

4TH MALAYSIAN TISSUE ENGINEERING AND REGENERATIVE MEDICINE SCIENTIFIC MEETING (MTERMS) 2012

“GENE, CELL AND TISSUE THERAPY”

3RD -4TH June 2012

Meritus Pelangi Beach Resort & Spa, Pantai Cenang, Langkawi, Malaysia

Organized by:

Tissue Engineering and Regenerative Medicine Society of Malaysia (TESMA)

Faculty of Medicine & Health Sciences, Universiti Putra Malaysia (UPM)

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MESSAGE FROM THE DEAN OF FACULTY



Dear colleagues and friends,

It gives me a great pleasure to welcome all participants to the 4th Malaysian Tissue Engineering and Regenerative Medicine Scientific Meeting (MTERMS) 2012. This meeting has become an established event in the calendar of prominent researchers in the field of tissue engineering and regenerative medicine in Malaysia. To all participants, my sincere thanks for your active participation, and to our foreign speakers, "Welcome to Malaysia".

I am certain that your deliberations over the next two days will be extremely fruitful. This meeting will serve not only as a platform for learning, but also to generate innovative ideas and establish research collaborations. I do hope that you take this opportunity to interact closely with the expert researchers to gain knowledge on various aspects of tissue engineering and regenerative medicine.

To the organizing committee, thank you for your tremendous effort and hard work to make this meeting a success. I would like to thank all speakers and participants again for the time and effort to attend this meeting. I wish all of you a successful and productive meeting.

Thank you.

A handwritten signature in black ink, appearing to be 'NORLIYAH OTHMAN'.

PROFESSOR DR. NORLIYAH OTHMAN
Dean
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia

MESSAGE FROM THE PRESIDENT OF TESMA



Assalamualaikum & Salam 1 Malaysia.

I would like to warmly welcome all guest speakers and participants to the 4th Malaysian Tissue Engineering and Regenerative Medicine Scientific Meeting (MTERMS) 2012.

'Gene, Cell and Tissue Therapy' has been selected as the theme for our meeting this year as the potential of related studies that can lead to the discovery of clinical applications for genetic and cellular therapies to alleviate diseases are vast. Stem cell study for instance, is likely to have a broad prospect for novel scientific innovations in the near future as long as it does not compromise ethical and religious issues. With that, we are very delighted to have eminent speakers and participants to share their views, experiences and findings during this meeting.

In Malaysia, research study on regenerative medicine is growing and with this informative event, I hope it will encourage our talented young scientists to explore new avenues of research in the related field. To the researchers, let's continue to work hard to produce significant discoveries. We are proud of our achievements so far, but there is still a long way to go. As what Michael Ehrenreich said, "It will take time, it will take money, it will take patience, there will be setbacks and there will be successes, but over time it is a certainty that biological methods of tissue repair, replacement and regeneration will come to dominate clinical thinking" (Ehrenreich, 2000).

I would like to express my utmost gratitude and appreciation to the members of the organizing committee to making this meeting a reality. No event can be a success without an enthusiastic and dedicated team. To all delegates, thank you for your participation and I hope this brief encounter will not end here but will continue to nourish for many years to come.
Thank you.

Thank you

PROFESSOR DR FAUZIAH OTHMAN
President
The Tissue Engineering Society Of Malaysia (TESMA)

MESSAGE FROM THE MTERMS 2012 CHAIRMAN



Assalamualaikum & Salam 1Malaysia,

It is my great pleasure to welcome you to the Malaysian Tissue Engineering and Regenerative Medicine Scientific Meeting (MTERMS) 2012.

Three MTERMS meetings have so far been organized, bringing together more than 300 academics, researchers and practitioners from different institutions in Malaysia. For the past 6 years, MTERMS meetings have developed rapidly from focusing on basic fundamental and applied research to generating wealth through science. Lately, a growing interest by the new generation of Malaysian scientists is to bring the outcome of their research to the clinics - a fast evolving domain known as translational research. Following this trend, a new and special theme for this year's meeting is "**Gene, Cell and Tissue Therapy**".

The aim for this meeting is to accelerate the translation of Malaysian biomedical research products into safe and effective therapeutic options looking to improve health and combat diseases. Many recent advances in this field globally have shown convincing evidences that clinical benefits can be achieved. Therefore, it is one of our goals to increase awareness of gene, cell and tissue therapy through not only this conference but also interaction with other societies and regulatory bodies.

Most products emerging from this research area are currently at either the preliminary stage, or being tested for safety and efficacy in animal models. Testing in humans, where robust, randomized clinical trials, will be in effect soon. Therefore, at this meeting, in addition to the sessions discussing the specific research details regarding gene, cell and tissue therapy, there will be sessions on pre-clinical research and clinical translation.

This year's conference abstracts consist of 65 papers, where 11 of the abstracts have been selected for oral presentation. I like to congratulate the authors of the abstracts that have made it to the oral -presentation session. Given that the delegates at this year's conference come from many nationalities and 18 different academic institutions, there are plenty of opportunities for cross boundary interaction and learning. We hope that this meeting provides a rich intellectual and multinational platform for networking and long term collaborations.

Welcome to Langkawi and do enjoy your stay here.

A handwritten signature in black ink, appearing to read 'Syahril Abdullah'.

DR. SYAHRIL ABDULLAH

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PROGRAM

DAY 1: 3rd June 2012	
08:00 - 12:00	Registration
08:45 - 08:50	Welcoming Speech by MTERMS Chairman: Dr. Syahril Abdullah (UPM)
08:50 - 09:00	Opening Speech by TESMA President: Prof. Fauziah Othman (UPM)
09:00 - 10:00	Keynote Address: Prof. Francesco Dazzi (Imperial College London, UK) "Mesenchymal Stromal Cells: Regulators of Inflammation"
10:00 - 10:20	Photography session / Refreshments
GENE THERAPY SYMPOSIUM	
Session Chairperson: Prof. Rozita Rosli (UPM)	
10:20 - 11:10	Plenary I: Dr. Stephen Hyde (Oxford University, UK) "Progress and Prospects for Gene Therapy"
11:10 - 11:40	Invited Speaker I: Dr. Teguh Haryo Sasongko (USM) "Therapy for Single-Gene Disorders; Initiatives by Malaysian Scientists and Some Preliminary Studies"
11:40 - 12:10	Invited Speaker II: Dr. Deborah Gill (Oxford University, UK) "Gene Therapy for Cystic Fibrosis Lung Disease"
12:10 - 12:25	Oral Presentation I: Dr Md. Ezharul Chowdhury (Monash University Sunway, Malaysia) "Extracellular matrix-embedded inorganic nano-particles for efficient gene delivery to embryonic stem cells"
12:25 - 12:40	Oral Presentation II: Prof. Mohamed Saifulaman Mohamed Said (UITM) "Targeted gene silencing of the MAPK pathway in Acute Myeloid Leukemia cells using RNAi as a possible novel treatment modality in the future"
12:40 - 14:00	Lunch / Poster Viewing

CELL THERAPY SYMPOSIUM	
Session Chairperson: Prof. Gabriele Froemming (UITM)	
14:00 - 14:50	Plenary II: Dr. John O Mason (Edinburgh University, UK) “Pax6: A Key Regulator of Forebrain Development”
14:50 - 15:20	Invited Speaker III: Dr. Rajesh Ramasamy (UPM) “Mesenchymal Stem Cells: Role in Immunity”
15:20 - 15:35	Oral Presentation III: Miss Shalini Vellasamy (UPM) “Umbilical cord tissue serves as an ideal source of human mesenchymal stem cells compared to placenta tissue”
15:35 - 15:50	Oral Presentation IV: Mr Ley Hian Low (The University of Melbourne) “Ndfip1 regulates nuclear PTEN import in vivo to promote neuronal survival following cerebral ischemia”
15:50 - 16:10	Refreshments
16:10 - 16:40	Invited Speaker IV: Prof. Francesco Dazzi (Imperial College London, UK) “Harnessing the Immunomodulatory Properties of Haemopoietic Stem Cell Transplantation”
16:40 - 17:10	Sponsored Talk I: Dr. Abhi Veerakumarasivam (BioTechCorp Malaysia) “DotScan Antibody Microarray Analysis of Cell Surface Markers”
17:10	End

Conference BBQ will be held 8:00 pm at the Meritus Pelangi beachfront.

DAY 2: 4th June 2012

TISSUE THERAPY SYMPOSIUM

Session Chairperson: Prof. Zabidi Azhar Mohd Hussin (USM)

8:30-9:20	Plenary III: Prof Lee Eng Hin (NUHS, Singapore) “Cartilage Tissue Engineering - Strategies to Optimize the Cartilage Phenotype”
9:20-9:50	Invited Speaker V: Dr. Angela Ng (HUKM) “Platelet-rich and Platelet-poor Plasma in Tissue Engineering”
9:50-10:05	Oral Presentation V: Mr Yap Zhen Wei (UNITEN) “MAH-g-HA/PLA composites for injection moulded scaffolds”
10:05-10:25	Oral Presentation VI: Dr. Md Enamul Hoque (University of Nottingham Malaysia Campus) “Development of PCL- peg hybrid tissue engineering scaffolds using extrusion based rapid prototyping system”
10:25-10:40	Refreshments / Poster Viewing

PRECLINICAL & ANIMAL RESEARCH SYMPOSIUM

Session Chairperson: Prof. Fauziah Othman (UPM)

10:40-11:30	Plenary IV: Assoc Prof Dr Daisuke Sugiyama (Kyushu University, Japan) “The Use of Animal Models in Hematopoiesis Research”
11:30-12:00	Invited Speaker VI: Dr. John O Mason (Edinburgh University, UK) “Exploring the role of Wnt Signalling in Cerebellum Development using Transgenic Mice”
12:00-12:30	Invited Speaker VII: Prof. Dr. Thuan D. Bui (IMU) “The Rationale For Selecting Animal Model In Preclinical Studies”
12:30-12:45	Oral Presentation VII: Mr. Ude Chinedu Cletus (UKM) “Comparison between chondrogenically induced adipose and bone marrow stem cells in osteoarthritic sheep model”

12:45-13:00	Oral Presentation VIII: Mrs. Nadine Nograles (UPM) “Biodistribution of oral dosed plasmid DNA-loaded alginate microparticles in mice”
13:00-14:00	Lunch / Poster Viewing
CLINICAL TRIALS SYMPOSIUM Session Chairperson: Prof. Mohamed Saifulaman Mohamed Said (UITM)	
14:00 -14:50	Plenary V: Prof. Dr. Zabidi Azhar Mohd. Hussin (USM) “The Perception & Reception of Novel Therapies – A Malaysian Perspective”
14:50 -15:20	Invited Speaker IX: Dr. Stephen Hyde (Oxford University, UK) “Translating Gene Therapy Research Ideas to the Clinical Trials”
15:20 -15:35	Oral Presentation IX: Mr. Law Jia Xian (UKM) “Characterization of the cell suspension harvested for rapid treatment of severe skin injuries: a preliminary study”
15:35 -15:50	Oral Presentation X: Prof. Surendra Kumar Verma (JIPMER, India) “Molecular tissue biomarkers of basal like breast cancers - an Indian scenario”
15:50 -16:05	Oral Presentation XI: Mr. Rani Abdulqawee (USM) “Codon-correction inframing approach for dystrophin gene mutation correction in malaysian patients with duchenne muscular dystrophy”
16:05 -16:45	Closing and Awards Ceremony
16:45 -17:30	Refreshments / End



Professor Dr Francesco Dazzi

Prof Dazzi is the head of Stem Cell Biology at the Kennedy Institute of Rheumatology, Imperial College London. His research focuses on mesenchymal stem cells. He researches into factors affecting stem cell recruitment and function, strategies to treat degenerative and inflammatory diseases and their immunosuppressive properties in transplantation and autoimmunity. Prof Dazzi has published extensively in the area of mesenchymal stem cell immunoregulation and has publications in esteemed journals.



Dr Stephen Hyde

Dr Hyde is a lecturer at the University of Oxford. His research focuses on the use of gene therapy to treat lung diseases. His research group was the first to demonstrate that the primary defect in cystic fibrosis mice could be corrected by gene therapy. Subsequently, he has run two gene therapy clinical trials involving patients with cystic fibrosis. He is a principle investigator of the UK Cystic Fibrosis Gene Therapy Consortium, an editor of the Journal of Gene Medicine and holds several lung gene therapy patents.



Dr John Mason

Dr Mason graduated in Molecular Biology from University of Edinburgh and did his PhD at the University of Cambridge. Following a stint as a postdoctoral fellow at the University of California, San Francisco, he returned to the University of Edinburgh. His current research interest is the molecular mechanisms that regulate embryonic development of the forebrain. In particular, he studies the roles played by several regulatory genes including the transcription factors *Gli3*, *Foxg1* and *Pax6* and the Wnt family of developmental regulators.



Assoc Prof Dr Daisuke Sugiyama

Dr Sugiyama's interest lies in the areas of haematopoiesis, stem cell development and cancer. He is medically qualified and trained at the University Pierre and Marie Curie, France and Dartmouth Medical School, USA. Currently he is working in the Department of Hematopoietic Stem Cells, Faculty of Medical Sciences, Kyushu University, Japan. He has published many articles internationally, refereed international journals and filed patents.



Prof Dr Zabidi Azhar Mohd Hussin

Prof Zabidi is professor of paediatrics, School of Medical Sciences, Universiti Sains Malaysia. He is a fellow of the Royal College of Paediatrics and Child Health and has received further training in paediatric neurology from the University of Sydney, University of Toho, and Texas Children's Hospital. Currently, he is a member of the International Council for Ethics Education, member of Ethics Committee, Malaysian Medical Council, President of Malaysian Paediatric Association, President-elect Asean Paediatric Federation, Deputy Chairman, National Professor's Council (Medical and Health sector) and Chairman, National Professor's Council (Child Health cluster).



Dr Deborah Gill

As a PhD student, Dr Gill discovered the defining member of the ABC superfamily of proteins, as published in Nature. Following post-doctoral work, she became an Assistant Scientist at the Weatherall Institute of Molecular Medicine in Oxford, undertaking two clinical trials to evaluate non-viral gene therapy for Cystic Fibrosis in the mid-1990s. She is now a University of Oxford research lecturer and co-director of the Gene Medicine Research Group. As part of the UK Gene Therapy Consortium, Deborah is responsible for the manufacture and procurement of gene therapy products for clinical trials.



Dr Teguh Haryo Sasongko

Dr Teguh is a senior lecturer at the Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia. His current research is on therapeutic approaches to manipulate exon splicing for restoring defective reading frames in Duchenne muscular dystrophy and spinal muscular atrophy. Dr Teguh has published many articles in national and international peer-reviewed journals and book chapters in human molecular genetics and bioethics. He also serves as an editorial board member and reviewer for international journals.



Prof Lee Eng Hin

Prof Lee is Professor of Orthopaedic Surgery at the National University of Singapore and Senior Fellow at the Agency for Science, Technology and Research, Singapore. He is an Emeritus Consultant at the National University Hospital and Senior Consultant the KK Women's and Children's Hospital. His research interest is in stem cells and musculoskeletal tissue engineering. He has over 150 publications in refereed journals and over 300 conference papers. He has co-authored a book entitled "Stem Cells: from Bench to Bedside" which is in its second edition, and has been adopted by many centres internationally as a standard text for stem cell courses.



Dr.Thuan D. Bui

Dr. Bui was a Wellcome Trust Ph.D. Scholar from U.K. and obtained a Ph.D. in Molecular Biology from the University of London, in 1994. He worked as a research fellow funded by the Imperial Cancer Research Fund and the Wellcome Trust, at Oxford University (1994-1997), followed by the Institute of Molecular and Cell Biology, Singapore (1998-2001). In 2001, he obtained an MBA from the University of MacQuarie, Australia. Between 2001-2, he worked in a Singapore government-funded start-up company, which subsequently merged to form Exploit Technologies Pte Ltd, the commercialization arm of A*STAR. He worked as an AVP of Commercialisation in the Biomedical Sciences Group until early 2004, where he left to start-up i-DNA Biotechnology Group of Companies in Singapore and Malaysia. In 2011, he was appointed as Adjunct Professor at the International Medical University (IMU).



Dr Angela Ng Min Hwei

Dr Angela is a Research Fellow at the Tissue Engineering Centre, Universiti Kebangsaan Malaysia. Her experience in tissue engineering and regenerative medicine is extensive including bone, cartilage, nerve, heart, bladder and cornea regeneration. She now specializes in bone tissue engineering and has since revolutionized the different approaches in bone regeneration including gene-modified cell therapy, fabrication of novel bone substitutes and cytokine therapy. Two of her works have been patented and she has numerous publications both locally and internationally.



Assoc Prof Dr Rajesh Ramasamy

Dr Ramasamy is currently an Associate Professor at the Immunology Unit, Faculty of Medicine, Universiti Putra Malaysia. His work focuses on the role of mesenchymal stem cell in immunity. He is also an active international journal reviewer as well as an editorial member of World Journal of Stem Cells, PULSE research bulletin and Regenerative Research, a TESMA online journal. He has published in high-impact factor journals and received numerous awards for his research including ITEX Gold medal, BioMalaysia, Malaysian Technology Expo, BioInno awards.

**SPONSORED TALK FROM BIOTECH CORPORATION:
DotScan Antibody Microarray Analysis of Cell Surface
Markers**

Presenter: Abhimanyu Veerakumarasivam

Abhimanyu Veerakumarasivam^{1,2,3} and Syahril Abdullah^{1,2}

¹ *Medical Genetics Laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan.*

² *UPM-MAKNA Cancer Research Laboratory, Institute Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan.*

³ *Perdana University Graduate School of Medicine, Perdana University, 43400 Serdang, Selangor Darul Ehsan.*



In the age of personalised medicine, profiling of individual disease states at a molecular level carries significant diagnostic, prognostic, predictive and therapeutic implications. The majority of disease-specific profiles have been conducted at a genomic level. Proteomic technology has traditionally lagged behind the genomics revolution due to the complexity, structure and stability of proteins. In most instances, relative mRNA expression is used as a surrogate measure of protein expression. However, as we begin to understand the plasticity of gene expression regulation, we realise that transcriptomic changes do not corroborate with corresponding proteomic variations. The utility to analyse proteins at a proteomic-scale is not limited to biomarker discovery, but also better understanding of molecular networks and regulatory circuits. Recent advances in antibody microarray technology have facilitated parallelisation, miniaturisation and automation of multiplex protein analyses. The use of antibody microarrays such as DotScan antibody microarray allows for the potential characterisation of disease states based on protein patterns obtained from parallel antigen expression data. In principle, the DotScan antibody microarray is printed as microscopic spots on a nitrocellulose-coated microscope slide. Cells of interest are subsequently incubated on the array to allow capture by the immobilized antibodies. Upon washing of unbound cells, a dot pattern is recorded with an optical array reader. The data is then converted into a quantitative and comprehensive report of the cell surface marker profile of the analysed cells. Amongst the cell-surface antigens that are targeted on this array is the human cluster of differentiation (CD) antigens that have significant immunomodulatory functions. Various other cell surface markers have also been included. The antibody microarrays can also be customised to include discriminating antibodies of choice. A DotScan microarray that contains a standard panel of 82 CD monoclonal antibodies has been used successfully to identify signatures associated with various disease states, such as HIV infection, cardiovascular diseases, leukaemia and colorectal cancer. The general utility of this technology could be extended to also profile autoimmune, metabolic, and degenerative diseases. Customised DotScan antibody microarrays can potentially be used to monitor disease progression, determine the efficacy of specific therapeutic options and more pertinently, for broader discovery-based research applications.

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Oral Presentations

GO 01-10. Extracellular Matrix-Embedded Inorganic Nano-Particles for Efficient Gene Delivery to Embryonic Stem Cells.E. H. Chowdhury*Jeffrey Cheah School of Medicine and Health Sciences, Monash University (Sunway Campus), Jalan Lagoon Selatan, Bandar Sunway*

Stem cells have the potential to be differentiated to a specific cell type through genetic manipulation and represent a versatile source of cells in regenerative medicine. F9 and embryonic stem cells were seeded at 50,000 cells per well into 24-well plates the day before transfection. A total of 3 ml of 1M CaCl₂ was mixed with 2 mg of plasmid DNA in the presence or absence of fibronectin and/or E-cadherin-Fc, respectively, in 1 ml of fresh serum-free bicarbonate-buffered (pH 7.5) medium (DMEM). The mixtures were incubated for 30 min at 37°C for generation of particles. Medium with generated particles was added with 10% FBS to the rinsed cells. After 4-h incubation, the medium was replaced with serum medium and the cells were cultured for 1 day prior to the assay of luciferase expression. We have developed a biofunctionalized DNA carrier of carbonate apatite by embedding fibronectin and E-cadherin chimera on the carrier, leading to its high affinity interactions with embryonic stem cell surface and accelerated transgene delivery for subsequent expression. In addition, we have developed an effective formulation process for such cell adhesive protein-embedded particles. We have established a strategy for the synthesis of biofunctionalized inorganic particles through their ionic interactions with DNA and "cell adhesive molecules". Moreover, the new approach has directly been applied for highly efficient delivery and expression of a transgene into embryonic teratocarcinoma stem cells—a success with tremendous future prospect for stem-cell-based therapeutic development.

GO 04-54. Targeted Gene Silencing of the MAPK Pathway in Acute Myeloid Leukemia cells using RNAi as a Possible Novel Treatment ModalityMohd Hafiz, MR, Rozita, MS & Mohamed Saifulaman, MS*Faculty of Applied Sciences, Universiti Teknologi MARA*

Acute myeloid leukemia (AML) is a type of blood disease resulting from an abnormal proliferation of myeloid cells in the bone marrow. Proliferation of the AML cells is usually linked to gene mutations, which lead to the activation of signal transduction pathways such as mitogen-activated protein kinase (MAPK) pathway. Protein inhibitors have been designed to target the MAPK pathway, attempting to block and stop proliferation. These synthetic drugs have demonstrated anti-tumor properties and increased survival of the patients. However, as with most synthetic drugs, they exhibit undesirable side-effects. RNA interference (RNAi) is a naturally occurring post-transcriptional gene silencing mechanism which has the ability to transiently inhibit the translation of specific mRNA in cells. However, RNAi-based therapy is at an early stage and has many critical issues, mainly in delivery to target cells. RNAi-based therapy is still considered an attractive prospect because it is induced by oligonucleotides such as short-interfering RNA (siRNA) which are subsequently degraded naturally by the cells. Hence, target-based therapies using RNAi shows promise for the development of novel treatment modalities for AML.

CO 02-59. Umbilical Cord Tissue Serves as an Ideal Source of Human Mesenchymal Stem Cells Compared to Placenta Tissue

Shalini Vellasamy¹, Sharmili Vidyadaran¹, Elizabeth George² and Rajesh Ramasamy¹

¹ Immunology Laboratory, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang Selangor

² Haematology Unit, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang Selangor

Mesenchymal stem cells (MSC) encompass a great therapeutic potential in regenerative medicine, tissue engineering and in immunomodulation. Hence, there is a growing demand for large scale and alternative production of MSC. This study has explored the feasibility of umbilical cord and placenta tissues as reliable and efficient sources of MSC in replacement of bone marrow MSC, the common source. MSC were generated from human delivery tissues by enzymatic digestion and mechanical dissociation method. Direct comparisons were made in opting for a better alternate source of MSC. These MSC were characterized for mesenchymal cell surface markers, embryonic stem cell (ESC) gene expression, mesodermal differentiation ability, and further assessed for their immunomodulatory properties through tritiated thymidine cell proliferation, transwell, apoptosis and cell cycle assays. Growth kinetics and doubling time were also measured and compared. MSC generated from human umbilical cord (UC-MSC) and placenta (PLC-MSC) appeared fibroblastic-like cells and expressed common mesenchymal surface markers and ESC transcriptional factors. Both MSC were able to differentiate into adipocytes, osteocytes and chondrocytes lineages when induced. However, UC-MSC showed higher degree of osteo and chondro differentiation than PLC-MSC. In comparison, UC-MSC demonstrated significantly rapid growth kinetic with higher cell numbers and a shorter doubling time as compared to PLC-MSC, ($p^* < 0.05$). Both UC-MSC and PLC-MSC profoundly exerted a dose dependent inhibition on T, B and NK 92-M1 cells proliferation interceded mainly via cell-to-cell contact than soluble factors, ($p^* < 0.05$). This inhibition was not mediated by apoptosis but was significantly due to cell cycle arrest of T cells at G_0 phase by down regulating Cyclin D1 and Cyclin D3 expressions. In comparison, UC-MSC exhibited a greater inhibition than PLC-MSC in all measured parameters. Overall, UC-MSC stands as an alternative and noncontroversial source of MSC as they closely resemble the mesenchymal and functional properties of MSC.

CO 01-2. Ndfip1 Regulates Nuclear PTEN Import *in vivo* to Promote Neuronal Survival following Cerebral Ischemia

Ley Hian Low¹, Jason Howitt¹, Alison Macintyre¹, Adam Naguib², and Seong-Seng Tan¹

¹ Brain Development and Regeneration Laboratory, Florey Neuroscience Institutes, The University of Melbourne, Parkville, Victoria 3010, Australia

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The neuroprotective Ndfip1 (Nedd4 family-interacting protein 1) is highly expressed in neurons following acute brain injury such as traumatic brain injury or stroke. Ndfip1^{-/-} mice suffer a larger infarct size in several brain injury models. In this paper, we investigated the Ndfip1 mediated neuroprotective pathway, and associated it with a tumor suppressor protein PTEN (phosphatase and tensin homologue deleted on chromosome 1EN), which is a negative regulator of phospho-Akt signaling pathway. Binding assays reveal that Ndfip1 interacts with PTEN, and enhances PTEN ubiquitination. The Ndfip1 mediated PTEN ubiquitination did not decrease the PTEN level, but involved in the PTEN nucleocytoplasmic transport mechanism. To prove this, we had used the hypoxia ischemia stroke mouse model, and showed that PTEN was translocated to the nucleus from the cytoplasm in stressed neurons. This nucleocytoplasmic transport event was dependent on a surge in the Ndfip1 as the Ndfip1^{-/-} mice failed to import PTEN in the stressed neurons. *In vitro* study with fluorescence recovery after photobleaching (FRAP) showed enhanced PTEN nuclear import rate when Ndfip1 was overexpressed, whereas, Ndfip1^{-/-} fibroblasts showed negligible transport rate. The PTEN nuclear transport had contributed to the suppression of phospho-Akt activation in neurons, which promoted neuronal survival. In cancer, PTEN nuclear transport inhibition due to its mutation is a major cause for tumor formation. However, in this study, our findings provided the first physiological example of Ndfip1 dependent PTEN nuclear translocation and their critical roles for neuron survival following acute brain injury.

TO 04-87. MAH-g-HA/PLA Composites for Injection Moulded Scaffolds

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Tissue Engineering is an interdisciplinary field that combines the knowledge and technology of cells, engineering materials and suitable biochemical factors to design & create artificial organ tissues and bone tissues or to regenerate damaged tissues. Bone scaffolds are the templates made of synthetic biodegradable polymers or bioceramics as substrates for 3D culture of osteoplast or other applicable cell types. The scaffold for Tissue Engineering provides support for the cells to grow and proliferate to form an extracellular matrix (ECM). It gives the structural stability and integrity to build tissues. An ideal scaffold should have the following characteristics: highly interconnecting pores; ability to transport oxygen and other nutrients; ability to provide structural stability and integrity; possesses compressive & flexural strength and stiffness; controlled biodegradability and biocompatibility. Maleic-anhydride (MAH)-grafted- Hydroxyapatite was reinforced with polylactic acid (PLA) using Haake Rheocorder. The composite mixture was then moulded to form the required tensile and flexural test specimens using Injection Moulding process have been utilised to fabricate three-dimensional (3D) scaffolds that consists of complex internal architecture with highly porous and interconnected channels. The composites with Maleic anhydride (MAH) 0wt%, 0.5wt%, 1.0wt% and 2.0wt% were fabricated. Tensile tests according to ASTM-D 638 standards were conducted and the fractured surface was analyzed using Scanning Electron Microscopy (SEM). The results showed that addition of maleic anhydride (MAH) increased the mechanical tensile properties. Properties of scaffold, such as stiffness and yield point, were evaluated from the results of tensile tests. For obtaining scaffolds with enhanced mechanical properties associated to a bioactive behaviour, i.e., by using the HA reinforcement, it is necessary to optimize the processing conditions in order to allow for the production of higher porosity composite scaffolds.

TO 01-38. Development of PCL- PEG Hybrid Tissue Engineering Scaffolds Using Extrusion Based Rapid Prototyping System

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In the field of regenerative therapy, synthetic biopolymers have attracted much attention due to their abilities to modulate biomechanical properties for targeted tissue engineering applications. This study aims at investigating the feasibility of processing poly(ϵ -caprolactone)- poly(ethylene glycol) (PCL-PEG) hybrid polymers into 3D porous tissue engineering scaffolds. The scaffolds were fabricated using a desktop-robot-based rapid prototyping (DRBRP) system with single and hybrid designs. The polymers were melted by electrical heating and directly extruded out by means of computer-controlled compressed nitrogen gas that built the 3D scaffold layer by layer. Both single (0-90) and hybrid (0-30-45-60-90) lay-down patterns were produced by using appropriate positioning of the robotic control system. Thermal properties of the individual polymers (PCL and PEG) before fabrication, and the hybrid polymer (PCL-PEG) after fabrication of the scaffolds were investigated by dynamic thermal analysis (DTA). The gross morphology and internal structure of the scaffolds were observed by scanning electron microscopy (SEM), which demonstrated the 3D honeycomb-like scaffold architecture with excellent fusion at the filament junctions, high uniformity and complete interconnectivity of the pore channels. Compression test and degradation study were also performed over both single PCL and hybrid PCL-PEG scaffolds. The compression test data obtained was in agreement with the typical behavior of a porous material undergoing deformation. The degradation study confirmed that the PCL-PEG hybrid scaffolds degraded faster than the single PCL scaffolds.

CO 03-92. Comparison between Chondrogenically Induced Adipose and Bone Marrow Stem Cells in Osteoarthritic Sheep Model.

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Autologous cartilage implants (ACI) have been successfully introduced into clinical practices, but there are still significant problems associated with them. Studies are now changing to using adipose stem cells (ADSC) and bone marrow stem cells (BMSC), which are multipotent, with the potential for cartilage regeneration. We compared chondrogenically induced ADSCs and BMSCs for cartilage regeneration in sheep osteoarthritic model. Osteoarthritis (OA) was induced at the right knee of sheep by complete resection of the anterior cruciate ligament and medial meniscus, followed by a 3-weeks exercise regimen. Stem cells from the experimental sheep were expanded and induced to chondrogenic lineage. Test sheep received 2×10^7 autologous PKH26-labelled, chondrogenically induced ADSCs or BMSCs as 5ml cell injections, while controls received the same volume of basal medium. ADSCs had significantly lower expressions of chondrogenic specific genes; collagen II, SOX9 and aggrecan compared to BMSCs ($p < 0.05$). Grossly, both knee joints showed regenerated de novo cartilages with BMSC having a better appearance. On the ICRS grading, BMSCs had a better score of 1.3 compared to 1.5 of ADSC but was not significant. Histological staining revealed loosely packed matrixes of de novo cartilages. Collagen II and SOX9 specific proteins for cartilage were positive via immunohistochemistry. There was fluorescence of PKH26 at the newly regenerated cartilages. Autologous ADSCs and BMSCs could be suitable cell sources for cartilage regeneration in OA which stand the chance of solving the complication involved with ACI. There was no significant difference between the regeneration capacities of the two cell types.

GO 02-6. Biodistribution of Oral Dosed Plasmid DNA-Loaded Alginate Microparticles in Mice

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Sodium alginate is a naturally occurring polysaccharide which has been used as encapsulating material in the production of beads, micro- or nanoparticles. *In vitro* characterization of alginate microparticles produced through the water-in-oil (w/o) emulsification method was conducted on unloaded and pDNA-loaded microparticles. Biodistribution of the oral pDNA dose suspensions of alginate microparticles as carriers of genetic material were examined in various tissues among BALB/c mice. Biodistribution was assessed through flow cytometric analysis to monitor reporter gene expression mediated from the plasmid vector entrapped within alginate microparticles. Alginate microparticles demonstrated size distributions, pDNA loading and release properties relevant for DNA delivery through the oral route into the intestinal environment. GFP was detected among intestinal cells, and some stomach cells, with appreciable levels for the 100µg dose as compared to the 50µg or 150µg dose. Inherent properties of the alginate material aids entrapment of DNA material within microparticles and release at appropriate conditions for the purposes of mucosal immunization and/or tissue-specific delivery of substances. Alginate microparticles further serve as potential oral carriers for drugs or vaccines into the intestinal environment where alkaline pH ensues and whereby absorption and antigen presentation occur. The oral route provides an attractive means for delivery of drugs or vaccines due to non-invasiveness of the approach; furthermore, this route stimulates humoral immune responses in the gut. Entrapment of plasmid DNA within alginate microparticles provides an attractive approach for oral delivery of antigens into intestinal mucosa cells as a means for mucosal immunization.

TO 03-67. Characterization of the Cell Suspension Harvested for Rapid Treatment of Severe Skin Injuries: A Preliminary Study

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The use of non-cultured autologous skin cells to promote wound healing is appealing as it possesses the desirable criteria of reducing preparation time, accelerate re-epithelialization and improve scar quality by reducing scarring and repigmentation. An ideal cells suspension for the treatment of deep partial thickness wound should contain a high number of fibroblasts and keratinocytes for the regeneration as well as melanocytes for skin pigmentation. The objective of the study was to characterize the cells suspension harvested using collagenase type 1 and trypsin EDTA through yield, viability and population balance. For this purpose, 2cm² of redundant skin from patient undergoing plastic surgery was collected and treated with 0.6% collagenase type 1 for 3-5h at 37°C, followed by 0.05% trypsin EDTA for 10min at 37°C to harvest the cells. Total cell count was determined using hemocytometer while trypan blue was used to differentiate live and dead cells. Cell type was determined with immunocytochemistry through the staining of harvested cells with anti-cytokeratin antibody for keratinocytes, anti-collagen type I antibody for fibroblasts and anti-tyrosinase antibody for melanocytes. From the results, the cell yield was 1.12 x 10⁶/cm² with a viability of 73.53%. Analysis of population balance revealed that the cell suspension was composed of fibroblasts (65%) and keratinocytes (33%) with a small portion of melanocytes (2%). In conclusion, the cell suspension harvested could be promising to promote deep partial thickness wound healing as it contains a high number of living fibroblasts and keratinocytes as well as a small portion of melanocytes that play an important role in skin pigmentation.

TO 02-48. Molecular Tissue Biomarkers of Basal-like Breast Cancers - an Indian Scenario.

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Breast cancer is the most common cancer affecting women all over the world. In India, it is the second most common cancer of women, particularly in affluent societies. It has been increasing in incidence, rapidly extending into the population of low socioeconomic status. In routine, for prognosis and treatment we do three markers - ER, PR and HER2Neu. Some of the cases are negative to all these three and have a different morphology called basal-like. These tumors are called 'Triple Negative'. These basal like breast cancers are associated with poor response to conventional chemotherapy and respond to taxanes. In this regard, identification of this subtype by basal markers like CK 5/6 and CK 14 are crucial for appropriate management of these patients.

We studied our breast cancer patients over two years, both retrospective and prospective from 2010 to 2011. Over 975 patients were studied and 111 of these patients were triple negative accounting for 11.4%. In our series, 30% were grade 1, 50% were grade 2 and 20% were grade 3 tumors. India is a large country with marked diversity in the population differing in culture, habits, food and race. This can explain the differences in the histologic grades of the tumors. In our series, triple negative cancers constituted 11.4% of the patients constituting a significant percentage of tumor burdens. Significant number of breast cancer patients belongs to this category of basal-like cancers and need special attention while diagnosing.

GO 03-47. Codon- Correction Inframing Approach for Dystrophin Gene Mutation Correction in Malaysia Patients with Duchenne Muscular Dystrophy

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Studies in various populations have shown about 90% of mutations that created premature stop codons in the reading frame (out-of-frame mutation) of *Dystrophin* result in a severe Duchenne Muscular Dystrophy (DMD) phenotype. This report describes an approach known as codon-correction inframing (CCI) to correct translation error due to out-of-frame mutation. CCI is basically a molecular approach to manipulate (artificially delete or insert) codon's nucleotides for restoration of aberrant reading frame. Our study showed that the most frequently deleted exon in our DMD patient cohort is exon 50. Based on this, we designed a codon correction approach within exon 51 to restore the reading frame of *Dystrophin*. In this study, we employed a *Dystrophin* deletion scenario from one of our patients having deletion of the entire exon 50. Three Minigenes were created using overlapping extension PCR in this study; the Patient Minigene consists of exons 49, 51 and 52, the Corrected Minigene consists of exons 49, 51 (with artificial deletion of 2 nucleotides) and exon 52 and the Wildtype Minigene consists of exons 49, 50, 51 and 52. Specific primers were used to generate these minigenes including their specific cis-acting elements. The effects of CCI approach on restoration of aberrant *Dystrophin* reading frame caused by deletion of exon 50 were evaluated using a cell-based reporter assay. In conclusion, CCI is a potential approach to rescue patients with out-of-frame mutation by restoration the open reading frame. This approach may provide the opportunity to transform the severe DMD phenotype into the milder Becker muscular dystrophy (BMD).

Poster Presentations

GP 01-3. Efficient Intracellular Delivery of a Tumor-Suppressor Gene and Small Interfering RNAs using pH Sensitive Carbonite Apatite Nanoparticles in Breast Cancer Cell Lines

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Gene therapy through intracellular delivery of a functional gene or a gene-silencing element is a promising approach to treat cancer. Unfortunately, gene therapeutic elements are not ideal drug-like molecules. Problems hindering their effective application mainly lie in their delivery, stability, and off-target effects. pH-sensitive carbonate apatite nanoparticles has recently been developed as an efficient tool to deliver gene therapeutic elements into the mammalian cells. In the present study we investigated the effectiveness of this vector system for the delivery of gene therapeutic elements into breast cancer cell lines. Fabricated carbonite apatite nanoparticle with p53 and siRNA complexes successfully delivered intracellular to MCF-7 and 4T1 cell lines. Cell viability and uptake of the particles were measured using MTT assay and fluorescence microscopy. The results showed that the new approach can be used as a highly efficient delivery system and expression of a transgene in breast cancer cell lines. More over the study showed that highly acid-soluble carbonate apatite crystals have wide and potential applications from laboratories to clinical medicine.

GP 02-4. Gene Expression Profiling of Time-Dependent Changes Associated with Regeneration and Repair of Airway Epithelium

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The airway epithelium has been demonstrated to be able to quickly repair itself following physical injuries. With the advent of molecular and bioinformatic tools and resources, the opportunity to extend the value of animal models of lung injury in defining the molecular pathways and interactions underlay the normal repair process is now possible. A large animal model was developed in selected areas of airway epithelium were subjected to bronchial brushing in an anaesthetised sheep. The process resulted in a physical perturbation of the normal pseudostratified structure of

the sheep airway epithelium at specific locations. The patterns of airway epithelial repair following bronchial brushing were studied in eight sheep at defined time points (6 hours, 1, 3 and 7 days) post-injury. Bronchial brushing resulted in acute removal of the epithelial cell layer and components of the underlying structures. Repair processes were rapidly implemented through initial epithelial dedifferentiation, proliferation and migration at the wound margins. Subsequent time-dependent changes in the proportion of subepithelial structures, including smooth muscle and blood vessels were monitored, as the epithelial surface moved towards repair. Transcriptome analysis revealed that over 13,000 probes showed evidence of differential expression at some point during the repair process ($p < 0.05$), whilst of these, 6420 probes had been annotated. Array results were validated using the conventional semi-quantitative RT-PCR for selected genes. Differentially expressed genes with previously characterized roles in epithelial migration, proliferation and differentiation were identified. In addition, the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases were queried and processes indicated the involvement of genes in various pathways as the epithelium exposed to injury towards repaired. The model of airway epithelial injury developed in this study generated features broadly consistent with those previously described in relation to various small animal model systems. This study defines the molecular features associated with repair in this model system and provides a useful resource to assess the comparative features of the airway response to physical injury at transcriptomic level. It is through such comparison, using analogous methodology, the fundamental pathways and interactions that underlie normal repair and regeneration can be identified and thereafter extended towards understanding the basis for variation associated with natural and experimental disease.

GP 04-8. *In Vitro* Characterisations and Gene Delivery Potential of Bio Functional Carbonate Apatite Nanoparticles into the Lung Cells

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Previous studies by our group have shown that carbonate apatite (CA) possesses remarkable properties for high-affinity binding of DNA for delivery into cells by endocytosis, either by specific or non-specific manner, and allows fast dissolution rate in endosomal acidic compartments to facilitate DNA release from the particles. However, there are scarce efforts to study the ability of CA in delivering plasmid DNA (pDNA) into lung cells for future prospect in gene therapy for lung disorders. Hence, this study focuses on characterising the CA and determining its potential to deliver pDNA (carrying Luciferase as the reporter gene) into human non small carcinoma lung cells (H1299). Our findings show that CA/pDNA complex prepared with serum protein exhibited significantly higher gene expression 4 hrs post transfection

with 10^{10} relative light units per milligram of protein (RLU/mg) when compared to the untreated cells and polyethylamine (PEI) group, with 10^5 and 10^8 RLU/mg, respectively. Significant reduction in gene expression was observed when CA/pDNA complex was prepared without serum protein. Morphological studies using scanning electron microscope (SEM) indicated that CA/pDNA complex prepared with serum protein were all in nano size, ranging from 150 – 400nm, and with clear spherical structure. Cytotoxicity study of the cells transfected by CA was also performed, with the results showing no significant difference when compared to the untreated groups.

GP 05-9. Transgene Expression from CpG-Reduced Lentiviral and Plasmid DNA Gene Delivery Vectors *in vitro*.

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Current viral and non-viral gene delivery vectors for gene therapy are inefficient due to transient transgene expression attributed to the cytosine-phosphate-guanine (CpG) motifs in the transgene. Here we assessed the effects of the reduction of CpG motifs in lentiviral (LV) and non-viral gene delivery contexts on the level and duration of reporter gene expression in Chinese hamster ovary (CHO) cells, human erythroleukemia (K562) cells and hematopoietic stem cells (HSCs). LV carrying Zero-CpG green fluorescent protein (ZGFP), LV/CMV/ZGFP, was transduced into the selected cells. The GFP expression was compared to its non CpG-depleted transgene LV (LV/CMV/GFP) counterpart. The LV/CMV/ZGFP exhibited prolonged transgene expression in CHO cells and HSCs up to 10 days and 14 days, in the respective cells. This effect was not seen in the transduced K562 cells. Transgene copy number analysis verified that the GFP expression was not from pseudo-transduction and the transgene remained integrated in the genome of the cells throughout the period of the study. The modest positive effects on the LV/CMV/ZGFP suggest that the reduction of CpG was not substantial to generate higher and more prolonged transgene expression. As for the non-viral delivery study, the selected cells were transfected with plasmid pMOD/CMV/ZGFP carrying ZGFP. The results showed no effect on the transfection efficiencies and duration of transgene expression when compared to its non-CpG depleted plasmid counterpart in all cell lines tested. Transgene copy number analysis confirms that the transient nature of gene expression was due to the loss of pDNA during cell division.

GP 06-11. Formulation and Characterization of Cellulose Acetate Phthalate Microcapsules Encapsulating Plasmid DNA for Delivery to the Intestines

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Oral administration of DNA requires protection from the gastrointestinal tract environment. Cellulose acetate phthalate (CAP) could be applied as polymeric carriers of DNA due to its enteric and nontoxic properties. Plasmid DNA needs to be protected against the acidic condition of the stomach and released gradually in the slightly acidic to alkaline conditions in the intestines; hence, the potential use of CAP as a carrier system. CAP microcapsules loaded with plasmid DNA were prepared using solvent evaporation method. CAP microcapsules were characterized in terms of size, morphology, release of plasmid DNA in acid/base, loading capacity, encapsulation efficiency, and DNA stability. The average size of the CAP microcapsules was $58.05 \pm 32.27 \mu\text{m}$, spherical in shape with smooth surfaces. The microcapsules were stable at acidic pH and showed gradual release of plasmid DNA in basic pH. The loading capacity of the microcapsules was low (4-18%) and the encapsulation efficiency was also low (7-20%). The CAP recovery was high which was around 45-96%. Plasmid DNA was found to be stable in the CAP microcapsules. High CAP recovery after extraction shows that CAP microcapsules were efficiently formed. However, the inefficiency in encapsulation and low loading capacity could be due to the anionic property of CAP. Nonetheless, the mucoadhesiveness of CAP could still be used to deliver the payload to the intestines. The charge interaction between CAP and plasmid DNA, and the effect of additional encapsulation material to offset the charge need to be studied further. CAP microcapsules encapsulating plasmid DNA could be further developed for application in gene therapies or DNA vaccines.

GP 06-19. Identification of Differentially Expressed Genes in Postnatal Dental Pulp stem Cells.

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GeneFishing technique™ using Annealing Control Primer (ACP) system is one of the techniques designed to identify differentially expressed genes (DEGs) in cells under various physiological stages and become important in modern pathology. Stem cells from human dental pulp represent a population of postnatal stem cells that are capable of extensive proliferation and multipotential differentiation. Thus, the gene expression profile of these stem cells needs to be evaluated to determine their biological activity and usefulness for cell-based regeneration therapy. GeneFishing™ DEG method was used to identify DEGs in Human Exfoliated Deciduous Teeth (SHED) and Dental Pulp Stem Cells (DPSCs) using the provided arbitrary primer pairs. DEGs were purified and sent for sequencing. Comparing the gene expression profiles between SHED and DPSCs, one gene was strongly expressed in SHED, one in DPSCs and one in both. Sequencing analysis revealed that they were *TIMP Metalloproteinase Inhibitor 1 (TIMP1)*, *NADH-ubiquinone oxidoreductase chain 2-like* and *Follistatin-like 1* genes. The gene expression patterns of SHED and DPSCs might be useful in determining the detailed functional roles of the relevant genes in stem cell therapies. These cells could also be used as a source for multipotent cells in genetic and tissue engineering applications.

GP 07-27. Identification of Mature microRNAs Differentially Expressed in Colorectal Cancer using Microarray Technology

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MicroRNAs (miRNAs) are highly conserved, small noncoding RNA molecules that have been shown to regulate various cellular processes by interfering with protein expression through posttranscriptional repression or mRNA degradation. More importantly, beyond their roles in physiological processes, many miRNAs are aberrantly expressed in various pathologies including cancer and regulate tumor- and metastasis-associated genes. The purpose of this study was to determine the deregulated miRNAs between Duke's stage B2, C and the lymph node metastasis tissue of colorectal cancer and compare them to the corresponding noncancerous tissues. Microarray-based hybridization has been proven to be a powerful technique for miRNA

profiling, thus, we use this platform to analyzed 70 cases of colorectal cancer using the Geniom RT Analyzer together with the Geniom Biochip MPEA Homo sapiens. The MPEA biochip shows an outstanding advantage for its sensitivity to reliably analyze nanogram amounts of total RNA coming from Formalin Fixed Paraffin Embedded (FFPE) tissue sample. The resulting detection pictures were evaluated using Geniom Wizard Software and 30 of the most deregulated miRNA were reported. The expression status of five selected miRNAs was then validated by real-time RT-PCR. In this study we use the TaqMan microRNA assays and U6 snRNA as the endogenous control in order to perform the qRT-PCR procedure. The TaqMan PreAmp Master Mix effectively increases the sensitivity of qRT-PCR analysis of the FFPE sample. Among the various miRNA detected, miR-145 was found to be downregulated in all tumour samples but not in the noncancerous sample. In addition, miR-143 was only significantly detected in sample with lymph node metastasis. Both miR-145 and miR-143 were successfully validated in this study. Although the result confirm the association of both miRNAs with colorectal tumour samples, further studies on the correlation between these miRNA with different tumour stages and metastatic status using a bigger sample size need to be carried out in the future.

GP 08-39. Culture Mediated Changes in Gene Expression Profile of Human Limbal Stromal Cells

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This study demonstrates the changes of gene expression profile of multipotent human limbal stromal cells and possible mechanisms underlying the changes when the cells were cultured in two different culture systems. Increasing attention has been given to the effect of changes in the *in vitro* microenvironment on the fate of the stem/progenitor cells. In this study, the effect of changes in the culture microenvironments of limbal stromal cells on the cells' gene expression profile was evaluated. Expanded limbal stromal cells were divided into two groups; each cultured separately on a matrigel coated plate in DMEM/F12 medium supplemented with bFGF and LIF and the other on normal plate in DMEM medium supplemented with 10% FBS. Total RNA was extracted from the cells and subjected to microarray experiments with Agilent platform by using Human GE 8x60k gene chips. Data analysis was carried out with GeneSpring software. Hierarchical clustering analysis revealed that two distinct clusters were formed. Statistical analysis showed that 871 genes were up-regulated and 538 genes were down-regulated when the cells were cultured on matrigel. Besides the long intergenic non-coding RNA, these genes represent gene ontology for cellular components, molecular function and biological process. Significant pathways such as Hedgehog, IL-1, IL-6 and IL-7 were suggested to be involved. Differences in culture

microenvironments had affected the gene expression profile of the limbal stromal cells. The DNA microarray analysis provides an important insight into the changes of the gene expression and aids in the understanding of molecular pathways implicated.

GP 09-42. Gene Expression Profile of Hematopoietic Stem Cells Transduced by Lentiviral Vector

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Hematopoietic stem cells (HSCs) hold great promises for gene therapy for blood disorders such as β -thalassemia and sickle cell diseases. Successful gene therapy relies on the high-level and sustained gene expression. Lentiviral vector (LV) is an attractive tool for gene delivery due to its ability to transduce HSCs efficiently. In this study, the level and duration of gene expression mediated by LV in HSCs were determined. First, bone marrow cells were harvested from femurs and tibias of BALB/c mice. The cells were subjected to Lin-cKit⁺ purification steps by EasySep Magnetic Nanoparticles Separation and subsequently verified by flow cytometry. The percentage of the isolated Lin-cKit⁺ cells from bone marrow was 0.32%. The isolated cells were verified as Lin-cKit⁺ cells with enrichment of 91.15%. LV carrying Green Fluorescent Protein (GFP) reporter gene was produced by co-transfecting 293FT cells with packaging mix and lentiviral expression plasmid. The titer of the LV produced was 1.31×10^6 TU/ml. The verified HSCs were transduced with the LV at different multiplicity of infections (MOIs). The gene expression increased from MOI of 3.2 to 12. However, the increment of MOI beyond 12 resulted in a reduction of gene expression. Multiple time-points study exhibited increased gene expression from 6 hours to day 2 post-transduction (PT), but rapidly declined to background levels by day 7. The phenomenon was due to DNA methylation and histone modifications. These results indicate that prolonged transgene expression in HSCs transduced by LV can be achieved by inhibiting DNA methylation and histone modifications.

GP 10-44. Effect of HDACis and Sirtuin Activator on the Inclusion of Exon7 within SMN2 Transcripts: An *in-vitro* Study towards Drug Therapy for Spinal Muscular Atrophy

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Spinal Muscular Atrophy is a common autosomal recessive neuromuscular disorder, caused by loss of *SMN1* gene and inappropriate function of its copy gene, *SMN2*. *SMN2* transcripts, which otherwise can compensate for the loss

of *SMN1*, produce 90% of truncated non-functional Mrna/protein due to skipping of its exon 7. Studies have shown that splicing of *SMN2* exon 7 can be modulated by small molecules and drugs. Several Histone Deacetylase Inhibitors (HDACis) have been shown to increase inclusion of exon 7 within *SMN2*. Sirtuin activators were known to increase expression of some genes. This study aims to elucidate the effect of a relatively novel HDACis and sirtuin activator on *SMN2* splicing. We designed an in vitro study by exposing SMA-patient-derived cell line to the drugs of interest and measuring the level of exon 7 inclusion within *SMN2* transcripts using RT-Qpcr. This presentation will outline preliminary data of drugs effect on overall *SMN2* expression and its exon 7 inclusions.

GP 11-49. Characterization of Molecular Roles of Novel Long Non-coding RNAs at SOX4 Gene Locus during Mouse Brain Development.

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The ENCyclopedia Of DNA Elements (ENCODE) project has revealed that as much as 90% of the analysed 30 million bases of the human genome sequences were actually transcribed, mostly into non-protein coding RNA. The landmark finding of the study suggested the previously termed 'junk DNA' has much more to offer predominantly in its long non-protein coding RNA (lncRNA) form. Although there are very limited evidences to support these lncRNAs as functional entities, their unique spatiotemporal regulation throughout embryogenesis and organogenesis suggests the importance of lncRNAs involvement in organismal development. Here, we report the discovery of novel lncRNAs at *Sox4* gene locus. These lncRNAs are antisense-complementary to the *Sox4* Mrna to form cytoplasmic dsRNA aggregates in various brain cells. Further investigations of the lncRNAs cluster at *Sox4* gene locus revealed that the formation of dsRNA aggregates led to the synthesis of a novel endogenous small interfering RNA with specific expression in the germinal layers of the developing brain, neurodifferentiating cells and developing liver. This is the prime investigation of lncRNAs' role in the cellular cytoplasmic compartment during brain development. The finding will change the way we interpret these emerging class of noncoding RNAs and may

explain the 36ncharacterized biogenesis of various nontypical noncoding small RNAs in the mammalian system.

GP 13-64. Functional Characterisation of a Novel miRNA, *Mir3099* in Neural Differentiation

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MicroRNAs (miRNAs) are 20-24nt small non-coding RNAs that can exert multilevel inhibition/repression at the post-transcriptional or protein synthesis level during development. We have showed that *Mir3099* is expressed in mouse embryonic stem cells, early embryonic development, cortical plate of the cerebral cortex during brain development and is upregulated in neurodifferentiating P19 teratocarcinoma cells. Based on the previous findings, we hypothesised that *Mir3099* has regulatory roles during neuronal cell development and function. We used the 46C mouse embryonic stem cell as an *in vitro* model to characterise *Mir3099* function during neurodifferentiation. 46C cells were induced and neurodifferentiation were carried out to assess the expression of *Mir3099* at different neurodevelopmental timepoints, from stem cell to mature neurons (3, 7, 11, 17 days after neural induction). Reverse transcription-Polymerase Chain Reaction (RT-PCR) analysis was performed on various markers for stem cells (*Oct4*, *Nanog* and *Sox2*), neural precursor cells (*Sox1* and *Nestin*), differentiating neurones (*Sox4*, *Sox11*, *Tuj1* and *Neurod1*), mature neurones (*NeuN*), glial cells (*GFAP*) and oligodendrocytes (*Olig1* and *CSG4*).

GP 14-70. The Phenotypic Effect of MiR-141 Modulation on the Aggressive Bladder Cancer Phenotype

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Due to the high prevalence from long-term survival rates and the need for life-long routine monitoring and therapy, the cost per bladder cancer patient from diagnosis to death is the highest of all cancers. The development of non-invasive biomarkers of recurrence and progression can increase survival time, decrease treatment costs and improve patient quality of life. However, to date, no tumour marker(s) tests can be endorsed with confidence for the use in the clinical management of bladder cancer, especially in predicting risk of progression. Differential expression of microRNAs (miRNA) in invasive cancers

indicates biomarker potential. We have previously identified miR-141 as a target of downregulation in invasive cancer cells. Hence this study aimed to investigate the phenotypic effects of miR-141 modulation. By using miRNA mimics, we ectopically overexpressed miR-141 in a low-expressing invasive cell line (EJ28). The relative expression of miR-141 was quantitated by real-time quantitative reverse transcription PCR (RT-qPCR) and normalized using RNU5A and RNU6B as endogenous PCR controls. MiR-141-transfected EJ28 cells displayed lower cell migratory, proliferative and invasive potential as compared to untransfected cells. Besides, invasion assay shows a significant decrease of invasiveness in miR-141-transfected EJ28 cells. These results corroborate with the postulate that inactivation or suppression of miR-141 elicits a pro-invasive signal in bladder cancers. Hence, the development of efficient reversal strategies of miRNA inhibition is a promising therapeutic avenue against aggressive bladder cancers. In addition, future validation studies on the expression of miR-141 and the identification of downstream targets of miR-141 across broader datasets will further determine its biomarker utility.

GP 15-71. Inhibition of MiR-21 Mitigates the Aggressive Bladder Cancer Phenotype

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MicroRNA (miRNA) expression dysregulation exerts a major impact on a plethora of developmental and physiological processes. MiRNAs have been found to be involved in cancer initiation and progression, acting as major regulators of oncogenes and tumor suppressors. This study was aimed at identifying key miRNAs involved in the conferment and/or maintenance of an aggressive bladder cancer phenotype. Microarray-based global gene expression profiling of RT112 (minimally invasive) and EJ28 (invasive) cell lines was performed to identify differentially regulated genes ($P < 0.01$). Gene ontology was assigned to the top dysregulated genes using GeneDecks V3 and DAVID v6.7. Genes linked to metastasis were identified as amongst the top dysregulated genes. MiR-21 was identified as one of the miRNAs postulated to target these genes based on in silico prediction. Several phenotype assays were conducted to characterize the phenotypic effects of siRNA-mediated miR-21 expression modulation. The relative proliferation rates of RT112 and EJ28 miR-21 knockdown cells decreased by 33.2% and 10.2% respectively as compared to untransfected cells. Relative to untransfected cells, the migratory potential of RT112 and EJ28 miR-21 knockdown cells was 30.4% and 47.4% respectively. In addition, the invasion potential of the RT112 and EJ28 miR-21 knockdown cells were decreased by 3.4-fold and 2.5-fold respectively. Hence, the inhibition of miR-21 decreases the proliferative, migratory and invasive potential of bladder cancer cells. MiR-21, as a pro-

invasive target in bladder cancer is a potential biomarker of prognosis and therapy in the future.

GP 16-86. Identification of Matrix Attachment Region (MAR) Element and Development of retroviral Vector for Gene Delivery

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Vector is a carrier to deliver genes into cells. In order to increase and stabilize the gene delivery system, modification of expression vector is needed. Matrix attachment regions (MAR) element are DNA sequence that can improve gene expression by acting as epigenetic regulator while retrovirus that consist of long terminal repeat (LTR) in its genome has a capability to integrate into host genome to stabilize the gene expression. Combination of these two elements may increase gene delivery efficiency into cells. List of 21 MAR element sequences was given by InnoBiologics Sdn Bhd. These MAR element were then analyzed using CLC-bio software to determine the most potential MAR element based on their characteristics amplifying it using PCR method. Potential retrovirus LTRs were identified from the NCBI data bank. To introduce restriction enzyme sites at both ends of these two elements in order to ligate them with expression vectors, extension PCR was performed. By using *in silico* analysis, two potential MAR elements located at 5' from the nearest gene were identified before being isolated and cloned. Three identified potential retrovirus LTR are from human T-cell lymphotropic virus type 1 (HTLV-1). These two elements were then ligated with expression vector pZAA_GFP, which consist of green fluorescence protein to serve as a reporter gene. Five modified expression vectors consist of MAR elements and retrovirus LTR were cloned and ready to be transfected into mammalian cell to analyze the efficiency of these vectors to deliver GFP into mammalian cell.

GP 17-93. Development of High Expression Vector System for Monoclonal Antibody Production

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Monoclonal antibody therapy against cancer is considered as one of the best approaches to overcome the disease at any stage of incidence. In the post-genomic era, most carcinogenic pathways and genes involved have been out ruled and understood. Using monoclonal antibody against specific antigen or target protein, the elimination of cancer cells is now depending on the immune system of patients. Despite of being specific and efficient in fighting cancer, the main drawback for antibody therapy remains at its production level. Low yield and transient expression of antibody by host cells

are among problems discovered during antibody production. Many strategies have been implied to increase yield and stability of protein including improving vector expression system with DNA elements integration. In this study, we constructed a high expression vector system using incorporated matrix attachment region (MAR) element for therapeutic monoclonal antibody (mAb) production. Generally, the expression vector system was integrated with a functional MAR element isolated from the host genome prior to gene sequence to enhance antibody production by the host cells. This approach is distinctive from previous studies as the MAR sequences were not predicted based on any available mammalian genome databases, but were isolated and sequenced from the host cell used, in this case, the CHO cell line. By integrating the MAR element originated from the CHO cell line into the expression vector, the random positional effects, which usually occur during vector-host genome integration, could be reduced. The integrated functional MAR element is assumed to increase the antibody production by host cells through up-regulation of gene transcription by adopting a DNA loop structure of nucleosomes which open the structure for specific DNA transcription factor binding.

GP 18-98. Analysis of microRNAs regulating α -Hb Modifiers in Normal Human Erythropoiesis

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MicroRNAs are small non-coding RNAs, regulating gene expression through inducing mRNA degradation or translational repression. Alpha hemoglobin stabilizing protein (AHSP) acts as molecular chaperone for α -globin by binding to free and nascent α -globin, stabilizing it from precipitation or pro-oxidation prior to HbA assembly. Nuclear factor erythroid-derived 2 (NFE2) is found associated with β -globin gene activation and in latter study; found as one of the erythroid regulators for AHSP expression. Expression pattern of microRNAs with AHSP and NFE2 gene were analyzed in erythroid progenitor derived from normal CD 34+ cells at different maturation stages. From our study, AHSP and NFE2 expression were most abundant at hemoglobin synthesis stage (Day 9) and down-regulated in enucleation process prior erythrocytes formation (Day 12). Three examined microRNAs (miR 361-5p, 146a and 125b), demonstrated contrary expression pattern to AHSP and NFE2 gene. It contributes to the hypothesis that these microRNAs might play a vital role in regulating AHSP and NFE2 gene expression. Elevation of miR 125b, 361-5p and 146a will suppress AHSP and NFE2 gene expression, and vice versa. Beta thalassaemia results from decreased or absence in β -globin chains synthesos causing anaemia. Its pathophysiology results from the consequence of precipitation of free excess α -globin chains leading to destruction of developing erythrocytes in the bone marrow (ineffective erythropoiesis) and mature red cells in the peripheral blood (hemolysis). Promoting of AHSP production through regulating of certain microRNAs might ameliorate the severity of β -

thalassaemia resulted from α -globin precipitation. MicroRNAs might be a potential target for therapy of β -thalassaemia patients.

GP 19-116. Identification and Characterisation of Flotillin-2 as a Target of Dysregulation in Breast and Bladder Cancers

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Invasive breast and bladder cancers are associated with poor clinical outcome and are characterized by a genotype that is distinct from superficial disease. Predicting the invasive and metastatic potential of tumor at the time of diagnosis remains a major challenge in cancer management. Exploiting a multi-component mining strategy on a set of high-throughput genome-wide experimental data, we probed for genes associated with an invasive phenotype. Fresh frozen bladder cancer tissues were profiled using global expression microarrays and array-based comparative genomic hybridization (aCGH). Differentially expressed genes were functionally annotated by gene ontology and compared to available expression datasets. We identified overexpression of a lipid raft associated protein, Flotillin 2 (*FLOT2*) in invasive cancers. The *FLOT2* locus (17q11-q12) was associated with copy number gains in 15% of the bladder tumors. *FLOT2* is an important lipid raft marker and is predicted to be involved in cell-matrix adhesion, cell migration and signal transduction. Immunohistochemistry was used to evaluate *FLOT2* expression in formalin fixed paraffin-embedded malignant and non-malignant breast cancers. *FLOT2* localization varied from a cytoplasmic distribution in normal cells to a more cell-cell contact distribution in malignant cells. A correlation was found between *FLOT2* overexpression in the invasive compartments of tumor tissues and clinical stages. The staining intensity in the invasive compartment increased with cancer progression. *FLOT2* protein expression was tested in an independent bladder cancer tissue microarray series by immunohistochemistry. *FLOT2* protein expression increased with bladder cancer progression as well. Subsequently, *FLOT2* was knockdown in bladder and breast cancer cells *in vitro* by siRNA. Migration and invasion assays were employed to determine the phenotypic effect of *FLOT2* inhibition. The inhibition of *FLOT2* expression in knockdown cells was confirmed by RT-qPCR and Western blotting. Knockdown of *FLOT2* lead to a significant reduction in the invasive and migratory cellular phenotypes. In concord, ectopic overexpression of wild-type *FLOT2* enhanced invasion in minimally invasive cells. The precise mode of action of *FLOT2* remains to be elucidated but it is predicted to play an important role in transmembrane signal transduction, cell adhesion and endocytosis. *FLOT2* overexpression has also been shown to enhance the spreading of cells, formation of filopodia as well as melanoma progression and metastasis. Thus, the functional targeting experiments and gene-dosage dependent *FLOT2* overexpression in invasive breast and

bladder cancers confirm a link between *FLOT2* and pro-invasive cancer phenotype.

GP 21-35. Newcastle Disease Virus as a Virotherapeutic Agent for Cancers

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The WHO Global Burden of Disease analysis estimates that more than 60% of cancer deaths occur in low- and middle-income countries (LMIC). The cancer burden in these countries is expected to rise due to population aging and growth, environmental degradation, as well as adoption of cancer-prone lifestyles such as smoking, physical inactivity and Western diets. Hence, novel interventions and treatment strategies will not only save lives but also improve economic development prospects. The discovery of targeted therapies such as monoclonal antibodies has raised expectations for improvement in the mortality and morbidity indices. However, the cost of and access to these 'magic bullet' therapies are already prohibitive to about two-thirds of cancer patients in high-income countries and this disparity is expected to be wider in LMIC. Hence, new, cheaper and more efficacious anti-cancer agents that include immunological manipulation and direct cellular-disruptive methods such as virotherapy are needed to combat the chronic escalation of cancer burden. Virotherapy is a treatment modality that includes reprogramming of viruses to attack and lyse cancer cells, while healthy cells remain relatively undamaged. This oncolytic viruses can also illicit a systemic effect by stimulating the own body's anti-cancer immunological response. Once inside the cancer cell, the virus propagates and has the propensity to infect the surrounding cancer cells. The virus also stimulates pleiotropic immune responses by induction of cytokines, interferon and cytotoxic T lymphocytes. Newcastle disease virus (NDV) is one of the candidates that are being investigated for its oncolytic properties. NDV is a paramyxovirus that infects more than 50% of the bird order and causes up to 100% mortality in infected birds. The virus, however, is not pathogenic to humans. Our group has been studying the sequence and phylogenetics of the local NDV strains. We have probed into the molecular biology of local NDV strains and its interaction with cancer cells. We have investigated various mechanisms underlying NDV-mediated oncolysis. NDV has been shown to induce cellular apoptosis upon infection in cancer cells but has no effect on normal cells *in vivo* and *in vitro*. NDV is currently being evaluated as a cancer vaccine in phase II clinical trials in late-stage and incurable tumours. Optimisation of the treatment regimens was extensively studied to determine the maximum tolerable dosage as well as reduce the side-effects. Complete and partial responses were observed in some treated patients. While the efficacy needs to be enhanced and improved, the potential of using NDV as a virotherapeutic agent against cancer is promising.

CP 01-1. α -Haemoglobin Stabilizing Protein Expression is Influenced by Mean Cell Haemoglobin and Hb F Levels in Hb E/ β -Thalassaemia Individuals

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The alpha haemoglobin stabilising protein (AHSP) acts as a molecular chaperone for α -globin by stabilising nascent α -globin before transferring it to waiting free β -globin chains. Binding of AHSP to α -globin renders α -globin chemically inert preventing it from precipitating and forming reactive oxygen species by products. The AHSP has been actively studied in the recent years, particularly in its relation to β -thalassaemia as studies have shown that AHSP is a modifier in β -thalassaemia mice models. We investigated the expression of AHSP in relation to selected demographic data, full blood count, HPLC results, Hb E/ β -thalassaemia genotype, *Xmn-1* G γ polymorphism, α -globin, β -globin and γ -globin expression. Simple linear regression analysis demonstrated no significant association between log AHSP expression with haemoglobin level, red blood cell, haematocrit percentage, red cell distribution width, reticulocyte count and Hb A₂ %. The red blood cell indices highly correlated with thalassaemia which are mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) has non-significant correlations with $p=0.085$ and $p=0.080$, respectively. Log AHSP expression has significant negative correlations with mean cell haemoglobin (MCH) value ($p=0.009$) and Hb F % ($p=0.002$). Both MCH and Hb F are indicators of functional tetrameric haemoglobin suggesting that AHSP is reduced when there are more functional haemoglobins. AHSP expression was found to be significantly correlated with α -globin expression ($p=0.003$), β -globin expression ($p=0.001$) and excess alpha globin expression ($p=0.004$). We concluded that AHSP could be a secondary compensatory mechanism in our red blood cells to counterbalance the excess α -globin chains in Hb E/ β -thalassaemia individuals.

CP 03-25. Cardiomyogenic Potential of Rat Full Term Amniotic Fluid Stem Cells

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Rat full term amniotic fluid stem cells (AFSCs) exhibit cardiac markers such as cardiac troponin (c-TnT) and α -actinin, upon directed differentiation with 5-azacytidine (5aza-C) and retinoic acid (RA). Thereby, AFSCs can be potential candidates for therapeutics purpose, to repair the damaged myocardium in patients with heart diseases. Cells transplantation has known to be the most promising treatment for myocardial regeneration. However, many problems have hampered it from being applied clinically, for instance, the difficulties in procuring and maintaining the cells *in vitro*. Hence, the ideal candidate cells should possess the angiogenic capability and cardiomyogenic potential in culture, plus scalable resources. Rat full term AFSCs which are non-tumorigenic and hassle-free in culture has been isolated by our group in the year of 2008. The cells were shown to exhibit mesodermal marker, Brachyury on day-5 upon spontaneous differentiation. Here, we aim to explore the cardiomyogenicity of AFSCs upon directed differentiation with various growth factors or synthetic chemicals, namely 5-azaC and RA. Embryoid bodies (EBs) were formed prior to 5-azaC and RA treatment on day-2. After 2 days treatment, the treated medium was changed with EB medium and EBs were plated into 0.1% gelatinized well at high density. EB medium were changed every subsequent 2 days until the cardiomyogenic-like morphology was observed. RNA were extracted from day-5,-10,-15 and day-20 EBs for reverse transcription PCR with mesodermal lineage associated genes (brachyury, chordin), early cardiomyogenic genes (GJA1), cardiac transcription factors (Nkx2.5, GATA4) and mature cardiomyocytes marker (SERCA2, TnnT2, MYL-2A). Simultaneously, immunocytochemistry (ICC) with brachyury and cTnT were done to the plated EBs. Mesodermal lineage associated genes was found in day-5 EBs, while the cardiomyogenic markers were found upon day-10 of directed differentiation. However, beating cardiomyocytes were not observed. Rat AFSCs possess admissible cardiomyogenic potential upon directed differentiation with 5-azaC and RA, suggesting the cells display interesting potential towards cardiac regeneration but the differentiation protocols need further evaluation.

CP 04-28. The Effects of Inhibiting Wnt Signaling during Neural Differentiation of Mouse Embryonic Stem (ES) Cells *in vitro*

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In the central nervous system (CNS), Wnt/ β -catenin signaling pathway involves in neural differentiation as well as proliferation of progenitor cells. Many studies found that the effects of Wnt/ β -catenin signaling pathway during embryonic neural development were stage depended. In order to explore the impacts of inhibiting Wnt/ β -catenin signaling pathway during neural differentiation at different embryonic developmental stage, an inducible Dkk1 expression of embryonic stem (ES) cell line has been

established. Dkk1 is a secreted glycoprotein which has the ability to inhibit Wnt signaling by binding to Wnt co-receptors LRP5/6 and Kremen. Combining Cre/loxp-based genetic recombination and ligand-dependent activation of Cre, the inducible Dkk1 expression system allows overexpression of Dkk1 transgene upon exposure of hydroxy-tamoxifen (4'-OHT). Inhibition of Wnt/ β -catenin signaling via overexpression of Dkk1 was carried out at three specific time points during neural differentiation process of mouse ES cells. The effects were evaluated based on the formation of neural precursor cells (NPCs) and the post mitotic neurons. Two specific markers; nestin and class III β -tubulin have been used to detect the NPCs and postmitotic neurons, respectively, by using both immunocytochemistry (ICC) and fluorescent-activated cell sorting (FACS) techniques, for qualitative and quantitative analyses, respectively. The formation of NPCs was inhibited when Wnt/ β -catenin signaling was inhibited at early stage during neural differentiation process. However, interestingly, the differentiation of NPCs into post mitotic neurons was promoted when Wnt signaling was inhibited both at early and late stages. The formation of NPCs and post-mitotic neurons was promoted when Wnt was constitutively inhibited (since undifferentiated ES cells). In summary, the inducible Dkk1 expression cell line allows us to analyze the effects of differentiation of ES cells toward neurons by inhibiting the Wnt/ β -catenin signaling pathway at different time points. Our results demonstrate the importance and complexity of Wnt signaling during neural differentiation. This may accumulate knowledge on neural development, and provide some basic supports for ES cell-based-therapies in the future.

CP 05-31. Differentiation of Transgenic Mouse Embryonic Stem Cells (46C) into Matured Neurons

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Embryonic stem cells (ES) have the potential for self-renewal, even after prolonged culture, and, under appropriate physiological conditions, capable of differentiating into all cells in the body, including neurons. Increasing number of neural differentiation protocols have been established, however, generation of pure population of homogenous neural cells remains a major challenge. Direct visualization of neural progenitor cells may provide a good indicator on the efficiency of neural differentiation protocol moving towards achieving higher number of homogenous cells. 46C is a transgenic mouse embryonic stem cell line that has been engineered to monitor the formation of neural precursor cells (NPCs). It carries a reporter construct of enhanced green fluorescence protein (Egfp) incorporated into the open reading frame of the specific marker for NPCs, Sox1. The expression of Egfp is silent in undifferentiated cells but activated upon neural induction. The aim of the study is to propagate good quality ES cells and differentiate them into

matured neurons. This was achieved by propagating the ES cells on gelatin-coated plate, prior to neural differentiation process, which was carried out through the formation of three-dimensional multicellular aggregates, embryoid bodies (Ebs) in bacterial grade dish upon withdrawal of LIF. Four-day Ebs were induced into neural lineage using all-trans retinoic acid (ATRA) and four days later were re-plated in poly-d-lysine/laminin coated flask. Neural differentiation success was monitored by observing the formation of NPCs by direct visualization of Egfp expression under fluorescence microscope, and quantitatively analyzed using fluorescent activated cell sorting (FACS) technique. In addition, we also examined the number of post mitotic neurons and matured neurons formed using immunostaining technique specific for class III β -tubulin and neurofilament, respectively. We found that the neural assay protocol produced promising results with high number of NPCs and neurons in our hands, which would be useful for future application.

CP 06-34. Establishment of Adipose Derived Mesenchymal Stem Cells Expressing TNF-Related Apoptosis Inducing Ligand / TRAIL as a Potential Anti-Tumor Target for Cancer Treatment

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In this study, *in-vitro* approach was designed to investigate the ability of transfecting human adipose derived mesenchymal stem cells (AD-MSCs) with the plasmid encoded for TNF-Related Apoptosis Inducing Ligand / TRAIL as a potential anti-tumor agent delivery for cancer. TNF-Related Apoptosis Inducing Ligand or TRAIL is a type II membrane-bound (MB) protein that can be processed by cysteine protease to generate a soluble ligand. Both MB protein and soluble ligand can rapidly induce apoptosis in a variety of cancers. The ability of mesenchymal stem cells to migrate towards the site of inflammation/tumor opens new opportunity of using AD-MSCs as a vehicle for anti-tumor agent delivery for cancer treatment. Adipose derived mesenchymal stem cells was purchased from ATCC, cultured and propagated in mesenchymal stem cell basal media. Semi quantitative reverse transcriptase polymerase chain reaction and immunostaining were performed to evaluate the expression of pluripotent markers in several passages. Karyotyping of AD-MSC was performed by G-banding technique. Positive mRNA expression was seen for OCT-4, REX-4, NANOG, NESTIN, GATA-2, BMP-4, SOX-2, TDGF and GATA-4. Immunostaining showed evidence of early differentiation protein markers. Normal karyotypes were demonstrated at early and late passage. Characterization of the AD-MSCs for pluripotency has been described from the positive mRNA expression and early differentiation protein markers. These cells have proven to maintain its genomic stability after long-term propagation. Further transfection studies and validation of the transfected genes of AD-MSCs encoded for TRAIL will be performed to evaluate the anti-tumor agent delivery to the tumor environment.

CP 07-36. Identification of Suitable Technique for the Isolation of Mesenchymal Stem Cells from Human Amniotic Membrane.

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The limitations of isolation of mesenchymal stem cells (MSCs) include invasiveness, ethical issues, immune rejection and low number of cells. The alternative source of MSCs is the MSCs from human amniotic membrane (hAM), which is usually discarded and can be found in abundance. Thus, the aim of this study was to compare the different isolation techniques of MSCs from hAM. Freshly obtained hAM was washed with PBS and minced. It was incubated in Dispase II followed by Type II Collagenase for different periods. The cells were filtered and cultured in two different media; Dulbecco Modified Essential Medium (DMEM) and Mesenchymal Stem Cell Basal Medium (MSCBM). The non-adherent cells were discarded after 5 days. The medium was changed every three days. The cells were observed daily under phase contrast microscope until it reached confluence. The stem cell surface marker, CD105 was later identified using flow-cytometry. The number of cell population as well as the expression of CD105 observed was higher in culture incubated with MSCBM compared to DMEM. The increase in the number of cells grown in MSCBM indicates that this medium is superior to DMEM for the isolation of MSCs from hAM. This could be attributed to the presence of specific amino acids and growth factors that may be contained in MSCBM which could facilitate the isolation and growth of MSCs from hAM. It can be concluded that MSCBM is suitable for the isolation and growth of MSCs from hAM.

CP 08-43. *TINOSPORA CRISPA* Inhibits Nuclear Factor- Kappa B Activation and Ameliorates Cisplatin Apoptotic Effects in Normal Breast Endothelial Cells

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NF- κ B is a transcription factor protein complex and it coordinates the activation of a large network of chemokines and cytokines. Several studies proved that inflammation plays an important role in the cisplatin-induced cell death in normal tissues. The aim of this study was to determine the gene expression of NF- κ B and apoptotic related genes in normal breast cells (MCF-10A) after treatment with cisplatin and *T. crispa*. qPCR was used to investigate the mRNA expression levels for NF- κ B, TNF-ICAM-1, caspase-3, caspase-8 and caspase-9 in MCF-10A cells. Our results showed significant up-regulation for mRNA expression levels of NF- κ B, TNF-ICAM-1, caspase-3, caspase-8 and caspase-9 in MCF-10A cells following treatment with cisplatin. TNF- and ICAM-1 are inflammatory genes and they are controlled by NF- κ B while the caspases play an essential role in apoptosis. Unlike cisplatin, *T. crispa* didn't show any significant change to the mRNA expression levels of these

genes in MCF-10A cells. More interestingly, our results showed significant down-regulation of mRNA expression levels for these genes in MCF-10A cells following the treatment with combination of cisplatin and *T. crista*. The combination of *T. crista* and cisplatin significantly down-regulated NF- κ B gene expression which in turn significantly decreased the inflammatory and apoptotic effect of cisplatin in normal breast cells.

CP 09-45. Establishment of Human HERTWIG'S Epithelial Root Sheath Cells (HERS) for Dental Regenerative Therapy

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Hertwig's epithelial root sheath (HERS) cells are a unique population of epithelial cells in the periodontal ligament compartment. To date, their functional role has not been fully elucidated.

Therefore, the aim of this study was to harvest, culture and establish of HERS. Normal human impacted third molars (n = 18) were collected from 12 adults (from 18-22 yrs old) at the Dental Clinics of the School of Dental Sciences, USM. Periodontal ligament (PDL) tissues were separated from teeth surfaces for HERS cells isolation. Selective digestion was done to facilitate culturing epithelial-like cells only. For characterization study, immunofluorescence staining analysis was done to evaluate the ability of HERS to express E-cadherin and pan-cytokeratin as epithelial markers. Initially, the primarily isolated HERS cells showed two distinct morphologies: a bipolar fibroblast-like appearance and a rounded epithelial cell-like appearance, and the epithelial-like cells were strongly attached to the surface of the culture wells than the fibroblast-like cells. Selective digestion was a successful procedure allowing the detachment of fibroblast-like cells. Consequently, HERS cells remained and formed colonies. Immunofluorescence staining analysis showed that HERS cells positively expressed the epithelial markers (E-cadherin and pan-cytokeratin). Establishment of HERS cells will have the potential to revolutionize the practice of regenerative dentistry giving high possibility of saving many teeth that would otherwise have a poor to hopeless prognosis. In conclusion, HERS cells were successfully isolated and will be useful for further analysis for the dental regenerative therapy.

CP 09-60. Dynamic Immunomodulatory Effects of Human Mesenchymal Stem Cells on Neutrophil Functions

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Mesenchymal stem cells (MSC) interact with innate and adaptive immune cells by modulating their functions *in vitro* and *in vivo*. Neutrophils are most abundant white blood cells, providing first line defence and mediate acute immune responses that enhance tissue repair and damage. However, their over activation can lead to detrimental effects hence it necessitates a careful modulation to maintain a normal tissue homeostasis. This study aims to investigate the immunomodulatory role of MSC on neutrophils' vital functions. Freshly isolated human neutrophils from peripheral blood were co-cultured with human umbilical cord derived MSC at various ratios and mitogens. Neutrophils' viability, cellular proliferation, chemotaxis, phagocytosis, respiratory burst and apoptosis activities were assessed in the presence or absence of MSC. Mesenchymal stem cells significantly (* $p < 0.05$) enhanced the viability of resting and phorbol myristate acetate (PMA) activated neutrophils and simultaneously rescued both resting and PMA activated neutrophils from apoptosis. MSC also significantly (* $p < 0.05$) inhibited opsonised zymosan (OZ) and lipopolysaccharide (LPS) induced neutrophil phagocytosis and inhibited resting, PMA, OZ, Fmlp (f-Met-Leu-Phe, N-formylated peptides) and *E.coli* activated neutrophils respiratory burst, while preserved the neutrophils mediated chemotaxis. Overall, MSC play a dynamic role in neutrophils immune response by enhancing neutrophil survival; reducing phagocytosis and respiratory burst activity; and preserving neutrophil mediated chemotaxis. These novel findings revealed the efficiency of MSC in limiting the hostile effects of neutrophils and the potential use of MSC as a therapeutic tool for the treatment of neutrophil mediated immune disorders.

CP 10-61. Assessment of Proliferation Activity of Rabbit Primary Epidermal Keratinocytes using Alamarblue® Assay

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Rabbit primary epidermal keratinocytes were cultured in CnT57 growth medium. The cell proliferation assessment using alamarBlue® assay from day 2 until day 8 showed a corresponding increase of 22.7, 36.0, 47.5, 71.5, 86.4, 88.5 and 102.9 per cent respectively. Keratinocyte is the predominant cell type in the epidermis, constituting 95% of the cells and could be found in the basal layer of the skin. The alamarBlue® assay is designed to measure the proliferation of various human and animal cell line which is simple to perform, stable in culture medium, non-toxic and does not alter the viability of the cells. A piece of skin approximately 2cm x 2cm was harvested from the dorsum of New Zealand White rabbits, (*Oryctolagus Cuniculus*) and placed in 0.6% Dispase solution at 4°C overnight. The keratinocyte cells were seeded at 1×10^4 in CnT57 growth media and the cell proliferation was assessed from day 2 to 8 using alamarBlue® assay. The percentage of reduction in alamarBlue® assay from day 2 until day 8 were 22.7, 36.0, 47.5, 71.5, 86.4, 88.5 and 102.9 per cent respectively. Assessment of proliferation is a fundamental measurement that can be made with cells in culture and the determination of proliferation activity enables researchers to optimize cell culture conditions as well as to quantitate the activity of cell growth factors. AlamarBlue® assay revealed a corresponding increase in cell proliferation of rabbit primary epidermal keratinocytes from day 2 to 8.

CP 11-63. Generation and Characterisation of Mesenchymal Stem Cells (MSCs) from Human Cartilage

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Cartilage damage has limited capacity for healing which made the treatment for cartilage lesions are difficult and lengthy. The current existing conventional treatments such as intra-articular injections and physiotherapy are only able to reduce the symptoms but none of them promotes cartilage repair. The limited capacity of cartilage regeneration at synovium necessitates an exploration for alternate therapies that overcome a non-conductive microenvironment that failed to trigger repopulation of tissue resident stem cells. In line with this, we have generated MSC from sport-injured, non-weight-bearing region cartilage biopsy and examined for the cells' primitive role to differentiate into mesodermal tri-lineage. Cartilage samples were finely minced and homogenised in enzymatic reaction mixture and plated in plastic culture-ware for colony formation. Once adherent cells confluent, cells were harvested and characterised based on standard guidelines outlined by International Society for Cellular Therapy. At morphological examination, cells were appeared as fibroblast-like morphology and able to differentiate into all three mesodermal lineages. Mesenchymal stem cells in respective induction media (adipo, osteo and chondro) analysed by biochemical staining and RT-PCR. Upon differentiation MSC express adipocytes (Lipoprotein-lipase), chondrocytes (aggrecan, collagen type-II) and osteocytes markers (parathyroid hormone, osteopontin, osteocalcin). Cell surface markers analysis was performed using flow

cytometry based immunophenotyping. In accordance to international standard, MSC expressed CD105, CD73, CD90, CD29, HLA-ABC molecules and negative for haematopoietic markers such as CD34, CD45 and CD14. Our results showed that MSC can be derived from human adult cartilage tissues thus recruiting and propagating such stem cells at injury sites would be as added advantage to address disorders related to cartilage deformation in future.

CP 12-76. Effects of Various Eye Toners on Cultured Rabbit Corneal Keratocytes.

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Nowadays, variety of commercially available eye toner products based on prophetic or natural resources can be obtained easily from any local health related shops. The products gain wide acceptance among the public due to its promising therapeutic claims. However, the use of this product is controversial due to some scientific inadequacy. This preliminary study aims to provide scientifically relevant evidence to either support or countering the hypothesis concerning product usage to the community. Specifically, the objective of this study is to evaluate the potential effects of various eye toners on cultured rabbit corneal keratocytes by means of XTT proliferation assay and wound healing study. Six (6) rabbits' corneal tissues which were dissected free from slaughtered rabbits were obtained from locally available slaughterhouse. The isolated corneal keratocytes were culture-expanded for three (3) passages in culture medium supplemented with or without Permata Hijrah (PHET), Dr. Eye (DEET) and Qurrotaaini (QET). XTT proliferation assay was used to assess cell growth and cytotoxicity effects of the eye toners. For wound healing study, a simple *in vitro* wound healing model was established by using monolayer cultured cells. The wound healing activity was observed every 4 hours and any significance changes were recorded as photomicrograph. All data were analyzed using Student's t-test, SPSS version 16 and values were expressed as mean \pm standard error of mean (SEM). A p value less than 0.05 were considered significant. The XTT proliferation assay has indicated that there was no significant difference between cell cultured with various eye toners and control, except for P2 cells where cells in PHET, DEET and QET showed a significantly lower proliferation than control. This can be an indication that the eye toners may have some toxicity effect on corneal keratocytes. From the two histograms generated for the 'time taken for wound closure' and the 'wound healing rate', there were trends indicating that PHET, DEET and QET may accelerate wound healing. However, the differences between cell cultured with eye toners and control were not statistically significant. This preliminary analysis has indicated that PHET, DEET and QET have no significant effects on cultured corneal keratocytes.

Upcoming research therefore shall include a systematic dose-response study to clearly identify any potential effects of the eye toners. This will also include more parameters and other relevant details as the outcome of this experiment will serve as a basis that may influence the perceptions of our community towards eye toner products; the conditions for its acceptance or the grounds for its rejection.

CP 13-82. Characterization of Human Sterna Bone Marrow Derived Stem Cells as a Source of MSc

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Bone marrow stem cells (BMSCs) have been used widely for cell therapy. The most common source of BMSCs is from the femur or iliac crest. In cardiac surgery during the sternotomy, the sternal bone marrow is always wasted via the bleeding from the borders of the opened sternum. These cells can be carefully harvested and utilized for cell therapy and research purposes. The aim of this study is to assess the feasibility of using sternal bone marrow derived mesenchymal stem cells for use in tissue engineering. Bone marrow samples were aspirated by salah bone marrow puncture needle from 3 patients with a mean age of 49.3 ± 26.3 undergoing open heart surgery. The collected volume of bone marrow was 12 ± 3 ml. Cells were isolated by density gradient centrifugation using ficol paque and cultured in DMEM (high glucose). Selected surface antigens specific for MSCs were analyzed by flow cytometry study. Cytometric analysis of sternal derived Mesenchymal stem cells showed that human MSCs are positive for CD13, CD29, CD44, CD73 and CD105 which are considered as markers of MSCs, and negative for hematopoietic lineage markers CD10, CD 34, CD45 and HLA-DR. Mesenchymal stem cells can be isolated from the human sternum during open heart surgery and this bone marrow has a potential source of cells for tissue engineering researches and cell therapy.

CP 14-85. Cytotoxicity Study of Carrageenan using Human Hepatocellular Carcinoma Cells Line (HepG2)

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Carrageenan is a linear sulphated polysaccharide extracted from red seaweed of the class *Rhodophyceae*. The red algae *Kappaphycus* (kappa-carrageenan) and *Euchema* (iota-carrageenan) are now the most important sources of carrageenan, commercially used as food additive for gelling, thickening, and emulsifying purpose. The concentration used in food industrial range from 0.2 - 1.5% (w/v) which is equal to 0.2 g/100mL – 1.5 g/100mL. Experiments were performed to investigate the cytotoxicity of undegraded *iota* and *kappa* carrageenan on human hepatocellular carcinoma cell lines. HepG2 cells were treated with undegraded food grade *iota* and *kappa* carrageenan, which purchase from Tacara, Sabah. The concentration tested range from 3.13 – 1500 µg/mL. Cell viability was determined by MTT assay where IC₅₀ value was used as the parameter for cytotoxicity. *Iota* carrageenan showed IC₅₀ value on HepG2 cell after 48 hours and 72 hours treatment at concentration 440 µg/mL and 1400µg/mL respectively. Meanwhile, *kappa* carrageenan also showed IC₅₀ value on HepG2 cells after 24, 48 and 72 hours treatment at concentration 440 µg/mL, 1000 µg/mL and 1160 µg/mL respectively. From the study, we found that the longer the HepG2 cell treated with carrageenan, the higher the concentration needed to inhibit cell growth at 50%. The criteria of cytotoxicity activity for the crude extracts is an IC₅₀ value less than 30 µg/mL in the preliminary assay, as established by the American National Cancer Institute (NCI). In our study, IC₅₀ value of both *iota* and *kappa* carrageenan was more than 30 µg/mL. So, the cytotoxicity activity of carrageenan is very low.

CP 17-104. Autologous Immune Enhancement Therapy (aiet): Ex vivo Expansion of Natural Killer Cells and T-Lymphocytes from Patients with Metastatic Cancers

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Autologous Immune Enhancement Therapy (AIET) is a form of cell-based therapy that has emerged as a promising utility to treat cancers due to its safety and feasibility. It involves *ex vivo* expansion and activation of Natural Killer (NK) cells and Cytotoxic T Lymphocytes (CTLs). Both these cells play important roles in maintaining the stability of the immune system. This study reports the successful *ex vivo* expansion and activation of NK cells and CTLs from 14 patients with different types of stage-four cancers. Peripheral blood was collected and mononuclear cells were isolated and seeded onto anti-CD3 and anti-CD16 coated flasks. NK cells and CTLs were expanded for 14-16 days. Pre- and post-evaluation of the CTL and NK cell populations were done by flow cytometric analysis. The CTLs and NK cells were expanded at an average of 300-fold and 160-fold respectively. All 14 patients' expanded samples displayed a heterogeneous phenotype of CD3+, CD3+CD4+, CD3+CD8+ and CD3-CD56+ populations. Hence, the *ex vivo* manipulation did not alter cell-cell interactions and enabled the *in vitro* activation and expansion of both immune cells. The presence of populations of cells of interest indicates that *ex vivo* expanded cells were not associated with phenotypic changes, lineage deviation and thus, potentially possessed an intact anti-tumor function. Further refinement and improvement of this protocol is highly expected to facilitate large-scale production of NK cells and CTLs.

CP 16-101. Novel Molecular Pathways Mediate Retinoic Acid-induced Cardiac Stem Cell Differentiation

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To suppress neoplastic transformation and promote integration of stem cell grafts for replacement of diseased myocardium, cardiac stem/progenitor cells (CSC/CPC) must be programmed to differentiate and exhibit a cardiomyogenic phenotype prior to transplantation. In this study, we tested the effects of all-trans-retinoic acid (atRA), a potent inducer of cardiac differentiation, on cultured CPC and discovered putative signaling molecules involved in regulation of cardiac differentiation. Cardiac progenitor cell line (h9c2 cells) was exposed to atRA and characterized for expression of cardiomyocyte-restricted biomarkers. The activity of Mef2C, a cardiac-specific gene regulatory factor, was determined using luciferase reporter assays and a dominant negative Mef2C construct. The activity of p38MAPK, a known activator of Mef2C, was also determined using antibodies against phosphorylated p38MAPK, pharmacological inhibitors, and dominant negative p38MAPK plasmids. Treatment of h9c2 cells with atRA induced morphological changes, p38MAPK phosphorylation, Mef2C activity, and expression of cardiac myosin heavy chain (cMHC) and ventricular myosin light chain (vMLC2). Interestingly, atRA activation of p38MAPK and Mef2C occurred in the presence of RA receptor antagonists suggesting involvement of non-classical signaling pathways. Overexpression of dominant negative mutants of p38MAPK and Mef2C inhibited atRA-induced cMHC and vMLC2

levels in a dose-dependent manner. Moreover, inhibition of p38MAPK activity suppressed atRA-induced Mef2C activity. Our study shows atRA promotes cardiomyogenic differentiation in CPC via p38MAPK and Mef2C signaling. Moreover, retinoic acid and other retinoids may prove useful in programming stem cells to undergo cardiomyogenic differentiation prior to transplantation. Our study identifies p38MAPK and Mef2C as potential targets in cardiac stem cell therapy.

CP 18-94. Cultivation Of Hematopoietic Stem Cell For Cell-Based Therapeutic Applications

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The clinical potential of mobilized peripheral blood derived stem cell has been demonstrated in various animal and human transplantation studies. This analysis describes a bioprocess that generates human hematopoietic stem cell (HSC) with a conserved hematopoietic activity, established analysis criteria for *in vitro* HSC expansion studies, thus serves as a foundation to test the therapeutic utility of cultured HSC in large animals and humans. However, the need for increased number of appropriate HSC continues to limit the development and success of these therapies. Hence, it is a priority to improve the cultivation methods, driven by the fact that many potential therapeutic applications are limited by the availability of stem cells or their derivatives. This is due to donor-derived cell samples which consist of only small numbers of cells despite the fact that large amount of cells are needed for research and especially cell therapies. The objectives for this research are to determine the factors that will affect the *in vitro* growth of HSC and to study the efficient cell cultivation system that will supply enough HSC for cell-based therapeutic applications. For the *in vitro* expansion of HSC, culture conditions were optimized using cytokine cocktails and media change method. Media change method enhanced the number of HSC from $2.02 \times 10^6/\text{ml}$ at day 4 of cultivation to $4.28 \times 10^6/\text{ml}$ at day 6 of cultivation.

TP 01-17. Polyphenol-Rich Oil Palm Leaves Extract Enhances Bone Mineral Density during Estrogen Deficiency

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Postmenopausal estrogen deficiency often causes bone-density loss and osteoporosis. This study reports on the *in vivo* bone mineral density enhancing effects of oil palm leaves extract (OPL) in estrogen-deficient ovariectomised rats as compared to green tea supplemented ovarioectomised or intact rats. The five experimental rat groups were: (1) intact rats (control N); (2) ovariectomised rat (OVX control); and OVX rats supplemented with either (3) 2% w/v green tea (OVX+GT); (4) 150 mg OPL/kg body weight (BW); or (5) 300 mg OPL /kg BW in the drinking water. After three months, the OVX control rats had the lowest femur and tibia bone-density; calcium content, ash weight and total serum alkaline phosphatase (T-ALP). The phytoestrogenic OPL dose dependently enhanced OVX bone-density and structure, bone calcium content, ash weight and T-ALP to higher levels than in the OVX+GT or intact rats. This study showed the polyphenol-rich OPL significantly enhanced bone-density and prevented bone-calcium loss.

TP 02-23. Quercetin-Induced Inhibition- A Chemotherapeutic Strategy for Nasopharyngeal Carcinoma

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Nasopharyngeal carcinoma (NPC) is a tumour of epithelial origin with distinct geographical distribution, genetic predisposition, and environmental as well as dietary influences as aetiological factors. NPC is treated with radiotherapy and concurrent chemotherapy with cytotoxic drugs, such as cisplatin and 5-FU, which are known to be associated with significant toxicity. We investigated the therapeutic potential of quercetin, a polyphenolic flavonoid widely distributed in fruits and vegetables. The effects of quercetin on cell proliferation, cell cycle events and apoptosis of NPC cells were studied. The effects of combining of quercetin and cisplatin on human NPC cells were explored. Cell proliferation was examined by a real-time, impedance-based cell analyzer (xCELLigence system) and the MTS assay. Ki67 levels were measured by real-time quantitative PCR and Western blotting. Flow cytometry was also carried out to study the effects of quercetin on cell cycle and apoptosis status. At 100 μ M, quercetin displayed anti-proliferative activity and arrested cell growth in the G2/M phase in NPC cells. Addition of quercetin reduced the IC₅₀ value of cisplatin against NPC cells. The CI value of quercetin-cisplatin combination was < 1, indicating synergism. Our study shows that quercetin displays synergistic effects with cisplatin against NPC cells. This suggests the possibility that the dosage of cisplatin required to treat

NPC could be reduced with the addition of quercetin. In turn, this could reduce the risk of cisplatin associated toxicity. The potential of combining quercetin with cisplatin as a chemotherapeutic strategy for treatment of NPC should be explored further.

TP 03-30. Analysis of Human Amniotic Membrane as a Scaffold for Dental Stem Cells

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Attachment, spreading and growth of cells on a scaffold are important fundamentals in ensuring success in tissue regeneration. Amniotic membrane (AM) is known to have special structures and properties to be used as a scaffold. Hence, the aim of this study was to examine the behavior of dental stem cells seeded on human AM preserved by two different techniques. Dental stem cells were seeded on both sides of the chemically de-epithelialized glycerol and cryo-preserved human AM, namely stroma and basement. The samples were fixed and analyzed using scanning electron microscope (SEM) at 6 hours and day 1, 3, 7 after seeding of cells. Filopodial-like processes of the stem cells were observed at 6 hours. Later, the attachment as well as the number of cells increased with time. Attachment was also noticed between cells through the filopodial-like processes. All these results were observed on both sides of the AM, but were better on the stromal side and of the cryo-preserved AM compared to the glycerol preserved. The growth of cells on the stromal side implied that there was infiltration of cells across the membrane but not the basement side. Besides, the cells on the stromal side of cryo-preserved AM were uniformly spread. It can be concluded that based on the current findings, the attachment, spreading and growth of cells was better on the stromal side of the cryo-preserved membrane.

TP 04-32. Effect of Sintering Time on the Microstructure of Bioceramic Bovine Bone

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Sintering is the heat treatment process that has widely used in the development of hydroxyapatite (HA) from natural calcium precursor in biogenic materials. The extraction of HA from natural bone from bovine is biologically safe and economic since it is easy to obtained. Sintering time can promote the unknown effects on the microstructure of bioceramic bovine bone. The purpose of this study is to observe the microstructure of bioceramic bovine bone sintered in various sintering time points. Bovine femoral heads

were cleaned and cut into rectangular shape of 10mm x 10mm x 10mm. The samples were sintered at 800°C at a rate 10°C/min by using Carbolite furnace and were taken out at 2 h, 4 h, 6 h and 8 h after the temperature reach at 800°C. The microstructure observation was done using the environmental electron microscope (ESEM), Quanta FEG 450. The results indicated that all the bioceramic bovine bone specimens preserved the spongy structure of natural bone, which has high porosity and interconnecting macro and micro porous structure. High microporosity was observed when sintered for 2h and decreased with the increment of sintering time. Uniformity of grain size increased while the grain boundaries became clearer with increasing sintering time from 2 to 6h. Flat grains structure observed at 8h sintering time revealed molten hydroxyapatite. As a conclusion, sintering for 2h at 800°C resulted in the higher microporosity and quality of bioceramic bovine bone. Microporosity enhances bone regeneration by modulating osteogenic differentiation.

TP 05-33. Chemical Composition and Biological Evaluation of White Portland Cement of Different Origins for Potential Use in Dentistry

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White Mineral trioxide aggregate (WMTA), a Portland cement (PC) based material is a successful endodontic biomaterial. However, high cost and delayed setting times are the main disadvantages. As such, modified PC formulations are required to serve as a cheaper substitute, while maintaining an acceptable biological response. Calcium chloride dihydrate was added as a setting accelerator to WMTA (Dentsply, Switzerland) and sieved WPC manufactured in Malaysia (M) and Egypt (E) by the same company (Aalborg). Three samples of each material were prepared for SEM/EDX micro-analysis. For biological evaluation, human periodontal ligament fibroblasts were applied and incubated for 24 and 72 hours. The SEM images were established after the samples were processed. Accelerated formulations of WMTA, MWPC and EWPC showed a different surface morphology. EDX micro-analysis revealed that these materials had in common most of the main elements including calcium, oxygen, silicon, aluminum and chlorine. While bismuth was only expressed in WMTA, sulphur, magnesium and potassium were scarcely detected in MWPC. Potassium was not identified in EWPC. For the attachment behavior, the cells were viable and attached to all materials with prominent cytoplasmic processes after 72 hours. Despite MTA being a PC based material, the surface morphology and elemental composition of accelerated WMTA, MWPC and EWPC showed some detectable variations. Nevertheless, all materials favored cell attachment. This advantageous finding, however, requires further studies to validate the potential use of both MWPC and EWPC for clinical applications.

TP 06-37. Edible Bird's Nest Extract Promotes *in vitro* Corneal Wound Healing of Stromal Keratinocytes

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Injury or infection to the cornea perturbs stromal organization resulting in corneal opacity, thus compromising vision. Recent researches focused on natural products as the alternative treatment to the conventional approach. To date, the effect of Edible Bird's Nest (EBN) on corneal cells has never been elucidated. Rabbit corneal keratocytes were cultured into four different groups using two different media; the serum-containing medium (FDS) and serum-free basal medium (FDB) with or without supplementation of 0.05% EBN extract. A four millimetre-wound was created over the confluent keratocytes monolayer culture to mimic the corneal ulcer. Cell migration study, gene expression of α -smooth muscle actin (SMAA), matrix metalloproteinase 12 (MMP-12), aldehyde dehydrogenase (ALDH) and immunocytochemistry were performed to determine the extent of corneal wound healing. Cell migration study revealed fastest wound closure in FDS supplemented with 0.05% EBN extract. Gene expression analysis of keratocytes cultured in FDS with 0.05% EBN showed significant reduction in MMP-12 expression but significant increment in SMAA expression. ALDH expression was maintained. Immunostaining of SMAA and ALDH corresponded to gene expression analysis. There was a synergistic effect of EBN and serum during the process of *in vitro* corneal wound healing. The changes in SMAA and MMP-12 expressions were in accordance to the initial stage of wound healing. Supplementation of EBN at 0.05% concentration in medium of cultured stromal keratocytes induced cell migration, proliferation as well as promoted genes and proteins associated with corneal wound healing.

TP 07-46. Establishment of a Growth Medium for Primary Rabbit Epidermal Keratinocytes *in vitro*

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The use of primary rabbit keratinocyte cultures in a wound healing model is uncommon. Thus, growth medium that are suitable for these types of cultures remains undetermined. Full thickness skin biopsy was obtained from adult male New Zealand white rabbits (*Oryctolagus Cuniculus*). Primary rabbit epidermal keratinocytes were isolated from the skin tissues using dispase (at 4°C for 20hrs). Three types of culture media were validated on the primary

rabbit epidermal keratinocyte cultures, namely the Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), the CELLnTEC 07-Progenitor Cell Targeted media (Human/Mouse Keratinocytes-defined) and the CELLnTEC 57-Progenitor Cell Targeted media (Human/Mouse Keratinocytes-Low BPE). DMEM with 10% FBS retarded the growth of keratinocyte cultures. The CELLnTEC 07 medium resulted in 50% growth of keratinocytes whereas 90% of cell growth was observed with the CELLnTEC 57 medium. CELLnTEC 57 is the most suitable growth medium for the culture of rabbit epidermal keratinocytes in this study. This could be due to the low levels of BPE in the culture medium that favour the growth of rabbit epidermal keratinocytes. CELLnTEC 57-Progenitor Cell Targeted is a suitable growth medium for primary rabbit epidermal keratinocytes *in vitro*.

TP 08-50. Bioactive Fractions Isolated from a Plant to Improve Wound Healing in a Hyperglycemic Animal Model

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The presence of wound and hyperglycemic conditions are complicated combinations. Following hyperglycemia, a small wound tends to become chronic which subsequently leads to permanent morbidity. Thus, the search for efficient wound healing products continues alongside the growing practice of traditional herbal medicine. The study was conducted to investigate the improvement of wound in hyperglycemic models following the application of bioactive fractions from *Moringaceae* species. Crude extract was prepared in a powder form and various *in vitro* tests were conducted to evaluate the action of the fractions. Following isolation of the active fractions, active compounds were identified. The fraction was then tested on a wound-induced hyperglycemic animal model and the action of the fraction was evaluated. Following the closure of the wound, as compared to the control, the fraction was confirmed to have an active wound healing activity in a diabetic animal model.

TP 09-51. Analysis of Pathophysiological Changes in Regeneration and Repair of Rabbit Airway Tracheal Epithelium Response to Induced-Injury

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Understanding the mechanisms that operate during airway repair processes is fundamental to developing cell-based therapeutic approaches towards better treatment of lung-related diseases. The airway epithelial layer undergoes well-defined repair stages such as cell migration, proliferation, and redifferentiation in response to injury. In order to study the cellular mechanisms of response to injury and repair, we imposed an injury on the rabbit's trachea by performing a newly invented procedure called blinded-brushing technique that does not require specific surgery skills, time-efficient and less risk of infection as compared to induced epithelium injury. Rabbits were exposed to tracheal brushing and euthanized at different time points – 30 min, 1 hr, 6, 12, 24, 48, 72, 96 hrs and 7 days (n=3 for each time point including naive control animals). The length of the induced-injuries was measured between the edges of the remaining epithelium bordering the lesion. The results found that the length of the injured area was gradually decreased over the time points as compared to 30 min following injury. Decreases in the length of the injuries indicate that the regeneration process was activated to restore the normal epithelial layer. Various histopathological responses were observed from 30 min to day 7 post-brushing, from completely removal of the epithelium layer and its basement membrane until some of injured areas were covered by a series of single-cell layers at day 7. We have successfully developed a more practical and time-efficient brushing technique with less infection risks and thus could be a very useful technique in order to study cellular and molecular mechanisms during airway regeneration and repair that is comparable to studies that are carried out on larger animal models such as sheep and calves.

TP 10-58. Biocompatibility of Tobramycin-Incorporated Calcium Phosphate as Local Drug Delivery System

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The development of calcium phosphate as drug carrier is an important breakthrough in the field of bone repair biomaterials. Calcium phosphate minerals such as β -tricalcium phosphate (β -TCP) and dicalcium phosphate dehydrate (DCPD) have been shown to be suitable materials for local drug delivery in orthopaedic applications. The mineral component of calcium

phosphate consists of calcium ions (Ca^{2+}), orthophosphates (PO_4^{3-}), metaphosphates or pyrophosphates ($\text{P}_2\text{O}_7^{4-}$) and occasionally hydrogen or hydroxide ions which are similar to the mineral components of bone. Calcium phosphate ceramic was used for tissue engineering applications as a template for cell adhesion, proliferation and ultimately for tissue repair. In this study, the cytotoxicity of tobramycin-incorporated calcium phosphate on osteoblasts was carried out using cytotoxicity assay (MTT); while cell adhesion and morphology of osteoblasts on tobramycin-incorporated calcium phosphate were analysed by scanning electron microscopy (SEM) and confocal laser scanning microscopy (CSLM). Our results showed that the material did not demonstrate any cytotoxic effect on osteoblasts in all concentrations ranging from 1.6 mg/ml to 25 mg/ml. Moreover, osteoblasts were well attached and grew nicely on the material for day 1, day 3 and day 5 of incubation. In conclusion, calcium phosphate incorporated with tobramycin showed good biocompatibility with osteoblasts and has potential to be a new formulation for local drug delivery systems.

TP 11-68. Quality Evaluation of Human Tissue Engineered Respiratory Epithelium Constructs (hTEREC)

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Study of cells characteristics in a tissue engineered construct is important to assess functionality and quality to ensure superior therapeutic outcomes. In our previous study, tissue engineered respiratory epithelium construct (TEREC) was shown to treat tracheal mucosal defects in a sheep model. This study was focused on the investigation of human TERC (hTEREC) to understand the quality of constructs *in vitro* by the means of cellular proliferation, differentiation and distribution. For this purpose, respiratory epithelial (RE) cells derived from human nasal turbinate were co-cultured with fibroblasts, subsequently separated at 80–90 % confluency by differential trypsinization. Approximately 2 million RE cells/ml of plasma were used to prepare hTEREC. The immunocytochemical analysis was performed on the hTEREC for day 1 and 4 using anti-MUC5AC and anti-Ki67 to investigate the presence of mucin-secreting and proliferative cells, respectively. In addition, DAPI counterstaining was performed to understand the cellular distribution and total population. It was found that cells were homogeneously distributed and actively proliferating in hTEREC. Percentages of proliferative cells were measured at 26.4% and 50.3% at day 1 and 4, respectively, and 4 times increase in total cell number was achieved at day 4 as compared to day 1. In addition, the population of mucin secreting cells was increased with time, and percentages were measured at 32.1% and 44.3% at day 1 and 4, respectively. These results indicate that RE cells were functionally active by the means of cellular proliferation and differentiation properties in the hTEREC.

However, further studies are required to investigate the presence of goblet and epithelial cells, two other cell types that exist in the native trachea that will facilitate the use the hTEREC for clinical applications.

TP 12-78. Fracture Fixation Plates for Fracture Healing: Comparing 316L Stainless Steel by Metal Injection Molding Technique Plate with Conventional Plate in Rabbit Fracture Model

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Metal injection molding (MIM) combines the material flexibility of powder metallurgy and the design flexibility of plastic molding. With properties comparable, or better than, those of wrought steel, the MIM process are ideally suited to producing small and complex-shaped parts with outstanding mechanical properties. In addition to that, implants produced via this process have high final density and close porosity. This is an innovation to produce new orthopaedic fracture implants and it also can be used to device new special orthopaedic implants. The cost for the technique is cheaper when compared to the conventional technique. The objective is to evaluate the plate function in fracture using New Zealand White (NZW) rabbits. Evaluation was done at three, six, nine, twelve and twenty six weeks by using radiograph evaluation. The procedure commenced with pre-operation, intra-operative and post-operation sessions. There were three groups of experimental studies; Group O Sham group as control, Group 1; (fracture midshaft tibial implant with conventional plate by Synthes), Group 2; (fracture midshaft tibial implant with MIM plate). Both showed similar results as a method to hold the fracture fragments. X-Rays showed that callus formation occurred in both groups (Conventional and MIM groups) of fracture site at three, six, nine and twelve weeks. Bridging was noted at six weeks. There is no infection noted in both groups with no adverse effects. Fracture union noted in all groups. These results revealed that MIM showed comparable function capabilities as the conventional plate to hold the fracture fragments and promote immobilization for fracture healing process.

TP 13-79. Development of a Novel Hybrid Skin Construction for Drug Diffusion Studies.

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The limited availability of human skin for *in vitro* drug diffusion studies has driven researchers to seek skin alternatives. The stratum corneum is the rate-limiting barrier to topically applied formulation and is crucial for *in vitro* drug diffusion studies. In this study, two types of skin substitutes were evaluated for their drug permeation profile, i.e. a bilayered human skin construct consisting of an upper layer of keratinocytes with fibrin and a lower layer of dermal fibroblasts with fibrin, and a novel hybrid skin construct, composed of harvested human stratum corneum, layered with a bilayered human skin construct. Stratum corneum is used as control in this study. Histological and immunohistochemistry staining showed that the bilayered skin constructs were made up of two distinct layers which confirmed the presence of the keratinocytes layer, positively stained with Cytokeratin 14 (CK14) and fibroblast layer, positively stained with Collagen Type III (Col III). The drug permeation profile was evaluated using Franz diffusion cells. A commercial formulation of 1% diclofenac sodium hydrogel was employed. The rate of drug diffusion across the artificial skin construct was the highest (85.5 $\mu\text{g}/\text{cm}^2/\text{h}$), followed by the skin hybrid (53.1 $\mu\text{g}/\text{cm}^2/\text{h}$) and stratum corneum only (22.1 $\mu\text{g}/\text{cm}^2/\text{h}$). The results showed that the skin hybrid does offer some resistance to drug permeation. However, it cannot fully replace the human skin for *in vitro* drug diffusion studies. Further investigation is required to improve the structure and integrity of the novel hybrid skin construct in order to mimic biological human skin.

TP 14-80. Effects of Seeding Density on Morphological and Functional Properties of Human Chondrocytes in 3D Fibrin Construct: A Preliminary Study

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Seeding density has been found to be a parameter affecting the quality of tissue engineered cartilage. Therefore, human chondrocytes embedded in plasma-derived fibrin were evaluated to investigate the cell behavior by means of morphological and functional properties in an early culture phase. Culture expanded chondrocytes were mixed with plasma-derived fibrin and polymerized to form 3D constructs with two different seeding densities of 2.0×10^6 and 7.0×10^6 cells/ cm^3 , and incubated in F12:DMEM with 10% FBS for 7 days. Spatial distribution and fluorescent staining for F-actin and Collagen Type II were evaluated. The chondrocytes seeded at 2.0×10^6 cells/ cm^3 demonstrated homogenous distribution of cells and Collagen Type II. Meanwhile the cell seeded at 7.0×10^6 cells/ cm^3 exhibited heterogeneous distribution where dense aggregates were seen at the periphery of the construct. Collagen Type II was produced predominantly at the periphery of the constructs. In the high density constructs, cells predominantly developed actin cytoskeleton with elongated morphology, whereas no actin was detected in the low density construct. This indicates that cellular migration

occurs in high density constructs. However further studies with live imaging are needed to be conducted. These preliminary results suggest that lower seeding density of 2.0×10^6 cells/cm³ is suitable for maintaining the quality of native chondrocytes. In conclusion, seeding density is an important factor that affects the functionality of tissue engineered cartilage.

TP15-83. Fabrication of PMMA Nanofibers using the Electrospinning System and its Effects on Fibroblasts Morphology

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Modulation of surface with nanotopographic structures has evolved as an exciting strategy in regulating cell morphology and consequently intracellular signaling mechanism in determining cellular fate. This study was conducted to establish the electrospinning system for fabricating nanofibers of polymethylmethacrylate acid (PMMA) and to evaluate its effect on the morphological features of human dermal fibroblasts. The electrospinning system was assembled with a gamma high voltage power supply with dual voltage supply, a syringe pump and static collector. Various parameters that include the concentration of polymer, distance between syringe pump to collector, voltage and flow rate were tested to fabricate nanofibers with continuous morphology. Fibroblasts were cultured on the nanofibers for 7 days followed by F-actin staining. It was found that PMMA with lower concentration (3-5%) at 15 cm distance with voltage of 5Kv (both positive and negative) and 7ml/h flow rate is a suitable condition to fabricate continuous fibers at a nanometer scale (on average 350nm and 780nm for 3% and 5%, respectively). The cultured fibroblasts on 3% and 5% PMMA nanofibers demonstrated that cell morphology can be regulated into both round and elongated shapes, while fibroblasts in the control case generally appeared spindle shaped. The change in cellular morphology was probably due to the alignment of fibers and the availability of binding sites. These observations demonstrated that the nanotopographic structure acts as a binding site for cells and their morphology can be regulated by using defined arrangements of nanostructures.

TP 16-83. Development of Novel Wounding Technique for Evaluation Wound Healing Properties

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The conventional scratch wound healing assay was developed to study cell migration *in vitro* that mimics *in vivo* healing process. The wound created mechanically by a pipette tip in a confluent monolayer of cells results in a high degree of variability in wound size (up to 33%). This study was designed to develop a new prototype for creating wounds with consistent size. This prototype was used for testing wound healing properties of natural and chemical products. Silicon rubber was attached to the culture surface before inoculation of human dermal fibroblast. After confluence, the silicon rubber was peeled off to create a wound. Wound size and migration efficiency was compared between the wound created by silicon rubber and pipette tip scratching. It was found that silicone rubber created a regular wound size of 0.900 ± 0.001 mm vs 0.38 ± 0.03 mm by pipette tip scratching without affecting migration efficiency. Thus, we used it to test wound healing properties of natural product X. Supplementation of product X significantly reduced the attachment and proliferation of fibroblasts, which is respectively 50% and 62% of control. Moreover, significant reduction of migration was also calculated with live imaging, suggesting that product X is not effective for early phase wound healing, but may be helpful for scar reduction at late stage of healing. Together, all these results indicate that this newly developed prototype can produce constant width for wound healing assay without affecting migration. This model is simple and effective for investigating effects of various substances on wound healing. This product can reduce animal testing in drug discovery.

TP 17-84. The Effective of Tissue Engineering Approaches in Articular Cartilage Restoration.

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Damage and degeneration of articular joints is a major healthcare concern. Restorative techniques, mainly cell-based therapies and autologous or allograft transplants continue to expand, giving surgeons more options for biological reconstruction of the articular surfaces. However despite this great evolution, therapeutic uncertainty in the restoration of damaged cartilage using tissue engineering approaches still remains unclear for the surgeon treating patients to make evidence-based decisions. The purpose of this

review is to assess the hierarchy of studies dealing with tissue engineering strategies applied to restore the articular cartilage while providing a systematic narrative that will enable clinicians to become familiar with the most recent developments in the field. Relevant publications on PubMed from the year 2006-2010 were identified using the keyword "articular cartilage restoration" with the following terms: "tissue engineering", "cell based therapy" and "systematic review". This includes online searches of journals, references and citations to identify English-language literature for restoration of articular cartilage via tissue engineering. 117 articles were identified via PubMed search. Non English language article (n=9) and 32 other studies unrelated to the topic were excluded. Common cartilage restorative techniques include microfracture, osteochondral fragment repair, osteochondral allograft, osteochondral autograft, and autologous chondrocyte transplantation. Current approaches are now focusing on the promising third generation cell therapy which delivers cultured chondrocytes via cell carriers or cell seeded scaffolds together with growth factors and mechanical stimuli for improving cartilage tissue repair. Significant evidence exists now supporting the idea that tissue engineered articular cartilage represents a potentially valid approach to effectively treat cartilage injury or trauma.

TP 18-115. HA-ZrO₂ Composite Characterization for Dental Implant

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Hydroxyapatite (HA)- $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ is a type of biomaterial ceramic which has chemical and mineralogical similar to the component/substance of natural bone. This study was conducted to produce and characterise the composite of HA-ZrO₂. Hydroxyapatite has been synthesized by using precipitation method and further mixed with commercial yttria stabilized zirconia. The effect of ratio percentage composite HA-ZrO₂ mixture has been investigated. Zirconia percentages at 40% and 60% were mixed with HA. The different times of milling process for all samples also have been investigated. From our observation, the peak of phosphorus group $[(\text{PO}_4)^{-3}]$ bending is higher for HA-ZrO₂ with ratio 40:60 (ranging from 1025.97 cm^{-1} to 1033.21 cm^{-1}) compared to HA-ZrO₂ with ratio 60:40 (ranging from 1024.59 cm^{-1} to 1026.26 cm^{-1}). This might be due to the presence of higher ratio of zirconia phase in the mixture, which may affect the nature phase of hydroxyapatite. The property makes the porous hydroxyapatite suitable for a variety of potential biomedical applications including materials for bone repair and matrices for tissue engineering. It is important to prepare the mixture powder of HA- ZrO₂ in nano size so that the product can be used in dental application, especially for teeth implantation.

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