic Surgery

Background: For knee cartilage regeneration, cell therapies using undifferentiated mesenchymal stem cells (MSCs) or autologous chondrocytes suffered from undesired fibrocartilage formation or the lack of integration between implants and articular cartilage.
Methods: Subjects with medial femoral condyle cartilage defect were recruited. Their autologous bone marrow MSCs were isolated, expanded, and then induced to chondrocyte precursors (CPs). CPs is a unique population of chondrogenic MSC, which could secrete glycosaminoglycan without forming lacunae. In our previous study, CPs improve the integration of implants to native cartilage and prevent fibrocartilage formation. CPs were implanted in the cartilage defect through arthrotomy. The knee function was evaluated with the International Knee Documentation Committee (IKDC) subjective knee form. X-ray and MRI examinations were done before and after the operation periodically. After one year of CPs administration, the efficacy was also addressed by the International Cartilage Repair Society (ICRS) visual histological assessment scale. A group of subjects received microfracture as a control group.
Results: This trial comprised 10 subjects who received CPs implantation, and 5 subjects received microfracture. In the CPs treatment arm, subjects' cartilage defects were implanted with 1.6-3.3 × 10^6 cells /cm^2. Total 7 treatment-emergent adverse events (TEAEs) were reported from 5 subjects: 3 TEAEs from the CPs-treated group and 4 TEAEs from the microfracture group. All TEAEs are grade 1 and recovered/resolved during the study. In terms of therapeutic efficacy, IKDC scores were significantly improved in subjects after CPs implantation compared to the scores before treatments. While there was no significant difference of IKDC between the CPs-treated and microfracture groups at Week-28, the CPs-treat group demonstrated better surface domain in the ICRS visual histological assessment scale.
Discussion: Sufficient cell seeding density in the cartilage defects would be critical for the recovery. Rather than by the one-step strategy, our approach involves *in vitro* expansion of MSCs, which allows the generation of numerous chondrogenic cells from a minute number of MSCs harvested from subjects. Our cell seeding density is in the range of conventional autologous chondrocyte implantation (ACI), 0.5-12 × 10^6 chondrocytes/cm^2. However, ACI is limited by the risk of donor site morbidity. There is also a concern regarding the integration of implanted mature chondrocytes into the recipient site cartilage.
Conclusion: This study demonstrates that a single articular operation of CPs is safe for subjects with medial femoral condyle cartilage defects. The knee function and the surface domain of the recipient site were significantly improved in the CPs treated group.

Name： **張至宏**
Position： 台灣再生醫學學會 理事長
亞東紀念醫院骨科部 部主任/主治醫師
Affiliation： 新北市板橋區南雅南路二段 21 號
亞東紀念醫院 7 樓骨科部
E-mail： orthocch@mail.femh.org.tw
Specialty： 臨床醫學、骨科學、醫學工程、膝關節重建、軟骨修復、創傷骨科

Current Position
台灣再生醫學學會 理事長
台灣骨科研究學會 常務理事
亞東紀念醫院骨科部 部主任/主治醫師
元智大學生物科技與工程研究所 教授
國立台灣大學附設醫院骨科部 兼任主治醫師
International College of Surgeon 國際外科學院 院士 (F.I.C.S.)

Education
2000-2005 國立台灣大學醫學工程研究所 博士
1985-1992 國立台灣大學 醫學士

Professional Experience
2016-2018 台灣再生醫學會 秘書長
2016-2018 台灣骨科研究學會 秘書長
2014-2016 台灣骨科創傷醫學會 常務理事
台灣骨科研究學會 常務理事
2010-2014 台灣骨科研究學會 理事
元智大學生物科技與工程研究所 副教授
2008-2016 台灣再生醫學會 理事
2006-2010 元智大學生物科技與工程研究所 助理教授
2006-2007 亞東紀念醫院外科部 代理部主任
2005-2007 亞東技術學院通識教育中心 兼任助理教授
2006-2010 亞東技術學院材料與纖維系 兼任講師
2003-2004 亞東技術學院機械工程系 兼任講師
2000-2005 國立台灣大學醫學院醫學系骨科 兼任助理教授
2005-2007 Mayo Clinic Department of Orthopedics Visiting Clinician
1999-2005 亞東紀念醫院外科部骨科 主治醫師
1994-1999 國立台灣大學附設醫院骨科部 住院醫師

Honors and Awards
2018 2nd Annual Summer Meeting of Korean Society of Cartilage and Osteoarthritis 受邀演講
2018 Annual Meeting of Taiwan Arthroscopy and Knee Society 受邀演講
2017 JMBE Annual Excellent Paper Award

2015 International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine (ISAKOS 2015 Lyon, France) 受邀演講
2014 3rd New Taipei Medical Charity Award : Educational Research Award (新北市醫療公益獎教育研究獎)
2014 Asian Cartilage Repair Society (ACRS) 2nd Annual Congress 受邀演講
2014 第九屆亞太生物醫學工程會議(APCMBE 2014) 受邀演講
2014 1st Global Conference on Biomedical Engineering (GCBME) 受邀演講
2014 Asian Mayo Clinic Morrey Elbow Club Facaulty
2014 台灣骨科研究學會 SCI 論文獎第三名
2013 台北國際發明暨技術交易展：鉑金獎
2011 第八屆國家新創獎
2010 第八屆有庠科技獎：傑出教授獎
2010 亞東紀念醫院第八屆年度卓越研究論文獎：銅牌獎
2008 第五屆國家新創獎
2008、2009、2010、2011、2012、2016 國際外科醫學會優秀論文獎
2006、2008、2014 中華骨科醫學會優秀論文獎

14:20-14:45

I-09

以膝關節脂肪墊幹細胞治療退化性關節炎的簡介與初步臨床成果

張至宏
亞東紀念醫院骨科部，元智大學生技所

***Background*:** Infrapatellar fat pad–derived mesenchymal stromal cells (IFP-MSCs) have not yet been used in a human clinical trial. In this open-label phase I study, patients with knee osteoarthritis (OA) received a single intra-articular injection of autologous IFP-MSCs. The safety was assessed through physical examination of the knee joint, vital signs, laboratory tests, and adverse events. The efficacy was evaluated on pain and function through X-ray and magnetic resonance imaging (MRI). Indoleamine-2,3-dioxygenase (IDO) expression in IFP-MSCs primed with interferon-γ was used as an *in vitro* potency measurement in investigating the correlations of clinical outcomes.
***Materials and methods*:** Twelve patients with symptomatic knee OA were recruited. IFP adipose tissue was harvested from each patient's knee through surgical excision for IFP-MSC manufacturing. Cryopreserved IFP-MSCs (5 × 107cells) were injected into the knee joint immediately after thawing.
Results: No significant adverse events were observed. Patients who received IFP-MSCs exhibited clinically significant pain and functional improvement at a 48-week follow-up. The MRI Osteoarthritis Knee Score average was also significantly reduced, from 100.2 before injection to 85.0 at 48 weeks after injection. The IDO expression of the primed IFP-MSCs of the 12 patients was correlated with clinical outcomes after injection.
Conclusion: A single intra-articular injection of IFP-MSCs appears to be a safe therapy for treating knee OA and may improve disease symptoms. IDO measurement of primed IFP-MSCs has potential as a potency marker of MSC products for immunomodulatory therapy.

Clinical Applications under Specific Medical Management Regulation

After proved by government, we started the IFP-MSCs clinical applications. Here we report 4 cases. Case 1, 43 y/o female, received both knees HTO (high tibia osteotomy) + multiple drilling + IFP-MSCs. Case 2, 76 y/o male, received left knee HTO + DFO (distal femoral osteotomy) + both knee multiple drilling + IFP-MSCs. Case 3, 63 y/o, constitutional varus knee who did not want to receive HTO, received multiple drilling + IFP-MSCs. Case 4, 60 y/o female received right total knee and left HTO in other hospital with left knee still being painful. We performed multiple drilling + IFP-MSCs in left knee. All patients got satisfied and dramatic improvement.

Curriculum Viate

Name: Yuan-Kun Tu
Title: MD. PhD, Professor.
Medical Education:
Taipei Medical University; School of Medicine
Mayo Clinic, Medical Graduate school, Research fellow.
Cheng Kung University, Graduate School of Medical Engineering. PhD

Professional affiliations:
1. *Professor in Orthopedic & Medical Engineerings*, E-DA hospital/ I-Shou University
2. *Professor, Department of Medicine,* I-Shou medical school / University
3. *Superintendent,* E-DA hospital / I-Shou University.
4. *President,* Taiwan hand surgery society (2006-08)
5. *President,* Taiwan orthopedic trauma society (2010-12)
6. AO Trustee, (2012-2016)
7. *President* APFSSH (2014-17)
8. *Chairman,* Committee of Brachial plexus injury, International Federation for the Society of Surgery of Hand (IFSSH). (2014-17)
9. CEO, E-Da Health Care Institutes

Surgical Specialty:
(1). Orthopedic trauma, such as open fracture, mangled extremity.
(2). Microsurgery in adult & children, flaps, toe-to-hand transfer.
(3) Brachial plexus reconstruction
(4) Hand & wrist surgery
(5) Treatment for osteomyelitis
(6) Spine surgery (Cervical spine & Tetraplegia)

Academic works (Attached references)
153 scientific SCI papers in *JBJS, JHS, JOR, JRM. J Trauma, Acta Orthop, CORR, Spine, Orth Clin NA, Injury, Biomaterial, Microsurgery, Bioengineering, etc.*

Reviewer & Board of editor: Microsurgery, The Scientific World Journal, Journal of Hand Surgery, Clinical Biomechanics, JOS, JBMS, J Wrist surgery, Injury, Biomedical J..
12 Chapters author in orthopedic and microsurgery textbooks.
85 research projects (1991~2019) focus on biomechanics of hand, nerve, vessel, endothelial cells, stem cell, and implants (spine, fracture, hand & wrist).

****** International Invited Lectures: **more than** 300 **invited speeches in international conferences.**

** **Honors & Awards:**
1. The **"Whole National 10 Most Outstanding Youth Award"**, Taiwan, 2000.
2. The "**Distinguished best 100 doctors in Taiwan**" Award (2009-2010)
3. **The National Outstanding and the Best Doctor in Taiwan Award,** 2012
4. **The Taiwan National Special Contribution & Dedication Award ,** 2013

5. **The Distinguished Outstanding Alumni Award, Taipei Medical University,** 2015
6. **The Distinguished Outstanding Alumni Award, National Cheng Kung University,** 2016
7. **The Distinguished honorable Citizen Award, Peng-Hu City,** 2017
8. **The International College of Surgeon: Special Contribution & Dedication Award ,** 2018
9. **The Distinguished Outstanding Citizen Award, Kao-Hsiung City,** 2019

14:45-15:10

I-10

細胞療法在軟骨重建與神經再生的最新發展

杜元坤
義大醫院院長/骨科部

Abstract:
台灣於西元 2018 年 9 月在衛福部及醫療先進們的推動下，通過了『特定醫療技術檢查檢驗醫療儀器實行或使用管理辦法』修正條文（底下簡稱特管辦法），以嘉惠病人並且推動醫療生技的發展。義大醫院和三顧公司合作申請的第六項：自體軟骨細胞移植術，已於西 2019 年 12 月 18 日正式通過，應用在膝蓋軟骨缺損的病患，並且為全台灣一個通過該項目的醫院。透過軟骨細胞層片的專利技術，成功的重建患者缺損的軟骨，再合併膝蓋矯正手術，克服了長久以來無法解決的膝蓋關節軟骨缺損問題。截至目前為止，義大醫院已經收案了 29 位病人，完成了 24 為病人的軟骨層片植入，病人年紀在 26 到 69 歲，11 位男性、18 位女性，最久的病人已經追蹤滿 10 個月。所有的病人都有一致性的功能進步、疼痛減輕，甚至在追蹤評估的核磁共振（MRI）中，看到軟骨再生的證據，是目前的特管辦法中，收案最多、成功率最高的項目。2021 年 02 月，衛福部更增修了特管辦法的條文，開放除了表訂六項之外的細胞治療項目，更進一步的將細胞治療在台灣往前推進。下一階段的軟骨重建將發展到異體軟骨移植，透過胼指症的小朋友提供的軟骨，可以大幅度增加培養出來軟骨的品質、數量，使病人無需二次手術，透過一階段手術就可以完成治療，重建缺損的軟骨。目前義大醫院也已經著手安排人體試驗，以及後續的特管辦法申請，以期更多的病人受惠。

脊髓損傷造成患者嚴重的肢體功能喪失，胸腰椎神經損傷者，會造成下肢半身癱瘓，而頸椎神經損傷者，會導致四肢全身癱瘓，患者會完全喪失自我照顧能力，大小便失禁，生活沒有尊嚴。義大醫院透過複雜的顯微重建手術，使用肋間神經進行神經繞道，重建受損的脊髓神經，可以使得脊髓損傷的病人獲得更好的功能恢復。這一技術發展至今已經使許多癱瘓病人重新站起來。除細胞療法之外，近幾年細胞外泌體被證實其於各項疾病治療成效，是極具潛力的治療方式。在這次研討會當中，本人也將報告近期細胞外泌體於調控神經幹細胞與改善軟骨細胞老化治療軟骨缺損初步的研究成果。

Curriculum Viate

中文姓名	林 欣 榮	英文姓名	Shinn Zong Lin	出生日期	1955年04月02日

一、學　歷（擇其重要者填寫）			
學校名稱	學 位	起迄年月	科 技 專 長
國防醫學院	學士	1973/08 至1980/07	醫學系
杜蘭大學	碩士	1998/10 至2000/05	醫院管理學
紐約州立大學石溪校區	博士	1986/09 至1989/08	神經外科及生理學

二、經　歷（請按服務時間先後順序填寫與現提計畫有關之經歷）		
服務機構及單位	職 稱	起迄年月
現任： 花蓮慈濟醫院	院長	2016/07-迄今
佛教慈濟醫療財團法人創新研發中心	研發長	2016/07-迄今
經歷：中國醫藥大學附設醫院	院長	自2016/02-2016/06
中國醫藥大學	特聘教授	自2015/08-2016/06
中國醫藥大學北港附設醫院	院長	自2009/02-2016/02
中國醫藥大學附設醫院	副院長	自2007/08-2016/01

傑出成就

◎林欣榮院長榮獲美國神經治療及再生學會最傑出獎

2010 年，林欣榮院長榮獲美國神經治療及再生學會最傑出獎殊榮，這個獎是第一次頒給華人。林欣榮院長是是神經外科及生理學博士，他是開創臺灣將胚胎幹細胞成功移植在巴金森病人身上的第一人。

美國國家衛生研究院藥物濫用研究所主席 Barry Hoffer 教授(2007年該獎項得主)在頒獎時指出，林欣榮教授的得獎事蹟是：(1)共發表超過 180 篇文章，包括基礎、臨床及轉譯醫學的研究論文；(2)已獲得九項專利；(3)成功主辦「泛太平洋國際幹細胞研討會」等各類大型國際會議，包括 2005 年的世界神經移植及再生會議；(4)成功的進行幹細胞治療神經疾病的轉譯醫學，引領世界風潮。

◎美發明家院士 林欣榮院長臺灣第一人

2012 年，已獲得二十多項國際發明專利，並長期投入研發惡性腦瘤新藥的林欣榮院長，獲選 National Academy of Inventors (NAI)「美國國家發明家學會」發明家院士。九十八名獲獎院士中，有八人為諾貝爾獎得主，林欣榮是臺灣第一位獲此榮銜的大學教授，可說是科學界的「臺灣之光」。

林欣榮獲頒發明家院士的主因，是在於他發明廿多項國際專利，還長期投入治療惡性腦瘤的新藥研發，並將藥物轉化為產品，且從臨床經驗研發腦部外科手術用的導航定位系統(BPS)，讓腦外科手術更為準確。

◎研發腦瘤新藥有成　林欣榮院長研發團隊榮獲第十一屆國家新創獎

2014 年，林欣榮院長帶領的研發團隊，成功研發治療惡性腦瘤的標靶藥物，榮獲社團法人國家生技醫療產業策進會第十一屆國家新創獎表揚。

原發性腦瘤世界上約有二百二十五萬人，然而治療人類惡性腦瘤的藥物種類少且效果不彰，傳統的外科手術治療及術後放射線治療，很少能活過一年，林欣榮院長帶領韓鴻志教授、邱紫文教授、詹子民助理研究員等，創新研究找出新的標靶藥物，增加治療效果及減少大腦副作用，又製成置入大腦的緩釋劑 HK-001-Wafer，能有效去除手術後殘存的惡性腦膠質瘤細胞，延長壽命。

◎林欣榮獲頒美國科學促進會院士

2015 年，林欣榮院長以幹細胞治療神經疾病的轉譯醫學研究，與研發標靶新藥治療惡性腦瘤，在國際上獲得高度肯定和推薦，榮獲美國科學促進會 (American Association for the Advancement of Science，簡稱 AAAS)院士(AAAS Fellow)榮譽，並於 2016 年2 月在美國華盛頓特區舉辦的美國科學促進會年會中接受表揚。

美國科學促進會是世界最大的非營利科學組織，下設二十四個專業分會，包括數學、物理、化學、天文、地理、生物、醫學等領域，也是科學雜誌(Science)的出版者。美國科學促進會在 2015 年選出三百四十七位院士，他們都是在專業領域對發明創新、教育和科學有卓越貢獻的頂尖人才，同時對豐富人類生活質量，促進科學發展與教育有顯著的貢獻和深遠的影響。

15:30-15:55

I-11

Stem Cells and Combination Therapy with Rehabilitation for Chronic Stroke

林欣榮 蔡昇宗 邱琮朗
SHINN-ZONG (John) LIN, M.D., Ph.D., CPI, Sheng-Tzung Tsai, M.D., Ph.D., Tsung-Lang Chiu, M.D., Ph.D.
Department of Neurosurgery, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation

Stroke is the leading cause of neurological disability in adults worldwide and involves significant impairment of sensory-motor function caused by cerebral ischemia and subsequent neuronal death. Owing to a lack of medical or surgical treatments to improve neurological function and neurogenesis, chronic stroke places a huge burden on patients, their families, and society. Over the past twenty years, increasing evidence from translational and clinical research has demonstrated the potential effectiveness of growth factors and stem cells to improve neural plasticity and neurogenesis. These treatments include intravenous or intra-arterial hematopoietic growth factors and stem cell administration or intra-cerebral transplantation for the disability of chronic stroke. In particular, these studies have included granulocyte colony-stimulating factor, umbilical cord blood stem cells, neural stem cells, mesenchymal stem cells, such as autologous CD34+ peripheral blood stem cells, and adipose-derived mesenchymal stem cells. Each different type of stem cell has various advantages on clinical application and stroke recovery; however, mesenchymal stem cells are currently the most widely used cell type for stroke therapy. In addition, multi-modality treatment including intensive rehabilitation are also very important.
Given the growing evidences to show the benefit of combination with stem cell and rehabilitation therapy, we could anticipate that patients with chronic stroke will get better quality of life in the near future. In addition, it is also important to consider the safety of these putative therapies whilst achieving the maximum benefit for patients with chronic stroke in terms of the route of administration and stem cell numbers.

Curriculum Viate

戴念梓 教授

國防醫學院三軍總醫院整形外科

最高學歷

學　　校： 英國阿斯頓大學

科　　系： 健康與生命科學院

畢業年度：2007

級　　別：■研究所

	單位名稱	職稱	教學年資	實務年資	研究年資
現職	國防醫學院三軍總醫院整形外科	主任	18	22	16
經歷	美國史丹佛大學整形外科	臨床研究員			
	英國伯明罕大學附設醫院燒傷中心	臨床研究員			
	國防醫學院三軍總醫院整形外科	主治醫師		22	
	國防醫學院三軍總醫院整形外科	總醫師		1	
	國軍金門 820 醫院	住院醫師		2	

專 長　燒燙傷臨床醫學與研究、組織工程研究、整形外科、醫學美容、雷射除斑及疤痕治療、手外科、臨床試驗。

特殊成就
1. 榮獲第九、十三、十六屆國家新創獎
2. 榮獲衛福部八仙樂園粉塵暴燃事件有功人員表揚及獎狀

15:55-16:20

I-12

細胞治療案例分析與成果報告

戴念梓醫師 Niann-Tzyy Dai, MD, PhD
國防醫學院 三軍總醫院 外科部 整形外科
Division of Plastic and Reconstructive Surgery, Department of Surgery, Tri-Service General Hospital, National Defense Medical Center, Taiwan, R.O.C.

目標

讓學員透過實際細胞治療案例之分析與成果以了解所應考慮的臨床實務問題，進而在醫學倫理原則的考量下，能重新審視計畫書內容的合理性及可行性，以利細胞臨床試驗的執行。

課程綱要

本文內容包含：細胞治療特管辦法所公告之細胞治療項目、細胞的功能、細胞的種類、細胞產品與藥品的區別、細胞治療可能發生之風險、體細胞醫藥品製配作業、細胞臨床治療/試驗計劃注意事項、並且以實際細胞治療案例之分析與成果來說明細胞治療計畫之合理背景、細胞治療的可能療效、合理且必要的收案條件及排除條件、同意書的內容、治療的步驟及規劃、試驗進行的合理期程及追蹤時點、細胞臨床治療應特別考慮事項等。最後再分享多種細胞操作與臨床治療之實務與成果。

Curriculum Vitae

洪士杰，Shih-Chieh Hung

EDUCATION
M.D. National Yang-Ming University, 1990
Ph.D. The University of Tokyo, 1997.

ACADEMIC APPOINTMENTS
Distinguished Professor, Department of New Drug Development, China Medical University;
Joint Investigator in Institute of Biomedical Sciences, Academia Sinica

SERVICE
Director, New Drug Development Center; Integrative Stem Cell Center, Department of Orthopaedics, China Medical University Hospital

HONORS & AWARDS
科技部傑出研究獎 2012, 2016
科技部特約研究畫主持人, 2019
第16屆永信李天德醫藥科技獎
中華民國生物產業發展協會年度創新獎 2015
臺北生技獎-技轉合作獎之優勝 2015

Selected Publications: (selected from ~180)

1. CH-Liu, Sengupta Raj, CH-Chen, CT-Chou, IH-Chen, JT-Chien, IY-Lin, SY-Yang, Takashi Angata, WC-Tsai, James CC-Wei, IS-Tzeng, **SC-Hung***, KI-Lin*. HLA-B-RARB-TNAP pathway promotes syndesmophyte formation in ankylosing spondylitis. **Journal of Clinical Investigation**, 129(12):5357-5373, 2019.
2. WC-Shen, YC-Lai, LH-Li, Kolin Liao, HC-Lai, SY-Kao, John Wang, CM-Chuong, **SC-Hung***. PTEN activation and methylation are associated with lineage commitment and imply tumorigenesis in mesenchymal stem cell. **Nature Communications**, 10(1):2226, 2019. (recommended by Faculty 1000-Prime)
3. CC-Liu, SP-Lin, HS-Hsu, SH-Yang, CH-Lin, MH-Yang, MC-Hung, **SC-Hung***. Suspension survival mediated by PP2A-STAT3-Col XVII determines tumour initiation and metastasis in cancer stem cells. **Nature Communications**, 7:11798, 2016.
4. KC-Lee, HC-Lin, YH-Huang, **SC-Hung***. Allo-transplantation of mesenchymal stem cells attenuates hepatic injury through IL1Ra dependent macrophage switch in a mouse model of liver disease. **J Hepatology**, 63(6):1405-12, 2015.
5. CC-Tsai, PF-Su, YF-Huang, TL-Yew, **SC-Hung***. Oct4 and Nanog directly regulate Dnmt1 to maintain self-renewal and undifferentiated state in mesenchymal stem cells. **Molecular Cell**, 47: 169–182, 2012. (recommended by Faculty 1000-Prime)
6. CC-Tsai, YJ-Chen, LL-Chen, TL-Yew, JY-Wang, CH-Chiu, **SC-Hung***. Hypoxia inhibits senescence and maintains mesenchymal stem cell properties through downregulation of E2A-p21 by HIF-TWIST. **Blood** 117(2):459-69. 2011.
7. KS-Tsai, SH-Yang, YP-Lei, CC-Tsai, HW-Chen, CY-Hsu, LL-Chen, HW-Wang, Stephanie A. Miller, SH-Chiou, MC-Hung*, **SC-Hung***. Mesenchymal Stem Cells Promote Formation of Colorectal Tumors in Mice. **Gastroenterology**, 141(3):1046-56, 2011.

16:20-16:45

I-13

Epigenetic Regulation of Self-renewal, Differentiation and Oncogenesis of Mesenchymal Stem Cells

Shih-Chieh Hung
Distinguished Professor & Director, Drug Development Center, Institute of New Drug Development, China Medical University;
Integrative Stem Cell Center, China Medical University Hospital
Adjunct Research Fellow, Institute of Biomedical Sciences, Academia Sinica

Human bone marrow-derived mesenchymal stem cells (MSCs) have emerged as a promising tool for clinical application, however, the mechanisms that regulate MSC properties have not been well studies. We demonstrate the important roles of Oct4 and Nanog in maintaining proliferation and differentiation potential and suppressed spontaneous differentiation, via DNA methyltransferase (DNMT)1 upregulation through direct binding to its promoter, thereby leading to the repressed expression of p16 and p21 and genes associated with development and lineage differentiation (1). Furthermore, the role of retinoblastoma (RB) protein in regulating MSC properties has also been demonstrated. We show that RB levels are higher in early-passage MSCs compared with late-passage MSCs, which upregulates DNMT1 expression and inhibits senescence in early-passage MSCs (2).
Lineage commitment and tumorigenesis, traits distinguishing stem cells, have not been well characterized and compared in mesenchymal stem cells derived from human dental pulp (DP-MSCs) and bone marrow (BM-MSCs). Here, we report DP-MSCs exhibit increased osteogenic potential, possess decreased adipogenic potential, form dentin pulp-like complexes, and are resistant to oncogenic transformation when compared to BM-MSCs. Genome-wide RNA-seq and differential expression analysis reveal differences in adipocyte and osteoblast differentiation pathways, bone marrow neoplasm pathway, and PTEN/PI3K/AKT pathway. Higher PTEN expression in DP-MSCs than in BM-MSCs is responsible for the lineage commitment and tumorigenesis differences in both cells. Additionally, the PTEN promoter in BM-MSCs exhibits higher DNA methylation levels and repressive mark H3K9Me2 enrichment when compared to DP-MSCs, which is mediated by increased DNMT3B and G9a expression, respectively (3). The study demonstrates how several epigenetic factors broadly affect lineage commitment and tumorigenesis, which should be considered when developing therapeutic uses of stem cells.

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1. C. C. Tsai, P. F. Su, Y. F. Huang, T. L. Yew, S. C. Hung, Oct4 and Nanog directly regulate Dnmt1 to maintain self-renewal and undifferentiated state in mesenchymal stem cells. Mol Cell 47, 169-182 (2012).
2. S. P. Lin et al., RB maintains quiescence and prevents premature senescence through upregulation of DNMT1 in mesenchymal stromal cells. Stem Cell Reports 3, 975-986 (2014).
3. W. C. Shen et al., Methylation and PTEN activation in dental pulp mesenchymal stem cells promotes osteogenesis and reduces oncogenesis. Nat Commun 10, 2226 (2019).

Curriculum Vitae

張毓翰 MD, PhD

最高學歷

台灣 長庚大學 臨床醫學研究所 醫學博士

經歷

台灣 長庚紀念醫院 骨科部 住院醫師

台灣 長庚紀念醫院 骨科部 總醫師

美國 俄亥俄州 克里夫蘭 凱斯西儲大學附設醫院 骨科部 關節重建科 研究員 (師事 Prof. Victor M. Goldberg)

美國 俄亥俄州 克里夫蘭 凱斯西儲大學 生物系 骨骼研究中心 研究員 (師事 Prof. Arnold I. Caplan)

美國 Biominetic Pharmaceutical, Inc. 生物科技公司研究顧問

現任

林口長庚 骨科部 關節重建骨科 主任

長庚醫院 骨科部 教授級主治醫師

林口長庚醫院 生物資料庫 主席

林口長庚醫院 研究審查會 副主席

林口長庚醫院 臨床試驗中心 副主任

台灣關節重建醫學會 常務理事

台灣骨科醫學會雜誌. 主編

16:45-17:10

I-14

細胞治療在退化膝關節的應用

張毓翰 MD, PhD
林口長庚醫院 關節重建骨科 主任
林口長庚醫院　骨科部 教授
長庚大學 醫學系 教授

Knee osteoarthritis (OA) has become the most popular disease in an aging society. Every year about 27,000 Taiwanese get knee replacements, a number that could rise to 3-fold by 2035. Until recently, treatment options were either temporary or surgical. Knee arthroplasty is highly successful, there are still inherent risks associated as an invasive procedure. The use of biological therapies for knee OA management is a growing interest. Throughout the world, including Taiwan, stem cell therapy is being touted as a miracle cure for everything from wrinkles to spinal repair. The use of stem-cell therapies for the treatment of various musculoskeletal conditions, especially knee osteoarthritis, is rapidly expanding, despite only low-level evidence to support its use. Ongoing research is striving to determine which stem cell knee therapy techniques, cell choices, and dosages yield the most effective and consistent results. Stem cell therapy for knees is still very new and the TFDA is proceeding with caution, but studies so far are promising. The current evidence of using stem cell for the treatments of knee OA will be reviewed in this talk. Meanwhile, the preliminary results of using allogenic adipose derived stem cell for the knee OA treatment which has been completed in my institute will be presented.

Poster Paper

P-01

生物活性鈣鈦礦量子點/明膠納米顆粒，用於監測眼睛中的藥物輸送應用
Bioactive Perovskite Quantum-Dots/Gelatin Nanoparticle for Mornitoring Drug Delivery Applications in Eyes

Le Ngoc Hoang [1] 林群哲 [2,*] 曾靖孋 [1*]
臺北醫學大學生醫材料暨組織工程研究所 [1]
國立臺北科技大學分子科學與工程系暨有機高分子研究所 [2]

Introduction：The perovskite quantum dots (Perv-QDs) have shown significant promise for a range of optical technologies with unique structure. Basing on bright photoluminescence with ultra-narrow bandwidth of Perv-QDs, an emission wavelength that can be changed by adjusting the intensity of excitation, chemical composition and also emit much faster than Perv-QDs made from other materials. However, the strong toxicity of metal molecules in QDs is still the biggest limitation of the nano-type application in biomedical field. Therefore, the use of gelatin to create biocompatible shell is necessary. In this study, we synthesize Perv-QDs coated with gelatin (Perv-QDs@GNP) as a bioactive nanoparticle system for mornitoring drug delivery applications in eyes.

Materials and Methods：Gelatin type A, Cesium (II) bromide, Copper (II) bromide and all solvent were purchased from Sigma-Aldrich. Following typical formula of perovskite quantumdot as Cs_2BX_4 (X = Cl, Br, I), we created non-toxic Perv-QDs are by replacing Lead (Pb) in its composition with Copper (Cu), and also shell coating with gelatin to enhace the biocompatibility of the nanosystem.

Results：The Perv-QDs, GNP, and Perv-QDs@GNP were successfully prepared and their sizes were 10.0 ± 2.3 nm, 140.7 ± 46.1 nm, 136.2 ± 35.4 nm, respectively. The structure of the functional groups, size and morphology of particles were characterized by TEM, DLS, NTA, FTIR and XRD, which showed the homogeneous crystal structure of Perv-QDs still remained before and after encapsulation. The Photoluminescence (PL) method shows high luminescence efficiency of Perv-QDs before and after gelatin coating, and great intensity is also the advantage of Perv-QDs when tested with Optical Coherence Tomography (OCT) and Micro-CT.

Discussion：In this study, we have created an ophthalmic dispersible nanosystem that can be monitored in living animals (without sacrifice required). The fluorescence properties of perovskite quantum-dot have been widely used in optics or solar cells, but because of the many toxic limitations of the metallic elements present in Perv-QDs, they have not yet been applied in the biological field. The Perv-QDs@GNP nanosystems significantly reduced the toxicity of Perv-QDs, while also pioneering the biomedical application of Perv-QDs, especially in monitoring dispersion of the nanosystems in mammalian eyes.

Conclusions：The Perv-QDs@GNP nanosystem is one of our pioneering efforts to create a nanosystem that can track nanomedicine dispersion in the eyes over long periods of time. In the future work, in vitro and in vivo tests will be done continuously in order to prove the potential of Perv-QDs@GNP for tracking of nanomedicine distribution in the eyes and other organs.

P-02

於酸鹼應答混摻材料之細胞脫附:開發具高再生能力之脂肪幹細胞連續生產系統
Cell Detachment on pH-responsive Blended Surfaces: The Development of Continuous Cell Harvest of Human Adipose-derived Stem Cell with Enhanced Regenerative Capacity

顏嘉祥[1] 楊台鴻[1] 鄭乃禎[2]
國立台灣大學醫學工程研究所[1] 國立台灣大學附設醫院外科部[2]

Introduction：During the *in vitro* expansion of ASC monolayer culture, the quick loss of primitive stemness properties and the insufficient cell number for clinical demands have become a crucial issue for ASC-based target therapy. To improve the efficiency of stem cell therapy, the current study aims to develop a high-yield continuous cell harvest system for producing ASCs with enhanced regenerative capacity on pH-responsive polymer blends.
Materials and Methods：The human ASCs attachment and detachment in response to subtle pH variation were investigated on different ratios of chitosan/nylon polymer blends, where the chitosan component is to mediate cellular functions and control cell detachment, and the nylon component is introduced to facilitate cell adhesion.
Results：ASCs were capable of performing at least four repeated cycles of cell confluence and cell detachment on the pH-responsive blended surfaces.
In vitro studies proved: the healing potential of the harvested cells was increasing as the working cycle increased.
In vivo wound healing model showed prominent tissue regeneration of harvested cells derived from the blended surfaces.
Discussion：Due to the microphase separation, polymer blends with the coexistence of pH-responsive and pH-nonresponsive domains could be applied to facilitate the repeated pH-dependent cell harvest cycle and be considered a smart material.
It is rich in the potential to design polymer composites with desired physical properties while preserving each capacity to facilitate cellular behaviors.
Conclusions：The rapid cell production of ASCs in the continuous cell harvest system was demonstrated with enhanced stemness and regenerative capacity, including self-renewal, multi-lineage differentiation, cell proliferation, and pro-angiogenic ability, expression of CXCR4, as well as more resistant to the cellular senescence.

P-03

利用血小板釋放的細胞外囊泡包覆山奈酚以抑制血管新生之藥物
Use the Platelets Extracellular Vesicles to loading the Kaempferol for Anti-angiogenic

張哲禕　白台瑞　曾靖孋 [1]
台北醫學大學生醫材料暨組織工程研究所

Introduction：Extracellular vehicles (EVs) are physiological membrane-secreted nanosized particles released by cells. With a size of ca. 50 to 300 nm, and membrane markers, EVs can be up-taken by cells and can cross biological barriers. EVs are expected to exhibit limited side effect for *in vivo* application as targeted drug delivery system. Human platelets are known to be safe and reliable source materials and to generate numerous EVs. In this study, we used platelet derived EVs (PEVs) as a carrier for loading kaempferol (KA), an herbal polyphenolic compound with anti-angiogenic properties. We hypothesized that KA-loaded PEVs can be used as a new nanomedicine for enhancing drug bioavailability and providing anti-angiogenetic effect for inhibit neovascularization.

Materials and Methods：Human platelet concentrates were centrifuged and separated to get platelets EVs (PEVs). The KA solution was loaded into PEVs thereafter, and then we used dialysis method to remove the unloaded KA and then recover the KA-loaded PEVs. Dynamic light scattering (DLS) was used to determine the mean size population and zeta potential. High-performance liquid chromatography (HPLC) was used to quantify the KA concentration within PEVs. The angiogenesis effect of this KA-loaded PEVs (PEVs-KA) was assessed by co-culture with human umbilical vein endothelial cells (HUVEC cell).

Results and Discussion：The mean population size of the unloaded and KA-loaded PEVs was 193.9 ± 5.6 nm and 259.5 ± 30.1 nm, respectively. HPLC result confirmed the successful loading of KA within PEVs and indicated a loading rate was over 70%. We co-cultured with KA at 10 μg/mL, the HUVECs migration capacity was inhibited by PEVs-KA obviously.

Conclusions：KA can be loaded within PEVs at a high drug-loading rate. The KA-PEVs exhibit a suitable size range for drug delivery. Furthermore, the PEVs-KA can inhibit the HUVEC cell effectively when compare with the KA solution only. We also used the cornea endothelial (BCE) cell to check the safety for using KA-PEVs in eyes. At 10μg/mL KA concentration of PEVs-KA and KA, BCEs could not be affected. These data provide preliminary indications on the potential of KA-PEVs to be used in eyes for anti-angiogenetic treatment.

P-04

人類間葉基質細胞在調節周邊 B 細胞上的組織特異性
Resident vs. Non-resident Multipotent Mesenchymal Stromal Cell Interactions with B Lymphocytes Result in Disparate Outcomes

李瑋[1,2] 王麗姿[3] 嚴孟祿[3] 徐珮茹[2] 李雨薇[2]
劉柯俊[4] 林國儀[5] 蘇郁文[6] 司徒惠康[7] 顏伶汝[2]
國防醫學院生命科學研究所[1] 國家衛生研究院細研所[2] 台大醫學院婦產科[3]
國衛院癌研所[4] 中央研究院基金體中心[5] 國衛院免疫所[6] 國衛院感疫所[7]

Multipotent human mesenchymal stromal cells (MSCs) from multiple organs including the bone marrow (BM) and placenta harbor clinically relevant immunomodulation best demonstrated towards T lymphocytes. Surprisingly, there is limited knowledge on interactions with B lymphocytes, which originate from the BM where there is a resident MSC. With increasing data demonstrating MSC tissue-specific propensities impacting therapeutic outcome, we therefore investigated the interactions of BM-MSCs—its resident and 'niche' MSC—and placental MSCs (P-MSC), another source of MSCs with well-characterized immunomodulatory properties, on the global functional outcomes of pan-peripheral B cell populations. We found that P-MSCs but not BM-MSCs significantly inhibit proliferation and further differentiation of stimulated human peripheral B populations *in vitro*. Moreover, while BM-MSCs preserve multiple IL-10-producing regulatory B cell (Breg) subsets, P-MSCs significantly increase all subsets. To corroborate these *in vitro* findings i*n vivo*, we used a mouse model of B cell activation and found that adoptive transfer of P-MSCs but not BM-MSCs significantly decreased activated $B220^+$ B cells. Moreover, adoptive transfer of P-MSCs but not BM-MSCs significantly decreased overall $B220^+$ B cell proliferation and further differentiation, similar to the *in vitro* findings. P-MSCs also increased two populations of IL-10-producing murine Bregs more strongly than BM-MSCs. Transcriptome analyses demonstrated multifactorial differences between BM- and P-MSCs in the profile of relevant factors involved in B lymphocyte proliferation and differentiation. Our results highlight the divergent outcomes of tissue-specific MSCs interactions with peripheral B cells, and demonstrate the importance of understanding tissue-specific differences to achieve more efficacious outcome with MSC therapy.

P-05

利用表面形態控制幹細胞分化探討應用於視網膜修復之可行性
The Possibility of Using Binary Colloidal Crystals Arrayed on Surface to Modulate Stem Cells Differentiated in to Retinal-like Cells Epithelium Cells for Retina Repair

張哲禕[1] 陳盈汝[1] 張睿[1] 范育睿[1] 王鵬元[2,3] 曾靖孋[1]
台北醫學大學[1] 澳洲國立旋濱科技大學[2] 中國科學院大學[3]

Introduction：視力下降和失明是一個嚴重的問題，特別是在台灣人口持續老化與許多 3C 產品重度使用者，發展幹細胞用於視網膜再生與分化將有助於疾病治療。人類骨髓間充質幹細胞（Human bone marrow derived mesenchymal stem cell, MSCs）是一種具有分化潛能的細胞群，目前已有許多研究指出，特殊的表面結構可誘導 MSCs 分化成脂肪、骨骼。而目前尚未有研究藉由表面結構誘導 MSCs 分化成視網膜色素上皮細胞(retinal pigment epithelium, RPE)，此部分值得深入探討。本研究是利用一種名為雙顆粒單層膜(Binary colloidal crystals, BCC)新型材料，透過表面改質誘導 MSCs 分化為 RPE 細胞，對於推動 RPE 走向臨床應用具有極大的潛力。

Materials and Methods：微米矽粒子(SiO_2)和帶有官能基的奈米聚苯乙烯粒子(polystyrene, PS)，會以自組裝的方式製備出 BCCs (BCC4 和 BCC8)。BCCs 會先測定材料表面的親疏水性、表面元素分析、以及表面型態。MSCs 培養於特製 BCC 培養盤上觀察型態變化、免疫螢光染色用於分析細胞骨架結構、細胞分化程度以即時聚合酶鏈式反應分析 RPE 特異性基因表現。不同分化時期所表現之基因 Nanog, Oct-4, VSX2, OTX2, MITF, Pax6, CRALBP, RPE65, BEST-1 會於本實驗分析其表現。

Results and Discussion：分析 PS 粒子表面特徵其結果顯示，BCC4 不具有羧基而 BCC8 具有羧基，SEM 的結果顯示 BCC4 和 BCC8 有相同的微米結構。親疏水性接觸角分析結果顯示，與 TCPS 相比，BCC8 為相對親水表面，BCC4 則為相對疏水表面。表面元素分析顯示，氧元素含量於 BCC8 高於 BCC4，此部分可能是源自於羧基。細胞活性測試顯示 BCC4、BCC8 兩者具有相似細胞活性；BCC 並不會造成細胞毒性。細胞影像則可觀察到 BCC4 上的細胞具有明顯較細長的骨架結構，而培養於 BCC8 的細胞具有較大攤開面積。細胞培養 15 天後分析 RPE 特異性基因，相較於普通細胞培養盤，BCC4 和 BCC8 培養下 RPE 特異性基因表現量皆有上升趨勢，其中又以 BCC4 最為明顯。我們認為這可能和 BCC4 有著讓細胞更容易拉長的特性有關，因 RPE 細胞是和神經高度相關的細胞，且先前有研究指出，幹細胞在被拉長的情況下有機會進行走向神經分化，因此我們認為 BCC4 相對其他表面更有機會促進 RPE 分化。

Conclusions：本研究建立在新型材料雙顆粒單層膜 BCC4 培養條件下，可誘導 MSC 分化為 RPE，BCCs 可做為創新性誘導細胞分化之基底材料。

P-06

超音波與壓電刺激促進人類骨髓間質幹細胞的遷移、增生及軟骨分化
Ultrasound and Piezoelectric Field Stimulate Human Mesenchymal Stem Cells Migration, Proliferation and Differentiation

劉禹呈 黃文顥 林若梅 王兆麟
國立台灣大學 醫學工程研究所

Introduction： 一直以來，較低再生能力之軟骨修復問題是個難以克服的障礙，所以近年來以可分化為多種細胞的人類骨髓間質幹細胞(MSC)作為分化軟骨之研究，可視為解決方法之一。

臨床使用超音波歷史悠久，研究表示使用低強度脈衝超音波可誘導軟骨、肌腱組織的再生。而軟骨也因形變產生電場，造成細胞遷移、聚集等現象，研究亦表示電刺激可誘導 MSC 的軟骨分化。

Materials and Methods： 實驗中使用本實驗室開發的超音波刺激載台做傷口復原實驗、增生及分化聚合的超音波與壓電刺激，觀察骨髓間質幹細胞在不同刺激原下的傷口復原速度、增生程度、與分化聚合現象。超音波與壓電刺激分別是種於玻璃片與石英片上，石英為一壓電材料，藉由機械震動使石英片產生微小型變，進而產生電場造成電場刺激。

Results： 在傷口復原實驗中可觀察到超音波與壓電電場刺激皆可提升傷口復原速度，超音波刺激恢復速度為未刺激組別的 1.2 倍，而壓電刺激則是未刺激組別的 1.4 倍。在增生實驗中，壓電刺激中的細胞數量增生多於超音波刺激與未刺激組約 60%，經由西方點墨法和免疫螢光染色標定磷酸化的細胞外調節激酶(phospho-ERK)蛋白，兩種方法皆可觀察到壓電刺激組別的 phospho-ERK 上升大於未刺激組。在分化與聚合實驗中也可看出壓電刺激確實可以促進分化及聚合過程，並使用艾爾遜藍(Alcian blue)成功染色軟骨、和使用西方墨點法標定細胞內的 SOX9 濃度提升來確認軟骨分化成功以支持此論點。最後以初級纖毛角度分析及 β-環連蛋白(β-catenin)濃度探討細胞極性與 WNT 傳遞路徑。

Discussion： 在分化聚合實驗中，可以觀察到 MSC 受壓電刺激聚合時有極性產生，認為 MSC 對電場較敏感，但在軟骨細胞的元代細胞培養上並未觀察到此現象，認為可能是 MSC 內之電敏感原在完全分化成軟骨後減少或消失，需再進行後續實驗才能確認之。而壓電環境確實是個有潛力的刺激方法，在臨床實踐可運用的潛力值得深入研究。故蛋白質的調控及其細胞膜分佈皆為目前蛋白質體分析的研究重點。

Conclusions： 本實驗說明並提出有效論證，超音波與壓電環境可影響幹細胞增生及遷移，並很有可能在人體內形成軟骨的過程中起作用，包括遷移、聚合以及在軟骨分化因子作用下進行分化。

P-07

超音波刺激促使小鼠腦神經細胞新生
An in Vivo Study of Inducing Neurogenesis in Mouse Brain Upon Ultrasound Stimulation

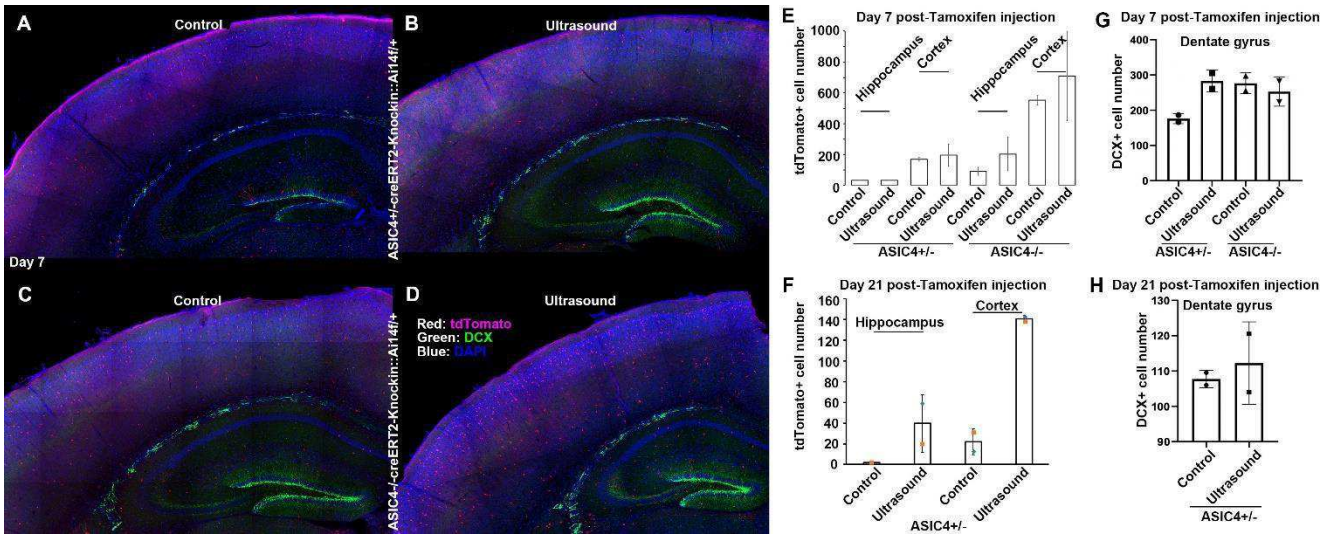
戴小芯　林若梅　朱亞成　王兆麟
國立台灣大學 醫學工程研究所

Introduction：Applications of ultrasound are anticipated to provide a non-invasive therapeutic intervention. The ultrasound stimulation inducing ERK phosphorylation in neuronal cells was observed in our previous study. Here we use a genetic mouse labeling method to investigate the long term effect of ultrasound stimulation in brain. Lineage tracing is used to monitor the neurogenesis in this study. We used tamoxifen to induce reporter gene expression and mapped the expression of ASIC4 lineage in brain. Due to the exclusive expression of doublecortin (DCX) in developing neuroblasts, DCX as a marker for the potential of neurogenesis was assayed upon ultrasound stimulation.

Materials and Methods：The $Asic4^{-/-}$ ($Asic4^{CreERT2/CreERT2}$) mice and the heterozygote $Asic4^{+/-}$ ($Asic4^{CreERT2/+}$) mice received intraperitoneal injection of 3 mg tamoxifen a day before ultrasound stimulation (US). US was applied to mice brain at 1 MHz for 3 days (5 minute/day). The 900mVpp (duty factor 1%) input voltage results in an I_{SPTA} of 4.2mW/cm^2. The mice were sacrificed at day 7 and day 21 after tamoxifen injection. To observe the tdTomato signals, 100μm thick slides were sectioned from mouse brain by using vibratome. Slides were subjected to immunofluorescent staining of DCX (Cell Signaling Technology, USA) to detect the effect of US.

Results：ASIC4 lineage (tdTomato positive cells) increased in hippocampus and cortex upon US in $Asic4^{-/-}$mouse while remained unchanged upon US in $Asic4^{+/-}$ group (fig. E). In addition, tdTomato positive cells in US group were much more than in control group at the time point of 21 days after tamoxifen injection (fig. F). It seems that ASIC4 lineage survives longer if the mice are treated by US. In dentate gyrus, DCX was up regulated upon US in $Asic4^{+/-}$ mouse (fig. G), whereas there was no significant difference in 21 days sacrificed group (fig. H).

Discussion：Our results indicate that there are at least two kind of effects upon transcranial US. First, the DCX levels are upregulated within one week and second, the ASIC4 lineage remains surviving better at the time point of day 20 after initial US treatment. Moreover, the ASIC4 gene deletion may enhance the US effects.


A
Control
B
Ultrasound
ASIC4+/-creERT2-Knockin::Ai14f/+
Day 7
C
Control
D
Ultrasound
Red: tdTomato
Green: DCX
Blue: DAPI
ASIC4-/-creERT2-Knockin::Ai14f/+
E
Day 7 post-Tamoxifen injection
tdTomato+ cell number
Hippocampus
Cortex
Control
Ultrasound
ASIC4+/-
ASIC4-/-
F
Day 21 post-Tamoxifen injection
G
Day 7 post-Tamoxifen injection
Dentate gyrus
DCX+ cell number
H
Day 21 post-Tamoxifen injection

P-08

結合放射線照射及經腫瘤內注射自體 CD16⁺樹突狀細胞與 PD-L1 抗體之三合一療法對乾癬患者的末期鱗狀上皮癌治療

Intratumoral Injection of Autologous CD16⁺ Dendritic Cells and Anti-PD- L1 Antibody Combined with Radiotherapy: The Triple-regimen Therapy in a Psoriatic Patient with Advanced Cutaneous Squamous Cell Carcinoma

楊秉恆[12] 李玉鳳[3] 王麗姿[4] 黃文彥[5] 劉峰誠[6] 楊崑德[789]
國防醫學院醫學科學研究所[1] 三軍總醫院病理部臨床病理科[2] 國防醫學院醫學系[3]
台大醫院婦產部[4] 三軍總醫院放射腫瘤部[5] 三軍總醫院內科部風濕免疫科[6]
國防醫學院微生物及免疫學科暨研究所[7] 國立陽明大學臨床醫學研究所[8]
馬偕紀念醫院兒童過敏免疫風濕科[9]

Introduction：Cutaneous squamous cell carcinoma (cSCC), which accounts for 20% of cases of non-melanoma skin cancer, has a strong metastatic capability and thus a high mortality rate. Surgical excision is primarily adopted for localized cSCC, and radiotherapy or chemotherapy is used for locally advanced cSCC with nonresectable tumors or metastatic cSCC. Immunotherapy uses immune checkpoint inhibitors (ICIs) such as anti–programmed cell death protein 1 (anti–PD-1) antibody, which is known to enhance antineoplastic immune responses by reducing T cell exhaustion and then restimulating T cells. Anti–PD-1 antibody has been tested in a phase 1 trial for advanced cSCC and a phase 2 trial for metastatic cSCC as an alternative management strategy for patients with cSCC and surgical contraindication. Moreover, the efficacy of dendritic cell (DC)–based immunotherapy has been demonstrated in clinical trials for cancers other than cSCC. However, the limitations of immunotherapy include low PD-ligand (PD-L) expression levels in some patients and high costs for immune cell processing. In this article, we describe, to the best of our knowledge, the first protocol for enriching $CD16^+$ myeloid DCs (mDCs) from the peripheral blood (PB) of a patient with psoriasis who developed advanced cSCC.

Materials and Methods：The triple-regimen therapy (TRT) was carried out on a 63-year-old male patient with psoriasis vulgaris, plaque type (BSA~60%) for decades and complicated with advanced cSCC in right inguinal region. 2 ml product was collected through the leukapheresis procedure by using automation COBE Spectra Apheresis System (PBSC collection mode) which composed of autologous peripheral mononuclear cells with enriched DCs. The procedure was conducted without previous G-CSF mobilization and injected intratumoraly twice with 24hr interval within two consecutive days for three times. Meanwhile, three times of durvalumab (anti-PD-L1) injection with 40mg, 60mg, 120mg, respectively, were also intratumoral (iT) administrated concurrently with DCs injection. RT of right inguinal region was performed with a total dose of 60 Gy in 3 treatment courses and interrupted for 5 days after each conduction of iT injection. To uncover the mechanisms underlying this TRT protocol, we compared the transcriptomes between preintervention and postintervention biopsies using Metascape.

Results：In these DC-enriched PB samples, $CD16^+$ mDCs were the major subset in both the overall DC population and mDC subpopulation, with approximately 80% of the subpopulation being $CD16^+$ and only approximately 15% and less than 1% being $CD1c^+$ and $CD141^+$, respectively. The amounts of injected $CD16^+$-predominant mDCs were approximately 7.3×10^4, 2.5×10^6, and 1.7×10^7. Among the top 20 clusters with corresponding representative enriched terms in the Gene Ontology Biological Processes Kyoto Encyclopedia of Genes and Genomes Pathways and Reactome gene sets, the activation of both innate and adaptive immunity was

significantly enriched in postintervention biopsies. The efficacy of the TRT was encouraging in shrinking tumor mass with remarkable SUVmax reduction (approximately 42%) on FDG PET-Scan despite relatively low-dose DCs were available. Besides, normal subcutaneous tissues and muscular mass regenerate slowly with neoformation of vascular tissue enclosing the artificial artery stent.

Discussion：We present a case in which combined radiotherapy and immunotherapy was applied for treating a patient with cSCC and contraindications to surgery and chemotherapy, including vascular encasement by the tumor and risk of HBV reactivation. We first characterized PBSC apheresis samples derived from the PB of the patient, who had abnormal immune responses due to psoriasis and high levels of $CD16^+$ mDCs. Because the activation of peripheral $CD16^+$ mDCs has been reported to be upregulated by the secretion of inflammatory cytokines in patients with other autoimmune diseases, such as systemic lupus erythematosus, we assessed whether $CD16^+$ mDC-enriched cells isolated from the patient were potent for eliciting T cell response through intratumoral injection. DC-based immunotherapy was supplemented with anti–PD-L1 and radiation because both measures have been demonstrated to disrupt the growth of tumor bulk and improve the efficacy of immunotherapy through the enhancement of immune responses. In addition, our protocol could produce more tumor antigen–specific DCs than traditional DC-based immunotherapy could because the mutated tumor antigens are unrecognizable during long-term DC expansion *in vitro*.

Conclusions：In this study, we found that most DCs harvested were $CD16^+$-predominant (approximately 80%) mDCs and the activation of both innate and adaptive immunity was significantly enriched in post-TRT biopsies. This is significant because it is critical to demonstrate the success of our novel TRT could be attributed to T cells elicited by mDCs from peripheral circulation. Although prior research has identified a lot of combinational cell therapies for advanced cancers, almost DC-based immunotherapy is still cost-prohibitive and time-consuming. We develop the easily accessible combinational regimen with both time-saving and cost-effective benefit for treating cSCC patients, especially for those with contraindication of surgery and chemotherapy. Moreover, the combination of local injection of anti-PD-L1 is a genuine choice for a patient with autoimmune disease, such as psoriasis in our case who has a contraindication to use systemic administration of anti-PD1, anti-CTLA4 or anti-PD-L1.

P-09

幹細胞與體細胞形成三維球體之共同的生物路徑
3-Dimensional (3D) Sphere Formation in Stem Cells vs. Somatic Cells: Involvement of Conserved Pathways

張家齊[1,2,4] 江士昇[3] 李雨薇[2] 徐珮茹[2] 顏伶汝[2] 嚴孟祿[4]
國防醫學院生命科學研究所[1] 國家衛生研究院細胞及系統醫學所[2]
國家衛生研究院癌症研究所[3] 台灣大學醫學院醫學系婦產科[4]

Introduction：Novel cell culture methods such as 3-dimensional (3D) culture for spheroid formation is increasingly used to better mimic physiological states. However, the biological consequences of 3D spheroid formation could be drastically differed for diverse cell type. For embryonic stem cells (ESCs) which are pluripotent stem cells, spheroid formation—termed embryoid bodies (EBs)—mimics the natural process of embryo development, and thus is known to result in loss of pluripotency with a concomitant occurrence of differentiation and lineage commitment. In contrast, spheroid formation in somatic cell types may allow for selection of higher 'stemness' possessing cells, i.e. selection of somatic stem cell (SSC) population as has been done with neural stem cells and mammary stem cells. Despite the biological differences in the starting cells, however, there may be a "core process" governing the ability for 3D sphere formation. We therefore were interested in studying the conserved processes in 3D sphere formation.

Materials and Methods：We performed transcriptome analysis on conventionally 2D-cultured and 3D spheroid ESCs and SSCs to explore the "core process" for 3D spheroid formation.

Results：Pluripotency markers such as Oct4, Sox2, Nanog, & Klf4 were significantly down-regulated in ESCs after 3D sphere formation, as expected; however, these 4 markers were not significantly upregulated in somatic cells after spheroid formation. Convergent processes for both ESCs and SSCs include hypoxia and cell cycle arrest. Other highly expressed pathways include metabolism and oxidative phosphorylation. We found a metabolic pathway can mediate cytoskeleton rearrangement to regulate spheroid formation across diverse cell types.

Discussion：3D spheroid formation is a process for lineage differentiation on ESCs but a strategy to maintain SSC stemness. While the mechanisms of spheroid formation involved in of these two types cell is not due to the pluripotency, but a metabolic pathway.

Conclusions：Our data implicates pluripotency, hypoxia, and cell cycle arrest are not playing a vital role in 3D spheroid formation across diverse cell types. One metabolism pathway mediate spheroid formation via cytoskeleton rearrangement.

P-10

以臍帶血間葉幹細胞分化而成之類許旺細胞所建立之三維幹細胞球體應用於周邊神經損傷之修復

Development of 3D Cell Spheroids of MSC-derived Schwann-like Cells for Treating Peripheral Nerve Injury

李筠蔚 [1] 林郁婕 [1] 張哲瑋 [1,2] 黃玠誠 [1*]
國立清華大學生物醫學工程研究所 [1] 國立清華大學醫學科學系 [2]

Introduction：Peripheral nerve injury is one of the most frequent causes of disability globally, with significant clinical and socioeconomic effects. It has been reported that the microenvironment established by Schwann cells at the damaged tissue may play a crucial role in promoting the survival of neurons and the ultimate prognosis. In this study aims to transplant cbMSC-derived SC-like cells (SCLCs) raised using a non-genetic approach in the configuration of multicellular three-dimensional (3D) spheroid, which is known to recapitulate the in vivo physiological conditions and improve the cell retention and survival after injection.

Materials and Methods：

1. Differentiation of cbMSCs Into SCLCs

The differentiation process was initiated by replacing the growth medium of cbMSCs with αMEM containing 1 mM β-mercaptoethanol for 24h. Cells were then incubated for 72 h with medium containing 35 ng/mL RA. Subsequently, growth medium with RA, 5.7 mg/mL forskolin, 10 ng/mL bFGF, 5 ng/mL platelet-derived growth factor-AA and 126 ng/mL glial growth factor-2 was employed for the cultivation of cbMSCs for 2 weeks to establish SCLCs.

2. Preparation of 3D Spheroids of cbMSC-Derived SCLCs

The cbMSC-derived SCLCs were assembled into three-dimensional (3D) cell spheroids by using a methylecellulose hydrogel system.

3. Effects of SCLCs on SH-SY5Y Cell Neurite Formation

Two methods are applied: 1) Indirect coculture, the differentiated SH-SY5Y cells and SCLCs were inoculated into different wells in a μ-Slide 2 Well Co-Culture. 2) Direct coculture, the differentiated SH-SY5Y cells and SCLCs were mixed and plated into a μ-Dish for cultivation. After coculture, the cells were fixed and processed for immunofluorescence staining.

4. Rat Model of Sciatic Nerve Crush Injury and Cell Transplantation

An incision was made on the right thigh posteriorly to create a crush injury. Immediately after establishment of the crush injury, saline, cell suspensions of undifferentiated cbMSCs or cbMSC-derived SCLCs, or 3D spheroids of cbMSC-derived SCLCs were transplanted into the injured site (5 × 105 cells in 20 μL saline).

Results：The cell–cell and cell–extracellular matrix interactions were well-preserved within the formed 3D SCLC spheroids, and marked increases in neurotrophic, proangiogenic and anti-apoptotic factors were detected compared with cells that were harvested using conventional trypsin-based methods, demonstrating the superior advantage of SCLCs assembled into 3D spheroids. Transplantation of 3D SCLC spheroids into crush-injured rat sciatic nerves effectively promoted the recovery of motor function and enhanced nerve structure regeneration. In summary, by simply assembling cells into a 3D-spheroid conformation, the therapeutic potential of SCLCs derived from clinically available cbMSCs for promoting nerve regeneration was enhanced significantly. Thus, these cells hold great potential for translation to clinical applications for treating peripheral nerve injury.

Discussion：we collected the CSC aggregates, which were fabricated using the MC hydrogel

system, and plated them into a culture plate. The CSC aggregates were able to adhere to the surface of the plate, migrate out, and proliferate continuously as time progressed. Meanwhile, GFAP, the marker of Schwann cells, was still highly expressed. Comparing to the traditional mono-layer cell culture, the cell-cell or cell-ECM interactions are well-preserved in the 3D cell aggregates during the procedure of transplantation, thus resulting to their better therapeutic effect. Therefore, the 3D CSC aggregates may hold great promise to be used in treating peripheral nerve injury in the future.

Conclusions：In this study, the present findings demonstrate the therapeutic potential of cbMSC-derived SCLCs for promoting the functional recovery and structural repair of injured peripheral nerves can be significantly enhanced by assembling the cells into a 3D-spheroid conformation. Such an approach may have great potential for translation to clinical applications for treating peripheral nerve injury.

P-11

可注射式三維複合細胞球體作為腎小球足細胞補充之平台
Injectable 3D Hybrid Cell Spheroid as a Platform for Replenishing Glomerular Podocyte

陳立騏[1] 楊文妤[1] 許翔皓[2] 黃玠誠[1*]
國立清華大學生物醫學工程研究所[1] 林口長庚醫院腎臟科[2]

Introduction：足細胞為一種特別的上皮細胞，主要貼附於腎絲球內血管並與蛋白交織成一個過濾系統，提供腎臟對大分子蛋白的滲透選擇性。然而因足細胞為一種不再生的細胞，若成人體內足細胞因高血壓的機械力或是藥物造成傷害後，會造成腎臟永久性的傷害，進而導致蛋白尿等腎臟功能異常。因此本研究透過外源性的足細胞、血管內皮細胞與間葉幹細胞形成三維複合細胞球體去補充受損的足細胞，解決蛋白尿的症狀，並探討三種細胞在腎臟修復上所扮演的角色。

Materials and Methods：我們利用甲基纖維素作為 3D 細胞球體的平台，將永生化的小鼠足細胞(podocyte, P)、人類臍帶血間葉幹細胞(human umbilical cord-derived MSC, cbMSC, M)與人類臍靜脈內皮細胞(human umbilical vein endothelial cells, HUVEC, E)製成三維複合細胞球體，並以 P/PM/PE/PME 四種組別來了解細胞所扮演的角色。透過免疫螢光染色、基因與蛋白質的分析技術我們發現不同組別所釋放生長因子的種類及含量的不同，對於足細胞的成熟與存活等特性都有不同的影響。體內實驗我們將腎臟上下半部各一點注入 PME 細胞球，並用組織免疫染色去證實細胞球的留存性，最終透過自發性高血壓小鼠來證實細胞球對於腎損傷修復之效果。

Results：我們透過四個組別的免疫螢光染色圖發現 MSC 能夠調和 podocyte 與 HUVEC 之間的交互作用，進而影響細胞在球內細胞分布的現象。接著利用 real-time PCR 發現在 podocyte 本身小鼠促生長因子 Vegfa 的基因表現量中，三維細胞球體組別都顯著高於二維細胞，另外 PM/PE 兩個組別在人類的 VEGFA 基因表現量比較中發現 MSC 扮演主要分泌 VEGFA 的角色。然而，在另一個幫助足細胞成熟與存活的生長因子—HGF 的表現量則相反，我們發現 HUVEC 為主要分泌的細胞並且在 PME 的組別有加乘的作用，代表 podocyte 分化基因 Synpo 的表現量同樣也看到 HUVEC 扮演重要的角色。根據上述實驗結果我們將 PME 作為後續細胞球的條件。我們以免疫螢光染色去觀察 PME 球體內 VEGF 和 IGF-1 等生長因子的表現，同時也分析構成腎小球基底膜的細胞外基質成分如 Collagen type Ⅳ和 Laminin 等，發現這些物質在細胞球中有很高的表現。體內實驗我們以 WT-1 免疫組織螢光染色證實兩周後細胞球仍然留存於腎皮質區。最後量測自發性高血壓小鼠的 Serum creatinine 和 Urine albumin 當作腎功能的檢測指標，發現在打入細胞球 15 天後 Urine albumin 的含量相較於第 0 天下降了將近六成，也代表著蛋白尿的症狀得到改善，證實 PME 三維細胞球體對於腎損傷之修復潛能。

Discussion：此研究發現 MSC 與 HUVEC 分別對 podocyte 的特性有不同影響。MSC 藉由旁分泌作用釋放 VEGFA 並增加細胞外基質中 Laminin 和 Fibronectin 的分泌量來增進 podocyte 的生長與腎小球基底膜的形成，至於 HUVEC 則能夠釋放 HGF 來幫助 podocyte 的成熟和分化。因此在 PME 所組成的三維複合型細胞球體能夠兼顧各方面的優勢並且對於 podocyte 整體能力有所提升。並且也可從體內實驗看到 PME 能夠長時間地留存於注入的區域，且有效改善尿蛋白的問題。

Conclusions：我們成功透過三維細胞培養的方式製備出含有足細胞、人類臍帶血間葉幹細胞與人類臍靜脈內皮細胞的三維複合型細胞球體，並且能夠有效提升足細胞成熟、分化以及存活的能力，同時也在體內實驗證實了三維細胞球體在施打後擁有良好的細胞活性與留存性，最終在腎功能分析上，也能夠明顯改善蛋白尿的情形，證實此細胞球體擁有改善腎損傷之療效與潛能。

P-12

以層光顯微鏡對哺乳類組織進行活體影像
Intravital Imaging of the Mammalian Tissues By Lightsheet Microscopy

吳岳峰[1] 張煒塋[2] 譚欣媛[3] 潘明楷[4] 陳壁彰[5#] 江安世[2#] 朱麗安[2,6#] 林頌然[1, 2, 7, 8#]
台大醫學工程學系[1] 清大腦科學研究中心[2] 林口長庚醫院眼科[3]
台大藥理學科暨研究所[4] 中研院應用科學研究中心[5] 清大生醫工程與環境科學系[6]
台大醫院皮膚科[7] 台大發育生物學與再生醫學研究中心[8] #Contributed equally

Introduction：Intravital imaging approaches have emerged as a powerful technique to study cellular behaviors in the natural environment. Due to optical design limitation, conventional lightsheet microscopy only allows intravital imaging of tiny biological species embedded in gels. Here, we demonstrate new lightsheet microscopy that enables live imaging in mice. Furthermore, we highlight the imaging of the corneal endothelium whose detailed healing process remains unclear.

Materials and Methods：For illumination, the beam from a laser combiner equipped with 488, 561 and 637 nm is expanded to 1/e2 diameter of 3mm by two lenses, and each laser is combined with a mirror and two dielectric filters. The water-immersion objective lens (16x NA0.8) is used for imaging acquisition. We apply this microscopy on fluorescent reporter mice for live imaging of eyes through designed holder.

Results：Large-scale 3-dimensional images of ocular surfaces, including the full thickness of corneas and limbus, and the intraocular lens can be visualized. We create wounds with precise, controllable size of 50μmx50μm by multiphoton femtosecond laser ablation in corneal endothelium to characterize the 4-dimensional wound healing dynamics. After wounding, cells on the wound edge exhibited a latent period for about 9 hours before they started migrating as a sheet toward the wound center. This migratory phase lasted from 10 hours to about 40 hours after wounding and closed the wound.

Discussion：Large-scale imaging shows that endothelial cells across the entire cornea remained arrested in G1-phase during the entire healing process without division, which authenticates that cell enlargement and migration are the major means of endothelial repair.

Conclusions：This study not only broadens the application of lightsheet microscopy to intravital imaging in mammals but also provides key insights into the healing process of corneal endothelium *in vivo.*

P-013

可釋放一氧化氮之雙亞硝基鐵錯合物應用於增進間葉幹細胞移植後的存活與治療潛能
Mesenchymal Stem Cells Primed with Nitric Oxide-Releasing Dinitrosyl Iron Complex Exhibit Enhanced Post-engrafted Survival and Therapeutic Potential

王歆雯 謝麗虹 魯才德 黃玠誠*
國立清華大學生物醫學工程研究所

Introduction：間葉幹細胞具有自我增生、分化等能力，應用在醫療中是具有修復損傷組織潛力的療法之一。然而，現行的幹細胞療法面臨植入體內後存活率低落的問題。一氧化氮為生物訊息傳遞分子之一，在適當的濃度下具有可以調節細胞行為的功能，並具有提升細胞存活率、增加細胞因子表現與抑制過度免疫反應發生等作用。因此，本研究使用以雙亞硝基鐵錯合物所製備而成的新型長效一氧化碳供體，探討人類臍帶血間葉幹細胞受其刺激後，是否同樣會產生正面生理調節，或促進其有利損傷部位恢復的相關因子表現，進而提昇後續細胞移植後的治療潛能。

Materials and Methods：本研究使用已轉染 DsRed2 紅螢光蛋白以及端粒酶反轉錄酵素(telomerase reverse transcriptase)之臍帶血間葉幹細胞(Cord-Blood Mesenchymal Stem Cell, cbMSC)，分別透過雙亞硝基鐵錯合物(Dinitrosyl iron complexes, DNIC)和市售的一氧化氮供體 S-nitrosoN-acetyl-D, L-penicillamine (SNAP)處理，並以 cbMSC 的生長型態、DAPI 染劑定量等方式確認特定濃度之一氧化氮對於細胞無毒性；接著以即時定量聚合酶連鎖反應(Real-time PCR)及西方墨點法(Western blotting)分析其抗氧化、血管新生之基因與蛋白質表現量。DNIC 是由與本研究合作的清華大學生物醫學工程研究所魯才德老師及該團隊合成並提供；SNAP 則由 Cayman 購入。

Results：本研究首先以 Griess assay 確認 DNIC 能夠長時間釋放 NO 的效果，其中 DNIC 可持續釋放 12 小時，而 SNAP 僅 4 小時。接著驗證所使用之一氧化氮供體濃度對於 cbMSC 無毒性後，以 Real-time PCR 分析，經 DNIC 刺激不同時間長度之 cbMSC 所提升抗氧化基因 Heme oxygenase-1(HO-1)的表現程度，皆相較於 SNAP 為佳。而在西方墨點法中，可以觀察到經 DNIC 刺激之 cbMSC，有效提升 HO-1 蛋白質表現的能力優於 SNAP 刺激。為近一步評估 HO-1 之蛋白質表現是否有助於細胞於氧化壓力下的存活，本研究事先以一氧化氮供體處理 cbMSC 後，再以雙氧水刺激細胞，並使用 LIVE/DEAD Cell Viability/Cytotoxicity Assays (Invitrogen)分析其存活情形，可以觀察到 DNIC 降低細胞凋亡的數量最為顯著。此外，在促血管新生相關的基因表現，經 Real-time PCR 分析亦顯示出 DNIC 能夠有效提升 VEGF、ANG1、IL-6 的表現。

Discussion：由於過高濃度之一氧化氮供體會使細胞老化凋亡、低濃度時才有利細胞增生及存活，但濃度過低時將會失去細胞保護的效果，因此適當濃度之一氧化氮供體的選用至關重要。本研究中，cbMSC 在 100μM DNIC 與 SNAP 的刺激下皆有明顯凋亡的現象；而在 10μM 時則不影響其細胞存活率與型態，推測此濃度對於細胞並無毒性，因此選用此濃度作為後續基因以及蛋白質分析之基準。

Conclusions：本研究成功證實 DNIC 相對於市售之一氧化氮載體 SNAP，具有更長時間釋放 NO 的能力；並能夠促進 cbMSC 在基因與蛋白質等層面，提升抗氧化以及促血管新生的表現。上述 DNIC 調節 cbMSC 的能力，將有助於未來體內實驗的進行。

P-014

三維幹細胞球體衍生之多功能基質支架系統於促進組織再生之應用
A Versatile 3D Stem Cell Spheroid-derived Matrix Scaffold System for Promoting Tissue Regeneration

何肇庭 江承恩 方怡喬 王鈺婕 黃玠誠*
國立清華大學生物醫學工程研究所

Introduction：近年來，組織工程學的許多研究結合細胞、細胞支架、訊息因子進行組織修復，提供細胞良好生長環境，進而恢復受損區域。本研究所使用甲基纖維素水膠系統將間葉幹細胞進行三維培養並使其分泌細胞外基質，利用界面活性劑之方式進行去細胞，製備去細胞支架，並使用大分子擁擠現象原理，增加間葉幹細胞之細胞外基質之分泌及細胞激素與生長因子含量。

Materials and Methods：本研究所使用之細胞為經非病毒式轉染 DsRed2 紅螢光蛋白以及人類端粒酶反轉錄酵素基因 (human telomerase reverse transcriptase)臍帶血間葉幹細胞 (cord-blood MSC, cbMSC)。製備細胞球的方法為重量百分濃度為 12%的甲基纖維素粉末配置於 0.5 倍磷酸鹽緩衝溶液 (phosphate buffered saline, PBS)中，於低溫塗佈於 96 孔盤底部，置於 37°C 培養箱內 30 分鐘成膠，再將細胞懸浮液注入 96 孔盤，培養兩天後收集細胞球體。而大分子擁擠分子的濃度分別為，Ficoll 70：37.5 mg/mL、Ficoll 400：25 mg/mL、Dextran sulfate：10 μg/mL，Ficoll cocktail：Ficoll 70 與 Ficoll 400 混和，於製備細胞球時溶於細胞培養基中。本研究使用兩種去細胞方式，分別為冷凍回溫以及界面活性劑兩種方式來進行去細胞工作，冷凍回溫的方法為放置於–80°C 冰箱內 30 分鐘使其結凍，之後將其置入於 37°C 培養箱內回溫，使其解凍，重複上述步驟三次；使用界面活性劑的方法為加入含有 0.5% Triton X-100 以及 20 mM NH_4OH 之去離子水中，靜置於室溫一小時。最後皆加入 1 kU/mL DNase I 溶液於 37°C 低速率搖晃反應四小時。

Results：從 PicoGreen assay 的結果可知，冷凍回溫組別之殘餘 DNA 含量約 27%，而界面活性劑組別只剩原有的 1.7%。ELISA 結果顯示，兩種去細胞方式之 VEGF 含量皆約為未去細胞的一半且兩者差異不大。Western blot 結果顯示，經過去細胞後，Ficoll 70、Ficoll 400、Dextran sulfate 之間 Fibronectin 含量沒有顯著的差異，相較於未加大分子組別約提升了 2 倍，而 Laminin 含量以 Ficoll 400 最高，相較於未加大分子的組別提高約 1.2 倍。ELISA 結果顯示，於去細胞過後，Ficoll 400、Ficoll cocktail 及 Dextran sulfate 之 VEGF 含量較未加大分子組別提升約三倍。CCK-8 assay 結果顯示，於常氧，加入去細胞支架後細胞活性約提升 1.2 倍；在缺氧，控制組的活性下降至 78%，而加入去細胞支架後回復至 97%。由螢光顯微鏡可以觀察到，cbMSC 與人類臍靜脈內皮細胞(human umbilical vein endothelial cell, HUVEC)分別與去細胞支架共培養 1 小時後，兩種細胞皆能貼附於支架表面上。動物實驗為皮下移植，去細胞支架混入 Matrigel 植入皮下，一個星期後取出移植物進行 H&E 染色，結果可以看到有加入支架的組別，內部細胞核的數量有明顯上升，此外也有紅血球的出現。

Discussion：首先，確認了界面活性劑去細胞方法能有效將 DNA 去除，使得於移植體內免疫排斥反應能降到最低，且亦能保留 Fibronectin、Laminin 以及 VEGF 等 ECM 成分與生長因子；接著確認大分子擁擠現象分子 Ficoll 400 能有效增進去細胞後 Fibronectin、Laminin 及 VEGF 的含量，說明能增加細胞外基質的分泌，而與細胞共培養可以發現，此支架對於細胞增殖、細胞活性皆有提升的效果。此外，進行再細胞化，於結果可觀察到細胞具有貼

附以及重塑去細胞支架之能力，動物實驗中細胞支架的組別，可見細胞核數量提升與紅血球出現，說明宿主細胞能遷徙進去細胞支架上，並有血管新生的現象。
Conclusions：本研究藉由甲基纖維素水膠系統與大分子擁擠分子成功製備保留細胞外基質與生長因子之去細胞支架，且此支架對於細胞之遷移、貼附、增生有良好的特性。

P-015

脂肪幹細胞裝載入 HAMA/GelMA 光固化混合水膠並結合 3D 生物陶瓷成為骨與軟骨仿生支架應用於軟骨組織修復

Adipose-derived Stem Cells Laden Into HAMA/GelMA Photo-cured Based Hydrogel to Build a 3D Biomimetic Scaffold for Cartilage Tissue Reengineering

Swathi Nedunchezian[1,2] 王志光 [1,2]
高雄醫學大學醫藥暨應用化學系 [1] 再生醫學與細胞治療研究中心 [2]

Introduction: The prime function of articular cartilage is to provide a smooth surface and facilitates load bearing with a low friction capacity. The avascular nature of articular cartilage limits the healing and self-repair. Cartilage tissue engineering (CTE) helps in repairing or regenerating damaged cartilages by using a combined strategy which involves cells, growth factors, and biomaterial scaffolds. In our research study we will develop a 3D biomimetic hybrid hydrogel and examine its potential for cartilage tissue regeneration.
Materials & Methods: HA from Kikkoman, Japan. Bovine pituitary extract (BPE) was from Gibco thermo fisher scientific, USA. Trypan blue solution was from Sigma-Aldrich. Live/Dead viability/cyto toxicity kit was from Invitrogen, FBS from Thermo Fisher scientific, USA.
Results & Discussion: The cell proliferation & live/dead assay shows the suitable environment for the hydrogel for cartilage tissue regeneration(fig 1 a&b). Furthermore, the hydorgels were tested for the amount of sGAG present in the ECM(Fig 2 a&b).The results shows that our hydrogel system can help to enhance the chondrogenic differentiation of ADSCs. The cartilage gene such as Aggrecan, SOX-9,COL-2 also shows the enhanced expression in our hydrogel system.

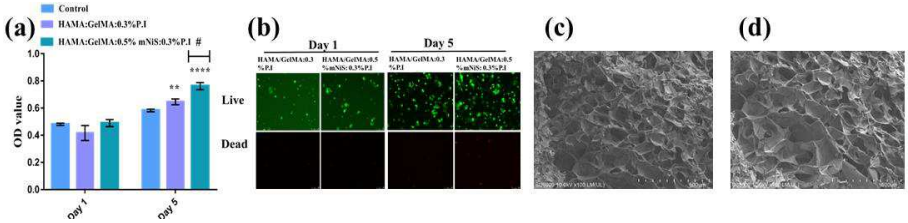


Figure 1. MTS assay (a), live/dead assay (b), SEM images (c,d).

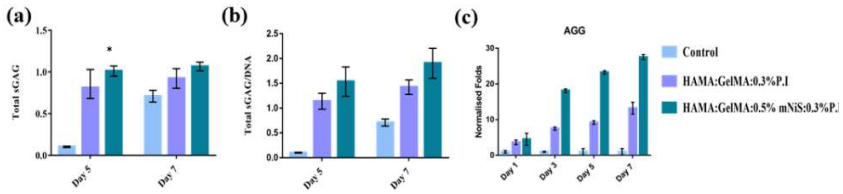


Figure 2. Quantification of sGAG using DMMB assay (a,b), Cartilage gene Expression (c).

Conclusion : The HAMA/GelMA/mNiS showed promising cell viability and chondrogenesis and could bring a suitable microenvironment to enhance ADSC chondrogenesis for articular cartilage tissue engineering applications in future experiment of animal study.

P-016

肌母細胞 C2C12 分化前與分化後之超音波刺激對鈣離子訊號的反應
Calcium Signaling Reaction between Myoblast and Myotube by Ultrasound Stimulation

黃粵軍 朱亞成 林若梅 王兆麟
國立台灣大學醫學工程研究所

Introduction：對於體外細胞、組織培養一直都是再生醫學重要的一環，肌肉再生及修復的過程中我們可以藉由觀察體外培養之肌母細胞（C2C12），來了解其中的再生以及修復的機制。

低強度脈衝超音波對於骨癒合及近年來細胞培養的研究，發現細胞受低強度脈衝超音波刺激後有加速其增生的現象，但本實驗室發現低強度脈衝超音波刺激對肌肉修復最重要的一環—**分化肌管**(myotube)上卻無顯著的幫助。

有鑑於此，我們研究 C2C12 分化前後的鈣離子通道差異以了解超音波刺激 C2C12 的機制，以利於開發對分化肌管刺激的方法。

Materials and Methods：C2C12 種在玻片上培養到 70%玻片滿後經染色(Oregon Green™ 488 BAPTA-1, AM)將培養液更改成 HHBS buffer 後進行活細胞影像攝影(live cell imaging)，以確認其 Calcium 反應為受激後的即時反應。

透過實驗室內研發的極細管玻璃針將超音波能量傳導進 HHBS buffer 中對細胞進行刺激，再將刺激過後肌母細胞與肌管細胞得到的影像紀錄，透過影像量化處理其灰階值的變化量(F/F_0)，比較兩者的不同。

刺激的方式以聲壓刺激及聲流刺激為主，細胞內的離子通道(ion channel)會在兩種刺激下產生 Calcium 反應而有灰階值上升的改變，再以不同的抑制劑去阻斷特定的離子通道觀察肌母細胞與肌管細胞在相同刺激條件下，灰階值的變化是否受抑制。

Results：在聲流刺激實驗中可發現肌管細胞加了 G 蛋白偶合受體抑制劑(GPCR antagonist)後其 Calcium 反應可被抑制(F/F_0 降低 3 倍)，而肌母細胞則不被抑制；聲壓刺激中也可發現肌管細胞加了 Piezo1 離子通道的抑制劑後 Calcium 反應也可被抑制(F/F_0 降低 2 倍)，而肌母細胞則不被抑制。

Discussion：由聲流刺激及聲壓刺激實驗推論當肌母細胞分化成肌管細胞後特定的受體和離子通道會因為其功能的改變而增多，以至於當受體或通道被阻斷後 Calcium 的反應減弱。

Conclusions：透過超音波刺激後 Calcium 的反應來了解 C2C12 分化前後離子通道的不同，可得知藉由調高 G 蛋白偶合受體和 Piezo1 離子通道，來促進 C2C12 分化成肌管的趨勢。

P-017

載有薑黃素之多功能脂質／PLGA 複合型微米粒子與明膠支架於角膜內皮再生之應用
Gelatin Scaffold with Multifunctional Curcumin-loaded Lipid-PLGA Hybrid Microparticles for Regenerating Corneal Endothelium

蔡孟妤[1] 陳思靜[1] 李佩蓁[1] 沈柍君[1] 陳宏吉[2] 黃玠誠[1*]
國立清華大學生物醫學工程研究所[1]
林口長庚醫院眼科部[2]

Introduction：人類角膜內皮細胞在受損後不具有再生之功能，而在臨床上，角膜移植是目前改善患者視力的唯一方法。但受限於捐贈來源不足，以及移植後角膜可能因其本身品質不佳或受贈者免疫排斥而導致移植失敗。因此，藉開發一組織工程支架，用於培養角膜內皮細胞並支持其移植到前房，做為捐贈角膜之替代品，進而解決捐贈角膜短缺及手術失敗之風險。本研究利用明膠結合包覆有薑黃素之藥物緩釋系統，做為角膜內皮細胞培養與移植的支架，期望藉由薑黃素之抗氧化及促進細胞增生等特性，改善移植細胞的存活率，並進一步提升移植成功率。

[aterials and Methods：利用 oil-in-water 單乳化製備含有薑黃素的 Lipid/PLGA 複合型微米粒子（Cur@Lipid-PLGA MPs，此稱 MPs）。將製得的 MPs 與 20%的明膠水溶液混合均勻後倒入模具，於 4°C 下固化，最後以 EDC/NHS 進行交聯。在細胞實驗中，以人類角膜內皮細胞株 B4G12 細胞分析 Cur@ MP/Gelatin 支架之生物相容性及抗氧化特性，更以人類臍靜脈內皮細胞(HUVEC)和小鼠白血病巨噬細胞(Raw264.7)分別測試 MPs 之抗血管新生作用及抗炎症分析。在動物實驗部分也初步建立了化學灼傷之兔眼模型來觀察 MPs 對傷口癒合之效用。

Results：以 CCK-8 測試確定薑黃素對 B4G12 細胞無細胞毒性，接著製作 Cur@ Lipid-PLGA MPs，並確保 MPs 有將薑黃素包覆於其中。同時，量測 MPs 釋放薑黃素之情形，我們發現釋放情形較緩慢且曲線趨於線性。將 MPs 與明膠製成 Cur@MP/Gelatin 後，其支架於可見光波段之穿透率雖略低於未包覆微米粒子之支架，但其穿透率仍可達 90%。為了研究其生物相容性，將 B4G12 接種在 Cur@MP/Gelatin 的表面上，根據螢光量化結果，B4G12 細胞存活率高於在一般細胞培養皿中培養。更加入 H_2O_2 使細胞存在高氧化壓力環境，經定量分析後，其存活率高於對照組，證實 Cur@MP/Gelatin 具有良好的生物相容性及抗氧化的能力。在 HUVEC 管狀形成實驗中，可看到 MPs 在 24 小時內即具有明顯的抑制血管新生效果，而經由 Raw264.7 的炎症反應測試也證實了 MPs 能有效增強細胞的抗發炎能力。在角膜灼傷之動物實驗中，經由 MPs 治療之組別傷口癒合速度確實是優於對照組的。

Discussion：Cur@MP/Gelatin 具良好的光線穿透度，表示 MPs 的添加不會在移植後造成透明度不足的問題。於型態分析中，發現 Cur@MPs/Gelatin 表面具有 MPs 之顆粒狀結構，進而推測我們有成功將 MPs 與 Gelatin 結合。細胞實驗結果中，可發現於 Cur@MP/Gelatin 中，B4G12 細胞的增生情形有逐漸上升的趨勢(薑黃素濃度於 40 μM 以下)；但若濃度持續增高，則會使細胞量逐漸下降，於抗氧化實驗結果亦是如此，而兩者實驗結果之細胞存活率均高於對照組。經由與 MPs 共培養後，HUVEC 的管狀生成及 Raw264.7 的炎症反應也有被抑制之效果。在角膜化學灼傷實驗中，由於 MPs 能控制炎症反應及抗血管新生，因此促進傷口的癒合。故本實驗之 Cur@MP/Gelatin 可做為一細胞移植載體，讓細胞良好貼附，亦能藉由薑黃素的緩慢釋放，提升角膜內皮細胞的活性與增生能力，並減緩血管生成現象和降低發炎反應。

Conclusions：我們成功製備出包覆有薑黃素的 Lipid-PLGA 複合型微米粒子，並證明其可緩釋薑黃素，延長藥物在生理環境下的作用時間。由細胞實驗結果可知，由於 MPs 可使薑黃素緩慢釋放，其對於提升 B4G12 細胞增生的效果較未包覆的薑黃素更加明顯。將 MPs 與明膠薄膜結合成為 Cur@MP/Gelatin 後，發現其光線穿透性良好。此外，B4G12 細胞亦能於支架表面貼附與生長；而支架內部的 MPs 亦能提升細胞的抗氧化、抗血管新生及抗炎症能力；在動物實驗中也驗證了 MPs 具有促傷口癒合的能力。

P-018

超臨界二氧化碳去細胞血管支架用於體內血管重建之動物模式
Recellularization of Supercritical Carbon Dioxide Decellularized Blood Vessel: An *in Vivo* Approach

宋世英[1] 蔡建松[1] 蔡宜廷[1] 林怡彣[2] 林豐彥[3,4] 顏克中[5] Periasamy Srinivasan[5] 謝達仁[5]
三軍總醫院外科部心血臟血管外科[1] 國立陽明大學口腔生物所[2]
台北醫學大學醫學系內科[3] 台北醫學大學附設醫院心血管研究中心及心臟內科[4]
亞果生醫股份有限公司[5]

Introduction：Tissue-engineered blood vessel grafts are made by employing numerous decellularization technique, leaving behind the mechanically robust extracellular matrix. We propose to use supercritical carbon dioxide (SCCO2) technology to decellularize the rabbit blood vessel and implant directly into rat artery to evaluate the main concept of tissue engineering, the decellularization-recellularization approach.

Materials and Methods：Rabbit abdominal artery was harvested from a healthy New Zealand white rabbit. We used proprietary SCCO2 extraction technology to decellularize the rabbit blood vessel. In brief, the rabbit blood vessel was washed in saline followed by subjecting the heart to SCCO2 at 100-350 bar carbon dioxide pressure, 30-40°C and 0.5-2 h finally washed with sodium hydroxide and water. The decellularized blood vessel was implanted in ACI rats.

Results：Decellularized blood vessel sections were stained by hematoxylin and eosin depicted complete removal of cells. Implanted decellularized blood vessel showed normal blood flow in all examinations after 12, 42 and 58 days by Doppler ultrasonography. Hematoxylin and eosin staining depicted complete recellularization. Endothelial marker staining showed the presence of endothelial cell distribution in the recellularized blood vessel. Inflammatory markers were minimally expressed.

Discussion：Decellularized blood vessel showed normal blood flow in all 12, 42 and 58 days indicating the normal function of the blood vessel. Cell distribution was noticed in all three layers of the blood vessel, with the expression of alpha-smooth muscle actin. Endothelial markers such as CD31 and vWF were expressed on the implanted graft.

Conclusion：To conclude, we successfully decellularized rabbit blood vessel by SCCO2 technology and implanted in rats, which showed excellent recellularization with normal function.

P-019

An Intermediate Concentration of Calcium with Antioxidant Supplement in the Culture Medium Enhances the Proliferation and Decreases the Aging of Bone Marrow Mesenchymal Stem Cells

陳崇桓[1,2,3,4,5,6,7] 莊淑君[1,2] 鄭琮霖[1,2,8] 林松彥[1,2,3,4,5,6]
林怡珊[1,2] 何美泠[1,2,8,9,10], 張瑞根[1,2,3,4,5]
高雄醫學大學骨科學研究中心[1] 高雄醫學大學再生醫學及細胞治療研究中心[2]
高雄醫學大學醫學系骨科[3] 高雄醫學大學附設醫院骨科[4] 高雄市立大同醫院[5]
高雄醫學大學醫學研究所[6] 中山大學醫學科技研究所[7] 高雄醫學大學生理學科[8]
高雄醫學大學臨床醫學研究部[9] 國立中山大學海資系[10]

Introduction：Human bone marrow stem cells (HBMSCs) were isolated from bone marrow. Stem cells can self-renew and differentiate into various types of cells. They are able to regenerate kinds of tissue that are potentially used for tissue engineering. To maintain and expand of these cells in culture condition is difficult. These cells on culture condition are easily triggered for differentiation or death. In this study, we described a new culture formula to culture isolated HBMSCs.

Materials and Methods：This new formula was modified from NCDB 153, a media with low calcium, supply with 5% FBS, addition with extra growth factor and supplemented with N-acetyl-L-cysteine and L-ascorbic acid-2-phosphate for maintaining cells in a steady stage. The proliferation potential of HBMSCs was measured. Induction of multilineage stem cell differentiation and cell surface marker were also tested. We also examined the array comparative genomic hybridization (array-CGH), 53Bp1 immunostaining, telomerase activity assay and telomere length assay.

Results：Cells can keep these characteristics as primarily isolated HBMSCs. Moreover, our new formula keeps the HBMSCs with high proliferation rate and multiple linage differentiation ability, such as osteoblastogenesis, chondrogenesis and adipogenesis. This formula keeps HBMSCs with stable chromosome, DNA, telomere length and telomerase activity even after long-term culture. Senescence can be minimized under this new formulation and carcinogenesis of stem cells can also be prevented.

Discussion：Our new formula keeps the HBMSCs at the state as they were originally cultured with high proliferation rate and multiple linage differentiation ability with stable chromosome, DNA, telomere length and telomerase activity even after long-term culture.

Conclusions：These modifications greatly enhance the survival rate, growth rate and the basal characteristic of isolated HBMSCs which will be very helpful in stem cell research.

P-020

脂肪幹細胞分泌之細胞外囊泡對於成骨細胞之功能
Effects of Extra Cellular Vesicles Secreted from Adipose Derived Stem Cells on Osteoblastic Functions

歐彥廷 [1#] 陳振偉 [2] 吳順成 [2*] 邵佩琳 [3*]
高雄醫學大學生物科技學系 [1] 再生醫學與細胞治療研究中心 [2] 亞洲大學護理學系 [3]

Introduction：Bone injury is one of the most common traumatic injuries. To date, adipose-derived stem cells (ADSCs) has been studied for bone regeneration. Besides the osteogenic differentiation capacity of ADSCs, it has been suggested that ADSCs mediate bone regeneration via its secretome including growth factors, cytokines, and extracellular vesicles (EVs) . ADSCs can secrete EVs. EVs are shown to play an important role in cell-to -cell communication because they carry a cargo of proteins, miRNA, and even organelles from ADSCs to the recipient cells. We hypothesize that EVs released from ADSCs enhance functions of osteoblasts.

Materials and Methods：The ADSCs are purchased from StemPro®. The human osteoblast cell line was purchased from Elabscience®. CM-DiI-labeled ADSCs were cultured in DMEM for 48hr to induce EVs release. After induce EVs release, the conditioned medium (CM) were isolated. EVs were isolated by ultracentrifugation of CM. The characterization of EVs were performed following the guideline recommended by International Society for Extracellular Vesicles (ISEV). To test the effect of EVs on osteoblastic functions, the calcium deposition, alkaline phosphatase activity and collagen type I synthesis of osteoblasts were tested after EVs treatment.

Results：The round spherical shape structure of the EVs was observed by TEM analysis. The EVs were positive for CD9, CD63, CD81, TGS101, and Alix, whereas α-tubulin and β-actin were not detected. After EVs treatment, CM-DiI-labeled EVs that is derived from ADSCs were intake by osteoblasts shows that intercellular communication occurs through EVs. The calcium deposition, alkaline phosphatase activity, and collagen type I synthesis of osteoblasts are significantly enhanced after EVs treatment.

Discussion：To date, ADSCs has been studied as cell-based therapy for bone regeneration. Based on these findings, we show that ADSC's EVs enhance osteoblastic function.

Conclusions：EVs released from ADSCs enhance osteoblastic functions.

P-021

脂肪幹細胞分泌之細胞外囊泡可促進關節軟骨細胞之功能
Extracellular Vesicles Released from Human Adipose-derived Stem Cells Enhance Articular Chondrocyte Function

吳順成 [1,2] 張玲華 [1,2] 陳振偉 [1,2] 伍哲緯 [1,2] 陳崇桓 [1,2,3] 張瑞根 [1,2,3#] 何美泠 [1, 2,3,4*#]
高雄醫學大學再生醫學與細胞治療研究中心 [1]
骨科學研究中心 [2] 骨科 [3] 生理學科 [4]

Introduction：Although autologous chondrocyte implantation (ACI) has used as the "gold standard" for articular cartilage repair, a major part of treated patients shows fibrocartilage in cartilage defect site rather than native hyaline cartilage. The in vitro monolayer expansion process of human articular chondrocytes (hACs) during ACI is shown to cause chondrocyte de-differentiation. To date, mesenchymal stem cells (MSCs) including adipose-derived stem cells (ADSCs) have been studied to treat articular cartilage damages. It has been widely evident that ADSCs mediate tissue regeneration via secretion of trophic factors including growth factors, cytokines, and extracellular vesicles (EVs). Recent studies also show that EVs plays an important role in cell-to-cell communication. We hypothesize that EVs released from ADSCs can be used for enhancing functions of hACs.
Materials and Methods：ADSCs were labeled with CM- DiI and then pre-cultured in DMEM supplemented with 2% FBS for 48hr to induce EVs release. After induce ADSCs-EVs release, the conditioned medium (CM) derived from pre-cultured with ADSCs were isolated. The CM was used for ADSCs-EVs isolation by ultracentrifugation of CM. The characterization of ADSCs-EVs were performed following the guideline called Minimal Information for Studies of extracellular Vesicles 2018. There were four groups in the study: 1. Control group: hACs without any treatment, 2. EVs (10^7-10^9) group: hACs treated with EVs at concentrations of 10^7/ml, 10^8/ml or 10^9/ml, respectively. The protein expressions of Collagen type II&I by hACs were quantified by ELISA. Dimethylmethylene blue (DMMB) assay were used to quantify sulphated glycosaminoglycan (sGAG) deposition. Statistical significance was evaluated by a one-way analysis of variance (ANOVA), and multiple comparisons were performed using Scheffe's method. A $p<0.05$ was considered significant.
Results：The result showed that the mean diameter of ADSC-EVs is 170 $\pm$ 17.4 nm. The morphology of ADSCs-EVs detected by transmission microscopy showed that ADSCs-EVs are rounded sphere. The ADSCs-EVs are positive for CD9, CD63, CD81, Alix, but negative for α-tubulin. ADSCs-EVs were intake by hACs in the EVs group at day 5 after treatment. There was no CM-DiI-labeled ADSCs-EVs uptaken by hACs in the Control group. The protein level of collagen type II and sGAG deposition of hACs are significantly enhanced in EVs group when compared with Control group. Moreover, the ADSCs-EVs also decreased the collagen type I expression of hACs. The results also show that ADSCs-EVs enhances cartilaginous matrix synthesis of hACs. Moreover, the ADSCs-EVs also contribute to reduce the fibrocartilaginous formation of hACs.
Discussion：ACI has used as the "gold standard" for articular cartilage repair. However, the de-differentiation of hACs is major challenge for a successful for articular cartilage repair during ACI. To reduce de-differentiation of hACs, and make re-differentiation of hACs remains an unmet need in ACI. Based on these findings, we show that ADSCs-EVs can be used for enhancing functions of hACs.
Conclusions：The ADSCs-EVs treatment may be an alternative method to prevent hACs from undergoing de-differentiation and can be used for improving drawback of ACI.

P-022

去細胞豬軟骨填料結合 PRP 可延緩大鼠十字韌帶切除誘發之退化性關節炎並重建膝蓋軟骨缺損

Decellularized Porcine Cartilage Graft with PRP Attenuated OA Progression and Regenerated Articular Cartilage in ACLT-induced OA Rats

Periasamy Srinivasan　葉怡君　賴意芊　謝達仁
亞果生醫股份有限公司

Introduction：Osteoarthritis (OA) is a common degenerative articular condition and one of the primary causes of pain and functional disability. Therefore, we hypothesized cartilage tissue engineering that combines the triads of decellularized porcine cartilage graft as a scaffold, PRP as signal and chondrocytes from rat as the cell to attenuate the OA progression and regenerate the cartilage injury in anterior cruciate ligament transection (ACLT)-induced OA model.
Materials and Methods：We used proprietary SCCO2 (100-350 bar carbon dioxide pressure, 20-40°C) extraction technology to produce decellularized porcine cartilage graft from porcine articular cartilage. The protective effect of decellularized porcine cartilage graft was evaluated by intra-articular administration in surgically induced ACLT rat OA model.
Results：Decellularized porcine cartilage graft (dPCG) characterized by hematoxylin and eosin and 4,6-diamidino-2-phenylindole (DAPI) staining and scanning electron microscopy (SEM) depicted complete removal of cells. SCCO2 technology completely decellularized the porcine cartilage. Intra-articular administration of dPCG with or without PRP is efficient in attenuating the progression and repairing the damaged cartilage in experimental OA model.
Discussion：Intra-articular administration of dPCG with and/or without plasma rich platelet (PRP) significantly reduced the ACLT-induced OA symptoms and attenuated the OA progression. dPCG reduced pain improved articular cartilage damage in the rat knee characterized by X-ray and micro-CT. Besides, the molecular mechanism of how dPCG repaired and attenuated the articular knee cartilage damage was explored by safranin-O, type II collagen, aggrecan and SOX-9 immuno-staining.
Conclusion：To conclude, dPCG can attenuate ACLT-induced OA progression with and/or without PRP by elevating the expression of type II collagen, aggrecan and SOX-9. This might serve as a potential therapy for OA patients and patients with cartilage defect caused by the sport-related injury.

P-023

創新超臨界二氧化碳去細胞豬軟骨3D半固體膠：*ex vivo*分析
Innovative 3D Semisolid Gel Engineered Using Supercritical Carbon Dioxide Decellularized Porcine Cartilage: An *ex vivo* Analysis

唐逸文[1] 史瑞尼[2] 葉怡君[2] 陳意芊[2] 謝達仁[2]
高雄榮民總醫院骨科部[1] 亞果生醫股份有限公司[2]

Introduction：Articular cartilage defects are one of the challenging clinical issues. The clinical interventions for cartilage repair techniques are not long-term clinical solutions, thus prompting for the development of regenerative medicine and tissue engineering approaches to restore articular cartilage. The aim of this study was to construct a novel 3D semisolid gel with decellularized cartilage for the treatment of cartilage defects.
Materials and Methods：Supercritical carbon dioxide ($scCO_2$) was employed to decellularize porcine articular cartilage. 3D semisolid gel was engineered using 50, 100, or 250 mg $scCO_2$ decellularized cartilage scaffolds with PRP, thrombin, and primary chondrocytes. SOX-9, collagen type II, and aggrecan in the 3D semisolid gel were evaluated by using immunohistochemistry staining. Moreover, 3D semisolid gel with agarose or without agarose combined with $scCO_2$ decellularized cartilage scaffolds was made to evaluate the effect of decellularized cartilage matrix for chondrocyte growth.
Results：Decellularized cartilage, 100 mg was suitable for chondrocyte growth compared to 50 mg and 250 mg. Immunohistochemical staining showed increased and consistent chondrocyte growth factor and the extracellular matrix proteins expression including the SOX-9, collagen type II, and aggrecan in 3D semisolid gel with the decellularized cartilage than without decellularized cartilage. Decellularized cartilage combined with agarose gel, depicted excellent chondrocytes growth, compared to agarose gel without decellularized cartilage.
Discussion：Decellularized cartilage scaffolds offer cells natural type II collagen microenvironment and good space to adhere to the decellularized cartilage for ECM production. The 3D semisolid gel composite maintained its shape due to the ECM substitute with thrombin and the engineering of 3D semisolid gel composite is done step by step *ex vivo* culture.
Conclusions：Decellularized cartilage-3D semisolid gel construct acts as a scaffold substrate for the chondrocyte with native type II collagen, thus facilitating ECM production by chondrocytes. This new cell-scaffold construct may provide the basis of a viable chondral graft suitable for *in vivo* implantation.

P-024

超臨界二氧化碳流體去細胞豬鼻軟骨材料應用於隆鼻手術之細胞治療可行性研究
Supercritical Carbon Dioxide Decellularized Porcine Nasal Cartilage Graft Cultured with Chondrocyte Derived a Novel Histotypic 3D Construct for Potential Rhinoplasty

Su-Shin Lee[1,2,3,4], Yi-Chia Wu [1,2,3], Shu-Hung Huang [1,2,3], Ying-Che Chen, Periasamy Srinivasan[5], Dar-Jen Hsieh [5], Yi-Chun Yeh[5], Yi-Ping Lai[5], Yun-Nan Lin[1]*
高雄醫學大學附設醫院整形外科 [1] 高雄醫學大學醫學院醫學部外科學系 [2]
高雄醫學大學再生醫學與細胞療法研究中心 [3] 高雄小港醫院外科部 [4]
亞果生醫股份有限公司 [5]

Introduction：Rhinoplasty, alters and restructures the defected nose caused by trauma, therapeutic operations including cancer, dermatological disease, congenital malformations and plastic surgeries, the most prevalent in the cosmetic field. Therefore, we developed a bioactive scaffold with 3D histotypic cartilage culture using decellularized porcine nasal cartilage graft (dPNCG).
Materials and Methods：We used proprietary SCCO2 (100-350 bar carbon dioxide pressure, 20-40°C) extraction technology to produce dPNCG from porcine nasal cartilage. An experimental 3D histotypic culture was engineered using dPNCG, rat adipose-derived stem cells (ADSC) and chondrocytes with different percentage of cells and cultured for 21 days.
Results：dPNCG was characterized by H&E, DAPI, alcian blue staining, scanning electron microscopy and residual DNA content, which demonstrated complete decellularization. dPNCG with 100% chondrocytes produced a solid mass of 3D histotypic cartilage with significant production of glycosaminoglycans.
Discussion：A solid mass of 3D histotypic cartilage with significant production of glycosaminoglycans in dPNCG with 100% chondrocytes culture. H&E, and alcian blue staining showed an intact mass, with cartilage granules bound to one another by extracellular matrix and proteoglycan, to form a 3D structure. In addition, phenotype chondrogenic markers, type II collagen, aggrecan and SOX-9 were elevated indicating chondrocytes cultured on dPNCG substrate synthesized type II collagen along with extracellular matrix to produce 3D histotypic cartilage.
Conclusion：To conclude, dPNCG is an excellent substrate scaffold that might offer a suitable environment for human nasal chondrocytes to produce 3D histotypic cartilage and a promising potential candidate for cartilage tissue engineering in rhinoplasty.

P-025

利用超臨界二氧化碳流體萃取技術製備各種組織器官膠原蛋白支架作為組織工程及 3D 生物列印材料
Supercritical Carbon Dioxide Extraction Technology-enabled Tissue and Organ Scaffold Production

Periasamy Srinivasan　謝達仁
亞果生醫股份有限公司

Introduction：Tissue engineering refers to the practice of combining tissue and organ scaffolds, cells, and biologically active molecules into functional tissues. Organ scaffolds are the key player in the game of 'Tissue engineering- Regenerative medicine'. Therefore, we propose to use supercritical carbon dioxide (SCCO2) technology to produce organ scaffolds for experimental and clinical tissue engineering and regenerative medicine.
Materials and Methods：We used proprietary SCCO2 extraction technology to produce dermis, whole skin, cancellous bone, heart, liver, brain, kidney, pancreas, blood vessel, ureter from a porcine source. In brief, the tissues were washed in saline followed by subjecting the tissues to SCCO2 at 100-350 bar carbon dioxide pressure, 20-40°C and 4-12 h finally washed with sodium hydroxide and water.
Results：Decellularized tissue and organ scaffolds were stained by hematoxylin and eosin and 4,6-diamidino-2-phenylindole (DAPI) staining and scanning electron microscopy (SEM) depicted complete removal of cells. SCCO2 produced organ scaffold is a bioartificial scaffold that maintains the structure of the extracellular matrix.
Discussion：Tissue and organ scaffolds not only support the cells for growth, proliferation and attachment. It also acts as a relay station for numerous signalling molecules. Our tissue and organ scaffolds are biologically mimetic scaffolds such as biologically active ECM thus creates a *in vivo*-like microenvironment mimicking biological entities and stimulating cell-specific responses to lead to tissue regeneration and repair. In the present investigation, SCCO2 organ scaffold is non-toxic, no solvent residue, off-odour, removal of lipids, biologically suitable with complete sterilization. Polar molecules such as proteins are preserved. Therefore complete preservation of ECM. The regenerative ability of SCCO2 produced organ scaffold is improved.
Conclusion：To conclude, we effectively produced tissue and organ scaffolds form porcine origin such as dermis, whole skin, cancellous bone, heart, liver brain, kidney, pancreas, blood vessel, ureter. The organ scaffolds can be used in experimental and clinical tissue engineering and regenerative medicine.

台灣再生醫學學會 (個人、贊助、準) 會員入會申請書

姓名		性別		出 生 年月日		出生地		身分證 ID or 統編	
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：			
通訊地址						專科醫師證書字號： (無者免填)			
電話	(公)		(宅)			傳真：			
其他連絡 方 式	(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									

感謝下列廠商對本次學術研討會之贊助 & 支持

謹 在此致上十二萬分的謝意！

台寶生醫股份有限公司
台灣安進藥品有限公司
向榮生醫科技股份有限公司
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（以字首筆畫數順序排列）