2nd CUHK International Symposium on Stem Cell Biology and Regenerative Medicine

19-20 November 2012

Chinese University of Hong Kong Shenzhen Research Institute, China

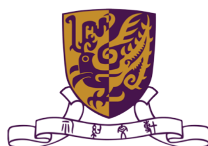
&

Prince of Wales Hosptial, Hong Kong

Website: http://scrm.ort.cuhk.edu.hk/

Organizers:

Stem Cell and Regeneration Theme, School of Biomedical Sciences, The Chinese University of Hong Kong
Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong
The Hong Kong Jockey Club Sports Medicine and Health Sciences Centre
Key Laboratory for Regenerative Medicine (Ji Nan University-The Chinese University of Hong Kong), Ministry of Education, China
Chinese University of Hong Kong Shenzhen Research Institute
School of Medicine, Shenzhen University



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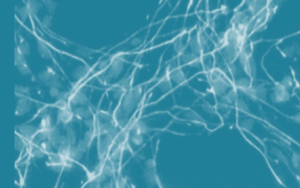

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Message from

Professor Joseph J Y Sung
Vice-Chancellor and President
The Chinese University of Hong Kong

It is with great pleasure that I welcome you all to the 2012 Stem Cell and Regenerative Medicine International Symposium.

Regenerative medicine enhances natural healing processes to work faster and better. Damaged tissues under normal circumstances would be unable to heal optimally, yet the technologies and techniques of regenerative medicine make it possible for these missing or damaged tissues to regrow and regenerate fully. This is an incredibly significant accomplishment! The developments include transplants of stem cells, manipulation of the patient's own stem cells, and the use of scaffold materials that emit biochemical signals to spur stem cells into action. Regenerative therapies have been demonstrated (in trials or the laboratory) to heal broken bone, severe burns, blindness, deafness, heart damage, nerve damage, Parkinson's disease, and a wide range of other conditions.

Although many momentous developments in the field of regenerative medicine have occurred, further work must be done to fully explore the potential of this exciting and promising field of medicine in order to continually bring benefits and improve the health of patients.

The importance of the field of Regenerative Medicine is continuously growing. Stem cells and Regenerative medicine have been identified by China's 12th Five Year National Plans as one of the nation's important areas of research.

In the last 3 years, CUHK have been expanding our research potentials and capacities in the field of regenerative medicine. Dedicated research teams and research projects are being set up and state-of-the-art research facilities are built to facilitate the needs of research and clinical applications in this field. I have no doubt that our innovative research will bring more great surprises and miracles into the applications of regenerative medicine to benefit our patients and society as a whole.

I am ecstatic to see that such a great number of respectable scientists and clinicians along with many young and energetic researchers have joined us to utilize this platform to exchange their latest results. I wish you enjoy your stay in Hong Kong and that the symposium will be very fruitful and successful!

Professor Joseph J Y Sung
Vice-Chancellor and President
The Chinese University of Hong Kong

Message from **Organizing Committees**

Dear colleagues and friends:

The 2nd CUHK International Symposium on Stem Cell Biology and Regenerative Medicine will be held at The Chinese University of Hong Kong Shenzhen Research Institute and Prince of Wales Hospital on 19 - 20 Novemeber 2012.

First, we would like to thank sincerely for all the guest speakers to join us on this occasion and show your support. Your presence has made this symposium a truly international one. Research on stem cell biology and regenerative medicine in Hong Kong develop very fast, more and more staff are interested or involved in the research fields of stem cell biology, biomaterials, tissue engineering under the broader term of regenerative medicine. We hope that the symposium will provide a platform for us to share and learn new research ideas, findings and techniques from each other.

The symposium is divided into 4 main parts: biology of tissue regeneration; topics of regenerative medicine; technological advancements and translational medicine related topics. Experts from USA, Taiwan, Singapore, China as well as from CUHK will share their latest discoveries, research ideas and techniques on various fronts of regenerative medicine research. This symposium is an ideal opportunity for researchers, students, clinicians and people who are interested in regenerative medicine to learn and to share.

On behalf of the symposium organizers, we warmly welcome you to join us and enjoy the symposium !

Organizing Committee
The 2nd CUHK International Symposium on Stem Cell Biology and Regenerative Medicine

Co-Charimen:

Prof. Gang Li
Professor
CUHK-ORT
CUHK-SBS-SCR

Prof. Wai-Yee Chan
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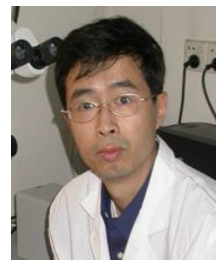
Chair Professor
CUHK-SBS

Prof. Kai-Ming Chan
Chair Professor
CUHK-ORT

Members:

Prof. Leung-Kim Hung
Chairman
CUHK-ORT



Prof. Dong-Qing Cai
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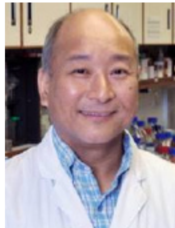
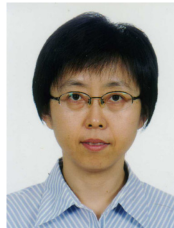
Prof. Guang-Qian Zhou
Medical School,
Shen Zhen University
China

Organizers

Stem Cells and Regeneration (SCR) Theme
School of Biomedical Sciences
The Chinese University of Hong Kong


Prof. Kenneth LEE


Prof. FENG Bo

Prof. LI Gang

Prof. Kingston MAK

Prof. WAN Chao

Prof. KM CHAN

Prof. Ling QIN

Prof. Pauline LUI

Prof. Faye TSANG

Prof. YUAN Ping

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Mission and Vision of SCR:

To co-ordinate and facilitate research, education, and clinical application of stem cells and regeneration technologies in the Faculty of Medicine, the Chinese University of Hong Kong and to implement a new, multidisciplinary, and sustainable program in translational research in regenerative biology, which will form the basis for incorporating clinical service with cutting edge technology into these disciplines.
More specifically we view as our missions:

- To provide a platform for interaction among investigators working on different aspects of stem cell biology and regenerative medicine in the Faculty of Medicine, CUHK.
- To enhance and facilitate collaboration between investigators.
- To serve as the representative body of all clinical and basic investigators in stem cell and regenerative biology at the Chinese University of Hong Kong when dealing with outside institutions.
- To provide a platform for collaborations with scientists in North America, Europe, Asia, Taiwan, Hong Kong and China mainland.
- To enhance international profiles of CUHK.

Research Focus of SCR:

The host reaction to tissue injury involves a complex interplay of local and systemic, cellular and hormonal responses. Mesenchymal stem cells (MSCs) present in many adult tissues can generate new cells either continuously or in response to injury/inflammation/cancer. The main research focus of this group is to understand the role of stem cells in diseases and development and to use MSCs for clinical translational research. The main research interests include:

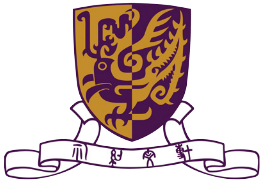
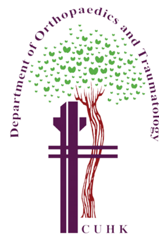
- Study the fundamental biological/mechanical factors that control/regulate MSCs proliferation, differentiation and fate.
- MSCs as a source for tissue engineering and regeneration such as bone-tendon healing, tendon repair, fracture healing, cardiac tissue repair, etc.
- The role of MSCs in cancer development and the use of MSCs as carriers for anti-cancer gene therapy.
- Reprogram the somatic cells into induced pluripotent stem cell (iPS) and the use of iPS as models for studying diseases and developmental process.
- To use GMP stem cell facility to carry out cell therapy clinical trials.

Core technology and research platforms of SCR:

The followings are some existing technologies that we have in the theme:

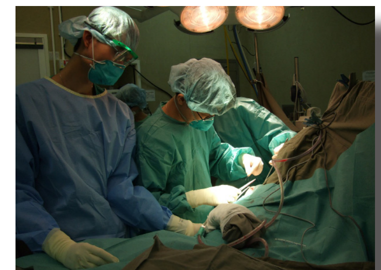
1. MSCs, iPS and embryonic cell culture techniques and standard characterization of various stem cells by flowcytometry, immunohistochemistry and morphology.
2. Multi-differentiation potential assays for stem cells, such as osteogenesis, chondrogenesis,adipogenesis, neurogenesis, angiogenesis and differentiation into cadivascualr muscles, b-cells of islets.
3. In Vivo imaging techniques to trace stem cell migration in vivo.
4. Chemotaxis analysis techniques and imaging techniques including microCT, VivaCT and ultrasound imaging.
5. Transgenic animal models of GFP rat, Luciferase mice, and BMP-4 promoter driver Luc-mouse.
6. Animal models of stem cell transplant, animal models of muscle, tendon, bone and cartilage, spinal cord injury and repair and assessments.
7. Bioreactor platform for stem cell culture.
8. GMP standard clinical grade clean room for human stem cell culture and clinical cell therapy applications.

For the research interests of each members, please check at the following website:
http://www.sbs.cuhk.edu.hk/Research_Scr.asp

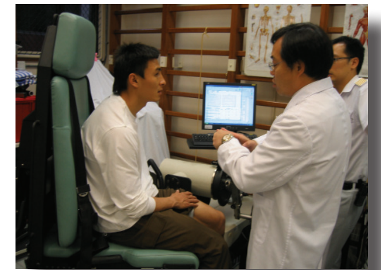



Department of Orthopaedics and Traumatology
The Chinese University of Hong Kong

The department was established in 1982 under the foundation Chairmanship of Professor PC Leung. The first batch of medical students started to have their clinical orthopaedic teaching in 1983.Through the years, the department has grown and developed under the clear Mission and Vision "to provide the highest quality service in patient care, research, education and teaching for medical students and postgraduate training".

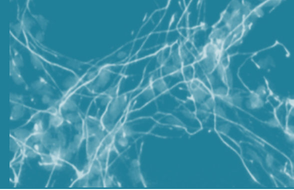


The department has grown from a single professor team to more than 40 clinical colleagues and 60 supporting clerical, technical and research staff now. It would be appropriate to divide the development of the department into three different phases, namely the establishment, the expansion and the consolidation phases. The initial establishment phase stretched from 1982 to 1990 and could be regarded as the infancy and childhood phase. This was followed by a rapid expansion phases from 1991 to 1996 by "hundred flowers blooming" phase which was quite similar to the pre - adolescent and adolescent phase. The past few years, from 1997-2001 featured the early consolidation and sustained growth of the department with the analogy of early and young adulthood phase.



On the clinical services, the department has developed along the major fields of subspecialties in orthopaedics, from Hand and Microsurgery, Sports Medicine, Traumatology, Paediatric Orthopaedics to Orthopaedic Oncology, Spinal injury, Orthopaedic Rehabilitation, Joint Reconstruction Surgery to the latest addition of Foot and Ankle surgery 3 years ago. Many of these subspecialties enjoy significant local, regional and international professional and academic recognition and achievements.

Commitment to quality teaching of medical students is one of the main keystones of the department. The department has been involving in the teaching of musculoskeletal system and orthopaedics in Med 3 and Med 5 students and with the introduction of the new curriculum in 2001, teaching has been extended further into year 1 and 2. With the setting up of a formal teaching committee and departmental teaching coordinator, the curriculum in musculoskeletal system is regularly reviewed and updated. Regular teaching quality assessment, meeting with students and annual curriculum review with honorary teachers has helped not only to update but continuous improvement of the quality of teaching as reflected by the evaluation results and recognition by the faculty and university.



Significant growth has been achieved in the research area. From purely clinical reviews and research, the department has steadily expanded in the years to cover different areas of basic and applied basic research that spread from soft tissue, bone and cartilage to biomaterials, osteoporosis and traditional Chinese medicine. The research committee and the musculoskeletal research laboratory structure now have clear responsibility and function to plan, advice and implement defined policies related to research. Three main focused research programs and functionalisation have been established to incorporate all teaching and research staff of the department. The research output and research grants have increased significantly over the years both in quantity and quality. Up to now, 50 Mphil, 23 PhD and 2 MD have graduated from the department. Active collaborations with other departments, universities and research institutions locally, regionally and with other countries have opened up many new and important areas of research.

The department has put great emphasis on the development of information technology and audiovisual supporting services to all staff from administration to training, teaching, research to clinical services. The whole department is now connected by a sophisticated system of high-speed Intranet. Active research and application of IT in enhancement of web-based interactive teaching is well supported. One of the most important highlights of the department is the establishment of the Orthopaedic Learning Centre from generous donations around 2 million US$ in total. Since it's opening in April 1999, over 5,000 local, regional and international participants have attended different courses and workshops conducted in the centre. The centre has also been recognised as advanced training centre by various societies and also a favorite center for visit by any outside guest to the Faculty of Medicine.

Throughout the years, colleagues of the department have and will continue to be actively committed to the university, the professional and specialty development, and play important roles in public services, voluntary services and services to the community.

With the support, spirit and dedication of colleagues at all levels, we can proudly look forward into the future, continue to strive, seek and develop "to provide the highest quality service in patient care, research, education and teaching for medical students and postgraduate training".

The Hong Kong Jockey Club Sports Medicine and Health Sciences Centre

About us

With The Hong Kong Jockey Club Charities Trust's generosity and support, The Hong Kong Polytechnic University (PolyU) and The Chinese University of Hong Kong (CUHK) jointly established The Hong Kong Jockey Club Sports Medicine and Health Sciences Centre with the aim of improving Hong Kong's quality and quantity of sports-related elite and public clinical services, "sports for health" promotional programmes, academic research and professional education. Each University already has impressive credentials in the fields of sports medicine, sports science, sports rehabilitation, and sports health. The Centre takes these tasks to a new level by maximizing the cost-effective usage of resources and enhancing the cooperation between the two Universities.

Background

Physical inactivity is associated with a myriad of chronic diseases including cardiovascular disease, colon cancer, breast cancer and diabetes. At least 60% of the global population fails to achieve the minimum recommendation of 30 minutes moderate intensity physical activity daily. As physical activity continues to be promoted as part of a healthy lifestyle, sports-related medical & health issues and injures are also becoming increasingly important public health concerns. Prevention effort aimed at reducing sports injuries by targeting high-risk activities, places and modes of occurrence need to go beyond the focus on athlete and also to consider the general public. This also applies to the provision of clinical services that should address the needs of both groups.

Mission

To recreate a healthy society by promoting exercise in the general public and to provide integrated sports medicine and health science services through evidence-based practice, training of sports medicine and health sciences professionals, supported by clinical, basic and applied scientific research related to improving the safety and efficiency of sport performance.

Vision

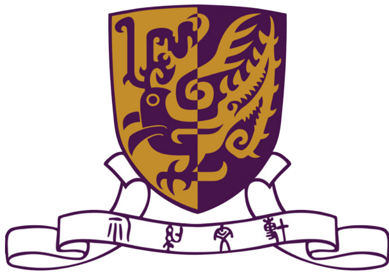
To become the leading hub in the Asia-Pacific region for promoting exercise for health and advancing sports medicine and health sciences-related education research and technology.

Our Units

The Centre comprises of 14 Units, each focuses on a specific area in sports medicine, sport science, sports rehabilitation, or sports health. They work independently and synergistically to provide integrated clinical services, professional training programmes, academic research and community services.

Key Laboratory for Regenerative Medicine
(Ji Nan University-The Chinese University of Hong Kong)
Ministry of Education, China

The Key Laboratory for Regenerative Medicine, Ministry of Education (Ji Nan University-The Chinese University of Hong Kong), was established by Ji Nan University, Guang Zhou, and the Chinese University of Hong Kong, Hong Kong, on the basis of the previously established Joint CUHK-JNU Lab for Regenerative Medicine in April 17th 2007. To further strengthen the expertise and resources of both universities, the Lab then applied for as a Key Lab of Regenerative Medicine, in the Ministry of Education, which was approved in Dec. 2007 to start building the Lab. Moreover, the Key Lab was approved in 2008 as an International Collaborative Base for Science and Technology, by the Department of Science and Technology, Guang Dong Province. In 2009, the key lab was further approved as International Collaborative Base for Science and Technology, by the Department of Science and Technology, P.R.China. Currently, the Key Lab has 31 permanent staffs with an average age of 45 years old. There are 20 high ranking members (Professor), 1 member with title in the “New Century National Hundred, Thousand and Ten Thousand Talent Project”, 1 member of Oversea Outstanding-Youth. Almost all of the principal investigators have been trained oversea. The expertise of the staffs includes almost all areas of regenerative medicine, which are medical regeneration, developmental biology, regenerative biology, cell and molecular biology, tissue engineering, physiology, and immunology etc. The total lab space is about 3600 m^2 , which includes laboratories for molecular biology, cell biology, stem cells, biological imaging, morphology, functional analysis, and up-to 1000-grade cell culture rooms. The labs are furnished with state-of-the–art equipment. The equipment and apparatus procured are worth about 50 million RMB. Post-graduate students from both laboratories move freely and conduct research at both sites. Our mission is to improve the lives of our community by conducting research to find cures for degenerative diseases, such as ischemic heart diseases, skeletomuscular degeneration, eye disease and tissue degeneration caused by cancer/aging. Stem cell- and small molecule- based therapies are currently being developed by principle investigators in the Key Lab to treat the various forms of degenerative diseases mentioned.



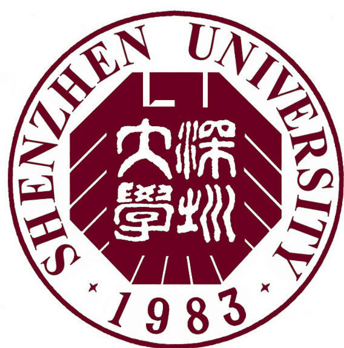
香港中文大学深圳研究院

香港中文大学深圳研究院成立于2007年5月，获深圳市政府的大力支持，由香港中文大学副校长、中国工程院院士徐扬生教授出任院长。作为香港中文大学在中国内地的重要平台，研究院兼顾高校优势与深圳经济发展需求，旨在将香港中文大学优秀的研究人才及成果与深圳的高科技产业相结合，推动科研成果产业化，加快深港创新圈和区域创新体系的建设。

根据国家科技发展规划结合深圳市科技发展需求，研究院重点推动生物科技、信息科技及环境与可持续发展等领域的建设。引入先进科研成果推动产学研合作，建立多个国家级重点实验室深圳研究基地及联合研发中心，发掘地方产业发展技术瓶颈，加大横向项目合作力度，推动技术成果高效转化；加强与内地业界的合作创新，从研究初期的项目合作开发的模式，逐步转变至以市场为导向的产品开发及高端人才输送的全方位合作模式；建设开放型科研平台，突出强调关键技术及科研设备优势资源互补，建立多领域的公共技术服务平台及开放性工程实验室；注重高层次人才培养，依托香港中文大学的优势师资资源和科研基础，开展高水平的教育培训课程，建设职业、专业的人才深造基地。

研究院主体大楼坐落于深圳虚拟大学园国家大学科技园内，建筑面积约25000平方米，为未来国家重点实验室、技术转移、教育及培训提供场地和设施。目前，已有328名中文大学教职员注册成为研究院成员，自2011年起至2012年间，承担国家、广东省及深圳市级科研课题近百项，科研经费约一亿元人民币。

香港中文大学深圳研究院将继续在深圳及珠三角地区积极开展科研、教育培训及有关产业化工作，为深港科技融合提供高效的平台，为粤港地区乃至国家的科技创新、经济及文化发展作出贡献。



深圳大學醫學院

深圳大学医学院于2008年12月由国家教育部、卫生部的评审通过，2009年9月招收首届临床医学学生。深圳大学医学院在深圳大学后海校区启动，将在西丽校区发展。目前的后海校区医学院综合楼建筑面积1.68万平方米，新规划的医学院院区将在西丽新校区建设，占地10万平方米，学生配套生活区占地5万平方米。深圳大学第一附属医院（深圳市第二人民医院）是集医疗、教学、科研和医疗急救于一体的三级甲等综合性医院，第二附属医院位于西丽校区附近，规划为面向国际、水平先进的综合型高端医院，正与医学院西丽校区同步建设。

目前，深圳大学医学院拥有3个本科专业（临床医学、生物医学工程、医疗器械工程）、1个生物医学工程一级学科硕士点和1个生物医学工程领域工程硕士点。拥有国家生化工程技术研究中心、医学超声关键技术国家地方联合工程实验室、广东省生物医学信息检测与超声成像重点实验室、深圳市生物医学工程重点实验室、深圳医学超声关键技术工程实验室、深圳市过敏反应与免疫学重点实验室、深圳合成生物学工程实验室等国家级和省市级重点实验室。

深圳大学医学院确立了“高端、精英、超前、精湛”的办学理念，和“小规模、精英化、研究型”的建院方针，努力与国际医学教育模式接轨。临床医学专业人才培养方面积极探索“1（理工学基础、医学预科）+5（医学本科）+4（硕博连读）”十年制精英式医学高端人才培养模式。深圳大学医学院积极推进对外合作，目前，已与爱尔兰都柏林大学医学院、香港中文大学医学院签署了教学科研合作协议，即将与南佛罗里达大学医学院、澳大利亚莫纳什大学医学院签订相关合作协议，为实现医学专业本科生毕业后高级学位（即1+5+4模式的PhD或MD）的培训和授予创造了良好的条件，并与都柏林大学联合共建“深圳-都柏林健康科学研究院”。同时，我院与深圳市孙逸仙心血管医院、罗湖医院、南山医院等深圳市大中型医院建立了非直属附属医院及教学、实习基地，并与翰宇药业、迈瑞、安科等十多家深圳市大型药业和医疗器械公司开展研发、实习基地、就业实践基地等方面的合作。

2009-2011年，我院累计承担各类科研项目109项，其中,国家“973”项目子课题4项、国家自然科学基金项目26项，实到科研经费累计3322万元;获2011年教育部科技进步二等奖1项、广东省科技进步一等奖1项、获广东省科技进步二等奖1项。目前，深圳大学医学院正在积极开展国内外合作和交流，尤其欢迎海内外的英才加盟或合作，力争向研究型医学院转变，成为在国内外具有一定影响的高水平医学院。

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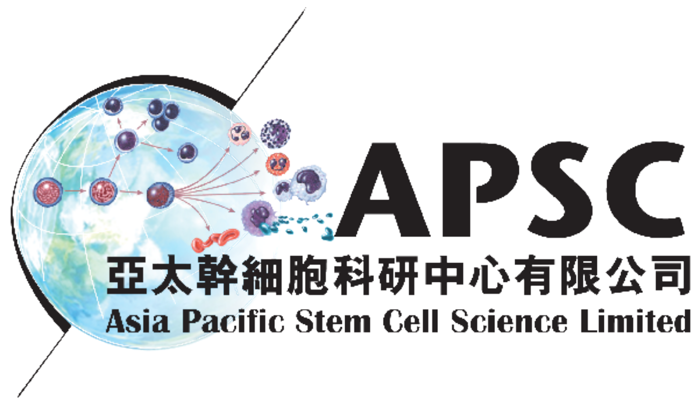

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Day 1 November 19, 2012 (Monday)

Section 1— Biology of Tissue Regeneration

Circulating stem cells and its clinical implications

Prof. Gang LI, M.D., Ph.D
Stem Cells and Regeneration Program, School of Biomedical Sciences
Department of Orthopaedics and Traumatology
The Chinese University of Hong Kong, Hong Kong

Mesenchymal stem cells (MSCs) have been found in cord blood and peripheral blood (PB) of mammalian species including human, guinea pig, mice, rat, dog, horse and rabbit. The number of MSCs in PB (PB-MSCs) is rare and their biological role was not fully defined. We have found increased numbers of circulating MSCs in peripheral blood in patients with long bone fracture, non-union and in patients with cancers. The number of PB-MSCs was approximately 9 times higher in the cancer patients, suggesting there is systemic recruitment of MSCs during cancer development. We have compared the difference between the circulating MSCs and bone marrow derived MSCs and found that they share similar phenotype in vitro, but the gene expression profile between the two cell populations was significantly different. cDNA microarray analysis and quantitative RT-PCR confirmed some genes that are differentially expressed with more than 10 folds difference, such as cellular retinol-binding protein 1 (CRBP1), N-cadherin, SRY-box containing gene 11 (Sox11), the aquaporin 1 (AQP1), et al. These genes are now being further investigated for their role in MSCs migration, homing and multiple-differentiation potential. In terms of potential clinical implications of PB-MSCs, we have demonstrated that allogenic PB-MSCs enhanced bone regeneration in rabbit ulna critical-sized bone defect model. We also demonstrated that BM-MSCs can be recruited via circulation toward the sites of bone fracture and participate fracture healing in rabbits. We have demonstrated that systemically administrated MSCs could home to tumor sites and participated tumor growth. We are now working on using MSCs as a systemic gene delivery vehicle for management of wound healing and cancer therapy, and the ways of enhancing the homing and recruitment of MSCs toward specific sites after their systemic delivery. In conclusion, PB-MSCs are new cell source of cells that may play very important roles in development, repair and disease progression. PB-MSCs may be used for disease monitoring, diagnosis, cell and gene therapy applications.

Key Reference (Prof. Gang Li's work in relation to PB-MSCs):

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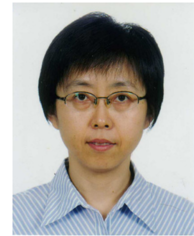
New development in iPS technology



Prof. Bo FENG
Stem Cells and Regeneration Program, School of Biomedical Sciences
Faculty of Medicine,
The Chinese University of Hong Kong, Hong Kong

Embryonic stem cells (ESCs) derived from early embryos can undergo indefinitive self-renewal in culture, and are able to differentiate into a multitude of cell types. Induced pluripotent stem cells (iPSCs), which were derived from somatic cells by overexpression of defined factors, highly resemble ESCs and possess the properties of self-renewal and pluripotency. Since conversion of easy-accessed patients' somatic cells into iPSCs has minimized ethical concerns, this technique has opened tremendous opportunities for development of autologous regenerative medicines as well as for human diseases modeling, i.e., patient-specific iPSCs can be differentiated into functional cell types for therapeutic applications, for modeling the occurrence of pathogenic process, or for drug screening.

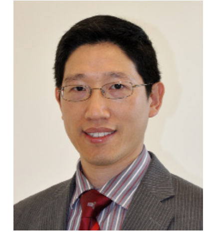
Harnessing the full potential of ESCs/iPSCs relies on efficient and specific differentiation into desired cell types. Neural induction represents the earliest step in determination of embryonic ectodermal lineage, thus it is important for the commitment of ESCs. In order to obtain insightful understanding of the molecular mechanisms that control the ESC commitment, we have set up experiments to induce early neural cells from ESCs. Our results showed that these early neural cells, could further give rise to more differentiated NSCs, which highly resembled NSCs isolated from embryonic mouse brain and can generate neurons and glias. Moreover, our global gene expression analysis revealed the unique transcription features of the early neural cells and provided further insight into the molecular basis that control the early events of neurogenesis.

The comprehensive roles of strontium in bone cells: a systems biology perspective

Prof. Guang-Qian ZHOU
Medical School, Shen Zhen University, China

As high-throughput biology advances, cells and organisms at all levels, from genome to protein, signaling, metabolism, and other cellular functions, can be scanned. We can move from a reductionist approach such as isolation of specific pathways and mechanisms to a more integrative approach, where biological systems are seen as a network of interconnected components. Bone is a complex biological system composed of several highly specialized cell types that are constantly derived from their progenitor cells. During remodeling and the pathological processes of ostoporosis, osteoblasts are regulated by multiple factors via crosstalks and feedback mechanisms, which form a network-like structure. The structure is so complex that systems biology analysis seems to be the only approach to display. As an initial step, we show that gross structure of cellular physiologic development of osteoblast can be obtained from a simple coarse grained core regulatory network. This network demonstrates that chronic low grade inflammation is a most prominent weakness of cellular molecular regulatory mechanism due to the wiring topology which is required for the normal development. The network was further applied in elucidating the mechanisms of the multilevel roles of Strontium-containing compounds in anti-osteoporosis. In comparison, the comprehensive effects of strontium on multiple cell types are also investigated.

Role of Insulin/insulin receptor signaling in chondrogenesis



Prof. Chao WAN
Stem Cell and Regeneration Program, School of Biomedical Sciences,
Faculty of Medicine, The Chinese University of Hong Kong
The Chinese University of Hong Kong Shenzhen Research Institute,
Shenzhen, China

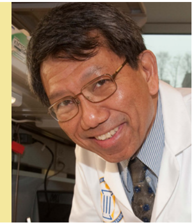
The insulin/insulin-like growth factor 1 (IGF-1) family of ligands and receptors controls key aspects of mammalian life, including growth, metabolism, and reproduction. Humans with mutations in the IR (Leprechaunism) exhibit diabetes with profound growth retardation. Insufficient insulin production or action associated with diabetic states is accompanied clinically or experimentally with impaired bone healing. Both IR and IGF-1 receptor (IGF-1R) exist in the cartilage, and IGF-I stimulates growth plate development. However, the function of insulin/IR in chondrocytes remains unclear. In this study, we investigated how insulin/IR signaling regulates chondrogenesis during skeletal growth and repair using conditional mutagenesis by Cre-loxp strategy where Cre recombinase is activated in chondrocytes. We first confirmed localization of IR in chondrocytes using immunostaining. In a metatarsal bone culture model, treatment with insulin increased the total length of metatarsals over that of untreated controls. Histological analysis shows that insulin increases the size of the proliferating zone in the cartilage rudiments, accompanied by increased cellular proliferation indexed by immunostaining for proliferating cell nuclear antigen. Deletion of IR in primary chondroctytes elevated IGF-1R mRNA and protein levels. IGF-1 stimulated Akt and ERK phosphorylation in the control cells, the effects were further enhanced in the IR mutant cells. The cell mass culture and Alcian Blue staining showed that deletion of IR decreased chondrogenic differentiation and proteoglycan synthesis, and the effects were rescued by IGF-1 treatment. The phenotypes were associated with altered Glut-1 levels and phosphorylation states of mTOR and P70S6K. In vivo phenotyping showed that the cell numbers in the proliferating zone of the growth plate is increased and the cell size is smaller in mice lacking IR in chondrocytes compared with the control littermates. In a fracture model, mice lacking IR in chondrocytes had decreased cartilage replacement by bone and delayed healing than that of the controls. The data suggests that deletion of IR in chondrocytes sensitizes IGF-1 signaling and action.

Tangential migration and proliferation of intermediate progenitor of GABAergic neurons in the mouse neocortex

Prof. Sheng-Xi WU
Department of Anatomy, Histology and Embryology,
K. K. Leung Brain Research Center, Fourth Military Medical University,
Xi'an Shaanxi, China

In the embryonic neocortex, neuronal precursors are generated in the ventricular zone (VZ) and accumulate in the cortical plate. Recently, the subventricular zone (SVZ) of the embryonic neocortex was recognized as an additional neurogenic site for both principal excitatory neurons and GABAergic inhibitory neurons. To gain insight into the neurogenesis of GABAergic neurons in the SVZ, we investigated the characteristics of intermediate progenitors of GABAergic neurons (IPGNs) in mouse neocortex by immunohistochemistry, immunocytochemistry, single-cell RT-PCR and single-cell array analysis. IPGNs were identified by their expression of some neuronal and cell cycle markers. Moreover, we investigated the origins of the neocortical IPGNs by Cre-loxP fate mapping in transgenic mice and the transduction of part of the telencephalic VZ by Cre-reporter plasmids, and found them in the medial and lateral ganglionic eminence. Therefore, they must migrate tangentially within the telencephalon to reach the neocortex. Cell-lineage analysis by simple-retrovirus transduction revealed that the neocortical IPGNs self-renew and give rise to a small number of neocortical GABAergic neurons and to a large number of granule and periglomerular cells in the olfactory bulb. IPGNs are maintained in the neocortex and may act as progenitors for adult neurogenesis.

Section 2 — Topics of Regenerative Medicine



Keynote Speech

Cell-Bassed Regenerative Therapies: Potential and Challenges
細胞基礎再生療法：潛能和挑戰

Prof. Rocky S. TUAN
Director, Center for Cellular and Molecular Engineering,
Arthur J. Rooney, Sr. Professor and Executive Vice Chair
Department of Orthopaedic Surgery
Associate Director, McGowan Institute for Generative Medicine,
Director, Center for Military Medicine Research,
Professor, Department of Bioengineering and Mechanical Engineering & Materials Science,
University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Biography

Rocky S. Tuan, PhD, received his PhD in 1977 from the Rockefeller University in New York, under the mentorship of the late Zanvil A. Cohn, MD. His postdoctoral research fellowship was at Harvard Medical School in Boston, first with Melvin J. Glimcher, MD in the Department of Orthopaedic Surgery at the Children's Hospital, and then from 1978 to 1980 with Jerome Gross, MD, in the Developmental Biology Laboratory at the Massachusetts General Hospital. In 1980, Dr. Tuan was appointed as Assistant Professor in the Department of Biology, University of Pennsylvania in Philadelphia, and was promoted to Associate Professor in 1986. In 1988, Dr. Tuan joined Thomas Jefferson University, Philadelphia, to be the Director of Orthopaedic Research and Professor and Vice Chairman in the Department of Orthopaedic Surgery with a joint appointment in the Department of Biochemistry and Molecular Biology. From 1992-1995, Dr. Tuan was the Academic Director of the MD/PhD program at Jefferson, and in 1997, he established the USA's first Cell and Tissue Engineering PhD program at Jefferson, with the mission of training the next generation of "cross-cultural" biomedical scientists committed to regenerative medicine and the development of functional tissue substitutes. In the fall of 2001, Dr. Tuan joined the Intramural Research Program of the National Institute of Arthritis, and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH), as Chief of the newly created Cartilage Biology and Orthopaedics Branch. In 2004, Dr. Tuan received the Marshall Urist Award for Excellence in Tissue Regeneration Research of the Orthopaedic Research Society. In the Fall of 2009, Dr. Tuan was recruited by the University of Pittsburgh School of Medicine to be the Founding Director of the Center for Cellular and Molecular Engineering, and as Arthur J. Rooney, Sr Chair Professor and Executive Vice Chairman of the Department of Orthopaedic Surgery, with a joint appointment as Professor in the Department of Bioengineering. Dr. Tuan is currently Co-Director of the Wake Forest University/University of Pittsburgh Consortium of the Armed Forces Institute of Regenerative Medicine, a Department of Defense funded, multi-institutional consortium focused on developing regenerative therapies for battlefield injuries. Two recent appointments at Pitt include (1) Associate Director of the McGowan Institute for Regenerative Medicine at Pitt in March, 2012, and (2) Founding Director of the Center for Military Medicine. Dr. Tuan has published over 400 research papers, has lectured extensively, and is currently Editor of the developmental biology journal, BDRC: EMBRYO TODAY, and the Founding Editor-in-Chief of STEM CELL RESEARCH AND THERAPY.

Dr. Tuan directs a multidisciplinary research program, which focuses on orthopaedic research as a study of the biological activities that are important for the development, growth, function, and health of musculoskeletal tissues, and the utilization of this knowledge to develop technologies that will regenerate and/or restore function to diseased and damaged skeletal tissues. Ongoing research projects are directed towards multiple aspects of skeletal and related biology, including skeletal development, stem cells, growth factor signaling, bone-biomaterial interaction, extracellular matrix and cell-matrix interaction, nanotechnology, biomaterials, 3D printing, mechanobiology, regenerative medicine, and tissue engineering, utilizing an integrated experimental approach combining contemporary technologies of biochemistry, cell and molecular biology, embryology and development, cellular imaging, and engineering.

Biomaterials for regenerative medicine

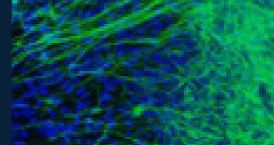
Prof. Peter X. MA
Department of Materials Science and Engineering,
University of Michigan, USA

Regeneration of biologically functional tissues is advantageous over restoration using artificial materials. In the regenerative approach, scaffolding materials provide three-dimensional environments for cells and serve as templates for tissue regeneration. Our lab develops biomimetic polymer scaffolds that recapitulate certain advantageous features of the natural extracellular-matrices (ECM) and impart engineering design to facilitate tissue regeneration. Novel phase separation techniques have been developed to create biodegradable ECM-mimicking nanofibrous scaffolds. Porous network design and computer assisted patient specific scaffold fabrication are intended to facilitate the regenerative restoration of both function and appearance. These novel scaffolds can deliver needed cells to the regeneration sites and have been shown to advantageously support various stem cells to regenerate bone and cartilage in a predetermined shape. To repair complexly shaped tissue defects, an injectable cell carrier is desirable to achieve accurate fit and to minimize surgical intervention. We recently developed star-shaped biodegradable polymers that can self-assemble into nanofibrous hollow microspheres as a novel injectable cell carrier. The nanofibrous hollow microspheres have been shown to efficiently accommodate cells and enhance cartilage regeneration over control cell carriers. To mimic the biomolecular activities in development, we have also developed scaffolds that can release various biological molecules in a controlled fashion to direct cell functions for regeneration. These results demonstrate that the biomimetic scaffolds advantageously support tissue regeneration.

Stem Cell-Based Therapy for Bone Repair: From Bench to Bedside

Prof. Ting-Ting TANG
Department of Orthopedic Surgery, Ninth People's Hospital of Shanghai,
Shanghai Jiaotong University, China

In recent years, we have developed different strategies or techniques to promote the bone repair. One strategy was the BMP-2 ex vivo gene medicine which used the implanted gene transfected bone marrow derived mesenchymal stem cells to achieve sustained secretion of BMP-2 protein in vivo. Such gene transfected cells were usually loaded in the big porous scaffold, but the cell survivor in the center of scaffold is poor due to the limited nutrition supply. One kind of perfusion bioreactor was used to promote the mass transport and stem cell survivor in the scaffold. We also demonstrated the fluid flow- induced shear stress was benificial to the osteogenic differentiation of stem cells in the scaffold. Though the effects of such stem cell-based therapy have been well demonstrated to repair the critical sized bone defects and treat the osteonecrosis in small and big animal models, the clinical trial of manipulated stem cells faced strict SFDA regulation and it is difficult to get approved. Thus we have developed one strategy to use the enriched bone marrow stem cells to promote the bone repair without any cell manipulation in vitro. Clinical trial with about 100 cases had demonstrated the high efficiency of enriched stem cells in the treatment of spine fusion and non-union.



In vivo mesenchymal stem cell proliferation regulated by mechanotransduction

Prof. Yi-Xian QIN
Department of Biomedical Engineering,
Stony Brook University, USA

Loading induced bone fluid flow (BFF) creates a pressure gradient that further influences the magnitude of the mechanotransductory signals. Our group has recently introduced a novel, non-invasive dynamic hydraulic stimulation (DHS) and found its beneficial effects on bone structural quality in a rat hindlimb suspension model. Mesenchymal stem cells (MSCs) have the abilities of self-renewal and differentiation into the cells that form tissues such as bone. The objective of this study is to evaluate MSC quantification on day 3, day 7, day 14, and day 21 in response to DHS as a longitudinal sensitivity of the BFF on MSCs.

Flow cytometric measurements indicated MSC population as represented by cells positive for CD29, CD49e, and CD90.1, and negative for CD45 and CD11b. After 3 days of treatment, %MSC was similar in all three groups. HLS for 7 days and longer significantly reduced MSCs' quantity. The data of day 7 and day 14 showed an interesting trend in the DHS group, as the %MSC seemed to elevate in response to the treatment. However, this elevation diminished after 21 days of treatment.

The change in MSC proliferation is highly time sensitive. As suggested in our previous work, bone growth was already accomplished by 28 days. As an earlier event, it is reasonable to see enhanced MSC proliferation in response to the mechanical signals of DHS at earlier time points, i.e. 7 to 14 days, which clearly explained downstream cellular effect of DHS and its potential mechanism on bone quality enhancement.

Section 3 — Technological Advancements

Hedgehog signaling, is it good or bad for bone?

Prof. Kingston King-Lun MAK
Stem Cell and Regeneration Program, School of Biomedical Sciences,
Faculty of Medicine,
The Chinese University of Hong Kong, Hong Kong

Hedgehog (Hh) signaling is one of the important signaling pathways for many developmental processes, including skeletal development. It has been shown that Hh signaling is required for both chondrocyte hypertrophy as well as osteoblast differentiation, both embryonically and postnatally. However, others and our recent findings indicate that the consequences by this signaling pathway may be very different during modeling and remodeling processes as compared to that of developmental processes. A tight regulation of appropriate Hh signaling activity is therefore critical for normal function of the skeleton. Dysregulated Hh signaling may be resulted for skeletal cancer development as well. Understanding the mechanism of these regulatory processes will inevitably advance the implication on regenerative medicine, leading to the development of new therapeutic interventions for treatment of common skeletal related diseases such as osteoarthritis and osteoporosis. In this talk, we will discuss its applications from the perspective of regenerative medicine using genetic engineered mouse models as examples.

Osteoblasts Control Lineage Commitment Of Mesenchymal Progenitor Cells Through Wnt Signaling

Prof. Hong ZHOU
Bone Biology Laboratory, ANZAC Research Institute,
The University of Sydney, Australia

Lineage commitment of mesenchymal progenitor cells is still poorly understood. Using a transgenic mouse model in which transgenic (tg) expression of 11b-HSD2, a glucocorticoid (GC) inactivating enzyme, under the control of a 2.3Kb collagen type I promoter (Col2.3-11bHSD2) abrogates intracellular GC signalling in mature osteoblasts, we demonstrate that Wnt signalling by osteoblasts is essential for mesenchymal progenitor cells to differentiate away from a default adipogenic into an osteoblastic lineage.

Dominant adipogenesis and reduced osteoblasto¬genesis were observed in calvarial cell cultures from transgenic mice. This phenotypic shift in mesenchymal progenitor cell commitment was associated with reciprocal regulation of early adipogenic and osteoblasto¬genic transcription factors, and with a reduction in Wnt7b and Wnt10b mRNA and b-catenin protein levels in transgenic vs. non-transgenic cultures. Transwell co-culture of transgenic mesenchymal progenitor cells with wild type osteoblasts restored commitment to the osteoblast lineage. This effect was blocked by adding sFRP1 to the co-culture. Treatment of transgenic cultures with Wnt3a resulted in stimulation of osteoblastogenesis and suppression of adipogenesis.

To define the individual roles of Wnt7b and Wnt10b in the control of osteoblast differentiation, we knocked down Wnt7b or Wnt10b expression in MC3T3-E1 cells. Knockdown of Wnt7b in MC3T3-E1 cells resulted in a complete failure of mineralized nodule formation, while Wnt10b mRNA was knocked down, the mineralized nodule formation was delayed and reduced by 75% compared to NT control cells.

Our findings suggest osteoblasts exert direct control over the lineage commitment of their mesenchymal progenitor through Wnt signaling. This glucocorticoid-dependent forward control function indicates a central role for osteoblasts in the regulation of early osteo¬blastogenesis. During this differentiation processing, Wnt7b appears to play an essential role.

FoxO3a Pathway in Senescence and Ischemic-Reperfusion Injury of Cardiac Microvascular Endothelial Cells

Prof. Xu-Feng QI
Key Laboratory for Regenerative Medicine of Ministry of Education,
Jinan University, Guangzhou, China

Dysfunction of cardiac microvascular endothelial cells (CMECs) plays important roles in cardiovascular diseases. Forkhead box O3a (FoxO3a), as an important direct target of the phosphatidylinositaol 3-kinase (PI3K)/Akt pathway, regulates the cell survival and the cell-cycle progression. Recent reports showed that FoxO3a is involved in myocardium infarction. The present study aimed to explore the involvement of FoxO3a pathway in senescence and ischemia-reperfusion injury of CMECs isolated from rat. We found that activity of FoxO3a decreased during CMECs senescence in parallel with cell survival inhibition, G1 phase arrest and p27(Kip) activation. Cellular reactive oxygen species (ROS) level increase and deactivation of antioxidants such as catalase and MnSOD were observed during CMECs senescence. Moreover, phosphorylation of Akt increased upon to senescence of CMECs. However, FoxO3a pathway was significantly activated in CMECs under ischemia-reperfusion conditions in parallel with cell survival inhibition. In addition, cell cycle was arrested in G1 phase in injurious CMECs in parallel with p27(Kip) activation. Furthermore, expression of cleaved caspase-3 was found to markedly increase. Taken together, these results indicate that senescence may arrest CMECs in G1 phase at least in part through inhibit activation of FoxO3a and FoxO3a-mediated down-stream targets including catalase and MnSOD, thereby up-regulating cellular ROS levels. Whereas, activation of FoxO3a may inhibit CMECs survival under ischemia-reperfusion conditions at least in part through inducing caspase-3-mediated apoptosis and p27(Kip)-mediated cell cycle arrest in G1 phase. These results may provide insight into the involvement and potential function of FoxO3a pathway in CMECs.

Acknowledgements
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Mechanobiology and Bone Adaptation

Prof. X. Edward GUO
Department of Biomedical Engineering,
Columbia University, New York, USA

Although it has been accepted that bone adapts to changes in the mechanical and chemical environment, the mechanisms by which bone cells detect or respond to these changes is still unclear. Degenerative diseases such as osteoporosis, bone loss due to microgravity, and failure of orthopaedic implants are largely due to disruption of normal bone adaptation processes. Therefore, increased understanding of the mechanisms by which bone cells sense and transduce mechanical stimuli, as well as the identification of factors that influence mechanotransduction may lead to treatments to mitigate or prevent degenerative bone diseases, as well as provide methods to improve the lifespan of implants. In vivo, osteocytes are embedded in the mineralized extracellular bone matrix, where their cell bodies reside in the lacunae and are interconnected to neighboring osteocytes through numerous intercellular processes. Osteocytes form gap junctions with other osteocytes as well as with osteoblasts on the surface of the bone. The 3-dimensional (3D) network positioning and ability to communicate with other bone cells make osteocytes ideal mechanosensors of bone. Thus the role of osteocyte network and intercellular communication between osteocytes and osteoblasts in response to mechanical stimulation may clarify the mechanisms behind normal bone remodeling. In this presentation, we will review the developments of two in vitro culture systems for studying the roles of osteocytes and osteocytic networks in mechanobiology of bone.

Central Tolerance and its Involvement in Regeneration

Prof. Jun-Wen QIN
Key Laboratory for Regenerative Medicine of Ministry of Education,
Department of Developmental and Regenerative Biology,
Jinan University, Guangzhou, China

Self–non-self discrimination is an essential prerequisite for against foreign pathogen while ignoring the body's own constituents, results in self-tolerance. Central tolerance is indispensable for the induction and maintenance of self-tolerance. Thymus is responsible for the central tolerance of T cells. In the thymus, central tolerance mechanisms mediated by medullary thymic epithelial cells (mTECs), which express peripheral tissue specific antigen (TSA) termed promiscuous gene expression, eliminate self-reactive T cells from the developing T cell repertoire before those T cells into the periphery. Recently, it has been demonstrated that RANK-CD40→TRAF6→AIRE signaling pathway is essential for the control of central tolerance. Deficiency of this signaling pathway results in abnormality of central tolerance, exhibits multiorgan autoimmune diseases including pancreas, which was likely to be related to the defective expression of insulin by thymic epithelial cells. Type I diabetes showed decreased -cells in pancreas and reduced insulin production, similar to that of AIRE knockout mice, suggesting that abnormality of central tolerance may be the direct reason leading to type I diabetes, and thus, recovering the abnormal central tolerance may be an ideal application for type I diabetes.

Bone Regeneration Mechanism of Advanced Biomaterials for Hard Tissue Repair

Prof. Xue-Nong ZOU
Orthopaedic Research Institute,
The First Affiliated Hospital of Sun Yat-sen University,
Guangzhou, China

Traditional biomaterials for hard tissue repair in composition and structure are hugely different with human bone tissue. The repairing process of these materials is basically a passive "filling" process when implanted in the body. Because new bone formation and material degradation rate do not match well enough, it is difficult to achieve the real "biological fusion", and this restricted the use of materials in the orthopaedic clinical application. Therefore, design and preparation of advanced third generation biomaterials for hard tissue repair with "active repair function" and "controlled biological response characteristics" has become the new demand of orthopaedic clinical application and the future research direction. This paper introduced main research methods for bone regeneration mechanisms of advanced biomaterials for hard tissue repair, and it also summarized the current research results of interactions between these biomaterials and the host cells in the host microenvironment, and interactions between the biomaterials and host defense responses in the process of bone regeneration. These sequential events after the implantation of advanced biomaterials for hard tissue repair are apparently controlled by gene expressions, which may be regulated by a series of epigenetic modification with many factors such as material itself and host microenvironmental factors. This paper puts forward new problems existed in the research of advanced biomaterials for hard tissue repair and development trend.

Keywords: biomaterials for hard tissue repair; bone regeneration; host defense; host microenvironment; epigenetics; gene regulation

Day 2 November 20, 2012 (Tueseday)

Section 1 — Biology of Tissue Regeneration

Size matters – Nanotopographical and biomaterial control of skeletal stem cell fate and function

Prof. Richard OREFFO
Bone and Joint Research Group, Center for Human Development, Stem Cells and Regeneration, Institute of Developmental Sciences, University of Southampton, Southampton, UK

Bone marrow derived skeletal stem cells and human embryonic stem cells have the capacity to maintain the stem cell state by self-renewal and the potential to differentiate to produce specialised cell types of osteogenic, adipogenic and chondrogenic lineages. However, the ability to harness these cells to replace or restore the function of traumatised or lost tissue as a consequence of age or disease is one of the biggest challenges facing an increasing ageing population.

We have developed protocols for the isolation, expansion and translational application of human stem cell populations, including enriched skeletal stem cell populations for skeletal repair. A number of areas of work will be presented including:

i) The development of nanopatterned substrates as biomimetic scaffolds in combination with stem cells to provide a unique approach to overcome current issues of stem cell attachment, expansion, lineage specification and directed differentiation in regenerative medicine.
ii) derivation of niche environments through combination of progenitor cells with tailored biomimetic scaffolds in an attempt to modulate the bone repair process and,
iii) translational studies to examine the efficacy of skeletal populations for orthopaedic application.

We demonstrate that defined nanoscale patterns can directly modulate differentiation of human adult skeletal stem cells and embryonic stem cells as well as maintain stem cell phenotype and cell expansion offering new strategies to overcome current issues of stem cell attachment, expansion, lineage specification and directed differentiation in regenerative medicine. Multi-disciplinary approaches that integrate nanotopography, stem cells, materials and clinical techniques for skeletal tissue regeneration offer exciting opportunities to improve the quality of life of many.

Biography

Richard Oreffo holds the chair of Musculoskeletal Science, is co-founder of the Centre for Human Development, Stem Cells & Regeneration and Associate Dean (International and Enterprise) within the Faculty of Medicine. He has held and holds positions on UK Research Council / Government bodies and serves on the editorial boards of seven journals. He leads a multidisciplinary group focused on understanding bone development and developing strategies to regenerate bone and cartilage. He led the group that won the 2010 Technology and Innovation Award and Grand Prix award for a stem cell concentrator. He is a Fellow of the Institute of Biology, has published over 160 peer-reviewed papers, holds 5 patents and is co-editor of “Epigenetic aspects of Chronic Diseases” published in June 2011.

Systemic Trafficking of Macrophages and Osteoprogenitor Cells

Prof. Stuart B GOODMAN, M.D., PhD, FRCSC, FACS, FBSE
Department of Orthopaedic Surgery and (by courtesy) Bioengineering,
Stanford University Medical Center Outpatient Center, USA

Inflammatory and traumatic stimuli are frequently seen in musculoskeletal conditions. For example, the biological response to by-products from joint replacements has traditionally been believed to induce only a localized inflammatory reaction. Recent research has demonstrated that wear particles evoke the systemic trafficking of macrophages and osteoprogenitor cells to the site of particle generation. The same phenomenon has been found for traumatic bone defects using rodent models.

We have recently shown that clinically relevant polymer particles (UHMWPE and bone cement) either injected or continuously infused into the femur of a nude mouse can cause reporter macrophages, injected into the tail vein, to migrate to the local site of particle deposition. This systemic migration of polymer particles is associated with decreased local bone density in the area of particle infusion. The migration of these cells and bone density changes can be mitigated by interference with the CCR2-MCP-1 chemokine-receptor axis. The tools to study these effects included bioluminescence, microCT, microPET, fluorescence microscopy and histomorphometry.

In addition to stimulating bone degradative processes, wear particles evoke biological events that attempt bone repair. We injected reporter MC3T3 osteoprogenitor cells into the left ventricle of nude mice in order track the migration of these cells systemically. The cells were injected into the arterial rather than the venous circulation because of sequestration of the much larger reporter MC3T3 cells in the lungs, when injected intravenously through the tail vein. Intra-cardiac injection of these reporter cells resulted in increased systemic trafficking to the particle site and enhanced bone formation. These effects could be blocked by systemic delivery of a competitive CCR1 inhibitor.

Taken together, the above studies suggest that interference with systemic migration of macrophages and promotion of the migration of osteoprogenitor cells may be viable strategies to prevent or treat developing particle-induced osteolysis associated with joint replacements, or other bone defects.

Modified Biomaterial Interfaces and Stem Cell Responses

Prof. Wilson WANG
Department of Orthopedic Surgery,
National University of Singapore, Singapore

One of the key challenges in bone healing and regeneration is the engineering of an implant that incorporates osseointegration to meet the metabolic demands of recovery. Despite the advances in current implant technology, there are still problems associated with their usage including loosening and tissue reaction. Under ideal conditions, implants could become permanently incorporated within the bone and survive under all normal conditions of loading. This process of osseointegration is highly dependent on the cascade of cellular and biological events that take place at the bone-implant interface. For the design of implant materials, cells and proteins at the implant interface play a critical role. Especially due to the scarcity of stem cells at such sites, biomaterials which regulate cellular functions such as adhesion, growth and differentiation are desired. Strategies to manage host-implant interfaces centre around three components: cells, structure and growth factors. Therefore the utilization of nanotopographical effects and biosignal proteins for development of biomaterial interfaces with structural and biological potential to manage bone healing and osseointegration holds great potential. In our studies we fabricated implant materials with nanotopographical features in conjunction with bioactive factors for enhanced stem cell functions to improve tissue integration capacity at the bone-implant interface.

Section 2 — Tendon Regeneration

Keynote Speech

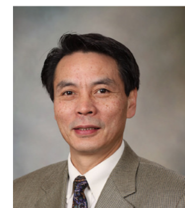
Are we trying to treat a failed healing response in Tendinopathy?

Prof. Christer ROLF
Department of Orthopaedics,
Karolinska University Hospital, Stockholm, Sweden

We speculate that the Tendinopathy process has a common denominator leading to deregulation of normal healing after injury and failed ligament reconstruction, due to hampered regeneration of collagen. In chronic cases it often presents as a focal pain condition, or in partial and complete ruptures. We further speculate that this process is involved in pathological processes in varying collagen components in the body including vascular structures, but in athletes presenting most frequently in relation to Achilles tendon, patellar tendon and rotator cuff injuries.

We do believe there is a rationale to suggest that this active "non-healing" process may have been caused or even controlled by infectious microbes, reprogramming tendon cells normal healing process, in particular in genetically predisposed individuals.

Tissue Engineering for Flexor Tendon Repair



Prof. Chun-Feng ZHAO
Orthopedic Biomechanics Laboratory,
Department of Orthopedic Surgery, Mayo Clinic, USA

Flexor tendon injuries in the hand are common, with primary repair of lacerated tendons being the gold standard treatment. However, restoration of tendon function is commonly hindered by postoperative complications, such as severe adhesions, repaired tendon gap formation, or rupture. Recent studies have demonstrated that surface modification of a repaired tendon with lubricating molecules, such as hyaluronic acid and lubricin, effectively decrease postoperative adhesion and improve digit range of motion. However, these positive effects are compromised by delayed repair healing. To overcome these adverse effects, we have developed cell and growth factor based engineering approaches to enhance flexor tendon repairs.

Tendon stem cells in tendon regeneration

Prof. Pauline Po-Yee LUI
Department of Orthopaedics and Traumatology,
Faculty of Medicine,
The Chinese University of Hong Kong, Hong Kong

Tendon and ligament injuries are common both in the workplace and sport activities. These injuries are difficult to manage and frequently result in long-term pain, discomfort and disability, placing a chronic burden on the healthcare system. Our group has focused in recent years to study the roles of tendon-derived stem cells (TDSCs) in tendon pathology as well as their application for the promotion of tendon and ligament repair. In my last year's presentation, I presented our findings on the superiority of TDSCs compared to bone marrow-derived stem cells (BMSCs) as an alternative cell source for musculoskeletal tissue engineering, the application of TDSCs for the repair of acute tendon injury, the effect of in-vitro passaging on the stem cell-related properties of TDSCs and the use of hypoxia for the in-vitro expansion of TDSCs. Only the use of allogeneic TDSCs with no immunorejection would justify their clinical use. Previous study also reported that transplantation of BMSCs at high concentration induced ectopic bone formation in an acute tendon injury model. We asked if tenogenic differentiation of TDSCs would reduce ectopic bone formation while promote tendon repair. In this presentation, I will present our recent findings related to the immunogeneicity of TDSCs, the use of TDSC sheet for the promotion of tendon healing and anterior cruciate ligament (ACL) reconstruction and the use of TDSC transfected with scleraxis (Scx) for the promotion of tendon repair.

Acknowledgement:
This results presented in this talk was supported by resources from the General Research Fund (project number: 460710, 471411, 470512), the Innovation Technology Support Program (ITSP) – Tier 3 (project number: ITS/156/11) and the Innovation Technology Fund Internship Program (project number: InP/164/11, InP/165/11, InP/070/12, InP/053/12).
goals of this work were to 1) evaluate the novel mechanical signal to promote osteointegration, and 2) deliver ultrasound at the vertebrae in OVX rat to mitigate of bone loss.

The mechanical signal regulation in tendon stem cells differentiation

Prof. Xiao-Ling ZHANG
Institute of Health Sciences, Shanghai Jiaotong University,
School of Medicine & Shanghai Institute for Biological Sciences,
Chinese Academy of Sciences, Shanghai, China

Chronic tendinopathy is a tendon disorder that is common in athletes and individuals whose tendons are subjected to repetitive strain injuries. The presence of ossification worsened the clinical manifestation of the disorder. The change of tendon loading due to mechanical overload, compression or disuse have been implicated as the possible etiologies, but the pathological mechanisms of tendinopathy remain unclear. In this study, we demonstrated that ossification in tendon tissue might be due to the osteogenesis of tendon-derived stem cells (TDSCs) induced by uniaxial mechanical tension (UMT) which mimics the mechanical loading in tendon. Rat TDSCs (rTDSCs) could be induced to differentiate into osteogenic lineage after treatment with elongation UMT as shown by the increased expression Runx2 mRNA and protein, Alpl mRNA, collagen type 1 alpha 1 (Col1a1) mRNA, ALP activity and ALP cytochemical staining. RhoA, an osteogenesis regulator, was activated in rTDSCs upon UMT

Abstracts (cont')

stimulation. Blockage of RhoA activity in rTDSCs by C3 toxin or ROCK activity, a downstream target of RhoA, by Y-27632 inhibited UMT-induced osteogenesis in rTDSCs. UMT up-regulated the mRNA expression of Wnt5a but not the other non-canonical Wnts. The inhibition of Wnt5a expression by siRNA abolished UMT-induced Runx2 mRNA expression and RhoA activation in rTDSCs and the inhibition of Runx2 expression could be rescued by addition of LPA, a RhoA activator. The results showed that UMT induced osteogenic differentiation of rTDSCs via the Wnt5a-RhoA pathway, which might contribute to ectopic ossification in tendon tissue due to mechanical loading. we further used miRNA microarray to examine the contribution of microRNAs in this progress. Several important microRNAs was screened and their function was tested. These findings reveal a significant additional mechanism by which miRNA control the differentiation of TDSCs which can be used in the treatment of tendinopathy or directional differentiation of TDSCs.the meanwhile, I would like to exemplify that excellent journal articles can be published in translational research as well as in basic research.

Lunch Time Seminar:

Development of nanofiber production equipment
Present Nanofiber application

Dr. Chikashi NAITO
Nanofiber division MECC co.ltd

We develop Electrospinning equipment to create nanofiber and it has been 8 years since we launched nanofiber business by electrospinning method.

As application of nanofiber, there are several application such as air filter, adsorption/ separation material,(Undesirable-elements removal, oil-water separation by a gydrofuge function), medical field (regeneration medicine, organ transplant, an antibacterial material,) water disposal, agricultural-chemicals medication, electrode material(capacitor, battery, sensor), an optical medium, a magnetic material, a solar call, and Channel of MEMS(minute machine electricity element).

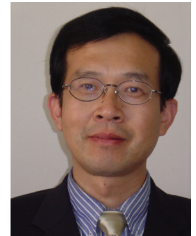
Nowadays Nanofiber for medical use is getting attentnion. Moreover, in medical field, research of a scaffold into which the cell of regeneration is grown up is performed. Reproduction of all the portions of persons, such as a brain, liver, the heart, skin, a cornea, a bone, a tendon, a tooth, and a blood vessel, is realized. In the medical field (regeneration medicine, organ transplant), there are application such as the medicine transfer system (drug delivery system) which can have an effect only the affected part, the wound dressing (Antibacterial material) which can cure a crack finely quickly, The market spreads out also as application as above.

MECC started nano fiber projects in 2004 and have developed electrospinnning setups based on the below concepts.
-possible to control various morphologies
-high reproducibility
-possible to spin a wide range of materials into nano fibers
-high flexible & safety design

Today I would like to introduce especially latest Nanofiber medical application and our Nanofiber Electrospinning equipment.

Section 3 — Technological Advancements of Stem Cell Biology and Applications

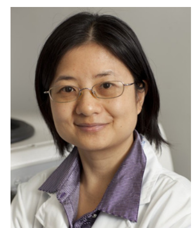
sRNA Induces the Large-scale Trans-determination of Mesenchymal Stem Cells into Hematopoietic Stem Cells in Human



Prof. James Q. YIN
Beijing Military General Hospital,
Institute of Biophysics, Chinese Academy of Sciences, China

Mesenchymal stem cells (MSCs) can differentiate into cells of bone, endothelium, adipose tissue, cartilage, muscle, and brain. However, whether they can transdeterminate into hematopoietic stem cells (HSCs) remains unsolved. We report here that a subpopulation of human MSCs that are CD44+,CD29+, CD105+, CD166+,CD133-,CD34- could differentiate into hematopoietic stem cells (CD150+/CD133+/CD34+) and their descending blood cells in vitro, when transfected with new endogenous shRNAs The sRNA was high-effectively delivered into MSCs by a novel peptide means. These induced MSC-HSCs could form different types of hematopoietic colonies as nature-occurring HSCs did. Upon transplantation into sublethally irradiated NOD/SCID mice, these MSC-HSCs engrafted and differentiated into all hematopoietic lineages such as erythrocytes, lymphocytes, myelocytes and thrombocyte. Furthermore, we demonstrated the first evidence that the transdetermination of MSCs was induced by acetylation of histone proteins and activation of many transcriptional factors. Together, our findings identify the sRNAs that dictates a directed differentiation of MSCs toward HSCs and open up a new source for HSCs used for the treatment of blood diseases and artificial stem cell-made blood.related diseases such as osteoarthritis and osteoporosis. In this talk, we will discuss its applications from the perspective of regenerative medicine using genetic engineered mouse models as examples.

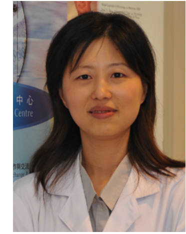
An underlying mechanism for the self-renewal of Smad3-/- ES cells



Prof. Ping YUAN
Department of Chemical Pathology, Faculty of Medicine,
The Chinese University of Hong Kong, Hong Kong

Disease associated gene knockout embryonic stem cells could provide valuable in vitro models to study the disease mechanism and screen drugs. Smad3 mediated TGF-β/Activin/Nodal signaling plays important roles in many biological processes and Smad3 -/- mice exhibit chronic inflammation and develop colorectal cancer. Till now Smad3 deplete embryonic stem cells have yet to be derived. To establish a new in vitro model to study inflammation and cancer, we derived Smad3-/- ES cells. Smad3-/- ES cells can form teratoma and differentiate into three germ layers. Microarray analysis shows that Rif1, a newly identified regulator of DNA replication time, is highly upregulated in Smad3-/- ES cells. Chromatin immunoprecipitation assay and luciferase assay confirms that Smad3 binds to Rif1 promoter region and directly represses its expression. Reduction of Rif1 by shRNA to wild type expression level in Smad3-/- ES cells leads to quick differentiation of Smad3-/- ES cells. Taken together, this study uncovers Rif1 as a novel target of Smad3 in ES cells and plays an important role in the self-renewal of Smad3-/- ES cells.

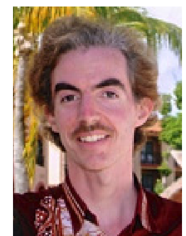
Reprogramming MSCs through in vitro differentiation and dedifferentiation for enhancing therapeutic potential in vivo



Prof. Xiao-Hua JIANG
School of Biomedical Science, Faculty of Medicine,
The Chinese University of Hong Kong, Hong Kong

Dedifferentiation is a cellular process often seen in more basal life forms such as worms and amphibians in which a partially or terminally differentiated cell reverts to a more primitive developmental stage, usually as part of a regenerative process. In mammals, the differentiation process was thought to be irreversible. However, recent studies have demonstrated that dedifferentiation may take place during mammalian wound repair in vivo or through reprogramming in vitro. Our recent studies show that after in vitro induction of neuronal differentiation and dedifferentiation, MSCs, which have already committed to neuronal lineage, revert to a primitive cell population (De-neuMSCs) exhibiting a reprogrammed phenotype distinct from their original counterparts. Of therapeutic interest, the De-neuMSCs exhibit enhanced cell survival and higher efficacy in neuronal differentiation compared to unmanipulated MSCs both in vitro and in vivo. Interestingly, significant upregulation of miR-34a was observed in De-neuMSCs and linked to enhanced cell survival and neural potentiality, hinting at the possible involvement of an epigenetic mechanism in the reprogramming. Recently, we have revealed that De-neuMSCs express significantly higher levels of chemokines and cytokines and display enhanced tropism to cancer, indicating its potential application in gene therapy for cancer treatment. Taken together, our findings have the potential to provide a novel and clinically practical method to overcome the hurdles faced by current MSC-based therapy.

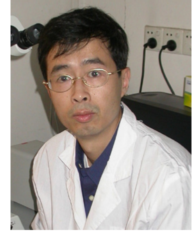
Readying adult stem cells towards the clinic: Incubation within cellular assemblies and sugar substrates



Prof. David W. GREEN
Faculty of Dentistry,
The University of Hong Kong, Hong Kong

There is considerable optimism that stem cells with their powerful regenerative potency can be used to permanently treat degenerative disease and tissue trauma. However, there are difficulties nurturing sufficient numbers of high quality stem cell populations in the tissue culture laboratory aimed at therapeutic use. Biomaterials can play a vital role in reinvigorating stem cell proliferation and differentiation activities in the petri dish. In light of this we introduce two biomaterial led strategies that have been devised to protect and maintain the regenerative potency of adult stem cells in the laboratory and for later transplantation. In our research we have been looking to harness human mesenchymal stem cells (hMSC) for application in bone regeneration. The first strategy is to create cellular assemblies that emulate bone stem cell niches- perivascular and endosteal with particular emphasis on adding niche cells. Our aim is to have executive control over hMSC proliferation and differentiation by modulating the molecular and cellular microenvironment. We have begun to fabricate modular constructs to arrange niche cells alongside embedded hMSC in patterns reminiscent of the perivascular niche. In a second strategy biomaterials can be used to selectively coat hMSC and provide them with a highly localised matrix for efficient targeted delivery and cellular uptake of vital factors in proliferation and differentiation. The hMSC-laden biomaterials provide the tissue engineer with transplantable packets that can be injected into the prospective patient. These biomaterial-led solutions are a small step towards resolving the continued problem of maintaining, protecting, controlling and guiding adult stem cells in proliferation and differentiation for eventual clinical use.

Cardiac Telocytes in Regenration of Infarcted Myocardium



Prof. Dong-Qing CAI
Key Laboratory for Regenerative Medicine, Ministry of Education,
Department of Developmental & Regenerative Biology, Ji Nan University
International Base of Collaboration for Science and Technology (JNU),
The Ministry of Science and Technology & Guangdong Province, China

Recently, a novel interstitial cell, named as telocyte, is found in myocardium. In our previous study, we reported that the density of cardiac telocytes in base part and the atrium-atria part was significantly higher than that in medium part. In addition, the density of cardaic telocytes in subepicardium was significantly higher than that in the endocardium. However, whether the distribution of cardaic telocytes is experienced change under the MI is still unclear. Our recent studies showed that the density of cardiac telocytes after MI was decreased significantly comparing with non-LAD ligated control group. In addition, it was found that the density of cardiac telocytes was decreased consecutively. In addition, our results revealed that intramyocardium injection of cardiac telocytes was able to improve the function of infarcted heart after MI. Our findings suggested that cardiac telocytes play an important role in integrity of myocardium and regeneration of MI.

ACKNOWLEDGEMENTS
This work was supported by the 863 grant (2007AA02Z105), the National Natural Science Foundation of China (30770886, 30570369, 30340038, 30973158, 81170324); Guangdong Key grant for Natural Science Foundation (04105826); Guangdong grant for Science and Technology Development (2004B30601007); International collaborated grant of Guangdong (2009B050900007); the Fundamental Research Funds for the Central Universities (21609408); Collaborated grant for HK-Macao-TW of Ministry of Science and Technology (2012DFH30060).
such as osteoarthritis and osteoporosis. In this talk, we will discuss its applications from the perspective of regenerative medicine using genetic engineered mouse models as examples.

Extracellular Matrix as Stem Cell Niche: Implications in Osteoarthritis

Prof. Qian CHEN
Department of Orthopaedics,
Alpert Medical School of Brown University, USA

Introduction: Matrilin-3 (MATN3) is an extracellular matrix (ECM) protein endogenous to cartilage tissue that may play important roles in protecting cartilage from degeneration. Deletion of the functional MATN3 gene leads to accelerated articular cartilage degeneration in mice while a point mutation (T298M) in the MATN3 gene is associated with hand osteoarthritis (HOA) in humans. While the mechanism underlying MATN3's chondroprotection remains to be fully elucidated, MATN3 has been shown to stimulate the expression of a potent anticytokine IL-1Ra, which inhibits IL-1 induced catabolism and promotes the expression of anabolic markers Col2a1 and aggrecan (Acan) in mature articular chondrocytes. In the present study we test 1) whether MATN3 modulates the expression of chondrogenic markers in chondroprogenitor cells, 2) whether such modulation depends on IL-1Ra, and 3) whether HOA MATN3 mutant affects modulation properties observed in the wild type MATN3.

Methods: Cell culture experiments were conducted using untransfected, WT-MATN3 or HOA-MATN3 transfected ATDC5 cells. For small interfering RNA-based gene silencing experiments, ATDC5 cells were transfected (48 hours) with murine IL1RN ON-TARGETplus siRNA using Lipofectamine 2000. A

Abstracts (cont')

non-silencing scrambled siRNA was used as control. mRNA was extracted for reverse transcription and gene expression analysis by real-time RT-PCR using primers specific soluble IL-1Ra (sIL-1Ra), Col2a1, Acan, and Sox9. Transcripts were normalized to rRNA 18S expression. Alcian blue staining was used to evaluate chondrogenesis of ATDC5 cells.

Results: To characterize the chondroprogenitor ATDC5 cell lines stably expressing WT-MATN3 or HOA-MATN3, we quantified mRNA expression levels of IL-1Ra and chondrogenesis markers. WT-MATN3, but not HOA-MATN3, stimulated IL-1Ra expression for 5.6 fold in comparison to the ATDC5 parental cell line. ATDC5 cells expressing WT-MATN3 exhibited enhanced Acan, Col2a1 and Sox9 mRNA levels in comparison to parental ATDC5 cells. In contrast, cells expressing HOA-MATN3 further reduced the expression of these chondrogenesis markers. Knocking down IL-1Ra abolished the stimulation of Acan, Col2a1, and Sox9 expression by WT-MATN3 in ATDC5 cells. Thus, WT-MATN3 stimulates chondrogenesis in an IL-1Ra dependent manner.

Discussion: WT-MATN3 modulates the expression of major chondrogenesis markers in ATDC5 chondroprogenitor cells. This modulation depends on its stimulation of IL-1Ra. Strikingly HOA-MATN3 loses its ability to stimulate IL-1Ra and also cannot modulate chondrogenesis in chondroprogenitor cells. This is the first time that a specific cellular deficiency has been identified with the HOA-MATN3 mutant. Our data suggest that the OA pathology associated with the HOA mutation may result from its deficiency in modulating chondrogenesis of chondroprogenitor cells within cartilage.

Bio-inspired approaches for bone tissue engineering

Prof. Peter Yun-Zhi YANG
Department of Orthopaedic Surgery,
Department of Materials Science and Engineering,
Stanford University, USA

Tissue engineering (TE) and regenerative medicine (RM) have been proposed to fabricate engineered tissues and organs to restore and improve the functions of diseased or traumatized tissues through the principles of life science and engineering. Cells, signals (or growth factors), and scaffolds (or microenvironment or extracellular matrix) are generally referred to as three key components of TE. Significant advances had been made in TE in the past two decades, however, limited clinical successes had been seen. Three grand challenges of TE are identified as limited cell sources, non-functional vascularization and inappropriate microenvironment. In this session, we will discuss how we use bio-inspired strategy and aim to (1) integrate microfabrication (bottom-up) with scaffolding (top-down) approaches to re-vascularize engineered cortical and cancellous bones at a large scale, and (2) to achieve temporally and spatially controlled signals that regulate tissue regeneration, leading to a functional tissue regeneration with biomimetic complexity and enhanced functionality.

Section 4 — Translational Medicine Related Topics

Keynote Speech

Experimental paradigms of tissue engineering and regeneration
組織工程和再生醫療的實驗範式

Prof. Rocky S. Tuan
Director, Center for Cellular and Molecular Engineering,
Arthur J. Rooney, Sr. Professor and Executive Vice Chair
Department of Orthopaedic Surgery
Associate Director, McGowan Institute for Generative Medicine,
Director, Center for Military Medicine Research,
Professor, Department of Bioengineering and Mechanical Engineering & Materials Science,
University of Pittsburgh, Pittsburgh, Pennsylvania

Biography

Rocky S. Tuan, PhD, received his PhD in 1977 from the Rockefeller University in New York, under the mentorship of the late Zanvil A. Cohn, MD. His postdoctoral research fellowship was at Harvard Medical School in Boston, first with Melvin J. Glimcher, MD in the Department of Orthopaedic Surgery at the Children's Hospital, and then from 1978 to 1980 with Jerome Gross, MD, in the Developmental Biology Laboratory at the Massachusetts General Hospital. In 1980, Dr. Tuan was appointed as Assistant Professor in the Department of Biology, University of Pennsylvania in Philadelphia, and was promoted to Associate Professor in 1986. In 1988, Dr. Tuan joined Thomas Jefferson University, Philadelphia, to be the Director of Orthopaedic Research and Professor and Vice Chairman in the Department of Orthopaedic Surgery with a joint appointment in the Department of Biochemistry and Molecular Biology. From 1992-1995, Dr. Tuan was the Academic Director of the MD/PhD program at Jefferson, and in 1997, he established the USA's first Cell and Tissue Engineering PhD program at Jefferson, with the mission of training the next generation of "cross-cultural" biomedical scientists committed to regenerative medicine and the development of functional tissue substitutes. In the fall of 2001, Dr. Tuan joined the Intramural Research Program of the National Institute of Arthritis, and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH), as Chief of the newly created Cartilage Biology and Orthopaedics Branch. In 2004, Dr. Tuan received the Marshall Urist Award for Excellence in Tissue Regeneration Research of the Orthopaedic Research Society. In the Fall of 2009, Dr. Tuan was recruited by the University of Pittsburgh School of Medicine to be the Founding Director of the Center for Cellular and Molecular Engineering, and as Arthur J. Rooney, Sr Chair Professor and Executive Vice Chairman of the Department of Orthopaedic Surgery, with a joint appointment as Professor in the Department of Bioengineering. Dr. Tuan is currently Co-Director of the Wake Forest University/University of Pittsburgh Consortium of the Armed Forces Institute of Regenerative Medicine, a Department of Defense funded, multi-institutional consortium focused on developing regenerative therapies for battlefield injuries. Two recent appointments at Pitt include (1) Associate Director of the McGowan Institute for Regenerative Medicine at Pitt in March, 2012, and (2) Founding Director of the Center for Military Medicine. Dr. Tuan has published over 400 research papers, has lectured extensively, and is currently Editor of the developmental biology journal, BDRC: EMBRYO TODAY, and the Founding Editor-in-Chief of STEM CELL RESEARCH AND THERAPY.

Dr. Tuan directs a multidisciplinary research program, which focuses on orthopaedic research as a study of the biological activities that are important for the development, growth, function, and health of musculoskeletal tissues, and the utilization of this knowledge to develop technologies that will regenerate and/or restore function to diseased and damaged skeletal tissues. Ongoing research projects are directed towards multiple aspects of skeletal and related biology, including skeletal development, stem cells, growth factor signaling, bone-biomaterial interaction, extracellular matrix and cell-matrix interaction, nanotechnology, biomaterials, 3D printing, mechanobiology, regenerative medicine, and tissue engineering, utilizing an integrated experimental approach combining contemporary technologies *of biochemistry, cell and molecular biology, embryology and development, cellular imaging, and engineering.*

Cell therapy for tendinopathy management

Prof. Ming-Hao ZHENG
Western Australia University, Australia

R&D and Validation of a Porous Scaffold Composite Material incorporating Osteopromotive Compounds for Bone Defect Repair in Osteonecrosis
骨坏死性骨缺损修复生物活性材料的研发与验证

Prof. Ling QIN
Musculoskeletal Research Laboratory,
Department of Orthopaedics & Traumatology,
The Chinese University of Hong Kong, Hong Kong

Our research group identified a phytomolecule Icaritin and proved its osteopromotive for prevention of osteoporosis and osteonecrosis. We further produced a bioactive poly (L-lactide-co-glycolide)/tricalcium phosphate (PLGA/TCP)-based porous scaffold incorporating osteopromotive phytomolecule Icaritin using a fine spinning technology. In vitro release of Icaritin from PLGA/TCP scaffold was quantified by high-performance liquid chromatography (HPLC). Both in vitro cytotoxicity test and in vivo test via muscular implantation were conducted to confirm its biocompatibility. The attachment, proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) on composite scaffold were evaluated. The results showed that the PLGA/TCP/Icaritin composite scaffold was porous with interconnected macro-pores (about 480 μm) and micro-pores (2-15 μm). The mechanical properties of PLGA/TCP/Icaritin scaffold were comparable to those of pure PLGA/TCP scaffold, yet was direction-dependent. Icaritin content was detected in medium and increased with time, confirming its sustained release from the scaffold. The in vitro cytotoxicity test and in vivo intramuscular implantation showed that the composite scaffold had no toxicity and good biocompatibility. The PLGA/TCP/Icaritin scaffold facilitated the attachment, proliferation and osteogenic differentiation of BMSCs. Animal experiments confirmed its treatment efficacy bone defect repair in a core-decompression model in steroid-osteonecrosis in qudrapedal rabbits and prevention of joint collapse in bipedal emu model. The underlying mechanisms are associated promotion of osteogenesis and anti-adipogeneis as well as promotion of BMSCs migration towards boen defect repair region with presence of bioactive scaffold. In conclusion, an osteopromotive phytomolecule Icaritin could be successfully incorporated into PLGA/TCP to form an innovative porous composite scaffold with sustained release of osteopromotive Icaritin and this scaffold had good biocompatibility and osteoinduction, suggesting its potential for orthopaedic applications.

Skeletal Progenitors-specific ablation of Cbfβ displays a novel function of Cbfβ in chondrocyte proliferation and Cartilage Repair

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Objectives: The role of Core binding factor beta (Cbfβ) in postnatal skeletal development and maintenance remains unclear. We hypothesized that Proteins that promote the chondrocyte proliferation and differentiation may stimulate the repair of damaged cartilage in Osteoarthritis and Cbfβ is essential to promotes stem cells to chondrocytes at the Cartilage Repair in osteoarthritis. To test the hypothesis, we investigated whether Cbfβ is required for the growth and maintenance of the skeleton in postnatal mice and identified a novel function of the transcription factor core-binding factor b subunit (Cbfβ) in chondrocyte proliferation and differentiation and Cartilage Repair induced by regulating the CBFb-RUNX1 transcriptional program.

Method: We used genetic approach to generate the skeletal mesenchymal cell-specific Cbfβ-deficient mice (Cbfβf/f Prx1-cre mice). Osteoarthritis (OA) is a degenerative joint disease that involves the destruction of articular cartilage and eventually leads to disability. Surgery based OA animal model was used to test whether Adeno-Associated Virus (AAV) mediated Cbfβ overexpression can repair cartilage damage and whether Cbfβ deficiency mice delay Cartilage Repair.

Results: Cbfβf/f Prx1-cre mice survive to adulthood with severe limb malformations. Our results showed that proliferation zones of the growth plate are dramatically shortened in the newborn stage of Cbfβ-deficient mice. Notably, the proliferation zone and the hypertrophic zone of the growth plates completely disappear and trabecular bone is absent in one-month-old mice. Loss of Cbfβ also impaired intramembranous bone formation, in vivo and in vitro. We found that Indian hedgehog (Ihh) was downregulated and parathyroid hormone-related protein (PTHrP) receptor (PPR) was upregulated in the postnatal growth plate of Cbfβf/f Prx1-cre mice, which indicates that the deficiency of Cbfβ disrupted the Ihh-PTHrP regulatory loop. Cartilage Repair was largely delayed in Cbfβ deficiency mice. Interestingly, our results demonstrated that Cbfβ regulates both chondrocyte proliferation and differentiation. Over-expression of Cbfβ shows chondroprotective effect and significantly enhanced Cartilage Repair in OA animal model.
Conclusions: Our study revealed, for first time, that Cbfβ is essential for both chondrocyte proliferation and differentiation in the growth and maintenance of the skeleton in postnatal mice, and essential to promotes stem cells to chondrocytes at the Cartilage Repair in osteoarthritis, which may ultimately lead to a novel gene therapy for osteoarthritis.

Keywords: Core binding factor beta (Cbfβ). stem cells, chondrocyte proliferation, Cartilage Repair, osteoarthritis

Development of an Human Fetal Mesenchymal Stem Cell Line Overexpressing Thymidine Kinase (TK) Gene for Anti-tumor Therapy

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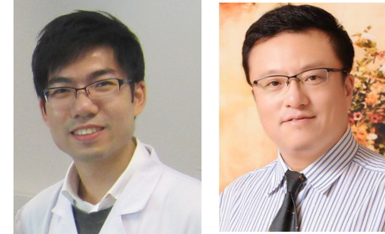

Cancer is one of the greatest health challenges facing the world today with over 10 million new cases of cancer every year. Based on the reported properties of self-renewal, tumor-homing ability and low immunogenicity, mesenchymal stem cells (MSCs) is a potential delivery candidate for sucidie genes for anti-tumor therapy. In this study, human fetal bone marrow-derived MSCs (hfBMSCs) transduced with herpes simplex virus thymidine kinase (TK) and SV40 large T antigen (SV40) by lentivirus, (SV40-TK-hfBMSCs) have retained proliferation, surface phenotype expression, mulitdifferentiation potential and tumor-tropic capabilities. The anti-tumor effect of SV40-TK-hfBMSCs in the presence of prodrug ganciclovir (GCV) was demonstrated in vitro and on nude mice bearing human prostate cancer cells, DU145 and PC3, which had been transduced with luciferase and GFP for imaging evaluation by in vivo live imaging system (IVIS 200). We demonstrated that repeated systemic injection of SV40-TK-hfBMSCs (1 x 106/kg) did not cause observable harmful side effect on vital organs (liver, lung, heart, kidney and spleen). Mixed lymphocye reaction demonstrated that SV40-TK-hfBMSCs did not induce significant proliferation of lymphocytes isolated from healthy adults. Taking together, the use of SV40-TK-hfBMSCs as tumor specific delivery vehicles with controlled prodrug treatment represents a safe and novel anti-tumor therapy approach.

Abstract Submission From Students

The Effect of Systematic Administration of Allogeneic Mesenchymal Stem Cells on Mouse Fracture Healing

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Introduction: Mesenchymal stem cells (MSCs) are multipotent stem cells that have the potential to give rise to a variety of specialized cell types such as osteoblasts, chondrocytes, adipocytes, myoblasts, fibroblasts, muscle and neural cells. MSCs have many advantages for clinical applications: 1). MSCs are easy to obtain by a simple routine bone marrow aspiration, and culture for expansion in vitro. 2). MSCs can target specific damaged tissues 3). MSCs are considered to have anti-inflammatory and immunologic characteristics and do not elicit immediate immune responses [1]. Therefore, MSCs have been considered as one of the most promising candidates for stem cell therapy, tissue engineering, and cell-based gene therapy. Previous studies showed that there is a systemic mobilization and recruitment of osteoblastic precursors to the fracture site via the peripheral circulation [2], therefore, we hypothesized that systemic administration of allogeneic MSCs promotes mouse fracture healing.

Methods: Bone marrow derived MSCs were isolated from the luciferase transgenic mouse, subcultured and characterized. Open transverse femoral fracture model with internal fixation were used in 24 twelve-week old male FVB mice. Following surgery, these 24 mice were randomly assigned into 3 groups: A. PBS injection group (0.15ml PBS was injected through heart puncture at 5 days following fracture); B. MSCs injection group (50x104 Luc-MSCs was injected through heart puncture at 5 days following fracture), C. MSCs fracture site injection group, (50x104 Luc-MSCs was injected through fracture site at 5 days following the fracture). The mice were monitored by IVIS 200 in vivo imaging system every 2 days until the signal disappeared. .8 mice from each group were sacrificed at 5 weeks following the fracture, 8 fractured femurs (right femurs) and 8 opposite femurs (left femurs) were carefully removed. fractured femurs were scanned by Micro-CT, 6 fractured femurs and 6 opposite femurs were used for three-point bending mechanical testing; 2 fractured femurs and 2 opposite femurs were fixed in 4% paraformaldehyde, decalcified with 9% formic acid and embedded in paraffin. 5 μm sections will be cut and stained with H&E and Safranin O. The localizations of the luc-MSCs in the fracture callus will be determined by Immunohistochemistry and Immunofluorescence using the antibody to Luciferase on the paraffin sections. At last, all quantitative data were analysed using SPSS.

Results: In Luc-MSC local injection group, the signal from injected Luc-MSCs can last 10-12 days. In systemic injection group, when Luc-MSCs were injected into left ventricle, we tracked the signal at once, and found that at first the signal showed on the whole body, but 2-4 minutes later, the signals only showed on the lung sites, and these signals can last 5-7 days. Very weak signal on the fracture site showed at 7 days following the Luc-MSC injection. And the experiment is still being carried out.

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The effect of systematic administration of allogeneic Mesenchymal Stem Cells in OVX rat

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Introduction: Osteoporosis is a progressive systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture [1]. Mesenchymal stem cells (MSCs) are multipotent stem cells that have the potential to give rise to a variety of specialized cell types such as osteoblasts, chondrocytes, adipocytes, myoblasts, fibroblasts, muscle and neural cells. MSCs have been widely being tested in many animal disease models for clinical applications. Objective: 1) To investigate the effect of systematic administration of allogeneic Mesenchymal Stem Cells in OVX rat, 2) To investigate the effect of repeated injection of MSCs from different rat donors in OVX rat.

Methods: For cell preparations, Bone marrow derived MSCs were isolated from the SD rat that overexpress green fluorescent protein (GFP-Rat), subcultured, characterized by flow cytometry for MSCs phenotypic markers and their multipotent differentiation potentials were also confirmed. Ovariectomised rat model were used in 18 twelve-week old female SD rats. The rat was anesthetized; bilateral ovariectomies were performed then incision was closed, Following surgery, these 18 rats were randomly assigned into 3 groups: A. PBS injection group (0.5ml PBS was injected through heart puncture at 10, 46, 91 days following OVX); B. MSCs injection group1 (2x106 GFP-MSCs from GFP-Rat donor 1 was injected through heart puncture at 10, 46, 91 days following OVX), C. MSCs injection group2 (2x106 GFP-MSCs from Rat donor 1 were injected through heart puncture at 10 days following OVX, 2x106 GFP-MSCs from donor 2 were at 46 days and 2x106 GFP-MSCs from donor 3 were at 91 days), This group was used to test if repeated injection of MSCs from different donors would trigger immunoresponses. The left tibial trabecular bone microarchitecture was analyzed using Scanco viva CT40 (in vivo) at 0, 45, 90, 135 days following the OVX, and 3D structural parameters were then calculated. All rats were sacrificed at 135 days following OVX, 10mL blood from each rat were collected before sacrifice. RNAs were extracted from half of the mononuclear cells to test the cytokine levels using RT-PCR; the rest of the mononuclear cells were used for mixed lymphocyte cell culture to compare the immunogenicity. Serum was used to test the cytokine levels by rat inflammatory cytokines Multi-Analyte ELISArray Kit. Peritoneal Mast Cells were harvested to test the effect of drug dose response. Femurs and tibias were carefully removed; left femurs were used for four-point bending mechanical testing; Bone marrow mononuclear cells were collected from the right femurs and then induced to osteoclasts to compare the ability of osteoclastogenesis. Left tibias are fixed in 4% paraformaldehyde, decalcified with 9% formic acid and embedded in paraffin. Seven µm sections will be cut along the long axis in sagittal plane and stained with H&E and Safranin O. And samples were also stained by ALP & TRAP to compare the numbers of Osteoblast and Osteoclast in the bone. The location of GFP-MSCs will be determined by Immunohistochemistry and Immunofluorescence using the antibody to GFP on the paraffin sections. At last, all quantitative data were transferred to statistical spreadsheets and analysed using SPSS.

Results: The VIVA CT data showed that following the OVX, BMD of TV, BV/TV, Trabecular number, Trabecular thickness in PBS group is significant higher than that in MSCs injection group2 ($p<0.05$); Trabecular Spacing in MSCs injection group2 is significant larger than that in MSCs injection group1 and PBS control group ($p<0.05$). And there is no significant difference between PBS group and MSCs injection group1. We cannot found any morphology difference from the 3D reconstruction pictures. The four point bending mechanical testing results showed that only the Energy between loading to maximum force in MSCs injection group1 is significant higher than that in MSCs injection group2 ($p<0.05$). And there is no any other significant difference between PBS group and MSCs injection group. The numbers of Peritoneal Mast Cells in MSCs injection group2 are more than the other two groups ($p<0.05$). And the experiment is still being carried out.

Discussion: The data so far demonstrated that there are no obvious positive effects of MSCs administration on osteoporosis development; and repeated different allogeneic MSCs injection group2 showed a faster bone loss. What are the reasons behind? Did the repeated injection trigger immunoresponses in rats so that the inflammatory cytokines promote osteoclastogenesis? We need further investigation.

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The role of Smad7 in bone development and BM-MSCs characterization

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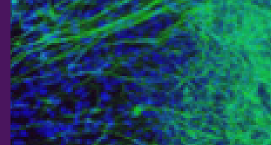
Key word: Smad7, Bone, development, MSC, Characterization

Aims: TGF-β (transforming growth factor β) controls proliferation, differentiation, apoptosis and other functions in most cell types during embryonic and postnatal life. Smad7 has been well demonstrated to be a negative regulator of TGF-β signaling which inhibits TGF-β signaling through multiple mechanisms in both the cytoplasm and nucleus. It serves as an important cross-talk mediator of the TGF-β signaling pathway with other signaling pathways. So the altered expression of Smad7 often leads to human diseases, such as cancer, inflammatory, diabetes, fibrosis and so on. To investigate the role of the TGF-β/Smad7 signaling in the process of bone development and MSCs characterization, we performed a serious of in-vivo and in-vitro experiments using wild-type (WT) and Smad7ΔE1 mice (KO), which has the translated part of exon I and part of intron I of Smad7 genomic sequence replaced by a PGKneobpA expression cassette, and the first half of the Smad7 protein is removed and its function is disrupted.

Methods: We use flow cytometry to detect the expression of positive MSCs markers of CD90, CD44, Sca□, and negative markers of CD34, CD45. After the confirmation of mBM-MSCs, multi-differentiation assays including osteogenesis, adipogenesis and chondrogenesis were performed to compare the characterization between the KO and WT mBM-MSCs; specific staining as well as quantitative acetic acid extraction methods were used and the mRNA expression of relative markers were also detected by quantitative real-time RT-PCR. We also used TRAP staining and bone resorption assay to compare the osteoclastogenic potential of the bone marrow macrophages (BMMs) from the two groups after stimulated with RANKL and M-CSF. The parameters of long bone development at 6, 12 and 24 weeks were assessed using digital X-ray, micro-CT, compression mechanical tests and histology methods. Data analysis was performed using SPSS software and Mann-Whiney U test, $p \leq 0.05$ was regarded as statistically significant.

Results: The adipogenic potential at day 7, 14 and 21 by Oil Red O staining (n=3) showed more and earlier lipid droplets formation, and the mRNA expression (n=6) of PPARγ and C/EBPα was significantly higher in the Smad7 KO group comparing to the control group. The osteogenic potential at day 7 and 14 using Alizarin Red S staining (n=3) and quantitation in the KO group showed less mineralized nodules, and mRNA expression (n=6) of collagen□ and RUNX2 were also lower. The osteoclastogenic potential of the KO mBMMs was significantly elevated than that of WT mice when induced by RANKL/M-CSF and detected by TRAP staining (n=3). The mRNA expression (n=6) of TRAP and CTR were significantly higher, and the osteoclasts were more and larger in the KO group than those in the WT group. Although no visible difference was seen between the long bones by digital X-ray among the KO and WT mice, micro-CT results showed that KO group has decreased of trabecular number (TbN), thickness (TbTh), and increased trabecular spacing (TbSp) in metaphysic region of the femurs at 6, 12 and 24 weeks (n=12) separately with statistical significance.

Conclusion: The in-vitro experiments showed that BM-MSCs from Smad7-null (KO) mice has enhanced adipogenic yet reduced osteogenic potential, and significantly higher osteoclastogenic potentials than the WT mice. The in-vivo MicoCT data showed that loss of Smad7 decreased TbN, TbTh and increase the TbSp in the mouse proximal femoral metaphysis, which supported the in vitro findings. The results suggest that Smad7 play a functional role both in vitro and in vivo, and it can regulate TGF-β signaling on BM-MSCs characterization, BMMs osteoclastogenesis, and also the bone remodeling. The precise function of Smad7 on skeletal development is still unclear and need further study, perhaps in disease models such as fracture healing and osteoporosis.



Three dimensions alginate/chitosan scaffolds for proliferation of mesenchymal stem cells and the potential application in tissue engineering

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Objective: To investigate the biocompability of three dimensions (3D) alginate/chitosan scaffolds and proliferation properties of mesenchymal stem cells (MSCs) in alginate/chitosan capsules.

Methods: 2% (w/w) alginate solution was prepared by dissolving 5.0 g alginate acid sodium in 250ml of 200mM kalium dihydrogen phosphate solution. 2% (w/w) chitosan solution was prepared by dissolving 5.0 g chitosan in 250ml of 50mM calcium chloride solution by adding 1.25ml acetic acid. Mesenchymal stem cells were harvested from the bone marrow of green fluorescent protein transgenic rats. The 2th passages of MSCs were used in this study. Cells were trypsinized and centrifuged and the alginate solution added to the cell pellet and vortexed to ensure thorough mixing and even distribution of cells throughout the alginate. Droplets (1ml containing approximately 4*104cells) were dispensed onto the surface of the chitosan solution by a 25G needle. Capsules, approximately 3 mm in diameter, were left in the chitosan in a covered Petri dish for 30min following self-assembly for the attachment of the chitosan shell to occur, and were subsequently washed 3 times in α-MEM media. Capsules were held with α-MEM media in media supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin-neomycin (PSN), and then placed in an incubator with gas phase of 5% CO_2 and 100% relative humidity at 37 ◦C for 10 days. The optical images were obtained by an inverted phase-contrast fluoromicroscope at day 1, 4, 7, and 10.

Results: When 2% alginate solutions without cells were dropped onto the surface of chitosan solution, the capsules with an average diameter of 3mm were formed (Fig1A). When 2% alginate solution without cells was dropped into the MSCs suspension (dispersed by 2% chitosan), then the capsules were coating with MSCs (Fig1B). Alginate/chitosan capsules showed good biocompatibility under this condition during 10 days' study. According to figure 2, MSCs encapsulated in the capsules maintained a property of proliferation and 3D clones were formed, which mimicked the proliferation of cells in vivo.

Conclusion: Alginate/chitosan capsules showed a good biocompatibility with MSCs. These capsules offer a 3D scaffold for the proliferation and organization of cells. This may offer a tissue engineering strategy for tissue regeneration and application.

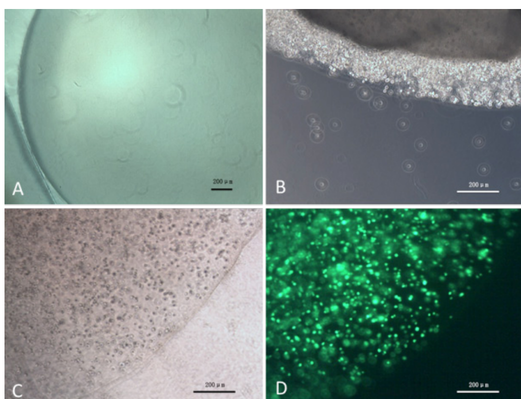

Figure 1. Alginate/chitosan capsules without MSCs (A) and coating with MSCs (B) were formed. The capsule with dispersed GFP MSCs was held with α-MEM media at day1 (C and D).

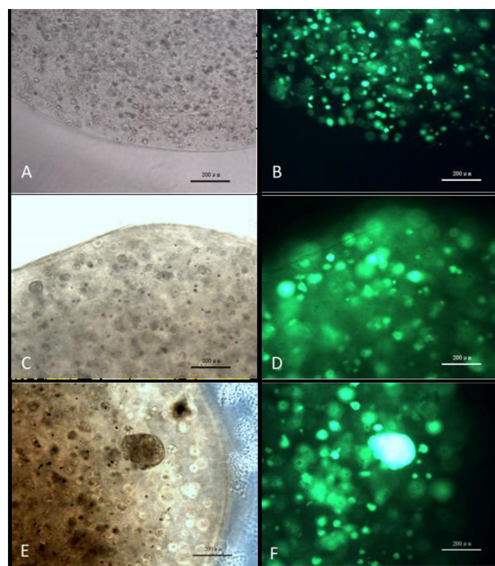

Figure 2. Alginate/chitosan capsules with dispersed GFP MSCs was held with α-MEM media at day3 (A and B), day7 (C and D), and day10 (E and F).

Effects of Sclerostin Antibody on Promoting Fracture Healing in Rats with Established Osteopenia

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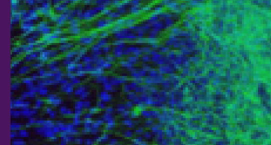
Liu, Yang and Rui, Yunfeng contributes equally to this work

Introduction: Osteoporotic fracture is a disease with high prevalence. About 50% of women and 30% of men will experience an osteoporotic fracture during their lifetime. Recently, studies have shown that inhibition of sclerostin by systemic administration of a monoclonal antibody (Scl-Ab) significantly increased bone mass and strength at both fractured and non-fractured bones in animal models of fracture healing (1-2). Previous data have suggested that ovariectomy (OVX) results in impaired healing in the rat closed fracture model (3), which may be associated with the loss of bone mass or higher rate of bone turnover. Scl-Ab has been shown to improve core defect healing in two OVX rat studies (3-4), models that heal primarily by intramembranous ossification. In this study, we examine the effect of a Scl-Ab in a closed fracture healing model in OVX rats, a complete fracture model that also heals by endochondral ossification.

Method: Eighty 4-month-old female Sprague-Dawley rats were obtained from the Laboratory Animal Services Center of the Chinese University of Hong Kong. Ethics approval was obtained for this animal experiment from the Ethics Committee of the Chinese University of Hong Kong. Rats were randomized to receive OVX or sham operation (Sham) at 4 months of age. At 3 months post-OVX, right femoral closed fracture was performed. One day after fracture surgery, sham and OVX rats received subcutaneous injection with 25mg/kg of Scl-Ab or saline, twice per week for 8 weeks. There were 20 rats in each group. Prior to the fracture surgery, proximal tibiae of 5 rats from each group were scanned by micro-CT (viva CT 40, Scanco Medical, Switzerland) to confirm the establishment of osteopenia induced by OVX. High-resolution digital radiography (Faxitron MX-20, Faxitron X-ray, Illinois, USA) was taken weekly to monitor the fracture healing. All rats were euthanized at 8 weeks post fracture (at 9-month of age), and the fractured femurs were harvested for micro-CT analysis (n=8 per group) and 4-point bending mechanical testing (n=12 per group, H25KS; Hounsfield Test Equipment, UK) to examine bone mass and bone strength at fracture site. Data was analyzed by two-way ANOVA with a LSD post hoc test. P values less than 0.05 were considered significant.

Result: At 3 months post-OVX, micro-CT data of proximal tibiae showed that bone volume fraction (BV/TV), Bone mineral density (BMD), Trabecular Thickness (Tb. Th.), Trabecular Number (Tb. N.) in the OVX group were significantly lower than in the Sham group (data not shown). These findings confirmed the osteopenia induced by OVX in these rats prior to fracture. Treatment with Scl-Ab for 8 weeks in OVX rats significantly increased BMD (22.0%) and BV/TV (10.2%) of the fracture region as compared with saline control. Similarly, mechanical testing at the fracture site showed that maximum load (35.7%) and energy to maximum load (42.9%) in Scl-Ab treated OVX rats were significantly higher than saline control.

Discussion: Patients with osteoporotic fractures may experience delayed union or nonunion due to low bone mass and other factors. Inhibition of sclerostin by systemic administration of Scl-Ab has been shown to increase bone mass and promote fracture healing in animal models of fracture repair (1-2). In this study, we performed femoral closed fracture on rats with established osteopenia induced by OVX to examine effects of Scl-Ab on osteoporotic fracture healing. The micro-CT data showed that Scl-Ab increased bone mass at the fracture site in both the OVX and Sham groups. Bone strength is one of the most important indicators of functional recovery of fracture healing. Eight weeks of Scl-Ab treatment



Abstract (cont')

significantly improved bone strength parameters in both ovariectomized and sham rats, and OVX did not appear to impair recovery of fractured femur strength in the control groups. These results suggested that Scl-Ab may have potential benefits in healing of osteoporotic fracture in patients.
Significance
This is the first report that demonstrated that Scl-Ab increased bone mass and bone strength at the fracture site in a closed femoral fracture model in rats with estrogen deficiency.

Acknowledgement
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Aquaporin 1 regulates MSCs cell migration *in vitro* and *in vivo*

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Objective: Administration of bone marrow mesenchymal stem cells (MSCs) is widely used in bone defect repair and fracture healing. Aquaporin 1(Aqp1) belongs to aquaporins family, water-specific, membrane-channel proteins, which is also proposed to promote tumor angiogenesis. To enhance the recruitment efficiency of MSCs to the injury sites, we manipulated the expression of Aqp1 gene in MSCs and explored its effects on MSCs migration both in vitro and in vivo.

Methods: MSCs were isolated from rat bone marrow, characterized, expanded in vitro. Aqp1 overexpression and knocking down stable cell lines were established by lentiviral transfection, which were used for cell migration assessment through transwell and wound healing assays. GFP labeled Aqp1 overexpressing MSCs and GFP-MSCs were then administrated systemically via tail vein injection in rats with experimental tibia fracture. The percentage of GFP expressing cells at fracture sites was quantified and compared statistically. Other experiments to address the underlying mechanisms were also conducted by western blot, co-immunoprecipitation and confocal image techniques.

Results: Knocking down Aqp1 had no effects on osteogenesis, adipogenesis, chondrogenesis and proliferation of MSCs. Overexpression of Aqp1 promoted MSCs migration, while knocking down Aqp1 impaired MSCs migration in vitro. Higher numbers of GFP-MSCs were found at the fracture site in the Aqp1-MSCs treated group compared to the GFP-MSCs group. The expression of beta-catenin and focal adhesion kinase (FAK) were upregulated in the Aqp1-MSCs, and down-regulated in the Aqp1 knocking down MSCs. Beta-catenin and FAK were co-immunoprecipitated with Aqp1, and the co-localization of FAK and Aqp1 was confirmed by confocal images.

Discussion: This study demonstrates that Aqp1 enhances MSCs migration ability by affecting expression of beta-catenin and FAK. Our findings suggest novel function of Aqp1 in governing MSCs migration, which may have therapeutic potentials.

GATA6 Promotes osteogenesis of human and rodent MSCs by regulating expression of Runx2 and Smad7 *in vitro*

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Introduction: Bone marrow derived mesenchymal stem cells (BM-MSCs) has multiple differentiation potentials. Enhanced osteogenic potentials of BM-MSCs may be beneficial for promoting bone formation. Using microarray our laboratory once compared the rat BM-MSCs and rat peripheral blood derived MSCs (PB-MSCs) and found many differential genes , among which GATA-binding factor 6 (GATA6), was highly expressed in the PB-MSCs. GATA-6 has been implicated in the transcriptional regulation of genes in heart, liver, gonad, gut epithelium, and lung. Runx2 is the master regulator for osteogenic differentiation, which is expressed exclusively in mineralized tissues and their precursors. Smad7 belongs to smad suprfamily, which is thought to regulate osteogenesis. However, the accurate role of Smad7 in osteogenesis is controversial. We hypothesized that GATA6 may play a role in regulating osteogenesis and interplay with Runx2 and Smad7 gene.

Methods: The animal experimental protocols were approved by the Animal Care and Use Committee of The Chinese University of Hong Kong, Hong Kong. Rat and human BM-MSCs culture was first established and their phenotypes were confirmed by flowcytometry analysis. GATA6 was then silenced and overexpressed in the rat and human BM-MSCs using lentiviral transfection technique. The osteogenic potential of rat and human GATA6-BM-MSCs was assessed using standard osteogenic induction assays. The expression of Runx2 and Smad7 was examined by qPCR and western blot methods.

Results: Overexpression of GATA6 both in rat and human BM-MSCs enhanced osteogenic differentiation, shown by alizarin red staining. The mRNA level of osteogenenic markers like Runx2, osteocalcin(OCN), osteopontin(OPN) and alkaline phosphatase(ALP) were highly upregulated in GATA6 overexpressing rat and human BM-MSCs. Overexpressing GATA6 in rat BM-MSCs could upregulate Runx2 and Smad7 at protein level, while knocking down GATA6 in human BM-MSCs downregulated expression of Runx2 and Smad7.

Discussion: The preliminary data suggest GATA6 participate osteogenic differentiation of MSCs both in rat and in human, partially by regulating expression of Runx2 and Smad7.

Sm51 promotes osteogenic differentiation of rat bone marrow mesenchymal stem cells by upregualting Runx2

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Introduction: Bone marrow derived mesenchymal stem cells (BM-MSCs) has multiple differentiation potentials. Cell Therapy using BM-MSCs have been applied in treating many musculoskeletal disorders such as bone defect, fracture delayed union or non-union, etc. Enhanced osteogenic potentials of BM-MSCs may be beneficial for promoting bone formation. Using microarray our laboratory once compared the rat BM-MSCs and rat peripheral blood derived MSCs (PB-MSCs) and found many differential genes , among which small nuclear ribonucleoprotein polypeptide N clone sm51 (Sm51), was highly expressed in the PB-MSCs. Sm51 is reported to regulate tissue-specific alternative splicing events.(Li, et al, 1989). Runx2 is master regulator for osteogenic differentiation, which is expressed exclusively in mineralized tissues and their precursors (Stein, et al, 2004). We hypothesized that Sm51 may play a role in regulating osteogenesis and interplay with Runx2 gene.

Methods : The animal experimental protocols were approved by the Animal Care and Use Committee of The Chinese University of Hong Kong, Hong Kong. Rat BM-MSCs culture was first established and their phenotypes were confirmed by flowcytometry analysis. Sm51 was then cloned and overexpressed in the rat BM-MSCs using lentiviral transfection technique. The osteogenic potential of rat BM-MSCs and Sm51-BM-MSCs was assessed using standard osteogenic induction assays. Ectopic bone formation was compared by implanting Sm51 overexpressing BM-MSCs and control BM-MSCs into nude mouse with HA/TCP scaffolds. Furthermore, Sm51 binding RNA was immune-precipitated with Flag antibody and amplified with specific primers including Runx2 and others by PCR. We also constructed a lentiviral vector that could knocking down Runx2 expression, and tested the effects of knocking-down Runx-2 gene in the Sm51-BM-MSCs. Values were presented for each group as means ± SEM. The Student's t-test and one-way ANOVA were used for comparison of mean values between different groups. P value was calculated with SPSS16.0, and $P<0.05$ was considered be statistical significant.

Results: Overexpression of Sm51 accelerated osteogenic differentiation of BM-MSCs by enhancing the bone mineralization rate and ALP activities in Fig.1. Ectopic bone formation data showed Sm51-BM-MSCs induced more osteoid bone formation than that of DsRed-MSCs control (Fig.2). The expressions of osteogenic markers such as Runx2, Osteocalcin (OCN), Osteopontin (OPN), ALP, type I collagen were up-regulated after overexpressing Sm51 with or without osteogenic induction at mRNA level (Fig.3). In addition, we proved that Sm51 up-regulated expression of Runx2 at protein level, and Sm51 bound to Runx2 RNA directly and specifically, no bounding was seen with other osteogenic genes such as OCN, OPN; adipogenic gene peroxisome proliferator-activated receptors gamma (PPARγ) and CCAAT-enhancer-binding proteins (C/EBPs) (Fig.4). Finally, knockdown of Runx2 abolished Sm51 effects on osteogenesis of BM-MSCs.

Discussion: Runx2 is indispensable for osteogenic differentiation of BM-MSCs and bone formation. It regulates many osteogenic marker genes like OPN, OCN and ALP. Our data demonstrated that Sm51 gene enhances osteogenic differentiation of BM-MSCs both in vitro and in vivo. Overexpression of Sm51 up-regulated expression of osteogenic mark genes via Runx2, and Sm51 directly bind to Runx2 gene. When depleting Runx2, the osteogenic potential of BM-MSCs overexpressing Sm51 was abolished, suggests Sm51 is an upstream regulator of Runx2 in osteogenesis process.

Significance: This study was first to report the novel role of Sm51 in regulating osteogenic differentiation of BM-MSCs, and identified Sm51 as a potential new target gene for enhancing bone formation.

Reference:

Li S, Klein ES, Russo AF, Simmons DM et al. Isolation of cDNA clones encoding small nuclear ribonucleoparticle-associated proteins with different tissue specificities. Proc Natl Acad Sci U S A. 1989 Dec; 86(24):9778-82.

Stein GS, Lian JB, van Wijnen AJ et al. Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression. Oncogene. 2004 May 24; 23(24):4315-29.

Computerized Quantification of Prussian Blue Staining in SPIO-Labelled Histological Tissues

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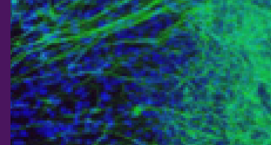
Objective evaluation of the colour and shade in stained images remains unsolved and is frequently and extensively encountered in biomedical studies. Most of the evaluations on the colour and shade in the stained images are currently performed by subjective grading, which is prone to be affected by inter-reader variation.

An established human osteosarcoma cell line (U2OS, ATCC number: **HTB-96™**, ATCC, Rockville, MD, USA) was used in this study. For Prussian blue staining, cells were fixed with 2.5% glutaraldehyde (Sigma-Aldrich, G4004). The micrographs were observed by an inverted microscope (Leica DM IRB, Leica Microsystems, Wetzlar, Germany) at x100 magnification. We proposed a novel approach to automatically quantify the colour and shade in the stained histological image based on its similarity map in the CIELAB colour space with respect to a user specified reference colour.

The proposed method was tested on 19 images of SPIO labelled U2OS cell culture or unlabelled controls. To validate the proposed automatic method, subjective rating was performed by two independent raters. The Pearson correlation coefficient between the automatic result and the first rater was 0.87, between the automatic result and the second rater was 0.91 respectively.

Results of this study demonstrated the consistency between this automatic method and subjective observation. The prominent advantage of the automatic method over the traditional subjective grading is its objectivity, which will not be influenced by inter-operators' variation.



Quantitative Tracking of Mesenchymal Stem Cell Changes in canine Model with cerebral ischemia

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This study aims to quantitatively analyze in vivo MR tracking of superparamagnetic iron oxide (SPIO)-labeled mesenchymal stem cells (MSCs) in a canine model of cerebral ischemia.

Twelve healthy adult beagle dogs were recruited in this study due to the structural similarity between dog and human brains. All of them were subjected to left proximal middle cerebral artery (MCA) occlusion first, and then followed left intracarotid artery (ICA) occlusion two hours later. All the stroked dogs were classified as three groups. Group A: complete MCA recanalization, group B: partial MCA recanalization, and group C: no recanalization. A series of in vivo MRI images were obtained before cell grafting, 1 and 24 hours after transplantation, and weekly thereafter until 4 weeks. The harvested MRI image were analyzed using the in-house developed MRI image post-processing pipeline for measuring the contents of SPIO-labeled mesenchymal stem cells through the following steps, ie, Brain segmentation, Image alignment, Segmentation of MSC and infraction, Quality assurance and Visualization.

The results showed that MSCs scattered widely in the left cerebral hemisphere in group A, while less engraftment was observed in group B and no cell was detected in group C.

In vivo molecular MR imaging was useful for tracking MSCs after SPIO labeling. The computational pipeline developed in this study enables automatic quantification volumes of MSCs and infracts in a repeatable manner.

The Research of Stem Cell Secretion on Differentiation of MSCs

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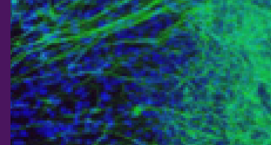
Mesenchymal stem cells in adult bone marrow are multipotent, they have an extensive proliferation capacity. Human bone marrow mesenchymal stem cells (hBMSCs) can differentiate into multiple lineages. MSCs synthesize a lot of growth factors and cytokines. Exosomes are the primary mediators of MSCs' paracrine effect, they help to reduce tissue injury and enhance tissue repair. Stem cell secretion can promote multiple proliferation and differentiation of hBMSC. We tested four types of stem cell secretions: adipose tissue stem cell secretion, dental pulp stem cell secretion, gingival stem cell secretion and umbilical cored stem cell secretion. Alizarin red S (ARS) staining showed the minimal effective concentration of 10ug/ml dental pulp stem cells secretion or 20ug/ml umbilical cord stem cells secretion could promote strong osteogenesis of hBMSCs after treated for 11 days. The in vivo bone defect healing study using rat calvarial defect model is in progress. Other differentiation induction effects of the stem cell secretions will be examined.



Systemic Administration of SOX11-modified Mesenchymal Stem Cells Improves Fracture Healing in an Open Femur Fracture Rat Model

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Objectives: Mesenchymal stem cells (MSCs) are valuable cell source for musculoskeletal tissue engineering. SOX11 belongs to the SOXC group of SOX transcription factors. The main function of SOX11 is involved in neural development and organogenesis during fetal development. The detailed role of SOX11 in MSCs differentiation and migration and the underlying mechanisms are still not clearly clarified.

Methods: Cultures of bone marrow-derived MSCs were established from 6-8 weeks SD rats. The gene encoding rat SOX11 was amplified and cloned. Pseudo-lentivirus was produced by transient transfection of 293FT cells. MSCs were transduced with lentivirus carrying SOX11 and stable cell lines were selected. The tri-lineage (adipo-, osteo- and chondrogenic) differentiation assays were performed as previous reported. Genes associated with the tri-lineage differentiation were assayed by quantitative RT-PCR or western blot. In addition, the effect of SOX11 on osteogenesis of MSCs was evaluated by ectopic bone formation carried out in nude mice. Finally, an open femoral fracture model was established in 8-week old SD rats, SOX11-modified MSCs were injected at four days after fracture. Five weeks after fracture, the femurs were collected for microCT, mechanical test and histological analysis.

Results: We demonstrated that SOX11 overexpression enhanced the adipo-, chondro- and osteogenic differentiation of BM-MSCs, through enhancing BMP signaling pathways. Using the open femur fracture model, we demonstrated that more bone formation was observed in the rats received SOX11-MSC injection compared with the control group. And quantitatively, the rats received SOX11-MSCs injection displayed a significant increase of the bone volume density-BVt/TV and BVl/TV, while no differences in bone volume and BVh/TV. The histological analysis also showed that the percentage of bone in calluses in the rats transplanted with SOX11-MSC was higher than that of the control, and so as the percentage of chondrocytes in the uncalcified calluses. Most importantly, the mechanical parameters were found to be improved in SOX11-MSCs group. There was significant difference found in relative maximum force and energy between the two groups, while E-modulus only showed a slight increase.

Conclusions: These findings suggest SOX11 plays important roles in regulating the trilineage differentiation of MSCs. Systemic administration of SOX11- modified BM-MSCs may be useful in promoting fracture healing.

N-cadherin Regulates Osteogenesis and Migration of Mesenchymal Stem Cells

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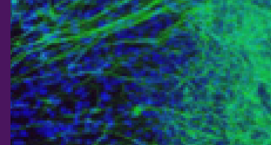
Objectives: N-cadherin, a calcium-dependent cellular adhesive protein, plays important roles during embryonic development and bone formation. The potential of mesenchymal stem cells (MSCs) in osteoblast differentiation and homing to the sites of injury make it a promising cell resource for tissue engineering. In the present study, we aim to investigate the role of N-cadherin in regulating osteogenesis and migration of MSCs.

Materials and Methods: Rat BM-MSCs were isolated from bone marrow of SD rats. N-cadherin overexpressing and silencing plasmids were constructed for producing lentiviruses. Osteogenic differentiation was performed as previously reported. Cell migration was examined using transwell insert culture system. The effect of N-cadherin on osteogenesis was also evaluated in nude mice.

Results: The results showed that prolonged N-cadherin overexpression inhibited osteogenic differentiation of MSCs through negatively regulating β-catenin and ERK1/2 signaling pathways. The mRNA expression levels of osteogenesis-related genes (OPN, OCN, Runx2, ALP and BMP2) were significantly inhibited by N-cadherin, as well as the ALP activity and calcium deposit as stained by Alizarin Red S. While, silencing N-cadherin using shRNA increased ALP activity by enhancing β-catenin and ERK1/2 signaling pathways. In addition, we also found that the N-cadherin overexpression could promote the migration potential of MSCs. Finally, ectopic bone formation conducted in nude mice verified that N-cadherin significantly inhibited ectopic bone formation of MSCs in vivo.

Conclusions: These findings reveal that N-cadherin inhibits osteogenesis but promotes migration of MSCs. The underlying mechanism of N-cadherin inhibiting osteogenesis may through suppressing β-catenin and ERK1/2 signaling pathways.



Bone Marrow-derived Mesenchymal Stem Cells Promote Vascularization and Growth of Breast and Prostate Tumors

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Objective: Mesenchymal stem cells (MSCs) are known to migrate to tumor tissues. This behavior of MSCs has been exploited as a tumor-targeting strategy for cell-based cancer therapy. However, the effects of MSCs on tumor growth are controversial. In this study, we attempted to assess whether MSCs derived from bone marrow stimulate the growth of breast and prostate tumors. The mechanisms underlie the effects will also be investigated.

Methods: BM-MSCs from mice were isolated and characterized. Effects of MSCs on tumor cell proliferation were analyzed in a co-culture system with mouse breast cancer cell 4T1 or human prostate cancer cell DU145. In order to exam the effect of BM-MSCs on tumor growth in vivo, tumor cells were injected subcutaneously either alone or coupled with BM-MSCs into the back of nude mice. The expression of cell proliferation and angiogenesis related proteins in tumor tissues were immunofluorescence analyzed. The angiogenic effect of BM-MSCs was detected using tube formation assay. The effects of the crosstalk between tumor cells and BM-MSCs on expression of angiogenesis related markers were examined by immunofluorescence and real-time PCR.

Results: BM-MSCs expressed cell surface markers characteristic of MSC and differentiated into mesenchymal lineages. Both co-culture with BM-MSCs and treatment with MSC-conditioned medium led to enhanced growth of 4T1 cells. Co-injection of 4T1 cells and BM-MSCs into nude mice led to increased tumor size compared with injection of 4T1 cells alone. Identical experiments using DU145 cells instead of 4T1 cells yielded similar results. Compared with tumors induced by injection of tumor cells alone, tumor vessel area was greater in tumors from co-injection of 4T1 or DU145 with BM-MSCs, which correlated with decreased central tumor necrosis. The proportions of Ki-67 positive proliferating cells and the expression of hypoxia inducible factor-1 alpha (HIF1-alpha), transforming growth factor-beta (TGF-beta) and alpha-smooth muscle actin (alpha-SMA) were increased significantly in the tumor tissues of mice co-injected with 4T1 cells or DU145 and BM-MSCs. Conditioned medium of BM-MSCs was able to induce angiogenesis in human umbilical vein endothelial cells (HUVEC). When BM-MSCs co-cultured with tumor cells, the expression of angiogenesis related markers (alpha -SMA, MIP-2 and VEGF) and tumor-promoting growth factors (TGF-beta and IL6) were increased as compared to BM-MSCs cultured alone.

Conclusions: These results indicate that BM-MSCs promote tumor growth and suggest that the crosstalk between tumor cells and BM-MSCs increased the production of cytokines, which may have induced tumor cell proliferation and angiogenesis thereby increasing solid tumor growth.

19 Nov 2012 (Mon)

香港中文大学深圳研究院大楼
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报告厅：位于香港中文大学深圳研究院二楼

20 Nov 2012 (Tue)

The Auditorium, 1/F Main Clinical Block and Trauma Centre
Prince of Wales Hospital, Shatin, Hong Kong

Day 1 November 19, 2012 (Monday)

	Time	Key Events	Speaker
Opening Ceremony **Moderators:** **Prof. Gang LI** *(HK)*	**08:30-09:00**	Opening Remarks	**Mr. Bin LIANG** **Prof. Wai-Yee CHAN** **Prof. Wen-Qi JIANG**
	09:00-09:15	Group Photo	
Section 1: **Biology of Tissue Regeneration** **Moderators:** **Prof. Dong-Qing CAI** *(CHINA)* **Prof. Chao WAN** *(HK)*	**09:15-09:30**	Circulating stem cells and its clinical implications.	**Prof. Gang LI** *The Chinese University of Hong Kong, HK*
	09:30-09:45	New development in iPS technology.	**Prof. Bo FENG** *The Chinese University of Hong Kong, HK*
	09:45-10:00	The comprehensive roles of strontium in bone cells: a systems biology perspective	**Prof. Guang-Qian ZHOU** *Shenzhen Univerisity, CHINA*
	10:00-10:15	Role of insulin/insulin receptor signaling in chondrogenesis.	**Prof. Chao WAN** *The Chinese University of Hong Kong, HK*
	10:15-10:30	Tangential migration and proliferation of intermediate progenitor of GABAergic neurons in the mouse neocortex.	**Prof. Sheng-Xi WU** *The Fourth Military Medical University, Xi'an, CHINA*
	10:30-10:45	Panel Discussion & Questions and Answer Session	
	10:45-11:00	Coffee Break	
Section 2: **Topics of Regenerative Medicine** **Moderators:** **Prof. Gang LI** *(HK)* **Prof. Guang-Qian ZHOU** *(CHINA)*	**11:00-11:30**	Keynote speech: Cell-Based Regenerative Therapies: Potential and Challenges. 細胞基礎再生療法：潛能和挑戰	**Prof. Rocky S. TUAN** *University of Pittsburg, USA*
	11:30-11:50	Biomaterials for regenerative medicine	**Prof. Peter X. MA** *University of Michigan, USA*
	11:50-12:10	Stem cells based therapy for bone repair: from bench to bedside.	**Prof. Ting-Ting TANG** *Shanghai Jiantong University, CHINA*
	12:10-12:30	In vivo mesenchymal stem cell proliferation regulated by mechanotransduction	**Prof. Yi-Xian QIN** *State University of New York, Stony Brook, USA*
	12:30-12:40	Panel Discussion & Questions and Answer Session	
	12:40-14:00	Lunch Break & Touring the CUHKRI Buildings	
Section 3: **Technological Advancements** **Moderators:** **Prof. Ling QIN** *(HK)* **Prof. Edward GUO** *(USA)*	**14:00-14:15**	Hedgehog signaling, is it good or bad for bone?	**Prof. Kingston King-Lun MAK** *The Chinese University of Hong Kong, HK*
	14:15-14:30	Osteoblasts Control Lineage Commitment Of Mesenchymal Progenitor Cells Through Wnt Signaling	**Prof. Hong ZHOU** *University of Sydney, AUSTRALIA*
	14:30-14:45	FoxO3a pathway in ischemic cardiac microvascular endothelial cells.	**Prof. Xu-Feng QI** *Jinan University, CHINA*
	14:45-15:00	Mechanobiology and Bone Adaptation	**Prof. Edward GUO** *Columbia University, USA*
	15:00-15:15	Central Tolerance and its Involvement in Regeneration	**Prof. Jun-Wen QIN** *Jinan University, CHINA*
	15:15-15:30	Bone regeneration mechanism of advanced biomaterials for hard tissue repair.	**Prof. Xue-Nong ZOU** *Sun Yat-Sen University, CHINA*
	15:30-15:45	Panel Discussion & Conclusion of the meeting	
	15:45-16:00	Board Crossing border coach to Hong Kong	
	18:00	Arrive Hong Kong, stay in Regal Riverside Hotel for invited speakers and guests	
	19:30	Welcome dinner at Regal Riverside Hotel for invited speakers and guests	

Day 2 November 20, 2012 (Tueseday)

	Time	Key Events	Speaker
	08:30-09:00	Opening Ceremony	**Prof. Joseph JY SUNG** **Prof. Tai-Fai FOK** **Prof. Kai-Ming CHAN**
	09:00-09:15	Group Photo	
Section 1: Biology of Tissue Regeneration **Moderators:** **Prof. Gang LI** *(HK)* **Prof. Edward GUO** *(USA)*	09:15-09:40	Size matters – Nanotopographical and biomaterial control of skeletal stem cell fate and function	**Prof. Richard OREFFO** *University of Southampton, UK*
	09:40-10:05	Systemic Trafficking of Macrophages and Osteoprogenitor Cells	**Prof. Stuart B GOODMAN** *Stanford University, USA*
	10:05-10:30	Modified biomaterial interfaces and stem cell responses	**Prof. Wilson WANG** *National University of Singapore, Singapore*
	10:30-10:45	Panel Discussion & Questions and Answer Session	
	10:45-11:00	Coffee Break	
Section 2: Tendon Regeneration **Moderators:** **Prof.Christer ROLF** *(Sweden)* **Prof. Kai-Ming CHAN** *(HK)*	11:00-11:30	Keynote Speech: Are we trying to treat a failed healing response in Tendinopathy?	**Prof. Christer ROLF** *Karolinska University Hospital, Stockholm, Sweden*
	11:30-11:45	Tissue Engineering for Flexor Tendon Repair.	**Prof. Chun-Feng ZHAO** *Mayo Clinic, USA*
	11:45-12:00	Tendon stem cells in tendon regeneration	**Prof. Pauline Po-Yee LUI** *The Chinese University of Hong Kong, HK*
	12:00-12:15	The mechanical signal regulation in tendon stem cells differentiation	**Prof. Xiao-Ling ZHANG** *Shanghai Jiao Tong University, CHINA*
	12:15-12:30	Panel Discussion & Questions and Answer Session	
	12:30-13:15	Lunch Break	
Lunch Seminar	13:15-14:00	Development of nanofiber production equipment Present Nanofiber application	**Dr. Chikashi NAITO** *MECC Co., Ltd, JAPAN*
Section 3: Technological Advancements of Stem Cell Biology and Applications **Moderators:** **Prof. Kenneth LEE** *(HK)* **Prof. Ting-Ting TANG** *(CHINA)*	14:00-14:15	sRNA Induces the Large-scale Trans-determination of Mesenchymal Stem Cells into Hematopoietic Stem Cells in Human	**Prof. James Q. YIN** *Chinese Academy of Sciences, CHINA*
	14:15-14:30	An underlying mechanism for the self-renewal of Smad3-/- ES cells	**Prof. Ping YUAN** *The Chinese University of Hong Kong, HK*
	14:30-14:45	Reprogramming MSCs through in vitro differentiation and dedifferentiation for enhancing therapeutic potential in vivo	**Prof. Xiao-Hua JIANG** *The Chinese University of Hong Kong, HK*
	14:45-15:00	Readying adult stem cells towards the clinic: Incubation within cellular assemblies and sugar substrates	**Prof. David W. GREEN** *The University of Hong Kong, HK*
	15:00-15:15	Cardiac Telocytes in Regenration of Infarcted Myocardium	**Prof. Dong-Qing CAI** *Jinan University, CHINA*
	15:15-15:30	Extracellular Matrix as Stem Cell Niche: Implications in Osteoarthritis	**Prof. Qian CHEN** *Brown University, USA*
	15:30-15:50	Bio-inspired approaches for bone tissue engineering	**Prof. Peter Yun-Zhi YANG** *Standford University, USA*
	15:50-16:00	Panel Discussion & Questions and Answer Session	
	16:00-16:15	Coffee Break	
Section 4: Translational Medicine Related Topics **Moderators:** **Prof. Ling QIN** *(HK)* **Prof. Yi-Xian QIN** *(USA)*	16:15-16:45	Keynote Speech: Experimental paradigms of tissue engineering and regeneration 組織工程和再生醫療的實驗範式	**Prof. Rocky S. TUAN** *University of Pittsburg, USA*
	16:45-17:00	Cell therapy for tendinopathy management	**Prof. Ming-Hao ZHENG** *Western Australia University, Australia*
	17:00-17:15	R&D and Validation of a Porous Scaffold Composite Material incorporating Osteopromotive Compounds for Bone Defect Repair in Osteonecrosis 骨坏死性骨缺损修复生物活性材料的研发与验证	**Prof. Ling QIN** *The Chinese University of Hong Kong, HK*
	17:15-17:30	Skeletal Progenitors-specific ablation of Cbfβ displays a novel function of Cbfβ in chondrocyte proliferation and Cartilage Repair	**Prof. Yi-Ping LI** *University of Alabama at Birmingham, USA*
	17:30-17:45	Development of an Human Fetal Mesenchymal Stem Cell Line Overexpressing Thymidine Kinase (TK) Gene for Anti-tumor Therapy	**Dr. Wayne LEE and Prof. Gang LI** *The Chinese University of Hong Kong, HK*
	17:45-18:00	Panel Discussion & Questions and Answer Session	
Section 5: Student Presentation	18:00-18:40	**Student Presentation & Award Announcement**	**Prof. Gang LI**
	18:40-18:50	Conclusion Remarks	**Prof. Kai-Ming CHAN** **Prof. Gang LI**

Conference Secretariat

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