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Topics
- Reprogramming and pluripotency
- Stem Cell and Cancer
- 3D Bioprinting and tissue engineering
- Biomaterials and Tissue Regeneration
- Transplantation and immunomodulation
- Cell and Gene Therapy
- Imaging and Pre-Clinical Model

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Abstracts for Oral Presentation

O-SRM 1

Awareness and perception on stem cells treatment among medical professionals in klang valley

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Purpose: The aim of this study was to assess the awareness and perception on stem cell treatment among medical professionals in Klang Valley.

Methods: A cross-sectional study utilizing a 14-item questionnaire to collect information about awareness and perception on stem cell treatment was conducted involving 100 randomly selected medical professionals.

Results: Majority of the respondents (99%) claimed to have heard of stem cells, courses/meeting/conference and media were the sources of information with highest selection, 54% and 46% respectively. However, only 31% of them self-perceived as knowledgeable in stem cell. Obstetrics and Gynecology specialists showed significantly higher (P<0.05) self-perceived level of knowledge on stem cells (53.8%) as compared to their peers (34.5%). This could be due to their expert knowledge in the science of reproduction, which including the pluripotent stem cells. No significant association was found between perception on stem cells and respondents’ religious or cultural background. In fact, a substantial number of medical professionals in Malaysia do support stem cells research whether on embryonic stem cell or adults adult stem cells (37% and 59% respectively).

Conclusion: There is a very high degree of awareness on stem cells among medical professionals in the Klang Valley, but the level of self-perceived knowledge is still inadequate.
O-SRM 2

Effects of ionizing radiation on chondrocytes growth

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Purpose: To evaluate the possible effects of ionizing radiation on cellular morphology and growth kinetics of monolayer cultured chondrocytes.

Methods: Following research approval (IIUM/IACUC Approval/2015/[5][24]), rabbit’s articular cartilage cells were isolated, cultured and divided into three groups; (1) control, (2) irradiated and (3) non-irradiated. Group 2 cells were subjected to irradiation after it reaches 80-90% confluency. While the control group was left in the incubator, groups 2 and 3 were taken out and put into a temperature-controlled container. They were transported to a typical X-ray examination room, some distance away from the incubator. After irradiating Group 2, both cells groups were returned into the incubator. The X-ray imaging parameters and the collimation used were recorded. Group 2 cells were subjected to irradiation at each different passage, from P0 till P3. Morphological observation and growth kinetics assessments were conducted on all groups after each passage.

Results: There are no differences in terms of cellular morphology in all groups throughout passages. The viability of all groups ranged from 94.1-98.6%. All groups showed reduction in growth rate after several passages. The non-irradiated group exhibited higher total cell yield than the other groups. The performance of growth kinetics of all groups can be appreciated as follows; non-irradiated group > irradiated > control.

Conclusion: Ionizing radiation may have certain effects on the growth of chondrocytes.
Mitochondrial changes associate with induction of human sternal mesenchymal stem cells by combination of basic fibroblast growth factor and hydrocortisone to cardiac lineage

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Purpose: Mitochondria play a central role in cell metabolism. Recent studies have highlighted the changes in mitochondrial morphology and function during the differentiation of stem cells. Combination of bFGF and hydrocortisone reported to induce MSCs into cardiac lineage. Aim of our study is to evaluate the effect of bFGF+Hydrocortisone on maturation of mitochondria in differentiated MSCs.

Methods: In this study, MSCs were induced into cardiomyocytes with 2 different cardiomyogenic induction methods; bFGF+Hydrocortisone and 5-Azacytidine. Immunocytochemistry staining (ICC) was performed to confirm the cardiomyogenic differentiation using cardiac troponin-C, cardiac troponin-I and Connexin43 as cardiac markers. Transmission electron microscopy (TEM) was performed after 7 days of induction to observe the mitochondrial maturation. Morphological properties of mitochondria were evaluated via image analysis.

Results: Our results on ICC showed that positive expression of all cardiac markers in both induced groups. TEM images revealed that mitochondria of induced MSCs were elongated, more dense and large in size, which was similar to cardiomyocytes. In contrast, mitochondria in non-induced group were immature, rare, and globular mitochondria and having poorly developed crista. Quantitative analysis demonstrated that mitochondria in bFGF+Hydrocortisone groups were significantly different (p<0.0001) in area, circularity, elongation and perimeter compare to non-induced. However, there is no significant difference between bFGF+hydrocortisone compare to human cardiomyocytes. bFGF+Hydrocortisone differentiate human MSCs to cardiac lineage and improved mitochondrial morphology in an in vitro study.

Conclusion: bFGF+Hydrocortisone might act as a safer inducer for mitochondria maturation during cardiomyogenic differentiation, which is promising for use in future cardiac regenerative strategies.
Presence of neural-transdifferentiated bone marrow derived stem cells alleviate neuropathic pain after sciatic nerve injury in athymic rats

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Purpose: Biological nerve conduit has been investigated to bridge peripheral nerve gap in animal model as an alternative method to standard surgical care i.e auto-grafting technique. This study was conducted to determine the efficiency of muscle-stuffed vein seeded with neural-differentiated stem cell in promoting functional recovery.

Methods: Male adult immune-deficient rat was used to create peripheral nerve injury model. Human muscle-stuffed vein was prepared from tissues harvested from diabetic-amputated patient with informed consent. A 15 mm of sciatic nerve defect was created and bridged with positive control, autograft (RA); human muscle-stuffed vein: seeded (SMSV), unseeded (MSV); a commercial conduit (NT) and without any treatment (ND) [n=3]. Footprint analysis, nerve conduction study, compound muscle action potential, CMAP and sensory test were evaluated from 2 to 12 weeks. Nerve grafts were harvested and subjected to histochemical staining (H&E staining).

Results: For all rats in MSV group, the footprint analysis was not measurable starting at 6 week due to severe autotomy observed compared to SMSV group while in NT group, observed in one rat. There is fluctuation of mean CMAP observed in SMSV, MSV and NT group resembles the presence of newly regenerated nerve from 2nd week until 12th week of implantation. However there is no significant difference compared to RA group. Histological analysis of nerve graft from MSV group showing haphazard pattern of neuroma which may be related to the severe self-mutilation behavior observed.

Conclusion: The implementation of cells may influence and alleviate severe self-mutilation behavior, which is a behavioral measure of neuropathic pain in animal model.
Establishment of acute lung injury model for cell therapy

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**Purpose:** This study was aimed to develop a model for acute lung injury (ALI) using brushing technique that damaged tracheal epithelium. Tracheal brushing is a practical method for creating injury to the whole respiratory tree without direct insult to the lungs.

**Methods:** An endotracheal tube was inserted into the mouth of the anaesthetized rabbit, and followed by interdental brush. Ten brushing strokes were performed on the ventral aspect of the trachea. H&E staining was performed 1 and 5 d after injury to observe the histopathology of the trachea and lungs. The severity of the injury was measured using a standardized tracheal and lung injury scoring.

**Results:** A proportion of tracheal epithelium was detached 1 d after injury (37.95 ± 0.92\%, \( P < 0.001 \)). The tracheal mucosa and submucosa were progressively thicker as the result of an influx of inflammatory cells (normal 315.66 ± 37.04 µm; injured 877.74 ± 30.82 µm, \( P < 0.001 \)). In lungs, alveolar damage was initiated by the infiltrating neutrophils that accumulate within the lung interstitium, which then resulted in a subsequent loss of the integrity of the alveolar capillary membrane that includes formation of hyaline membrane, alveolar septal thickening, oedema, and also haemorrhage (normal lung 0.03 ± 0.01; damaged lung 0.67 ± 0.02, \( P < 0.001 \)). These pathological hallmarks are termed as diffuse alveolar damage and reflect well the pathological hallmarks of ALI in human. The vigorous acute inflammatory tissue was accompanied by a high expression of IL-1\( \beta \), IL-6, IL-8, and VCAM pro-inflammatory cytokines 1 d after injury. Therefore, it is reasonable to conclude that ALI presented in this in vivo model is significant to clinical situation.

**Conclusion:** This finding demonstrated the feasibility of tracheal brushing that resulted in morphological damage to the lungs consistent with ALI.
**O-GRP 1**

**Generation of dental derived induced pluripotent stem cells under xeno-free conditions and modeling their epigenetic imprinting**

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**Purpose:** Induced pluripotent stem cells (iPSCs) are regarded as a versatile tool for cell therapy because of their embryonic like stemness properties leading to doable clinical contribution in terms of drug testing, disease modelling and personalized cell therapies. While conventionally used reprogramming induction using viral vectors results in higher efficiency, a shift towards nonviral and xeno-free methods is preferred for safer clinical applications.

**Methods:** We have demonstrated a robust reprogramming strategy for iPS generation from dental pulp derived stem cells by combining non-integration mode of episomal plasmids in combination with small molecules along with commercially available xeno-free reagents for generating clinical grade dental derived induced pluripotent stem cells.

**Results:** In our study we observed the reprogramming efficiency of dental pulp stem cells (DPSCs) using episomal vector was higher (0.19%) than the conventional viral methods (0.05%) thereby generating more number of iPS clones. Our results were consistent with the use of five different DPSCs donors. Additionally the robustness of DPSCs as starting cells provides an excellent model for iPS induction due to their exceptional inherent stemness properties, developmental origin from neural crest cells, specification for tissue commitment and differentiation capability.

**Conclusion:** Accordingly, in our study the supportive epigenetics profiling of DPSCs associated with pluripotent and developmental genes explains their favoured transition to dental derived induced pluripotent stem cells.
O-GRP 2

MicroRNA-22 regulates proliferation and differentiation of BMSCs-derived NPCs under the influence of IGF-1

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Purpose: We reported the addition of EGF, bFGF and IGF-1 enhanced cellular proliferation and survivability during differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) into neural progenitor-like cells (NPCs). To unravel molecular mechanisms governing such enhanced differentiation, microarray of microRNAs was performed. Among the microRNAs identified, miR-22-3p was the most down-regulated. Several mRNA targets have been proposed for endogenous miR-22-3p but the function and its targets remain to be elucidated. AKT1, regulating cell survival and proliferation via PI3K/AKT pathway, was up-regulated in IGF-1 treated NPCs. Since miR-22-3p is down-regulated while AKT1 is upregulated during neural induction, it is possible that miR-22-3p may regulate the expression of AKT1. Therefore, this study aims to validate the target of AKT1 by miR-22-3p.

Methods: BMSCs transfected with miR-22-3p inhibitor were differentiated into neural lineage in the presence of EGF and bFGF with or without IGF-1. Cellular proliferation and apoptosis were measured and the target of AKT1 by miR-22-3p was validated by dual-luciferase reporter assay in which BMSCs were co-transfected with miR-22-3p mimic and vector containing 3'UTR region of AKT1.

Results: Inhibition of miR-22-3p in the absence of IGF-1 significantly enhanced cellular proliferation and survivability of NPCs compared to control. Supplementation of IGF-1 further enhanced cellular proliferation and survivability of NPCs. Co-transfection of luciferase vector containing 3'UTR of AKT1 and miR-22-3p mimic resulted in the significant reduction in luciferase expression.

Conclusion: This study demonstrated that miR-22-3p suppresses AKT1 expression, which in turn affected the proliferation and survivability of BMSCs-derived NPCs.
O-GRP 3

Effects of SRY (Sex Determining Region Y)-Box 9 (SOX9) and Telomerase Reverse Transcriptase (TERT) genes transfection in chondrocytes seeded on three-dimensional scaffolds: gross observation and cell proliferation assay

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Purpose: To evaluate the formation of in vitro 3D tissue constructs engineered from the SOX9 and TERT genes post-transfected chondrocytes seeded on poly(lactic-co-glycolic acid) (PLGA) based scaffolds.

Methods: With the approval of Institutional Animal Care and Use Committee (IACUC, IIUM), the post-transfected rabbits’ chondrocytes were seeded on the prefabricated PLGA with and without fibrin scaffolds. The resulting constructs were cultured for three-week. This experiment setting examined the following groups: (1) non-transfected chondrocytes (control), (2) SOX9-transfected chondrocytes, (3) TERT-transfected chondrocytes and (4) SOX9/TERT-transfected chondrocytes; each seeded on PLGA and PLGA/fibrin scaffolds. All eight groups were evaluated for gross morphology and cells proliferation activity.

Results: Grossly, all constructs shrunk in size. The PLGA/fibrin constructs showed a glass-like appearance throughout the culture. The chondrocytes seeded in PLGA/fibrin number showed an increment pattern from day-1 to -21 compared to chondrocytes seeded in PLGA only. The SOX9/TERT-transfected chondrocytes seeded on PLGA/fibrin showed a steadily increased growth pattern comparable to that of control group. Both SOX9 and TERT genes may have synergistic effect in maintaining cell proliferation activity in 3D scaffolds. Overall cellular growth pattern seemed better in PLGA/fibrin than PLGA alone. Besides, PLGA/fibrin constructs exhibited a more stable macroscopic structure throughout the in vitro culture making it a suitable candidate for implantation purpose. Further in vitro study involving construct’s weight, histology analysis, genes expression, biochemical assessments and in vivo implantation are currently underway.

Conclusion: SOX9 and TERT genes transfected chondrocytes incorporated with 3D scaffolds may facilitate the formation of good quality cartilage in vitro.
O-BTR 1

Ovine tendon collagen I (OTC-I) sponge: A natural 3D biomaterial scaffold for skin tissue engineering

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Purpose: Collagen type I is commonly used as a biomaterial to fabricate 3D scaffolds for skin tissue engineering. However, low mechanical strength, faster degradation rate and undesirable structural properties of the collagen scaffolds limit the production of skin substitutes. This study aimed to fabricate OTC-I sponge and evaluate the physical, chemical, mechanical and biological properties of the scaffolds.

Methods: The OTC-I sponge was fabricated by freezing OTC-I solution at -80°C with subsequent freeze drying. The scaffold was crosslinked with genipin (GNP) and carbodiimide (EDC). Gross appearance, porosity, physicochemical and tensile strength were evaluated. The biocompatibility of OTC-I sponge towards human skin cells were also tested via live and dead assay.

Results: Grossly, GNP crosslinked OTC-I sponge appeared light brownish in colour, while other sponges appeared white. Moreover, crosslinking demonstrated significant changes in microstructural, physical and mechanical properties of OTC-I sponge, but no change in the chemical properties was detected. Electron microscopy analysis demonstrated presence of heterogeneous pores in all the sponges. However, non-crosslinked and GNP crosslinked OTC-I sponges has higher porosity and larger pore size compared to EDC crosslinked OTC-I sponge. Besides, GNP crosslinked OTC-I sponge demonstrated higher swelling ratio but lower degradation rate compared to other OTC-I sponges. Crosslinking with EDC and GNP significantly increased the mechanical strength of OTC-I sponge wherein EDC was superior to GNP. Finally, it was found that OTC-I sponge crosslinked with GNP exhibited biocompatibility towards skin cells, while EDC was cytotoxic.

Conclusion: These findings indicated that GNP crosslinked OTC-I sponge possess favourable properties for skin substitutes fabrication.
In vivo evaluation of 3-dimensional PLGA/Atelocollagen/Fibrin scaffolds for intervertebral disc (IVD) regeneration

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Purpose: Poly (lactic-co-glycolic acid) (PLGA) is a widely used synthetic biomaterial in tissue engineering. Incorporation of natural atelocollagen and fibrin may provide PLGA a better surface property for cellular adhesion and proliferation. This paper aims to evaluate PLGA/Atelocollagen/Fibrin hybrid scaffolds seeded with IVD’s annulus fibrosus (AF) cells after in vivo implantation.

Methods: Porous 3D PLGA were fabricated via solvent-casting and salt-leaching technique. PLGA was crosslinked with atelocollagen (PA). Fibrin was pipetted onto PLGA (PF) and PA (PAF) scaffolds. Rabbits’ AF cells were seeded onto each scaffold groups i.e. PLGA only, PA, PF and PAF. The “cells-scaffolds” constructs were cultured for three-weeks and implanted subcutaneously in athymic nude mice for four-week. All constructs were evaluated for gross morphology, histology, immunohistochemistry (IHC) against collagen I and II with PLGA construct as control group. Sulphated glycosaminoglycan (sGAG) and DNA contents were compared to that of monolayer AF culture.

Results: All groups exhibited significant reduction in size but showed increment in weight. Histology analysis indicated high cellular density, supported by high DNA content in all groups. The IHC showed presence of collagen I and II. The PAF showed a significantly higher sGAG content than the other groups. Degradation of PLGA did not hinder tissue formation. Atelocollagen and fibrin may contribute to a higher cellular proliferation and extracellular matrix production in PLGA. Further evaluation using gene expression shall be performed to study the intrinsic properties and possible mechanisms involved.

Conclusion: PLGA/Atelocollagen/Fibrin hybrid scaffolds may become potential candidate for tissue engineering application.
O-BTR 3

A bibliometric and thematic study of tissue engineering research and development in Malaysia by using online databases

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Purpose: The study aimed to examine Malaysian contributions in the field of tissue engineering (TE). This serves to fill the dearth of literature that systematically addresses the trend of TE research and development in Malaysia.

Methods: Using selected keywords, a bibliometric and thematic study of published articles indexed in Elsevier’s SCOPUS and Thomson Reuter’s Web of Science™ Core Collection related to TE were conducted. Publications from 1987 till 2015 were scrutinized. The study explored the publication productivity trends, authorship productivity, collaboration pattern, core journals used and areas of interest. The data was then filtered to identify contributions from Malaysian researchers. These data were examined in relation to the overall publications in the two databases.

Results: There are 224 journal titles that published documents related to TE out of 439 identified publications. Most publications were contributed by institutions of higher learning, involving multi-authors. Active collaborations between local and international researchers were noted. The active research areas and themes were identified with most studies are focusing on cartilage, stem cells and bone study. Researchers in TE in Malaysia seemed to be focusing on specific cell sources, biomaterials, signaling factors and organ systems. An increasing trend in these areas of interest is observed. Discussions were also directed to the current strengths, weaknesses, opportunities and obstacles in TE researches in Malaysia.

Conclusion: The present study can direct TE researchers in Malaysia to tailor their research in fulfilling the overall TE objectives as well as meeting the Malaysian community healthcare needs.
O-BTR 4

Desktop 3D-bioprinter: Printing evaluation of polycaprolactone

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**Purpose:** Currently the transplantation of vital organs such as kidney, liver, heart and lung is the only treatment for their end-stage failure. Because of the lack of donor organs, millions of patients are dying during the waiting period every year. In addition, ethical and religious problems in receiving organs from other people are also serious and still controversial. To close this transplantation gap, tissue engineering approaches offer the promise of restored tissues and organs through the combination of material scaffolds and a patient’s own cells. Recently, methods have been developed to spatially encode and design materials using 3D biofabrication method. 3D bioprinting of structured living cells/organs is relatively very new particularly in Malaysia. We have recently received a desktop 3D-Bioprinter, Biobots (USA) in our lab. Though the printing of cells encapsulated within hydrogel is feasible using this technique, the supporting host material need to be studied beforehand.

**Methods:** The supporting biocompatible material chosen in this study is polycaprolactone (PCL). Several parameters to print the PCL were studied such as pressure and speed. The print construct was designed to be a two layer with various thicknesses.

**Results:** It was demonstrated the system able to fabricate complex structure with controlled size. The printing stability correlates with the pressure and speed of the extrusion process.

**Conclusion:** Extrusion based 3D bioprinting was able to print PCL with defined structure with optimized parameters. We expect that this preliminary report would jumpstart the research on 3D bioprinting in Malaysia.
Toughening of biomimetic scaffolds for tissue engineering

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Purpose: Tissue engineering has great potential in offering solutions and transcending the limitation of current treatment of damaged tissue. A typical approach of tissue engineering involves seeding cells on a highly porous scaffold, which acts as a template of providing microenvironment and promoting the regeneration and proliferation of cells. Recent research shows an improvement of tissue regeneration by applying chemical and mechanical stimuli on the seeded scaffolds using bioreactor. Such external loading can induce failure, and in this regards mechanical properties of tissue engineering scaffolds become critical. However, many biomaterials such as hydrogel have poor mechanical properties and limit the use of bioreactors. This talk will review our latest development of tissue engineering scaffolds having mechanical properties comparable to that of native biological tissues.

Methods: Fish gelatin scaffolds having microstructures mimicking nanometer fiber and porous structure of native biological tissue were developed by using an electrospinning technique. Their microstructure morphology including fiber diameters and porosity was controlled by adjusting the process parameters. Further, natural small intestinal submucosa also was decellularized and reinforced in alginate hydrogel to form composite scaffold.

Results: The fracture toughness of the composite shows similar toughness of natural skin. The developed tissue engineering scaffolds not only have adequate mechanical properties that sustain mechanical loading from bioreactor but also have porous fibrous network structures ranging from nanometer to micrometer length scales mimicking native tissue structures.
Physicochemical and structural characterization of surface modified PMMA nanofiber

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Purpose: PMMA is a polymer that can be electrospun to mimic extracellular matrix (ECM). Surface of electrospun PMMA nanofiber is highly hydrophobic that cause lower cell attachment. The aim of this study was to modify PMMA nanofiber surface and to analyse the physicochemical and structural changes.

Methods: PMMA nanofibers (PMMA) were divided into several group. PMMA, collagen coated PMMA (PMMACOL), UV–irradiated PMMA (PMMAUV), collagen coated UV-irradiated PMMA (PMMAUVCOL), and collagen coated PMMA crosslinked with genipin (PMMACOLGEN). All tested group were subjected to scanning electron microscopy (SEM), atomic force microscopy (AFM), X-ray Powder Diffraction (XRD), and X-ray Photoelectron Spectroscopy (XPS). Respiratory epithelium were seeded on nanofibers and subjected to nucleus staining and SEM.

Results: All PMMA groups shows amorphous nature of the material through XRD test. Based on XPS results, amount of all functional group increased after UV irradiation. C=O and CH3 functional group increased in all collagen coated group. Roughness of scaffold is higher in PMMAUVCOL group (0.238µm±0.02) compared to others. Number of cells attached was observed to be significantly higher in PMMAUVCOL (6.24×10^4±2.77×10^3 cells/cm²) followed by PMMACOL group (3.58×10^4±2.5×10^3 cells/cm²). UV irradiation introduced functional group which caused increase in roughness. Higher amount of cells attached to PMMAUVCOL because of the roughness and the amount of functional group. However, presence of functional group also caused lower amount of collagen adsorption to PMMAUVCOL.

Conclusions: PMMA nanofiber’s physicochemical and structural properties were successfully characterised. UV irradiation and collagen coating were shown to increase PMMA’s functional groups and modify it surface, which contributed to the increased attachment of cells onto the inert PMMA scaffold.
O-BTR 7

Evaluation of human amniotic membrane as a scaffold for periodontal tissue engineering: An in vitro study

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Purpose: Human amniotic membrane (HAM) has many biological properties suitable for periodontal tissue regeneration. Its success as a suitable scaffold depends upon its biocompatibility to cells, which is reflected by good cell attachment and proliferation. This study was conducted to evaluate the ability of this membrane as a scaffold for human periodontal ligament fibroblasts (hPDLFs).

Methods: Commercially available hPDLFs were cultured in α-MEM till passage 6. The hPDLFs were seeded (5.0×10⁴ cells) on 1 cm² glycerol preserved HAM in 6-well plate at 37°C with 5% CO₂. HAM (without hPDLFs) was used as control. Proliferation test (alamarBlue® assay) and scanning electron microscopy (SEM) observation were assessed at day 1, 3, 7, 14 and 21.

Results: Proliferation assay showed that hPDLFs increased significantly (p<0.05) compared to control from day 1 to 7 and decreased from day 7 to 21. SEM demonstrated that hPDLFs attached on HAM on day 1 and became overlapping at day 7, while maintaining their flat shape. However the cells began to change their morphology and became rounded on day 14 and 21. HAM demonstrated good biocompatibility with hPDLFs within the first week of culture as the cells attached and proliferated on the surface of membrane. However hPDLFs’ proliferation declined from day 7 onwards. This phenomenon could be due to density dependent inhibition of growth which eventually leads to cell death and detachment.

Conclusions: The findings suggest that HAM could be a promising scaffold for periodontal regeneration. However, cells’ behavior in relation to the membrane over longer culture duration requires further investigations.
O-BTR 8

**Microvalve Bioprinting of Therapeutic Cells**

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**Purpose:** Three Dimensional (3D) printers are used for driving major innovations in various areas such as manufacturing, engineering, education and medicine. Advances in technology has enabled cells and biocompatible materials to be incorporated into 3D bioprinting. 3D bioprinting can be useful in dispensing therapeutic cells which will increase efficiency and decrease time and labour. It is very strenuous to manually pipette micro well dishes and it opens prospect to error. The idea of this project is to automate this activity with the use of a 3D printer and microvalves. Human Colorectal Carcinoma Cells (HCT116) are used for experiments for viability, consistency and variability.

**Methods:** The development of a systematic program was designed to dispense in 96 well plates. Additionally, the 3D printer has been modified to achieve accurate results.

**Results:** It was found that there was an improvement in variability and consistency and there was a high viability with cells dispensed by the system. The entire process of dispensing a 96 well plate with 100ul volume took 3 minutes and 12 seconds where manual took around 20 minutes.

**Conclusions:** This work demonstrates that the valve-based printing is able to dispense cells with high variability which allows the project to move to the next phase, testing on more delicate cells. The ability to dispense therapeutic cells will pave the way for new studies that require accurate and specific volume of cells.
Bilayer materials consisting chitosan film and gellan gum hydrogel for dressing application

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Purpose: Aim of this research is to develop bilayer materials using chitosan (CH) film and gellan gum (GG) hydrogel for wound dressing application. Norfloxacin is incorporated in CH film as an upper layer to combat the bacteria while the ibuprofen is included in GG hydrogels as an under layer to treat pain.

Methods: Norfloxacin is dissolved in CH solution (CH-NOR) and casted on acrylic plate and dried at 50 °C for 24 h as an upper layer. Then, GG solution containing ibuprofen (GG-IB) is poured on top of the dried CH film in the same acrylic plate, and further dried at 50 °C for 24 h, designated as under layer. The film and hydrogels are then characterized to examine the mechanical performances, drug release behavior, biocompatibility and antibacterial activities.

Results: CH-NOR films shown inhibition against Gram-positive and Gram-negative bacteria depending on the concentration used. GG-IB hydrogels showed improved mechanical property as compared to GG hydrogel with the capability to release the drug within 15 hours due to low swelling behavior. Water vapor transmission rates (WVTR) of GG-IB hydrogel were in the range of commercial wound dressing products. In-vitro cell study on normal human dermal fibroblast cells (CRL2522) indicated that the hydrogel formulation is biocompatible with the human cell line. The GG-IB hydrogel exhibited a slight antibacterial property towards S. aureus with inhibition zone measured at 9.7 ± 1.15 mm whereas

Conclusion: The formulation of GG hydrogel enhanced with an addition of ibuprofen can be a potential material for excellent wound dressing material.
O-NPR 2

Evaluation of *Centella asiatica* (L.) urban extract on nerve regeneration using organotypic model of spinal cord injury

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**Purpose:** *Centella asiatica* (L.) Urban (*CA*) is a well-known herb that has been investigated for effects on *in vivo* neural growth. The aim of this study is to evaluate its effect on spinal cord regeneration.

**Methods:** We evaluated effect of 0, 100, 200, 400 and 800 µg/ml of bioactive extract of *CA* (BECA) on a rat organotypic spinal cord injury model. After 8 days in culture, slices were fixed and immunostained with TUJ-1 to identify neurite outgrowths and GFAP for the astrocytes. Fluorescence image was taken and number and length of neurite outgrowth measured in 5 regions of interest per slice (n=6). Ratio of total area of neurite outgrowth and spinal cord slice to area of spinal cord slice alone was also calculated as the growth ratio.

**Results:** BECA up to 800 µg/ml did not exert any toxic effect to the tissue as MTT test showed 100% slice viability Results showed no significant difference between groups of different BECA concentration and also without any BECA. This can be because of low bioactive compounds in the extract used due to unsuitable extraction method or solvent. It has also been reported that the triterpenes in *CA* actually acts as a synergist to NGF in promoting neurite outgrowth rather than having neurotrophic properties on its own.

**Conclusion:** BECA in the tested concentrations do not show neuronal regenerative effect on organotypic model of spinal cord injury. Future studies can evaluate various extracts of *CA* and addition of NGF in potential synergistic relationship.
O-NPR 3

Cytotoxic effect of *Konjac Glucomannan* on HepG2 and WRL 68 cell lines

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**Purpose:** Liver cancer is the second leading cause of cancer-related deaths. Anti-cancer compound, konjac glucomannan (KGM) derived from *Amorphophallus konjac* has long been used as tumour suppressor. This project aims to determine the effect of KGM on normal (WRL 68) and liver cancer (HepG2) cell lines.

**Methods:** WRL 68 and HepG2 cells were treated with various concentration of KGM. Cytotoxicity assay (MTT) and morphological study were performed after 24, 48 and 72 hours of treatments.

**Results:** Selective inhibition of proliferation of KGM-treated HepG2 cells increased with IC50 of 3.626 mg/mL, while no changes in WRL 68 cells. KGM-treated HepG2 cells showed morphological changes associated with apoptosis. The interaction of mannosylated sugar, to mannose receptors, is believed to activate the downstream MAPK pathway in cells, resulting in apoptosis.

**Conclusion:** These results suggest that KGM is a potential cytotoxic agent selectively against liver cancer cell lines but its mechanism of action needs further investigation.
O-NPR 4

Biocompatibility evaluation of honey in skin cells

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Purpose: Efficacy of honey in wound healing has long been attributed to its physicochemical properties rather than on the cell behaviour. Hence, it is important to evaluate biocompatibility of honey to be used in vitro.

Methods: Pure honey was freeze-dried to powder, and diluted in culture medium. Monoculture of dermal fibroblast and epidermal keratinocyte cells from human skin samples were supplemented with different concentrations of honey ranging from 0.02% to 2% weight per volume. Cells cultured without honey was used as control. Viability of skin cells was evaluated using Presto Blue assay.

Results and Discussion: In dermal fibroblast, there is no significant difference in cell growth between different concentrations of honey up to 0.2% compared to the control. However, at 2% concentration, honey completely inhibits the cell growth. In keratinocyte, cell growth increases gradually with increasing concentration of honey up to 0.2% than the control. Similar to dermal fibroblast, at 2% concentration, honey completely inhibits the keratinocyte cell growth. The current study suggested that the honey effect on the skin cells is dose-dependent manner. However, this effect reaches its maximum at 0.2% concentration as cell started dying with higher concentration of honey. From our preliminary study, 0.2% concentration will be the suitable concentration to be used for cell treatment in the future.

Conclusion: The optimum concentration of honey that shows beneficial effect on cell proliferation was 0.2% and the cytotoxic concentration of honey was 2%.
O-NPR 5

The dose effect of *Centella asiatica* (L.) on the proliferation and neural differentiation of human mesenchymal stem cells *in vitro*

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**Purpose:** Since overdose of herbs can be harmful, herbal dose-effect relationship needs further investigations before realizing its applications into clinical practices.

**Methods:** In this preliminary study, standardized extract of *Centella asiatica* (L.) (SECA) was prepared in aqueous form at different concentrations; 400, 800, 1200, 1600 and 2000 µg/ml. Human mesenchymal stem cells (hMSCs) isolated from Wharton’s jelly umbilical cord and then were treated with SECA alone or SECA in combination of neurotrophic factors during neural differentiation. The cytotoxicity of SECA was evaluated by methyl thiazoly tetrazolium (MTT) assay and IC₅₀ value was determined from dose-dependent curve. The proliferation and neural differentiation of hMSCs were investigated by image analysis and immunocytochemistry, respectively. The neural differentiation assay was quantified using real-time PCR for neural markers expressions; GFAP, P75 NGFr, MBP and S100β.

**Results:** Our results revealed that SECA exerted effects on both proliferation and neural differentiation of hMSCs. SECA up to 1200 µg/ml reduced the proliferation of hMSCs. However, it was cytotoxic to hMSCs beyond 1600 µg/ml with IC₅₀ value, 1800 µg/ml. It showed that hMSCs induced with SECA alone below the IC₅₀ value expressed the neural cell-specific markers, though morphologically it appeared to be unhealthy. This suggests low concentration of SECA has potential to differentiate hMSCs to neural-like cells. Similar phenomenon was also seen, even if hMSCs treated with SECA in the combination of neurotrophic factors.

**Conclusions:** The proliferation and neural differentiation of hMSCs is dose-dependent to SECA. Further investigations are currently being done to unravel the effectiveness of SECA in neural differentiation hMSCs.
Abstracts for Poster Presentation

P-SRM 1

Medical practitioners’ awareness and knowledge on the use of hyaluronic acid in chronic wound care

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Purpose: This study is aimed to ascertain awareness and level of knowledge of medical practitioners on usage of HA in wound care.

Methods: A questionnaire with 47 questions is designed and distributed amongst medical practitioners. A total of 100 practitioners were recruited in Klang.

Results: Majority of participants are medical officers with 34% had 6-8 years of experience in medical field. Mean level of knowledge is 12.48± 3.03. There is no significant association between demographic factors and level of knowledge. About 79% respondents are aware of HA in general but only 44% are aware of its usage in wound care.

Conclusion: Moderate level of knowledge on HA for wound dressing and a high level of perception are observed amongst study participants.
Use of adipogenic biomarker CD10 to screen for adipogenic capability of adipose-derived stem cells from different adipose depots

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Purpose: Adipose tissue is a rich source of mesenchymal stem cells (MSCs), namely adipose-derived stem/stromal cells (ASCs), for regenerative medicine. ASCs from the “good” subcutaneous (S) and “bad” visceral (V) depots are inherently different. We previously reported that CD10 was predominantly expressed in S-ASCs versus V-ASCs in human and mouse. CD10 expression is also correlated with ASC adipogenic capability, consistent with the notion that the S-adipose tissue can effectively increase its triglyceride-storing capacity due to higher adipogenic capability of S-ASCs. The purpose of this study was to screen the adipogenic capability of ASCs, isolated from S- and various V-depots [gonadal (VG), mesenteric (VM) and perirenal (VP) regions] in mice, by using CD10 as an adipogenic biomarker.

Methods: S- and VG-, VM-, VP-ASCs from C57BL/6 mice were isolated and cultured in supplemented DMEM. Real-time qPCR analysis was performed to study CD10 expression level in these ASCs. In vitro adipogenesis was induced by standard adipogenic cocktail to confirm the adipogenic capability of these ASCs.

Results: As expected, the expression level of CD10 is markedly higher in S-ASCs relative to those in VG- and VM-ASCs. However, VP-ASCs expressed high level of CD10 comparable to that in S-ASC. In vitro adipogenesis confirmed the higher adipogenic capability of S-ASCs and VP-ASCs. Despite its visceral anatomical location, VP-ASCs showed biological features of S-ASCs. Further investigation is required to understand the metabolic function of VP adipose tissue.

Conclusion: CD10 is a robust biomarker for adipogenic capability of ASCs and the quality of adipose tissues.
**P-SRM 3**

**Human umbilical cord mesenchymal stem cells improve generation of spheres in human corneal epithelial cells and promote cell migration**

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**Purpose:** Transplantation of cornea limbal stem cells is the contemporary cellular therapy for corneal surface regeneration. Cells in three-dimensional (3D) cultures mimic the microenvironment *in vivo* thus improve the engraftment and survival rates. Umbilical cord-derived mesenchymal stem cells (UCMSCs) which possess regenerative capability and rich with paracrine factors were introduced to human corneal epithelial cells (HCECs) and sphere formation was induced.

**Methods:** Spheres were generated by hanging drop technique from either HCECs alone, or combination with UCMSCs using sphere induction medium (50% DMEM and 50% DKSFM supplemented with 10 ng/ml EGF and 20 ng/ml bFGF). Spontaneous sphere formation was performed on low cell-attachment plate and served as control. Spheres were harvested on day 4 and characterised. Cellular migration was evaluated by scratch migration assay in sphere cultures versus control.

**Results:** Sphere formation was improved by the presence of UCMSCs in coculture with HCECs compared to HCECs alone. Sphere cocultures could be generated after 1 day in 3D culture condition compared to 4 days required in 2D culture condition. Immunofluorescence staining revealed that HCEC in spheres maintained their epithelial stem cell marker p63 without expression of differentiation marker cytokeratin 3. Preliminary study on cell migration demonstrated capability for gap closure was enhanced in sphere cultures.

**Conclusion:** UCMSCs improves generation of sphere in HCECs. 3D sphere cultures showed a better condition for maintaining epithelial stem cell markers. Further functional studies to examine the effects of UCMSC co-culture in spheres for corneal regeneration will be investigated further. This project was funded by Fundamental Research Grant Scheme (203.CIPPT.6711508).
**P-SRM 4**

The effect of autologous human serum on culture and expansion of autologous dermal fibroblasts

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**Purpose:** The effect of human serum as bovine serum replacement in fibroblast culture has been studied and their benefit as fibroblast culture supplement was also proven. However, the possibility of disease transmission and immune reactions still remains even with the use of allogeneic human serum (HS). This study aims to compare the effect of commercially available Fetal Bovine Serum (FBS) and allogeneic human serum (HS) with autologous human serum (AS) on primary human dermal fibroblast (HDF) with respect to cell proliferation and secretion of growth factors.

**Method:** Primary HDF cells were cultured in FBS, HS and AS at various concentrations (2%, 5% and 10%). Proliferation rate were assessed using cell proliferation assay. Quantitative analysis of growth factors TGF-β1 and VEGF was analysed using ELISA.

**Results:** Proliferation rate is highly increased with the use of AS compared to other serum. Although 10% concentration is considered optimum, results shows that 2% of AS serum is comparable to FBS in sustaining HDF culture. Cells cultured in AS also secrete a high level of TGF- β1 and VEGF growth promoting factors compared to FBS and HS.

**Conclusion:** It was concluded that AS is highly beneficial to the proliferation and expression of cytokines in cultured dermal fibroblast. Although the ideal concentration to maintain HDF culture is 10%, the 2% of AS was demonstrated to be sufficient for the expansion of primary HDF cells *in vitro*. Using these preliminary data, further study of AS usage in dermal fibroblast culture for wound healing will be conducted.
**P-SRM 5**

**In vitro hepatocyte-like cells differentiation from human adipose-derived mesenchymal stromal cells – A preliminary study for potential therapeutics and drug discovery**

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**Purpose:** This study demonstrates the potential of human adipose-derived mesenchymal stromal cells (hADSCs) to differentiate into hepatocytes for use in drug discovery research and liver cell transplantation.

**Methods:** hADSCs were isolated from human adipose tissue as previously described. The MSC characteristics of the homogenous hADSCs were confirmed by flow cytometry analysis, and in-vitro multilineage differentiation potential assay. hADSCs were directed to differentiate into functional hepatocyte-like cells (HLCs) using a three-stage differentiation protocol. Briefly, hADSCs were cultured in serum-free medium with addition of activin A to promote the endodermic induction. Further treatment with FGF4 and BMP4 promotes the hepatic differentiation of the cells. In the final stage, the cells were further matured by sequential treatment of HGF, OsM, and dexamethasone. The hepatic functional characterization assays were performed to evaluate HLCs which includes the glycogen storage ability, urea production, and qPCR analysis of hepatic biomarkers (ALB and ApoF). HepG2 cells were used as positive control.

**Results:** After 4-5 weeks of differentiation culture, hADSCs changed morphology from spindle-shaped into polygonal or round-shaped cells resembling hepatocytes. The differentiated HLCs showed overexpression of ALB and ApoF genes, which predominantly expressed in mature hepatocytes as compared to the undifferentiated hADSCs. Moreover, urea production and glycogen storage ability were evident in differentiated HLCs, thus confirming the hepatocyte-specific phenotype.

**Conclusion:** These preliminary findings suggest hADSCs may potentially be induced into hepatic lineage; which will be a useful source for therapeutic purposes and drug discovery studies. Further investigations including hepatic functional assays are yet to be determined.
P-SRM 6

Interleukin-17A enhances osteogenic differentiation, matrix mineralization and alters OPG/RANKL ratio in stem cells from human exfoliated deciduous teeth (SHED)

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Purpose: In this study, the effects of rIL-17A on the differentiation and mineralization activity of stem cells from human exfoliated deciduous teeth (SHED) and SHED-derived osteoblast cells were examined.

Methods: SHEDs were cultured in 2 different conditions (complete alpha minimum essential medium (αMEM) and complete αMEM supplemented with osteogenic reagents. Both culture conditions were treated with 50 ng/ml of rIL-17A. The cells were then analyzed for mineralization activity by Alizarin and Von Kossa staining on days 1, 3, 7, 14 and 21. In addition, quantitative polymerase chain reaction (qPCR) was carried out for selected osteogenic genes such as alkaline phosphatase (ALP), collagen type 1 alpha 1 (Col1), osteopontin (OPN), osteocalcin (OCN), receptor activator of nuclear factor κB ligand (RANKL), osteoprotegerin (OPG) and RUNX2 for days 3,7 and 14.

Results: It was found that rIL-17A had increased mineralization activity by SHED and SHED-derived osteoblast cells. qPCR demonstrated enhanced osteogenic markers i.e. ALP, Col1, OPN, OCN, RUNX2 and OPG expressions following treatment with rIL-17A; however no changes to the RANKL expression could be detected. Interestingly, treatment with rIL-17A had improved OPG/RANKL ratio by the SHED and SHED-derived osteoblast cells.

Conclusion: These results demonstrate that IL-17A enhances osteogenesis by promoting osteogenic differentiation and mineralization activities, but suppresses osteoclastogenesis by altering OPG/RANKL ratio.
P-SRM 7

Proteomic analysis of dermal fibroblast conditioned medium (DFCM)

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Purpose: The aim of this study was to identify the proteins in DFCM, and determine their effect on keratinocyte migration during in vitro healing.

Methods: Confluent fibroblasts were incubated with serum-free keratinocyte-specific medium (KM) and fibroblast-specific medium (FM) to collect DFCM-KM and DFCM-FM, respectively. Concentrated DFCM was analyzed using mass spectrometry for protein composition. Subsequently, DFCM was supplemented to confluent keratinocyte monolayer with scratch wound, mimicking the healing process, and analyzed the expression of genes in wound healing pathway.

Results: Proteomic analysis demonstrated that the concentration and number of proteins were higher in DFCM-KM compared to DFCM-FM. The identified proteins were involved in different biological processes, molecular functions and signal transduction pathway (TGF and WNT signaling, kinases and cells surface receptors), which were important for wound healing. Besides, supplementation of DFCM to keratinocytes during in vitro healing shown to up-regulate the expression of genes involved in healing process, which includes ECM remodeling factors, inflammatory cytokines and chemokines, growth factors and signaling molecules, compared with keratinocytes in control culture medium.

Conclusion: The fibroblast secreted proteins in DFCM support in vitro wound healing. Further investigation is required to demonstrate its potential for in vivo wound healing.
P-SRM 8

The effects of preeclampsia and gestational diabetes mellitus on CD34+ umbilical cord blood: preliminary study of suitability and potency for cord blood banking

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Purposes: This study aims to determine the effects of preeclampsia (PE) and gestational diabetes mellitus (GDM) on cord blood (CB) volume, mononuclear cells (MNC) yield and CD34+ count. The ability of CD34+ cells from CB subjects to express pluripotency, self-renewal and multi differentiation was further assessed.

Method: 112 subjects (32 PE, 42 GDM and 38 controls) were recruited at UKM Medical Centre. The volume of UCB samples were measured before further investigations. MNC were then isolated using ficoll-plaque density gradient method, following CD34+ selection using column magnetic field separation method. MNC and CD34+ cells were counted using hemacytometer. A total of 5x10^3 of CD34+ cells were further plated for CFC analysis.

Result: There were significant reduction of MNC (p<0.005) and CD34+ cells (p=0.001) and CB volume (p<0.0005) in GDM subjects as compared to control. While PE subjects showed significant reduction in CB volumes (p=0.003), compared to control. Interestingly, the hematopoietic stem cells progenitors colonies from subjects with either PE or GDM were significantly lower compared to normal. An exceptional results for progenitor BFU-E, which showed significantly higher colonies number in GDM (94.19±6.21) as compared to PE (73.78 ± 3.06), and control (73.61 ± 2.73) subjects respectively.

Conclusion: These preliminary findings could be useful criterias for selecting both PE and GDM pregnancies for cord blood banking. Furthermore, CFC assays may benefit to predict the successful engraftment and incidence of graft versus host disease in stem cell transplant using UCB for future treatment.
P-SRM 9

Malaysian public awareness and perception on primary dental stem cells and therapeutical application

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Purpose: Stem cells (SCs) from human exfoliated deciduous teeth and dental pulp SCs are rich source of human postnatal SCs with multipotent differentiation potential. In contrast with the other sources, extraction of SCs from teeth is non-invasive with no ethical conflicts. However, the public is not commonly aware of primary dental SCs and its potential in medical applications. Therefore this research is aimed to assess the level of awareness and perception on primary dental SCs among Malaysians.

Methods: Along with information sheet a questionnaire were distributed among the parents who visited the dental clinic from November 2014 to January 2015. Data analysis was done using SPSS.

Results: A total of 60 respondents (38 male and 22 women) participated in this survey, their age range from 18 to 46, and majority (65.0%) of them had tertiary education. In average there were 2 to 3 children in one family. In general, 93.3% of the participants were aware of SCs but 26.7% of them heard about primary dental SCs. A low level of awareness (20.0%) was observed on potential of primary dental SCs for therapeutic applications. Overall education level played an important role on awareness of primary dental SCs (p=0.050), possibility of isolating SCs from primary teeth (p=0.027), support on SCs research (p=0.000), and willingness to conserve their child’s primary teeth in SCs bank (p =0.023).

Conclusion: This study shows a very low level of awareness on primary dental SCs and its therapeutic application and high level of perception on primary dental SCs.
**P-SRM 10**

**Comparison of explant-derived and enzymatic digestion-derived mesenchymal stem cells from Wharton’s jelly**

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**Purpose:** Mesenchymal stem cells (MSCs) with self-renewal and multi-lineage differentiation capabilities have emerged as a promising candidate for tissue engineering and regenerative therapeutics. Recently, explant culture is developed to isolate MSCs to overcome the shortage of enzymatic-digestion method that destroys the meshwork surrounding the cells. This project aims to examine the cell morphology, proliferation and osteogenic differentiation of MSCs isolated using different methods, which are enzymatic digestion and explant culture method.

**Methods:** For enzymatic digestion method, MSCs were digested with 0.1% collagenase for 2 hours at 37°C. For explant culture method, the Wharton Jelly was cut into pieces and transferred to petri dishes. Proliferation was assessed up till 9 days by using Presto Blue reagent. Osteogenic differentiation of MSCs was maintained up till 21 days and was stained with Von Kossa to visualize calcium deposits.

**Results:** The MSCs derived from both methods showed similar morphological features such as fibroblast-like and spindled-shape cells during primary culturing. However, they showed differences in terms of proliferation. Black stains indicating calcium deposits were observed after staining on both samples. The results were consistent to previous studies where explant culture method could acquire MSCs, but the growing conditions and osteogenic differentiation of explant-derived MSCs are still under optimization.

**Conclusion:** Our preliminary results showed that explant culture method could use to obtain MSCs, however, its application in regenerative medicine still require extensive studies.
**P-SRM 11**

**Muscle cells conditioned medium (MCCM) on angiogenesis**

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**Purpose:** Lack of vascularization is one of the major drawbacks for the success of engineered tissue substitutes. The development of new vessels in the engineered tissue will sustain the cell viability during tissue growth and efficient clinical outcome. This project aims to study the migration and network formation of human umbilical vein endothelial cell (HUVEC) using MCCM.

**Methods:** PMMA nanofiber was fabricated by electrospinning technique, and coated with collagen and laminin (PMMA-Col and PMMA-Lam, respectively). Muscle cells containing myoblasts and fibroblasts were cultured on PMMA nanofiber scaffolds and plastic surface (control) until confluence. MCCM was collected by incubating confluent cells with serum-free culture medium. MCCM were then supplemented (25% of culture medium) to HUVEC to evaluate the migration and network formation.

**Results:** The migration of HUVEC was significantly higher when supplemented with PMMA-Col-MCCM and PMMA-Lam-MCCM than Plain-MCCM and PMMA-MCCM. At 24h of culture, tubular-like structures in the network were apparent in HUVEC supplemented with PMMA-Col-MCCM and PMMA-Lam-MCCM. Whereas HUVEC supplemented with Plain-MCCM and PMMA-MCCM shows less elongated and form clumps rather than vessel-like structures. The sizes (means meshes and mean length) and topology (the number of meshes and the number of branching points) of the capillary-like network of HUVEC shows significantly higher for PMMA-Col-MCCM and PMMA-Lam-MCCM group than Plain-MCCM and PMMA-MCCM. Muscle cells cultured on collagen or laminin-coated PMMA nanofibers facilitate the production of factors that enhance HUVEC migration and network formation.

**Conclusion:** Collagen or laminin-coated PMMA nanofibers will facilitate angiogenesis.
Wharton jelly mesenchymal stem cells: proliferation and expansion

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Purpose: Wharton jelly has a rich source of mesenchymal stem cells (MSCs) and proven to be more proliferative, immunosuppressive and therapeutically active cells that have potential for cell-based therapy. The aim of this study was to compare the performance and effects between different types of animal-free culture medium with normal culture medium for human Wharton Jelly stem cells (hWJSCs) culture and expansion.

Method: The umbilical cord tissue that consists of Wharton jelly component was shredded into small pieces and was enzymatically digested using 0.6% of collagenase type 1 for 1-2 hour at 37°C. The isolated cells were cultured in three different animal-free MSCs culture media and DMEM low glucose with 10%FBS as a control in order to analyse the optimum culture condition, cells proliferation and expansion of the hWJSCs.

Results: hWJSCs showed fibroblast-like morphology in monolayer culture for all groups at the initial passages and subsequently, the morphological features of the cells become matured and bigger in size for later passages. However, the cells proliferation and expansion showed slightly higher and comparable for groups cultured in animal-free MSCs medium compared to normal culture medium. hWJSCs held a unique feature of MSCs such as the surface phenotype, the plastic adherence and multipotency. The morphology of fibroblast-like cell from hWJSCs is similar to bone marrow MSC.

Conclusion: The culture optimisation using animal-free mediums is comparable with the normal culture medium for the cells proliferation and expansion of the hWJSCs which important in developing future cell based therapy or clinical use.
P-SRM 13

Establishment of hydrogen peroxide-induced oxidative stress model on neurons derived from animal stem cell lines: A prospective in vitro neurodegenerative model

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Purpose: Oxidative stress and reactive oxygen species (ROS) have been associated with the development of neurodegenerative diseases, characterized by neuronal loss and dystrophic neurites as observed in Alzheimer’s disease (AD), Parkinson’s disease (PD), stroke and traumatic brain injury. Most in vitro neurodegenerative models were established from primary and cancerous cells which are not totally reflect the in vivo scenario as they usually generate only one type of neurons and are unstable. We aimed to establish in vitro neurodegenerative model using neurons derived from mouse embryonic (46C) and rat amniotic fluid (R3) stem cell lines. These cells have the ability to differentiate into neural lineage; an attractive feature to studyneurodegenerative diseases and neurodevelopmental processes. 46C is a transgenic mouse embryonic stem (ES) cell line that carries a green fluorescent protein (GFP) knocked-in in the open reading frame of Sox1, the marker for neural precursor cells (NPCs). The GFP expression indicates the presence of NPCs and the success of neural differentiation assay. R3 was isolated from amniotic fluid of full-term pregnant rats that exhibited a potent neurogenic property.

Methods: Neurons were obtained by two established neural induction protocols: adherent monoculture for R3 and multicellular suspension for 46C. These neurons were then exposed to hydrogen peroxide (H2O2) for 24 hours in a concentration-dependent manner.

Results: H2O2-induced neurons were evaluated based on morphology, cell viability (MTT) assay, and intracellular ROS production (H2DCF-DA assay). Our results showed that 46C and R3-derived neurons underwent apoptosis upon exposure to H2O2 and exhibited a significant decrease in cell survival at the concentrations of 1500 µM and 2000 µM, respectively, with increase in intracellular ROS activity, indicating oxidative stress phenomenon.

Conclusion: These findings strongly indicate that H2O2-induced 46C and R3 cells-derived neurons may be suitable for the establishment of neurodegenerative model.
Laminin and epidermal growth factor (EGF) preferentially enhances migration & proliferation of myoblasts

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Purpose: Human skeletal muscle contains contractile myoblasts and non-contractile fibroblasts. Expansion of myoblasts is still challenging due to slow growth rate of myoblasts compare to fibroblasts. Surface-coating with extracellular matrix (ECM) and/or supplementing growth factors were used commonly to regulate the growth of cells in culture. Laminin, a major ECM in skeletal muscle, and EGF were shown to act synergistically to facilitate myoblast growth and migration. The aim of the study was to compare the effect of laminin and EGF on myoblasts and fibroblasts during in vitro culture.

Methods: Human skeletal muscle tissue was collected from 3 consented patients. A mixed population of myoblasts and fibroblasts were isolated and cultured until passage 4 with F10: DMEM containing 10% FBS. In the co-culture system, a cell specific marker namely desmin was used to identify myoblasts via immunostaining, and unstained cells were identified as fibroblasts. The migration & and proliferation of skeletal myoblasts and fibroblasts on plain and laminin-coated surface with or without EGF were measured via image processing.

Results: The laminin-coated surface shown to enhance the myoblast migration and proliferation compare to that on the plain surface. Moreover, supplementation of EGF on the laminin-coated surface further increases the myoblast migration and proliferation. However, no changes in proliferation and migration were observed for fibroblast on the laminin-coated surface with or without EGF.

Conclusion: The results suggested that laminin and EGF preferentially enhance the proliferation and migration myoblasts by a synergistic effect. Enhancement of myoblast proliferation will enable the production of myoblast-enriched population during in vitro expansion for clinical applications.
P-SRM 15

Evaluation of the chondrogenic maturation of in vitro chondrogenically-induced BMSCs and ADSCs after implantation in nude mice

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Purpose: The body’s ability to confer maturity to in vitro induced cells/tissues after in vivo implantations was evaluated.

Methods: Bone marrow stem cells (BMSCs) and adipose stem cells (ADSCs) were differentiated to chondrogenic lineage in vitro. They were seeded in 2ml fibrin construct and implanted into the dorsum of nude mice. Constructs were harvested 6weeks post implantations.

Results: Both cells were positive to CD (44, 73, 90 and 105), and negative to CD (10, 34, 45 & HLA-DR). In vitro inductions showed characteristic features of chondrogenesis: positive to toluidine blue and safranin O; collagen I, collagen II, agrcencan and sox9 antigens. Pre-implantation gene analysis for cartilage oligomeric protein, sox5, pro-alpha collagen II, chondroadherin, agrcencan, collagen IX, sox9, collagen XI, collagen X revealed that BMSCs had (150, 15, 10, 10, 11, 2, 4, 0.2, 0.8, 31, 1500 and 2) mRNA fold increase, while ADSCs had (300, 35, 80, 30, 300, 2, 6, 0.6, 0.5, 150, 1000 and 60) respectively. Post implantation evaluations revealed further increases. BMSCs became (450, 100, 80, 350, 150, 900, 250, 250, 80, 100, 1000 and 100) of these chondrogenic genes, while ADSCs had (700, 100, 200, 400, 120, 1000, 300, 200, 40, 120, 500 and 80) respectively. SEM analysis of implants showed that both induced samples had close ultra-structures to native cartilage. Implanted chondrogenic induced BMSCs and ADSCs showed significantly higher cartilage qualities compare to their in vitro analysis.

Conclusion: In vivo implantation can provide the necessary clues for the maturity of newly engineered tissue.
P-SRM 16

Stem cell properties of chondrocytes harvested from less- and severely-affected human osteoarthritic (OA) cartilage

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Purpose: Chondrocytes isolated from less-and severely-affected OA cartilage demonstrated self-renewal ability. This study aimed to evaluate the stem cell properties of less affected chondrocytes (LA-C) and severely affected chondrocytes (SA-C).

Methods: Tissue samples from OA patients were graded according to Dougados classification (gross tissue) and OARSI (stained tissue). Cartilage tissue was classified into two groups; LA-C (Grade 0-1) and SA-C (Grade 2-3). Isolated chondrocytes from each group were cultured and compared their expression of MSC surface markers using flow cytometry at P1 and P4. The ability of LA-C and SA-C to differentiate toward adipogenic, osteogenic, and chondrogenic lineages was also evaluated.

Results: Both LA-C and SA-C showed the expression of MSC markers such as CD13, CD44 and CD105 at P1 and P4, however, the expression level decreases significantly at P4. Moreover, LA-C and SA-C were negative for CD14, CD34, CD45, and HLA-DR. LA-C and SA-C also demonstrated the multilineage potential, regardless of their passage number, upon induction to a specific lineage. Un-induce chondrocytes also shown the positive staining for toluidine blue, indicated that the cells were the chondrogenic origin. Whereas, negative staining of the adipogenic and osteogenic marker detected for un-induced cells. This study shows MSC-like cells present in OA cartilage have the ability to be differentiated into multiple lineages. The combined results from cell surface marker and multilineage differentiation shows that chondrocytes from OA patients have similar properties as MSC.

Conclusion: Chondrocytes from both less- and severely-affected regions demonstrate stem cell properties and have the ability to be induced into multilineage.
NF-κB pathway deactivation inhibits migration, mesenchymal transition and stemness of non-small cell lung cancer stem cells

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Purpose: Cancer stem cells (CSCs) are subpopulation of cancer cells that play a pivotal role in tumour development, invasion, metastasis, and recurrence. Our transcriptomic data analysis has identified a significant involvement of NF-κB in regulating the putative CSCs of non-small cell lung cancer (NSCLC). Thus the aimed of the study was to evaluate the effects of NF-κB deactivation on epithelial-mesenchymal transition (EMT), migration and stemness activities of lung CSCs and non-CSCs.

Methods: Different concentrations of BMS-345541, an inhibitor of NF-κB’s IKKβ molecule, were used to treat both positive population of lung CSCs (CD166⁺CD44⁺, CD166⁺EpCAM⁺) and its negative counterparts (*CD166⁻CD44⁻, CD166⁻EpCAM⁻) of A549 cells. The effects of BMS-345541 treatment on both populations of cells were evaluated by their ability to reduce migration and expression of stem cell transcription factors and EMT genes.

Results: NF-κB activation has shown to regulate EMT and cancer cells migration. In addition, it induced the CSCs population in lung cancer by increasing the expression of stemness genes (SOX2, NANOOG, OCT4, KLF4, and ATXN1). Our results have shown that inhibition of NF-κB reduced the expression of EMT transcription factors (SNAI1 and TWIST1) and expression of mesenchymal marker (N-cadherin and Vimentin), hence inhibited the migration of A549 putative CSCs and non-CSCs. Deactivation of NF-κB also reduced the stemness of putative lung CSCs as indicated by the expression of SOX 2, NANOOG, OCT4 and KLF4.

Conclusion: The inhibition of NF-κB had reduced the stemness and migratory capability, and the EMT expression of both CSCs and non-CSCs of NSCLC cells. Therefore our findings suggest that NF-κB could be used as a potential therapeutic target for lung cancer.
P-SRM 18

The effect of Activin-A on cell propagation and maintenance of highly potent stem cells isolated from human full term amniotic fluid

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Purpose: Among different types of stem cells that have been established, stem cells derived from amniotic fluid (AF) exhibit high capacity of self-renewal and have the ability to differentiate into endoderm, ectoderm and mesoderm lineages. While most studies to date emphasize on mid trimester AF cells, data on human full term AF cells are still lacking. Recent finding has reported the existence of highly potent stem cells from full term AF isolated from rat, hence suggesting the possibility of a similar outcome from human full AF. Activin-A, a member of transforming growth factor-beta superfamily has been demonstrated to have various roles in physiological processes including cell proliferation, cell death and cell differentiation. The activated signalling pathway of Activin-A has been associated with pluripotency and maintenance of undifferentiated state of human embryonic stem cells. However, the effect of Activin-A on human full term amniotic fluid stem cell has not been discovered. Thus, the aim of this study is to determine the effect of Activin-A on cell propagation and maintenance of highly potent stem cells.

Methods: AF from caesarean section deliveries at 38-40 weeks gestation was cultured in growth medium with and without Activin-A until passage 4. Growth kinetic, population doubling time and expression of stemness marker genes (Oct4, Nanog and c-Kit) were evaluated at RNA and protein levels.

Results: We show that culture medium supplemented with Activin-A increases amniotic fluid stem cell proliferation as shown by growth kinetic and population doubling time. RT-PCR and protein expression confirm the expression of Oct4, Nanog and c-Kit in cells cultured in culture medium supplemented with Activin-A.

Conclusion: Our results highly suggest that Activin-A promotes cell propagation while maintaining characteristic of highly potent stem cells from human full term amniotic fluid.
P-SRM 19

Aerosol-based stem cell delivery for treatment of ovalbumin-induced lung injury

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Purpose: The aim of the study was to investigate the effectiveness of aerosol-based mesenchymal stem cell (MSC) delivery on lung histopathology in an experimental ovalbumin-induced rabbit model of asthma.

Methods: The rabbits were sensitized and challenged with both i.p and inhalation of ovalbumin. The hAD-MSC was aerosolized into rabbit lungs using the MicroSprayer® Aerosolizer Model IA-1B 48 hours after injury. Post mortem was performed 3 days following cell delivery. Histopathological assessments of the lung tissues were quantitatively scored following treatments.

Results: Histopathological analyses revealed that aerosolized hAD-MSC resulted in improvement of the airway features. This has shown by the remodelling of the airway structures such as basement membrane, epithelium, mucosa, and sub-mucosa regions in the MSC treated group (p < 0.001). The airway inflammation score of MSC treated group indicated a significant reduction of the inflammation and granulocytes infiltration at the peribronchiale and perivascular regions (p < 0.05). Treatment with aerosolised hAD-MSC also has reduced the number of airway inflammatory cells present in bronchoalveolar lavage fluid and inhibited the goblet cell hyperplasia.

Conclusions: We have established an aerosol-based cell delivery as a feasible tool for cell therapy of asthmatic model using the MicroSprayer® Aerosolizer device. Our findings suggest that MSC cell delivery via aerosolization method promotes lung regeneration and repair, therefore can be a valuable tool for future therapy in the treatment of chronic lung injury.
P-SRM 20

The chemo-resistance activity and stemness features of triple positive (EpCAM+/CD166+/CD44+) markers facilitate in the selection of cancer stem cell (CSC) subpopulation in human non-small cell lung cancer (NSCLC)

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Purpose: The current study aimed to characterize a novel subpopulation of cancer stem cell (CSC) derived from non-small cell lung cancer (NSCLC) using a combination of three markers EpCAM, CD166, and CD44.

Methods: The triple positive (EpCAM+/CD166+/CD44+) and triple negative (EpCAM-/CD166-/CD44-) subpopulation were sorted from NSCLC cell line (A549) using fluorescence-activated cell sorting (FACS sorter) and the unsorted cells was used as control. Both the sorted and unsorted cells were subjected to chemo-resistant activity, self-renewal assay (clonogenicity and spheroid formation) and stem cell genes expression.

Results: The triple positive (EpCAM+/CD166+/CD44+) subpopulation was highly resistant to both 5-fluorouracil and cisplatin, showing 80% aldehyde dehydrogenase (ALDH) expression compared to only 67% expression detected in the triple negative (EpCAM-/CD166-/CD44-) subpopulation. The result suggested that high ALDH activity contributes towards greater chemoresistance activity in this subpopulation. Furthermore, the triple positive subpopulations exhibited better clonogenicity, consisted of mostly holoclones and markedly higher (p<0.001) spheroid formation indicating greater self-renewal capacity. Reliably, the expression of the stem cell genes such as REX 1, SOX 2, SSEA 4 and OCT 4 were higher in the triple positive subpopulation indicates that the subpopulation displayed a strong characteristic of pluripotency.

Conclusion: Our study is the first to confirm the existence of CSC based on the expression of the triple positive (EpCAM+/CD166+/CD44+) marker in NSCLC of which the findings could potentially in future leads to a better treatment efficacy in lung cancer patients.
P-SRM 21

Sterility Testing on Cord-Blood derived Cellular Therapy Product by automated culture system

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Purpose: This study aims to minimize the volume of Cord Blood derived Cellular therapy product required for sterility testing by automated culture system, Bactec 9240.

Method: Volume reduced Cord Blood derived Cellular therapy products (CBCT) were processed using automate UCB SEPAX-2 system. Aliquot CBCT were tested for sterility to identify the baseline sterility before qualified CBCT for sterility test. Total 6 strains of bacteria and 1 fungi strain were tested. The bacteria or fungi contaminated CBCT were simulated by spike of known CFU concentration into CBCT. Low volume contaminated CBCT were then inoculation in triplicate to aerobic and anaerobic culture bottles. The culture bottles then incubate at Bactec 9240 incubation system for at least 7 days or until the bottle flag positive in the system. The bottle then retrieve for bacteria strain identification.

Result: Results show that one millilitre is sufficient for detection within 48 hours after inoculation for all 6 bacteria strains and fungi. Assay reproducibility and time to detection (TTD) are consistent within the same bacteria or fungi concentration.

Conclusion: This study indicates that low volume of CBCT can be successfully used for sterility testing with the limit of detection as low as 1-10 CFU/ml. With this technique sterility testing can be achieved with less effect on the quantity of cells in cellular therapy products.
MicroRNomics analysis of in vitro aging of human mesenchymal stem cells

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**Purpose:** Significant progress has been witnessed in the last decade documenting the use of human Mesenchymal Stem Cells (hMSCs) as an alternative resource for cell replacement therapy owing to their intrinsic features of self-renewal and differentiation capacity into therapeutically valuable cell types. Expansion of MSCs in culture is a critical requirement to meet the clinical need. However, MSCs undergo in vitro aging, thus limiting their proliferation and differentiation potential in long term-culture. Cellular aging is the manifestation of a complex interplay of molecular pathways between the gene expression and micro niche which is govern by many processes including miRNA dysregulation. We aimed to determine microRNA signature of primary hMSCs isolated from deciduous pulp (SHED) and Wharton’s Jelly (WJ) associated with in vitro aging in culture.

**Methods:** hMSCs from SHED and WJ were isolated and cultured in vitro. Cells at passage 3 
& 6 were extracted for total RNA for profiling of microRNA.

**Result:** miRNA profiling showed a set of diverse miRNAs that were deregulated in hMSCs isolated from SHED and WJ. These miRNAs have been reported to modulate cellular activity by regulating many cellular processes including epigenetic, cell cycle, senescence, proliferative pathways and metabolism.

**Conclusion:** Our results suggest that further understanding of the regulatory role of miRNAs in cellular aging will not only improve our fundamental knowledge of the aging process but will also advance the development of a more effective and affordable targeted intervention approach for generating therapeutically valuable cell resources for treating many degenerative diseases.
**P-SRM 23**

**Isolation and characterisation of CD166⁺/EpCAM⁺ and CD166⁺/CD44⁺ as markers for cancer stem cells from non-small cell lung cancer of A549**

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**Purpose:** This study was aimed to isolate and characterise the putative cancer stem cell (CSC) populations from A549 non-small cell lung cancer cell lines and to compare the pluripotent capabilities between isolated cell populations.

**Methods:** The cell sorting was performed using flow cytometry analysis and the cells were sorted based on following cell surface markers; CD166⁺/EpCAM⁺, CD166⁺/EpCAM⁻, CD166⁺/CD44⁺ and CD166⁻/CD44⁻ from A549 cancer cell line. The isolated cells were tested for pluripotent capacities by clonogenic and differentiation assays and also expression of several self-renewal genes.

**Result:** Both CD166⁺/EpCAM⁺ and CD166⁺/CD44⁺ showed ability to form colonies and able to differentiate into adipocytes and osteocytes. Expression of transcription factor genes that critically involved in self-renewal of undifferentiated stem cell; SOX-2, NANOG, KLF4 and POU51 were up-regulated in both putative CSCs population when normalized to A549 parental. Among these four genes, NANOG showed the highest expression in both populations with 11.71 and 38.05-fold change, respectively.

**Conclusions:** Both isolated cell populations have the ability to self-renew and differentiate into adipocytes and osteocytes. Both cell populations also showed high expression of all four transcription factor genes. These results showed that both isolated cell populations have pluripotent capability of stem cells.
P-SRM 24

Long term effect of cryopreservation on human primary skin cells

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**Purpose:** To assess the effect of cryopreservation on cells and evaluate the performance of cells after 12 months of cryopreservation.

**Methods:** Redundant skin tissue samples were obtained from surgery with consent from patients. The tissue was cleaned, processed and cultured until passage 3. Upon confluency, cells were trypsinized and total cell yield and viability were determined before and after being cryopreserved. Besides that, sterility and Immunocytochemistry analysis for collagen type I (Col-1) and cytokeratin 14 (CK14) antibodies were also performed after cryopreservation for 1 month, 3 months, 6 months and 12 months.

**Result:** There is no significant difference in growth rate between 1 to 12 months after cryopreservation, except for fibroblast at 6 months. The cells viability drops with time for both types of cells (65%± 3.5% - 89%± 4.5%). Sterility testing showed no contamination after 12 months of cryopreservation and immunocytochemistry analysis shows positive expression for CK14 (keratinocytes) and Col -1 (fibroblast) after 12 months of cryopreservation. Morphologically, cells were able to retain its phenotype. The loss in viability is consistent in all samples and could be due to the possible thermal-cycling affect. The ability of the cells to express specific antibody markers using immunocytochemistry analysis and consistent cell growth indicates that cell is able to retain its characteristic in cryopreservation condition.

**Conclusion:** This preliminary data showed that primary skin cells can be stored via cryopreservation and still retain its characteristics but further investigation for a longer time frame study (e.g 24 months, 48 months) should be conducted.
**P-SRM 25**

**The effect of sticopus chloronotus aqueous extract on proliferation and phenotype maintenance in chondrocytes isolated from human osteoarthritis articular cartilage in vitro**

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**Purpose:** Tissue engineering encapsulated chondrocytes in carrier matrix have been widely used to repair cartilage defect and considered to be promising technique for regeneration of cartilage. However cell expansion is always accompanied by lost of chondrocyte phenotype and cellular dedifferentiation. Sticophus chloronotus, a marine sea cucumber invertebrate is rich in glucosamine, chondroitin and mucopolysaccharide, which can helps in the regeneration of the cartilage. In this study, we evaluated the effect of Sticophus chloronotus aqueous extract on human articular chondrocytes in-vitro.

**Methods:** Human articular chondrocytes were isolated from the knee joint cartilage of patients underwent total knee replacement surgery. Human articular chondrocytes were culture in different concentrations of sticophus chloronotus aquoes extract(SCAE). Cultured chondrocytes were evaluated by means cell morphology and proliferation, quantitative phenotypic expression and immunochemistry technique of collagen type 1, 11, aggrecan core protein and sox-9.

**Results:** Human articular chondrocytes cultured in various SCAE appeared polygonal feature with fibroblastic like features maintaining their chondrocytes characteristic. SCAE supplementation promoted chondrocytes proliferation and increased the expression of cartilage specific markers, collagen type 11, aggrecan core protein and sox-9 when compared to control. A prominent immunopositivity of collagen type 11, aggrecan core protein and sox-9 was observed in SCAE supplementation groups.

**Conclusions:** Supplementation of SCAE could effectively promote proliferation of chondrocytes, upregulating the expression of collagen type 11, aggrecan core protein and sox-9 while downregulating the collagen type 1 that could prevent chondrocyte dedifferentiation. This study showed that Sticophus chloronotus may be useful as a pro-chondrogenic agent for human chondrocytes.
P-SRM 26

Nasal fibroblast conditioned medium enhances cell attachment, proliferation and migration in the *in vitro* wound healing model

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**Purpose:** Mechanical harm, inhalation of toxic agents or micro-organisms infection resulted in denudation of respiratory epithelium and disorder of the barrier function which later required a repair process involved both cell migration and proliferation. Secretory proteins are known to enhance cell attachment, proliferation and increase cell migration. The objective of this study was to determine the effect of fibroblast-conditioned medium on respiratory epithelial cells (RECs) for attachment, proliferation and migration.

**Methods:** Nasal turbinate was obtained from consented patients underwent turbinectomy procedures. The cells were co-cultured in medium until reaching confluency. Fibroblast were differentially trypsinized and cultured separately either in DKSFM or F12: DMEM to acquire nasal fibroblast conditioned medium (NFCM) _DKSFM_ and NFCM_FD, respectively. Cell attachment and proliferation assay were performed on RECs cultured in DKSFM supplemented with 20% condition medium. Confluent respiratory epithelial cells were centrally scratched by using pipette tips, washed and cultured in medium supplemented with 20% condition medium. Wound closure was monitored by capturing images at 20 minutes interval with inverted microscope.

**Results:** The results showed significantly higher cell attachment in NFCM_DKSFM (3452.77±588.1 cell/cm²) as compared to NFCM_FD (2336.1±440.4 cell/cm²) and DKSFM alone (2819.8±509.5 cell/cm²), but the specific growth rate after 7 days of culture was higher in DKSFM (0.019±5.16×10⁻⁴ h⁻¹) as compared to NFCM_DKSFM (0.015±8.94×10⁻⁴ h⁻¹) and NFCM_FD (0.013±1.03×10⁻³ h⁻¹). The mean of migration rate was significantly higher in NFCM_DKSFM (4341.8±385.7µm²/hr) compared to other two conditions, NFCM_FD (1803.38±408.1µm²/hr) and DKSFM (1933.48±271.9µm²/hr).

**Conclusion:** Supplementation of NFCM in RECs accelerated cell attachment, proliferation and migration in the *in vitro* wound healing model.
Calcium in dermal fibroblast conditioned medium (DFCM) enhance keratinocytes wound healing \textit{in vitro}

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Purpose: Fibroblasts secreted factors were collected as DFCM, and shown to facilitate keratinocytes re-epithelialization \textit{in vitro}. Besides the secreted proteins, small molecules such as calcium play a vital role in keratinocytes biological properties. This study aimed to investigate the effect of calcium in DFCM on keratinocytes re-epithelialization process.

Methods: DFCM was prepared by incubating confluent fibroblasts with serum-free keratinocyte-specific (DFCM-KM) and fibroblast-specific (DFCM-FM) medium. DFCM was dialyzed to remove small molecules. The concentration of calcium in DFCM, before and after dialysis, was evaluated. Finally, DFCM was supplemented to keratinocytes to evaluate its proliferation, differentiation, migration and rate of healing.

Results: The concentration of calcium in DFCM-KM, DFCM-FM and DFCM-FM (dialysis) was 0.06, 1.08 and 0.01 mmol/L, respectively. Keratinocytes supplemented with DFCM containing high calcium (DFCM-FM and DFCM-KM+CaCl\textsubscript{2}) showed lower proliferation and migration compared to DFCM containing low calcium i.e. DFCM-KM, DFCM-FM (dialysis) and control. Moreover, supplementation of DFCM-FM and DFCM-KM+CaCl\textsubscript{2} leads to keratinocyte differentiation by forming multilayer, which was not detected in other conditions. The rate of healing of keratinocyte was also enhanced significantly when supplemented with DFCM-FM and DFCM-KM+CaCl\textsubscript{2} compared to other conditions. Calcium was known to induce keratinocyte differentiation. The presence of high level of calcium in DFCM also demonstrated the induction of keratinocyte differentiation process by reducing proliferation and forming multilayers. Besides, migration of keratinocytes during wound healing was increased significantly in the presence of high calcium in DFCM.

Conclusion: DFCM containing a high level of calcium could facilitate skin re-epithelialization.
Assessing human dermal fibroblast viability and shelf life in suspension to determine appropriate storage medium

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**Purpose:** Human dermal fibroblast will be used for clinical application in private clinics. Therefore, it is important to determine the optimal storage medium and shelf life of cells to ensure high cell viability (at least 70%) when delivered to customers.

**Methods:** Cryopreserved human dermal fibroblasts were revived and cultured in F-12:Dulbecco’s Modified Eagle Medium supplemented with 10% Fetal Bovine Serum (FD medium). After harvesting, the cells were stored in three different medium namely, Dulbecco’s Phosphate Buffered Saline (DPBS), normal saline and FD medium for 5, 24, 48, 72 and 96 hours at 4°C before cell viability was evaluated by trypan blue exclusion assay. The cell viability at 0 hour was used as a control. The shelf life of the cells in optimal storage media, selected from the above 3 buffers, was also determined by cell viability evaluation.

**Results:** Cells stored in DPBS, normal saline and FD medium at 96 hours showed 41.9%, 40.0% and 17.2% drop in cell viability, respectively, compared to the control. Further shelf life evaluation found the cell viability to be at 79.9±7.8% after 96 hours storage in FD medium. The results show that FD medium was able to maintain higher cell viability at 96 hours storage, which meets the release criteria for the product, compared to DPBS and normal saline.

**Conclusion:** This study found that FD medium was the appropriate storage medium for human dermal fibroblasts in suspension. Shelf life evaluation showed that the cells could be kept at 4°C for at least 96 hours.
P-GRP 1

Establishment of stable secretable TATκ-GFP fusion protein in 293T cell line: An alternative for transient expression of factors for iPSC reprogramming

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Purpose: Generation of iPSC through delivery of reprogramming proteins with the aid of protein transduction domain (PTD) such as trans-acting activator of transcription (TAT) peptide may serve as a safer alternative method without genetic modification. In this study, we aimed to establish a stable production of secretable fusion protein consisting of novel TATk which is an improved form of HIV-1 TAT domain and green fluorescence protein (GFP).

Methods: 293T cell lines were transfected with a plasmid encoding TATk-GFP fusion protein. The mixed populations of 293T cell lines were then analyzed at different culture time-points by western blot and flow cytometry for its stability in continuously expressing and secreting TATk-GFP fusion protein.

Results: Fluorescence microscope analysis showed the transfected 293T cells successfully expressed the TATk-GFP proteins. However, these stable mixed populations exhibited a decreased trend of GFP expression and secretion with prolonged period of culture. Interestingly, treatment of these transfected cells with DNA demethylation agent, 5-azacytidine (5-Aza) showed a slight increase of GFP expression but not the secretion, thus suggesting the possibility of methylation of the promoter that driving the gene expression.

Conclusion: In this study, stable mixed population 293T cell lines that are able to continuously expressed GFP was established. However, further experiments need to be conducted to confirm the methylation status of the transfected cells in prolong culture period. Preliminary data gathered from this study will lay a basis foundation for a safer approach of generating iPSC through delivery of TATk fusions proteins for direct reprogramming of targeted primary cells.
P-GRP 2

**In vitro transcriptional aberration of CpG-free plasmid DNA**

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**Purpose:** CpG-free plasmid DNA (pDNA) exhibited prolonged transgene expression and provide significant outcome in a recent clinical trial. However, we observed limited transgene expression in cell lines when extending this promising pDNA towards ex vivo approach. Hence, our objective is to determine the basis for the poor performance of the CpG-free pDNA expression *in vitro* and we postulate it is due to the CpG depletion.

**Methods:** Two novel pDNAs of identical CpG-free backbone with Green Fluorescent Protein (GFP) gene of variable CpG content were constructed. These pDNAs were transfected separately into HEK-293FT cell line using lipid-based transfecting agent and GFP proteins expression were compared up to 14 days. Following this, total DNA was extracted at Day 1 to evaluate transfection efficiency. Next, RNA distribution analysis was performed to study the influence of mRNA export by evaluating nuclear and cytoplasmic mRNA at Day 1.

**Results:** The constructed CpG-free pDNAs encode identical GFP protein but have different CpG content in the gene where pZGFP has 0 CpG and pRGFP has 60 CpGs. Upon transfection, pZGFP exhibited significantly lower GFP expression across all time-points when compared to pRGFP. This was not due to difference in transfection efficiency at Day 1. The low pZGFP expression was also not due to discrepancy at the mRNA export level as both pZGFP and pRGFP mRNAs have similar export rate. Instead, the rate of transcription in pZGFP was significantly lower than pRGFP.

**Conclusion:** CpG motif plays a role in improving *in vitro* transgene transcription and our data highly suggest that complete CpG depletion in CpG-free pDNA resulted in reduced rate of transcription.
Generation of neuron stem cells from human peripheral blood derived induced pluripotent stem cells

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Purpose: Induced pluripotent stem cells (iPSC) have recently evolved as a good cell source for disease modelling and therapy. However, most studies have targeted skin fibroblasts instead of easily accessible source such as peripheral blood for reprogramming. In order to harness the cell reprogramming technology, we produced iPSC lines from human peripheral blood samples and evaluated the differentiation potential of the derived iPSC towards neuronal stem cells.

Methods: Peripheral blood of three normal donors was reprogrammed with Sendai virus vectors carrying Yamanaka factors. We chose and maintained the best iPSC clones from each donor in E8 medium. These iPSC clones were characterized by immunofluorescence staining, flow cytometry, karyotyping, RT-PCR and microarray analysis. These clones were also assessed for embryoid body formation and in vivo teratoma formation by using mice model. The established iPS cells are then differentiated into neural stem cells and tested for the presence of neuronal markers.

Results: The iPSC resembled embryonic stem cells morphology with flatter cobblestone-like appearance and prominent nucleus. These cells expressed pluripotent markers such as NANOG, SOX2, OCT 4, Tra-1-81 and have similar gene expression profile compared to embryonic stem cells. Besides showing normal karyotype, the cells were free of Sendai virus and formed embryoid bodies and teratomas. Upon neural differentiation, the iPSC showed neural stem cell morphology and expressed neural stem cell markers.

Conclusion: Trangene-free iPSC clones were successfully generated and characterized in this study. The cells possessed differentiation potential towards neuron stem cells and could be used for neuron cells generation in the future.
P-GRP 4

Generation of hemophilia A mouse induced pluripotent stem cells using polycistronic lentiviral vector in serum- and feeder free culture conditions

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Purpose: The ability to reprogram somatic cells into embryonic stem (ES)-like state known as induced pluripotent stem (iPS) cells has made autologous transplantation therapy possible. iPS cells are not hindered by the ethical and practical dilemmas associated with embryonic stem cells. However, there are still many challenges to overcome; (1) multiple transgene integrations increase tumorigenesis of iPS cells, (2) serum- and feeder conditions introduces xeno-contamination in culture. To overcome these issues, we utilized polycistronic lentiviral vector (LV) carrying the Yamanaka’s factors (OSKM) for reprogramming of hemophilic A mouse tail-tip fibroblasts in serum- and feeder- free defined culture conditions.

Methods: Primary fibroblasts from hemophilic A B6;129S4-F8tm1Kaz/J and wild-type C57BL/6 mouse tail were transduced with an optimal MOI of the virus. Suspected iPS cell colonies were picked within 20 days post-transduction.

Results: iPS-like colonies were isolated from the wild-type mouse fibroblast. Unfortunately, the mutant fibroblasts transduced with the similar lentiviral vector exhibited neuronal-like morphology instead of the expected pluripotent cell colonies. The generated wild-type mouse iPS cells are similar to embryonic stem cells in many aspects including morphology, their properties of self-renewal and pluripotency, and in vitro differentiation into the three primary germ layers. For mutant fibroblast, the emerging of neuronal-like cells post-transduction is speculated as an incomplete reprogramming process. These partially reprogrammed cells could not maintain the stem cells-like characteristic and eventually undergo differentiation process.

Conclusion: Generation of iPS cells from mutant mouse fibroblast was unsuccessful. Instead, we obtained iPS cells from wild-type mouse fibroblast using the same reprogramming manner. These data could provide an insight of reprogramming process.
P-GRP 5

Reprogramming of peripheral blood from Malaysian beta thalassaemia patients to induced pluripotent stem cell

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Purpose: Beta (β)-thalassemia is one of the most common genetic diseases and inherited blood disorder in Malaysia. This disease is characterised by a reduction in the synthesis of haemoglobin subunit β caused by mutation of beta globin gene. Without treatment, affected patients fail to thrive and have a shortened life expectancy. Hence, this research aims to generate induced pluripotent stem cells (iPSC) from the peripheral blood of β-thalassaemia patients and characterize the iPSC cells for their potential use in regenerative medicine.

Methods: We reprogrammed iPSCs from three β-thalassemia patients peripheral blood using Sendai virus reprogramming method. After transforming peripheral blood cells into iPSC, the newly derived iPSC were further characterized through karyotype analysis, pluripotency gene expression, immunofluorescence staining, flow cytometry and microarray analysis. The iPSC were differentiated through multiple steps, including embryoid body formation, in vivo teratoma formation and cardiomyocytes formation. The cardiomyocytes formation was assessed by cardiomyocyte markers expression.

Results: Three iPSC clones from thalassemia patients were successfully propagated and maintained for this study. The iPSC expressed pluripotent markers such as NANOG, SOX2, OCT 4 and Tra-1-81 and free of viral vector. Hematoxylin and eosin staining of teratoma showed in vivo differentiation into three germ layers. The cardiomyocyte formation also expressed positive result on cardiomyocyte markers.

Conclusion: Our study demonstrated a reproducible protocol for reprogramming blood cells by using Sendai virus vector and subsequent differentiation of the iPSC into cardiomyocytes. Maintaining the iPSC would allow the development of autologous cells for the treatment of beta-thalassaemia in the future.
P-GRP 6

Stable lentiviral gene expression driven by constitutive promoters in mouse pluripotent stem cells

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Purpose: This study aimed to assess the duration of transgene expression in mouse embryonic stem cells (ESCs) and induced pluripotent stem (iPS) cells derived from the tail-tip fibroblast of C57BL/6 mice. Both cells were transduced with Lentivirus carrying Emerald Green fluorescent protein (EmGFP) driven by either Human elongation factor alpha (EF1α) or Cytomegalovirus (CMV) promoter.

Methods: The cells were transduced with optimal multiplicity of infection (MOI) of lentivirus. Primary tail-tip fibroblast cells from C57BL/6 mouse were used as a somatic cell control. The cells were sorted from non-GFP expressing cells two days post transduction using FACS Aria III. Time point analysis was started two days post sorting in order to avoid detection of transgene expression from nonintegrated lentivirus. Pluripotency markers analysis by immunocytochemistry was performed. Functional pluripotency analysis by embryo body formation was conducted.

Results: iPSCs present significant GFP expression (80% expressing cells) with persistent level of MFI for up to 30 days and beyond. Surprisingly, ES cells presented lower percentage of GFP expression, with LV/EF1α showing significantly higher MFI when compared to LV/CMV. Functional pluripotency analysis indicated that the cells were able to form good quality EBs.

Conclusion: The research findings might provide an insight for improvement of gene delivery and regenerative medicine using pluripotent stem cells, especially iPSCs, for persistent correction of genetic disorders.
P-GRP 7

**In vitro enrichment culture systems of hepatic cancer stem-like cells exhibit distinct cellular and molecular characteristics recapitulates tumour heterogeneity**

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**Purpose:** Accumulating evidence suggests that cancer stem-like cells (CSCs) which are subset of tumour population contribute to the therapeutic resistance, eventually leading to tumour recurrence and metastasis. Activation of various cellular processes regulated by different intrinsic and extrinsic factors/cues in the tumour microenvironment have been postulated to give rise to heterogeneous population, thus confers differential therapeutic responses and resistant nature of CSCs. We aim to investigate the differential cellular and molecular characteristics of CSC-derived from different in vitro enrichment culture conditions mimicking the heterogeneous nature of tumour mass.

**Methods:** Three in vitro culture conditions for CSC-enrichment were established, including: (i) 3D-tumoursphere culture (ii) TGF-β induced epithelial-mesenchymal transition (EMT) model and (iii) cisplatin-induced resistant CSC model using hepatocellular carcinoma cell line, HepG2. CSC derived from these models were characterised for expression of markers associated CSC characteristics.

**Results:** Different in vitro CSC enrichment methods confer the HepG2 cells distinct morphological and molecular changes associated with stemness, EMT and resistant signatures of CSCs when compared to the parental HepG2 cell.

**Conclusion:** Distinct cellular and molecular signatures in different CSC enrichment models recapitulate in vivo tumour progression, suggesting the heterogeneous phenotypes of CSC may have conserve regulators influenced by the different microenvironmental factors/cues. Discovery of such common CSC regulator(s) will pave a path to the development of drug(s) that can be effective in eradicating cancer.
**P-GRP 8**

**CD36 binding ability of selected peptide phages identified by using phage display technique**

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**Purpose:** CD36 is a class B scavenger receptor and a significant uptake route for oxidised low-density lipoprotein (oxLDL). Accumulation of lipids contributes to foam cells formation, which is the precursor to atherosclerotic plaque. Therefore, CD36-targeting peptides have the potential to be developed as a molecular intervention in treating athero-inflammatory disorders. In this study, we have identified several peptide phages that are able to bind CD36 receptor on cell surface.

**Methods:** Ph.D-12™ Phage Display Peptide Library (NEB) was panned against human recombinant CD36 protein. Subsequently, bound phages were eluted using oxLDL buffer. A total of 50 phage clones were selected and sent for DNA sequencing. The binding ability of selected phages towards CD36-expressing cell lines: CHO-CD36 and U937, was assessed by using anti-M13 phage-PE antibody staining and flow cytometry. Non-expressing CD36 HeLa cell line was used as the negative control cells. Specific streptavidin peptide phage was used as the irrelevant control phage.

**Results:** Sequence analysis showed similar peptide sequences in several selected phage clones. Thus, in total, 24 different clones with unique DNA sequences have been identified. In a binding test comprising of 10 randomly chosen clones, clone B6 showed higher binding ability towards CD36-expressing cell lines compared to HeLa cells. Clone A6 and B11 showed a lower CD36 binding ability while the rest of the clones showed similar binding pattern to that of the control phage.

**Conclusion:** Clone B6, A6 and B11 have different preferential binding pattern towards CD36 receptor on cell surface with clone B6 showing superior ability amongst the clones.
Transduction of human T-cells with Anti-ERBB2 Chimeric Antigen Receptor (CAR)

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Purpose: Breast cancer is one of the most common malignancy among woman. Decades of scientific study links the overexpression of ErbB2 antigen with aggressive tumours. To combat this, the Chimeric Antigen Receptor (CAR) technology can be utilised. Human T-cells are transduced with a gene sequence coding for a CAR that is specific to a tumour-associated antigens (TAAs). These genetically-engineered CAR-T-cells will be able to target the tumour antigen without the need for the major histocompatibility complex (MHC) recognition, rendering it a potentially universal immunotherapeutic option. In this study, we aimed to establish an anti-ERBB2 CAR-T-cells that are able to specifically target an ERBB2 overexpressing cancer cell line.

Methods: CD3+ T-cells were isolated from the peripheral blood mononucleated cells (PBMC). Constructed CAR gene was inserted into a lentiviral plasmid containing the green fluorescent protein (GFP) tag and produced as lentiviral particles. These lentiviral particles were used to transduce the activated T-cells. T-cells were activated using Dynabeads Human T-Activator CD3/CD28 and interleukin-2 (IL-2) priory to transduction.

Results: CD3+ cells were successfully sorted using magnetic beads section. Our results demonstrated presence of GFP expression, indicating successful transduction in the sorted T-cells.

Conclusions: We successfully demonstrated GFP expression of the transduced T-cells. Future studies will focus on in-vitro and in-vivo models to determine the efficiency of specific targeting of the CAR-T-cells on ERBB2 expressing cells.
Tissue engineering and regenerative medicine research and development in Malaysia: A scientometric study based on relevant conferences from 2004 to 2014

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Purpose: To examine the trend of tissue engineering and regenerative medicine (TERM) research progress. The study aims to fill the gap of literature dearth that systematically addresses the trend of TERM research and development in Malaysia.

Methods: A scientometric study of published abstracts presented in International Conference on Biomaterials and Tissue Engineering 2004, National Tissue Engineering and Regenerative Medicine Scientific Meeting 2006 and Malaysian Tissue Engineering and Regenerative Medicine Scientific Meeting 2008-2014 was conducted. The study explored the publication productivity trends, authorship productivity, collaboration pattern, sources of funding and areas of interest. These data were examined in relation to the overall publications.

Results: A total of 362 abstracts were published in 6 conferences from 2004 to 2014. Majority of publications were multi-authored involving public institutions of higher learning. The collaborations between local and international authors were noted. The active research areas and themes were identified. It can be appreciated that the conference participation expands with the coefficient of determination of $R^2 = 0.0775$. Thus, Malaysian researchers seemed to be focusing on various cell sources, biomaterials, signaling factors and organ systems. A declining trend in these areas of interest is observed. Based on the study, certain prominent researchers and institutions are actively upholding the TERM research.

Conclusion: The present study is hoped to shed some lights and serves as a reference towards the advancement of TERM research in Malaysia.
**P-BTR 2**

**Poly(lactic-co-glycolic acid) and atelocollagen hybrid scaffold seeded with annulus fibrosus cells enhances the formation of cartilaginous tissue engineered construct *in vitro***

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**Purpose:** To evaluate the *in vitro* formation of 3D tissue engineered constructs (TECs) using rabbits’ annulus fibrosus (AF) cells seeded on poly(lactic-co-glycolic acid) (PLGA) based scaffolds.

**Methods:** Porous disc-shaped PLGA was fabricated using solvent casting and salt leaching technique. It was crosslinked with atelocollagen to form “PA” scaffold group. Fibrin was added to PLGA and PLGA-atelocollagen composite to form “PF” and “PAF” scaffolds, respectively. The AF cells were seeded into the prefabricated scaffolds (1.0x10⁵ cells per scaffold) to form the following TECs groups: AF+PLGA (AFP; control), AF+PLGA+atelocollagen (AFPA), AF+PLGA+fibrin (AFPF) and AF+PLGA+atelocollagen+fibrin (AFPAF). The resulting TECs were cultured for three-week and evaluated for cells viability using MTT assay, cellular morphology and attachment using SEM, cartilaginous matrix production using sGAG assay and DNA content using PicoGreen® assay.

**Results:** Significant number of viable cells was observed in the AFPAF group (987,985.7±286,858.9 cells) when compared to other TECs (AFP: 373,319.0±5,456.9; AFPA: 547,763.4±66,038.2; AFPF: 463,763.4±46,160.8 cells). Cellular morphology and attachment were comparable in all TECs. The AFPA has the highest sGAG accumulation (0.279±0.117 mg/ml) but shows no statistical difference when compared to the other TECs (AFP: 0.083±0.038; AFPF: 0.237±0.131; AFPAF: 0.181±0.024 mg/ml). The AFPF has the highest DNA content (1,919.338±89.050 ng/ml) but shows no statistical difference when compared to the other TECs (AFP: 485.659±27.468; AFPA: 845.987±82.134; AFPAF: 1,575.007±307.174 ng/ml). Hence, atelocollagen seemed to provide better environment for cellular attachment and proliferation. This unique collagenous material also promotes sGAG production and DNA content in TECs.

**Conclusion:** The incorporation of atelocollagen into PLGA scaffold enhances the formation of TECs *in vitro*. 
P-BTR 3

In vitro and in vivo gene expression studies of cartilage-like tissue engineered construct using a combination of transiently transfected human osteoarthritic chondrocytes and tissue engineering technique

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Purpose: To evaluate the formation of cartilage-like tissue engineered constructs (TECs) using human osteoarthritic chondrocytes overexpressed with SOX9 gene seeded on poly(lactic-co-glycolic acid) (PLGA) with and without fibrin scaffolds through cartilaginous genes expression analysis.

Methods: Upon approval by the IREC18; NMRR-12-1383-14531 and IIUM/IACUC Approval/2015/[5][22], six cartilage samples were obtained from consented patients after joint replacement surgery. The cells were isolated, cultured and transfected with pcDNA3-SOX9 using Lipofectamine 2000™. Prefabricated disc-shaped porous PLGA with and without fibrin were used as scaffolds. The ‘cells-scaffolds’ TECs were formed and cultured for 3-week and implanted subcutaneously at the dorsum of athymic mice for 4-week. Collagens I, II, IX, X, XI, SOX9 and aggrecan expression were evaluated using a qualitative two-step reverse-transcriptase PCR. GAPDH and β-actin genes were used as internal controls.

Results: Presence of cartilaginous markers can be detected in all TECs with various expression intensity. Collagen II, the cartilage-specific marker was down-regulated in vitro but re-expressed in vivo. Collagen I, X, SOX9 and aggrecan were steadily expressed in all TECs. Although collagen IX and XI are closely associated with collagen II, their expressions were almost untraceable except for few cases. Presence of GAPDH and β-actin genes indicated the reliability of the analysis.

Conclusion: Cartilage-like TECs have been successfully formed based on genes expression analysis.
Decellularization of human umbilical cord artery for development of neural conduit for peripheral nerve injury

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Purpose: Various natural biological conduits have been investigated to bridge peripheral nerve injury especially in long nerve gap (greater than 3 cm in human). Autograph, the current gold standard, has several drawbacks including limited availability of donor graft, donor-site morbidity and mismatch in size in clinical practices. The aim of this study was to decellularize the human umbilical cord artery for development of neural conduit in bridging peripheral nerve gap.

Methods: Artery conduits of 1.5 cm were obtained from one human umbilical cord. Artery conduits were decellularized to remove all native cells (n=4). Then, the decellularized artery was characterized by DAPI staining, in vitro degradation assay and swelling assay. Then, 1.0 x 10⁶ mesenchymal stem cells (MSCs), obtained from human umbilical cord, were seeded into 1.5 cm decellularized artery conduit to study cell attachment onto the conduit.

Results: The characterization showed that decellularized artery conduit did not collapse and the lumen remained rigid. DAPI staining revealed that all cellular components of decellularized artery have been eliminated and it exposed extracellular matrix (ECM) collagen fibers that remain intact. In vitro degradation and swelling assay revealed that the conduits did not degrade and swell after 7 weeks.

Conclusion: We successfully developed a neural conduit from human umbilical cord artery, where the decellularization process successfully removed all residual blood, native cells, and other soluble components and currently the seeded MSCs attachment and proliferation on the conduit is being studied.
P-BTR 5

Optimization of nasal fibroblast-secreted proteins profiling for treatment of respiratory epithelium wounds in-gel proteomic approach

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Purpose: Analysis of secreted proteins from cells and the environment interactions could be disrupted by the production of salt and electrolytes and could be one of the limitations in secretome study. The aim of this study was to optimize the secretome profiling in nasal fibroblast-conditioned mediums (CMs) towards identifying proteins that enhance respiratory epithelium wound healing.

Methods: CMs protein from nasal fibroblast were estimated by Bichininoic Acid assay and subjected to pre-screening using SDS-PAGE at several proteins loading concentrations. Due to the presence of high salt concentration or other electrolyte, optimization on pre-treatment for two-dimensional electrophoresis (2DE) and isoelectric focusing (IEF) protocol was performed. Secreted protein profiling was conducted by performing 2DE.

Results: Both serum-free CMs were estimated to have higher amount of protein as compared to the control with total protein content of DKCM (1820 ± 0.01 µg/ml) and FDCM (1420 ± 0.01 µg/ml), respectively. The loading of 5µg protein in SDS-PAGE gel revealed clear and distinct protein bands. Dialysis and buffer exchange technique enabled successful focusing of the proteins at their respective isoelectric point, with total voltage hour of 16.8kVhrs. Protein loading at lower concentration exhibited better separation in SDS-PAGE analysis. Pre-treatment techniques applied in this study helps in perseverance of important protein(s) and successfully produced high resolution of 2DE gel secretome profiles for CMs.

Conclusion: This optimized technique is beneficial for identification of overall high abundance proteins in CMs using gel based proteomic approach and also it enhance maximal protein perseverance for protein-protein interaction (PPI) study between the secreted proteins and the respiratory epithelial cells.
Scale-up the production of dermal fibroblast conditioned medium (DFCM) using poly (methyl methacrylate) (PMMA) nanofiber mesh

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Purpose: DFCM, which facilitates wound healing, was collected by culturing dermal fibroblasts on the polystyrene surface. In this study, we explore the possibility of using nanofiber mesh mimicking the in vivo environment to scale-up the production of DFCM.

Method: Human dermal fibroblasts were cultured on polystyrene surface (control) and PMMA nanofiber mesh until confluence with F12:DMEM (FD) +10% FBS. Cells were then replaced with serum-free FD medium and incubate for 3 days. The waste medium was collected as DFCM. To overcome the starved conditions, cells were then incubated with FD+10% FBS for 3 days. This regime was repeated for five cycles. The DFCM from PMMA mesh (DFCM-P) and control (DFCM-C) was concentrated using a centrifugal filter. Protein concentration was determined using bicinchoninic acid (BCA) assay. Cells morphology were also evaluated during the whole process.

Results: DFCM-P shows higher protein concentration compare to the DFCM-C in all collection cycles, and the maximum concentration was achieved at the third cycle of collection. It was also found that viability of the cells was maintained throughout the process. Phalloidin staining shows that fibroblast became thinner and longer when cultured in serum-free condition, but returns to spindle shape when incubated with FD+10% FBS. The results suggested that nanofiber mesh facilitates the production of DFCM, which can further scale-up by revitalizing the same cells without affecting their viability and morphological feature.

Conclusion: The nanofiber mesh provides an advantageous environment to enhance the production of DFCM. However, further study is required to evaluate its wound healing potential.
P-BTR 7

A novel approach to optimize the fabrication of metal-ceramic scaffold and its regulation on MSC cell behaviour on molecular level for tissue engineering application

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Purpose: Our approach is to optimize the fabrication of metal-ceramic scaffold and its regulation on MSC cell behaviour on molecular level for tissue engineering applications.

Methods: The fabrication method involves the use of powder mixing of Titanium-Hydroxyapatite and Titanium-Wollastonite which is then compacted under a low force to produce a porous structure which is then sintered at high temperatures to produce a porous scaffold. Bone marrow stem cells were isolated and were cultured in several tissue culture flasks to be later expanded until the third passage. Quantitative assessment was carried out using flow cytometry for the identification of 11 cellular markers for bone marrow stem cells. SEM was also performed to obtain the microstructure of the scaffold and cell cytotoxicity was identified with the use of the PrestoBlue® solution. Qualitative assessment was also carried out through the use of a Live-Dead Imaging Kit.

Results: Our study showed BMMSC seeded onto the scaffold were positively expressed CD 73, CD 90 and CD 105. The Live-Dead Imaging test gave the Titanium-Wollastonite scaffold the best cell attachment quality. SEM results also show the scaffolds are porous with a pore size of 200 µm. Cell viability test using PrestoBlue solution revealed that the Titanium-Wollastonite scaffold display a high cell count at 4 week in culture. The Titanium-Wollastonite demonstrated superior cellular attachment in comparison to Titanium-Hydroxyapatite. This is because wollastonite consists of calcium which when combined with silicate provides a congruent transition of ions for cellular absorption. The quantity of cellular attachment also proves that Titanium-Wollastonite is less cytotoxic than Titanium-Hydroxyapatite which is further backed up by the live-dead test.

Conclusion: Further tests will be performed for confirmation such as immunological responses as well as in vivo studies will be conducted for future collection of appropriate biological data.
Three-dimension (3D) scaffolds for muscle tissue engineering

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**Purpose:** 3D scaffolds mimicking the microenvironment of native tissue are commonly used to produce engineered tissue substitutes. In last decades, nanofiber scaffolds fabricated via electrospinning techniques shown to facilitate cellular properties. Also, decellularized tissues became popular 3D scaffolds as it provides complex tissue microenvironment. The aim of the study was to fabricate biocompatible 3D scaffolds using nanofibers, decellularized human amniotic membrane (HAM) and their composite for muscle tissue engineering.

**Methods:** Electrospun fibers were fabricated using PMMA (5%) and PLGA (20%). Moreover, to enhance the biological functionality, PMMA fibers were coated with collagen and laminin. While, PLGA fibers were fabricated on top of HAM. The scaffolds were then characterized for physical, morphological and mechanical properties. Human skeletal muscle cells were cultured on scaffolds and evaluate cells proliferation and morphological properties.

**Results:** Electrospun produce straight and smooth fibers for both PMMA and PLGA. Coating of PMMA fibers with laminin and collagen had no effect on fiber diameter and mechanical strength. In contrast, PLGA fibers containing HAM shows higher mechanical strength than HAM alone and PMMA nanofibers. It was noted that there was no difference in muscle cells proliferation in 3D scaffolds compare to control. However, cells demonstrated more elongated and align structure on 3D scaffolds compare to control. 3D scaffolds containing nanofiber provides higher mechanical strength as well as cellular alignment, which plays a vital role in the development of engineered muscle tissue.

**Conclusion:** Electrospun fibers and decellularized tissue composite scaffold provide an improved microenvironment and structural feature for muscle tissue engineering.
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The potential of PCL/ Collagen nanofibers as drug carrier for gentamicin sulphate

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Purpose: This study aims to fabricate electrospun nanofiber from biodegradable PCL with the combination of collagen and loaded with gentamicin sulphate for topical application, to evaluate the drug release study, to examine fibroblast attachment, cell proliferation, and morphology of cell matrix interaction.

Methods: The polycaprolactone (PCL)/ collagen loaded gentamicin sulphate nanofiber was prepared by using electrospinning technique.

Results: Scanning electron microscopy (SEM) micrograph of the nanofibers showed the addition of gentamicin in the nanofibers resulted in an increase in fiber diameter. The addition of gentamicin in PCL/Collagen nanofibers showed good release until 2 weeks. The MTT assay demonstrated the high viability and proliferation rate of normal human fibroblast cells on PCL/collagen nanofibers with and without gentamicin sulphate. The confocal laser scanning microscopy evaluated the cell morphology and cell spreading pattern interaction of normal human fibroblast cell onto the nanofibers. The results suggest that the PCL/collagen loaded gentamicin sulphate nanofiber was successful in releasing the drug in slow rate, maintaining characteristic shape of fibroblast, besides good cell proliferation.

Conclusion: Therefore, PCL/collagen loaded gentamicin sulphate nanofiber might be potential tools for tissue engineering applications.
Commissural axon projecting direction for different regions of spinal cord during chicken embryonic development

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Purpose: To study commissural axon projection direction among different regions of chicken embryo spinal cord.

Methods: In ovo electroporation was conducted at E3 (3-day old embryo) to limit pCAGGS-GFP plasmids into different regions of chicken embryo spinal cord (cervical, anterior limb, anterior thorax, posterior thorax and posterior limb). Open-book was performed at E6 to observe axon projecting and Image J was used to quantify grey-level of axons projecting to different directions. Then paired-t test was used to estimate the significance between rostral projection quantity (RQ) and caudal projection quantity (CQ) for different regions. The analysis of variance followed by Dunnett’s T3 was used to estimate the significant difference of RQ/CQ among different regions. Difference was considered to be significant when $P$-value was less than 0.05.

Results: For the above five regions of spinal cord, most axons project rostrally and there was significant difference between RQ and CQ ($P<0.01$). For RQ/CQ, significant difference existed in the five regions ($P<0.01$), except for the comparison between cervical region and anterior limb ($P>0.05$). Furthermore, posterior limb region was the one most likely to project rostrally, followed by posterior thorax, while anterior thorax has the least partial to project rostrally, followed by anterior limb and cervical region.

Conclusion: Commissural axon projection direction preference was evident in different regions of chicken embryo spinal cord.
Polylactic acid microspheres loaded gentamicin using emulsion solvent evaporation technique

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Purpose: The use of antibiotic carriers in treatments can decrease microorganisms more efficiently. In this study, gentamicin was loaded onto poly(L-lactic acid) (PLLA) microspheres for a controlled release. Gentamicin is a very hydrophilic drug and tends to come out into the water phase when microspheres are fabricated using emulsion evaporation technique. Therefore, double emulsion evaporation (water/oil/water) technique were applied in fabricating PLLA microspheres.

Methods: Three different concentration of gentamicin; 1000 ppm, 2000 ppm and 10 000 ppm were loaded during fabrication of PLLA microspheres in this study. Surface morphology of loaded PLLA microspheres were evaluated using scanning electron microscopy (SEM). Particle size of microspheres were measured using Malvern particle size analyser. Drug loading and encapsulation efficiency were calculated from concentration data using UV-Vis spectrometer.

Results: SEM images showed that the microspheres had a smooth surface and the shape of the microspheres are almost spherical. Size of PLA microspheres of loaded and unloaded drug are is below 211 micrometre with PLA loaded gentamicin with highest concentration has smallest particle size average 40.1 µm followed concentration 2000 ppm with 58.9 µm and the lowest concentration 1000 ppm with size 66.9 µm.
Coating and surface treatments enhance scaffold degradation

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Purpose: Different compositions of materials in a structure determine the wear-strain of the devices during the regenerative and repair process. The drug delivery devices shall have high biocompatibility and patients’ compliance since they will be reserved in the body until fully degrade (non-removal devices) or until several time-point before being removed (removal devices).

Methods: In this study, different scaffold materials were tested, which were hydroxyapatite that was coated with different concentration of alginate (uncoated, 1%, 3% and 5%), beta-triphosphate with different surface treatment and coating (untreated and uncoated, 2% alginate, 4% alginate, 6% alginate, uncoated silane-surface treated, and silane- treated with 2% alginate), poly(lactic) acid microsphere with different surface treatment with NaOH (0.05, 1.0, 3.0, and 5.0) as well as hydroxyapatite/beta-triphosphate composite (20:80 and 70:30 ratios) were tested for the calcium release profile during degradation assay. The calcium can serve as a platform for osteoblast and osteoclasts homing and attachment during the bone regeneration process. All samples were immersed in phosphate buffer saline and the degradation assay was carried out for 21 days. At the end of the assay, each immersion was tested for the calcium content.

Results: The finding showed that the hydroxyapatite samples relase high profile of calcium, followed by the silane-treated; both uncoated and coated with 2% alginate and HA/TCP composite; both ratios. However, PLA microspheres release lowest calcium content.

Conclusions: These findings suggest that the degradation of the samples is dependent on the materials of the scaffold. The finding was also suggested that surface treatment and coating onto the scaffolds enhanced the degradation rate of the materials.
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Optimization on size of Poly(lactic) acid) microsphere

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Purpose: There is a need in modern therapeutic technique for safer and effective devices for drug delivery into targeted organs or tissues of interest. The main aim in drug delivery system is to provide the drugs or therapeutic agents a sufficient amount of drugs at the right time and at the right targeted area with optimized efficacy and high patient compliance, but with minimum side effects. The size of the nanoparticles plays important roles towards the effectiveness of the drug delivery process. This study is focused on optimization of the particle size of poly(lactic acid) microspheres.

Methods: During emulsion-solvent-evaporation technique, two different methods were tested which were stirring and sonicator methods. In stirring technique, the mechanical stirrer was tested in three different speed; 65 rpm, 150 rpm and 250 rpm. The resultant microspheres were then collected and tested for particle size distribution.

Results: There was no difference in particle size distribution among all the tested parameters in the stirring technique. The particle size of 65 rpm was 28-89µm, 28-94µm for 150 rpm and 56-118µm for 250 rpm. However, by using the sonicator technique, we were able to obtain microspheres with sizes ranges from 572-841µm.

Conclusions: Microspheres obtained using the sonicator technique will be able to serve as a carrier for mesenchymal stem cell during the stem cell treatment for life-threatening diseases such as diabetes mellitus, cancers as well as heart diseases. The effectiveness of microspheres obtained using the sonicator technique in homing of the stem cell will be tested in the future.
P-BTR 14

Effect of laminin coating on collagen type I scaffold onto human respiratory epithelial cell culture

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Purpose: Cell-cell interactions and the extracellular matrix (ECM) are key features contributing to unique cell/tissue-specific microenvironment. The aim of this project was to study the mucosalization capability of the laminin coated collagen sponge seeded with human respiratory epithelial cells by synergistic effect of combination of laminin and collagen.

Methods: Three dimensional collagen scaffold was fabricated by freeze-drying ovine tendon collagen, crosslinking it with genipin and coating it with laminin. Human respiratory epithelial cells (RECs) and fibroblasts were seeded on laminin coated collagen scaffold, maintained in submerged culture for two weeks and then changed to air-liquid interface culture for another two weeks. Thereafter, the construct was fixed and analysed by immunostaining and SEM. Cell morphology, distribution, proliferation and migration was determined by staining and analysis with confocal laser scanning microscope.

Results: The staining of fibroblast and RECs with Hoechst dye and CellTracker™ Red CMTPX dye, respectively, before seeding onto construct showed that the collagen scaffold contained interconnected pores and the cells were able to migrate into the collagen scaffolds. The ultrastructural study of the construct showed the cells had formed a confluent layer on top the scaffold and actively secreting extracellular matrix. The attachment and proliferation of both fibroblast and RECs was found to be the highest in laminin-coated genipin-crosslinked collagen sponge.

Conclusion: The laminin-coated genipin-crosslinked collagen sponge was found to be suitable for development of \textit{in vitro} respiratory epithelium model. This ongoing study now focuses on stratification of fibroblasts and RECs, formation of ciliated cells and subsequently a functional respiratory epithelium.
P-BTR 15

Fabrication and characterization of PLGA electrospun fiber-human amniotic membrane (EF-HAM) composite scaffold for cardiac patch

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Purpose: Tissue-engineered cardiac patch confers an advantageous benefit over direct cell transplantation by retaining large numbers of cells close to the infarct site. In this study, we fabricated a composite EF-HAM scaffold, and evaluated its physical characteristics as a potential supporting matrix for cardiac patch.

Methods: HAMs were decellularized using thermolysin followed by alkaline treatment. To confirm the complete decellularization, HAMs were counterstained with DAPI and observed under fluorescence microscope. PLGA fibers were subsequently spun on HAM via electrospinning to create a composite EF-HAM scaffold and subjected to scanning electron microscopy (SEM) for morphological evaluation of composite scaffold.

Results: Cells were completely removed by treating HAM with 18.75ug/ml of thermolysin for 10 minutes followed by soaking in 0.25 M of NaOH solution for 1 min. SEM analysis revealed the presence of fibrous collagen matrices in basement membrane of decellularized HAM without any detectable damage. Straight and beadless fibers were achieved when 20% (w/v) PLGA polymer (50:50) dissolved in dichloromethane:dimethylformamide (7:3) solvent were spun with 15 kV of applied voltage, 0.3 ml/hr of flow rate and 15 cm of deposition distance. Averaged fiber diameters of PLGA fibers significantly increased from 821.4 nm to 1,369.0 nm as we increased the deposition time from 3 mins to 7 mins.

Conclusions: A simple yet effective method was established to decellularize HAM without compromising the structural integrity of ECM. Electrospinning produced microscale PLGA fibers with favourable morphology expected to support the growth of skeletal myoblasts to be used for cardiac patch.
P-BTR 16

Potential of poly (DL-lactide-co-glycolide)/collagen nanofiber scaffold in cartilage tissue regeneration

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Purpose: To fabricate and characterize the collagen coated poly(DL-lactide-co-glycolide) nanofiber scaffold (PLGA/Col) for cartilage regeneration.

Methods: Electrospun PLGA nanofibers were fabricated using lactic acid/glycolic acid content ratio of 50:50. Immunogenicity of nanofibers was investigated using human peripheral blood mononuclear cells (PBMC) and T cells. PLGA nanofibers were further coated with collagen and evaluated its tensile strength (dry and wet condition) and degradation rate. To assess the biocompatibility, chondrocytes isolated from human osteoarthritic cartilage were cultured on PLGA nanofibers, and evaluated cell attachment and extracellular matrix (ECM) deposition.

Results: PLGA nanofibers did not provoke any immune response. In the dry condition, collagen coated PLGA nanofibers leads to a reduction in elongation at break, whereas there are no significant differences in Young’s modulus or tensile strength. In the wet condition, there is an overall decline in the tensile strength of all samples, while pure PLGA exhibits a higher reduction. In addition, PLGA/Col nanofibers degraded faster than PLGA nanofiber. However, no obvious morphological changes in both scaffolds were observed. The PLGA/Col nanofibers showed better cell attachment and proteoglycan production than PLGA alone. PLGA nanofiber was found immunogenically safe and biocompatible for chondrocytes. Coating the PLGA nanofibers with collagen provides a hydrophilic environment for better cell attachment and facilitate matrix production.

Conclusion: In conclusion, PLGA/Col nanofiber has potential to be utilized for cartilage reconstruction.
Physico-chemical and biological activity of Malaysia *channastriatus* and *micropeltes* water extract on human foetal lung cell (IMR-90)

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**Purpose:** Local fishes in Malaysia including *Channa* species, particularly *Channastriatus* (haruan) have been traditionally used for wound healing and reducing pain. For that reason, this study was carried out to determine the *Channaspecies* water extract physico-chemical and its bioactivity on human foetal lung cell (IMR-90).

**Methods:** *Channastriatus* (haruan) was used in this study. The sample was trimmed carefully to obtain the maximum fish fillet and extracted by using normal soaking extraction method (fish weight-to-solvent ratio = 1:4) as to procure the haruan aqueous extract. The extract was characterized for its physico-chemical properties such as pH, moisture content, rheological properties (*n* = 3), amino acid composition and fatty acid determination (arachidonic acid)(*n* = 2). The effective concentration (EC₅₀) of the extract was conducted via 2-dimensional (2-D) culturing system on a normal human fibroblast cell (IMR90).

**Results:** The fish’s essence physical properties are as followed: 1) pH 6.34 ± 0.01, 2) 97.3 ± 0.15% of moisture content and 3) viscosity of 1.50 ± 0.31 mPas. The extract contained essential amino acid, glycine and fatty acid (arachidonic acid), which could aid in the process of wound healing despite their low concentration. However, the presence of those compounds have shown insignificant growth as compared to the control (without treatment) (*p*>0.05). Moreover, the immobile and irresponsible IMR90 growth rate on different extract concentrations was consistent with the absence of mortality throughout the 3-day incubation period (*p*>0.05).

**Conclusion:** For that reason, the extract EC₅₀ values via 2-D cell culture system could not be determined conceivably due to the higher fish weight-to-solvent ratio which produced extremely low concentration during each cell treatment and shorter incubation time (stable post-treatment cell proliferation).
Effects of flaxseed extract on the viability of stem cells from human exfoliated deciduous teeth

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Purpose: To determine the effects different concentrations of flaxseed crude extract on the viability of stem cells from human exfoliated deciduous teeth (SHED).

Methods: Whole flaxseeds were ground and extracted using 99.8% ethanol in a Soxhlet chamber. The crude extract was kept in a closed container and stored at 4°C until further use. SHED (ALLCells, USA) was cultured and maintained in Alpha-MEM supplemented with FBS and antibiotics at 37°C in a CO₂ incubator. Cells were trypsinised and 1000 cells were seeded in a 96-well plate. After 24 hours of incubation, SHED were treated with different concentrations of Flaxseed crude extract (0.0-16 mg/ml). The cell viability was determined using MTT assay after 3 days’ incubation. The morphology of the cells was also observed.

Results: The SHED viability was more than 100% until a concentration of 8 mg/ml of flaxseed extract and an IC50 and IC25 values at 11.85 mg/ml and 10.82 mg/ml respectively which lead to cell death. Additionally, there were also morphological changes when treated with these different concentrations. Cells appeared healthy and maintained the fibroblast-like shape at 4 mg/ml. However, at 8 mg/ml, some of the cells cytoplasm started to demonstrate vacuolation and granulation and at 16 mg/ml, all cells appeared non-viable.

Conclusions: The cell viability of SHED was more than 100% until 8 mg/ml of the flaxseed extract consistent with the morphological changes indicating the potential of this extract to be used further in research relating to tissue engineering involving stem cells.
**P-NPR 3**

*Centella asiatica* enhances neuronal protein markers expression and dendritic thickness of neurons differentiated from animal stem cell lines

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**Purpose:** Neurons differentiated from stem cell provide a potential model that can be used in understanding brain-related diseases as well as for the search of adjunct for prevention or therapy of the diseases. *Centella asiatica*, locally known as Pegaga has a long history in traditional medicine, especially used in Ayurvedic medicine to enhance memory and clarity of thinking. Treatment of *C. asiatica* leaf extract at certain concentrations on rats showed a significant increase in dendritic arborisation, while *in vitro* treatment of *C. asiatica* on a neuroblastoma cell line enhanced the neurites outgrowth. However, the effect of *C. asiatica* on stem cell-derived neurons has not been documented. This project aims to evaluate the ability of *C. asiatica* extract called RECA in enhancing the formation of neurons when treated during the neural differentiation process of mouse embryonic (46C) and rat amniotic fluid (R3) stem cell lines.

**Methods:** Adherent monolayer and multicellular suspension protocols were used to generate neurons from R3 and 46C, respectively. MTT assay was used to determine the effect of RECA on the cell viability of both cell lines. The effect of RECA on the size of embryoid bodies (EBs) formed was assessed. Evaluation on the effect of RECA on mature neurons was carried out based on the observation on neuronal morphology, measurement of dendritic thickness, and quantification of neuronal protein markers expression (B-tubulin, MAP2, GFAP and ChAT) through flow cytometry analysis.

**Results:** Increase in number of viable cells to more than 100% was observed when undifferentiated as well as differentiated 46C and R3 were treated with certain concentrations of RECA in addition to bigger sized EBs. Treatment of RECA was also observed enhance the morphology of differentiated neurons, based on the analysis of dendrites formation and neuronal markers expression.

**Conclusion:** These findings highly suggest the neuro-enhancement effect of *Centella asiatica* on stem cell-derived neurons.
P-NPR 4

Effect of Qur’anic recitation on chondrocytes growth using scratch wound assay: work in Progress

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Purpose: This study aims to identify the potential effects of the Qur’anic recitation, particularly Surah Al-Fatihah on the wound healing activity of chondrocytes derived from rabbit articular cartilage.

Methods: A serially cultured and expanded chondrocytes was used in this study. A cellular model was established in vitro and divided into four groups. The first and second groups were exposed to recitation of Surah Al-Fatihah and an Arabic poem respectively. The third group was exposed to a Western poem recitation. The exposure duration of the recitations to all groups was standardized to 14 minutes. The fourth group was not exposed to any sound and serves as control. As the cells reach 80-90% confluency, a single line or scratch wound was introduced. Growth kinetics assessment was performed to study the healing activities within each group. Any significant changes were recorded as photomicrograph.

Results: Initial findings showed that the cells exposed to Qur’anic recitation showed faster and favourable healing effect compared to Arabic and Western poem. Growth rate, cell viability and total number of doubling were found to be increased with the Qur’anic recitation. Future work involving more samples will be conducted to validate these initial findings. It is found that the Qur’anic recitation was able to increase the proliferation and reduce the time to heal. The use of the Qur’anic recitation can be utilized to facilitate the cartilage regeneration in tissue engineering studies.

Conclusion: This preliminary study exerts the positive effect of Qur’anic recitation on the wound healing activity of chondrocytes.
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Polysaccharide based co-supplementation on cancer cells

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Purpose: Cancer is one of the leading causes of death worldwide. Various studies aimed for developing new substances for antitumor treatment with minimum toxic potential, and polysaccharide such as lentinan was investigated for this purposes. In this study, Konjac glucomannan (KGM), a type of natural polysaccharide was examined for its potential as anti-tumor treatment on breast cancer cells (MCF-7). The aim of this study was to investigate the anti-cancer properties of polysaccharide-based co-supplementation on MCF-7.

Methods: Experiments were conducted by treating cells with five different concentrations of KGM, D-mannose and co-supplementation of KGM and D-mannose. MTT assay and cell morphology study was done after 24, 48 and 72 hours treatments.

Results: Co-supplementation of KGM and D-mannose reduces the viability of MCF-7 after 72 hours treatment with IC50 of 1.8 mg/ml, meanwhile KGM alone acted on the cells at IC50 1 mg/ml after 72 hours. From morphology studies, spindle-like cells were resulted after 72 hours treatment. The reduction of MCF-7 viability resulted from the treatments can be due to the reaction of the cells with the receptors present on the cell’s surface. Treatments with KGM alone and the co-supplementation gave slight differences on the viability as the receptors are sensitive and acted differently on different types of sugar isomers of KGM and D-mannose.

Conclusion: The reduction of MCF-7 viability elucidates the anti-cancer properties of polysaccharide based co-supplementation. Extensive studies on the interactions of polysaccharide to the cell’s receptors need to be done to further understand this complex behavior.