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A002

Promoting Bone Regeneration in PLGA by the Addition of Calcium Sulphate and Fucoidan via the Synergistic Effect of In-Vitro Degradation Rate and Mechanical Strength

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ABSTRACT

Introduction: Poly (lactic-co-glycolic) acid is a polymer material that has received recognition from the FDA and is the choice of researchers for the preparation of bone-graft materials. Calcium sulphate (CS) exhibits excellent bone graft material properties due to its biodegradability, ease of shaping, good biocompatibility, and exhibit osteoconductivity. However, the low osteogenic activity and fast degradation rate of CS severely influence its bone regeneration efficiency. Although it is preferable in clinical use and has exceptional biocompatibility, it shows a weakness in degradability, which causes limitations to bone material. However, the degradation rates of PLGA and CS are significantly different. CS exhibits a rapid resorption rate and is unstable, while PLGA exhibits a slow degradation rate. The combination of these two materials may allow for the tailoring of their respective degradation behaviours. **Aim:** This study aimed to investigate the effect of incorporating Calcium sulphate with or without Fucoidan (PLGA wt 20%, CS 40%, Fu 40%, or PLGA wt 20%, CS 80%) on setting properties, in vitro degradation behaviour, and morphological characteristics of PLGA/CS/Fu and PLGA/CS. Herein, CS and Fucoidan were incorporated into PLGA to overcome these issues. The incorporation of CS significantly promoted the adhesion and proliferation of human bone marrow stem cells (hBMSCs). Therefore, the addition of CS and Fu together with PLGA microspheres is a promising way of modifying the osteogenic activity and degradability of PLGA via in-situ pore generation and promoting osteogenic differentiation. **Materials and Methods:** A composite comprising PLGA, CS, and Fu was prepared using the freeze-drying technique. The morphology and crystallinity of the material were examined through FESEM, EDS, and FTIR. The final weight of

the material after 30 days served as a test of the material's mechanical properties and in vitro degradation. **Results**: The results revealed that adding PLGA to the calcium and Fucoidan composite enhanced the strength and decreased the degradation rate of Calcium Sulphate. The new combination composite shows biodegradable material. The degradation test showed a fucoidan-dependent increase in the PLGA/CS/Fu pH compared to PLGA/CS. Whereas PLGA and CS showed an accelerated mass loss compared to that observed for PLGA, CS, and Fu. The results show that the material from CS with a combination of PLGA and Fu shows good compression results and a reliable degradation period. **Conclusion**: Based on the present in-vitro results, it can be concluded that CS and Fu can be successfully introduced into PLGA without exceeding the setting time beyond clinically acceptable values. In addition, material from CS and Fu added to PLGA showed good bone cell growth and calcium deposition. Future investigations should focus on translating these findings into in-vivo applications.

Keywords: Calcium sulphate (CS); degradation rate; Fucoidan (Fu); In-vitro Degradation; Poly (lactic-co-glycolic) acid (PLGA)

Acknowledgement: This work was supported by Monash University Research Grant (ECR-000002)

A003

Preparation and Characterization of Porous PLGA Microsphere with Simvastatin and Titanium Dioxide Nanotubes (TNTS) for Bone Scaffold

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ABSTRACT

Introduction: Porous structure of bone scaffold promote cell development while also providing mechanical support for bone tissue regeneration. Simvastatin (SIM) stimulates osteoblastic activity while suppressing osteoclastic activity. Titanium dioxide nanotubes (TNTs) favour in bone tissue engineering due to their biocompatibility, mechanical strength, unique chemical resistance, and physical behavior. This study aims to use TNTs to address the initial burst release and mechanical properties issue associated with porous SIM/PLGA microsphere that sintered at 37˚C. **Materials and Methods:** TNTs in the anatase form that produced by hydrothermal method, were combined with porous SIM/PLGA microspheres utilizing modified double emulsion solvent evaporation techniques. The desired porous morphology, drug release, and mechanical properties of SIM/PLGA/TNTs with different TNTs concentrations (0.02%, 0.06%, 0.10%) were evaluated using FESEM, HPLC and texture analyzer. Human fetal osteoblast cell lines (hFOBs) were used to evaluate the scaffolds' in vitro performance. **Results and Discussion:** SIM/PLGA/TNTs (0.02%, 0.06%, 0.10%) showed spherical porous morphology with lower porosity at 51.65%, 56.87% and 59.95%, respectively, compared to SIM/PMP with 87.71% porosity. The in vitro release behavior of porous SIM/PLGA/TNTs revealed no burst release, but rather controlled and sustained release for up to 21 days. Our study revealed that the incorporation of TNTs at lower concentration (0.02%), modestly enhanced compression strength at 0.18MPa, whereas higher TNTs concentrations (0.06% and 0.10%), resulted with reduced compression strength at 0.04 Mpa and 0.07 Mpa, respectively. Porous SIM/PLGA/TNTs (0.02%) scaffold induced proliferation and cell viability in hFOBs within treatment time of 24, 48 and 72 hours. **Conclusion:** The porous structure SIM/PLGA/TNTs microsphere successfully presented a slow drug release, however the compression strength of

porous SIM/PLGA microsphere scaffold being sintered at 37°C reduces with the present of TNTs. Our investigation confirmed that the incorporation of TNTs in a porous structure incorporated with SIM could be possibly induced the osteoblast proliferation for bone tissue regeneration.

Keywords: Microsphere; PLGA; porous; simvastatin; titanium dioxide nanotubes

A004

Organ-On-Chip: A Potential Biophysical Modelling Tool ToModulate Breast Cancer Metastasis

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ABSTRACT

Introduction: Breast cancer is the most prevalent cancer affecting women worldwide, with metastasis accounting for 90% of mortality. As breast cancer progresses, collagen is deposited to increase the surrounding extracellular matrix (ECM) stiffness, triggering breast cancer metastasis. However, existing *in vitro* models to study matrix rigidity in breast cancer are less physiologically relevant to investigate the underlying mechanisms, as they are typically conducted in conventional 2-dimensional (2D) monolayer models. Therefore, we aim to develop an organ-on-chip system using a microfluidic deviceconsisting of two compartments, including a spheroids compartment and a chemoattractant compartment, interconnected by invasion channels. **Materials and Methods:** We first embedded 3D breast tumor spheroids derived from MDA-MB-231 cell line into the alginate/matrigel composite matrix with both stiff (50 kPa) and soft (25 kPa) properties, before seeding them into our organ-on-chip platform. Subsequently, 20% fetal bovine serum (FBS) as a chemoattractant is introduced to initiate the first step of cancer metastasis, which involves cell morphological changes prior to invasion. The changes in spheroids are observed using an inverted microscope. **Results:** By measuring the morphology and circularity changes of our 3D breast tumor spheroids on-chip, we observed that the spheroids in the stiffer ECM exhibited a more spread-out morphology (circularity index: 0.22) compared to those in the softer ECM, which maintained a more circular shape with a circularity index of 0.35 by Day 2. **Discussion:** This result suggests that the stiffer ECM triggers the cancer metastasis initiation on Day 2. **Conclusion:** Given its ability to observe and perform real-time imaging on the breast tumor spheroids' morphological change and migration in different ECM stiffness, our organ-on-chip system holds great potential as a biophysical modelling tool for studying breast cancer metastasis.

Keywords: 3D spheroids culture; breast cancer metastasis, extracellular matrix stiffness; organ-on-chip

Acknowledgement: We thank Ministry of Higher Education Malaysia Fundamental Research Grant Scheme (FRGS/1/2022/SKK06/MUSM/03/1).

A005

Investigation of Cellular Traction Force as a Drug Testing Readout for In Vitro Cancer Metastasis

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ABSTRACT

Introduction: Metastasis is responsible for 90% of cancer-related deaths in solid tumors. However, the effects of metastasis in different anticancer drugs have been left largely unexplored. Existing preclinical models primarily focus on antiproliferative agents on the primary tumor to halt the cancer growth but not in metastasis. Unlike primary tumors, the process of metastasis requires cancer cells to exert sufficient traction forces through the actomyosin machinery to migrate away from the primary tumor site. Therefore, we aim to explore the potential of traction force as a novel readout for screening drugs that target cancer metastasis. **Materials and Methods:** We first established in vitro invasive and non-invasive cancer models using the MDA-MB-231 and MCF-7 cell lines, respectively. We then selected cisplatin and 5-fluorouracil (5FU), a paradigm antimetastatic drug and a non-antimetastatic drug respectively, to evaluate the potential of cellular traction force as a readout for in vitro cancer metastasis. Subsequently, we characterize and correlate the cell morphology, invasion assay, and their corresponding traction forces of MCF-7 and MDA-MB-231 following drug treatments. **Results:** We observed that the invasive cancer model, MDA-MB-231, displayed an elongated spindle-like morphology, contrasting with the more spherical shape of the non-invasive cell model, MCF-7. We also found that the MDA-MB-231 exhibit a higher average magnitude of force compared to MCF-7. When subjected to drug treatment, our results demonstrated a significant difference in the average magnitude of cellular traction force between MDA-MB-231 and MCF-7 in response to the drugs. **Discussion:** Through the correlation of cell morphology, cell invasion assay, and cellular traction force, we have demonstrated that cellular traction force can directly quantify the forces accountable for cell movement, to distinguish the antimetastatic drugs from the non-antimetastatic drugs. **Conclusion:** Our findings indicate that cellular traction force is an invaluable tool for drug testing, particularly in the context of cancer metastasis.

Keywords: Breast cancer metastasis; cellular traction force; drug testing readout; MCF-7; MDA-MB-231

Acknowledgement: We thank the Early Career Researcher (ECR) Grant Scheme 2022 (ECR-000043) supported by the School of Pharmacy (SOP), Monash University, Malaysia (SOP/SRG-ECR/04/2022) for providing the research funding for this study.

A007

Preconditioning of Baicalein-Enriched Fraction (BEF) Promotes Neural Stem Cell-Based Therapy for Ischemic Stroke Recovery via JAKMIP1-STAT6-Gabra 6 Pathway

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ABSTRACT

Introduction: Ischemic stroke is currently one of the leading cause of mortality and long-term adult disability worldwide. The only approved drug for ischemic stroke therapy is recombinant tissue plasminogen activator (rtPA), which has a narrow therapeutic window of approximately 3 to 4.5 hours and can potentially cause detrimental side effects if administered beyond this time frame. As a result, researchers have turned to stem cell-based therapy as a promising alternative for ischemic stroke treatment to offer wider treatment time frame and better repair of damaged brain tissue. **Materials and methods**: In this study, the therapeutic potential of neural stem cells (NSCs) preconditioned with baicalein-enriched fraction (BEF), derived from the leaves of *Oroxylum indicum*, was evaluated using *in vivo* ischemic stroke rat models. **Results:** The results demonstrated that rats treated with NSCs preconditioned with BEF at optimum concentration of 3.125 µg/mL for 48 hours exhibited significant improvements. These improvements included decreased brain infarct volume (9.79%), reduced neuronal degradation (47.26%) and inflammatory cells infiltration (50.81%) and increased blood vessel density (168%), compared to rats treated with non-preconditioned NSCs or the control group. Besides, the treated animals also showed significant improvement in neurological behavior within 24 hours after treatment and promoting beneficial genes expression. **Discussions:** These findings suggested that BEF can enhance the therapeutic potential of NSCs by promoting beneficial genes expression such as angiogenesis (ANGPT1), antioxidant activity (SOD2), anti-inflammation (IL-1Rn) and neuroprotection (JAKMIP1, STAT6, NGF, NFKβ) in the rats treated with BEF-

preconditioned NSCs. **Conclusion:** In conclusion, BEF shows promise as a potential neuroprotective drug, which could significantly enhance the clinical treatments for ischemic stroke in the future. Further researches and clinical trials are needed in order to validate these findings and explore the full potential of this approach.

Keywords: Baicalein; *Oroxylum indicum*; preconditioning strategy; stem cell-based therapy

Acknowledgement: We thank Ministry of Higher Education Malaysia for the grant funding from the Fundamental Research Grant Scheme with Project Code: FRGS/1/2019/SKK08/USM/03/7 and Universiti Sains Malaysia (USM) for the support in granting permission to the investigators to use the space and assets belonging to the university during the process of conducting the research.

A008

The Effects of Hif-1α Accumulation on the Proliferation and Tenogenic Differentiation of Human Adipose Derived Mesenchymal Stromal Cells *In Vitro*

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ABSTRACT

Introduction: Tendon injuries account up to 50% of all musculoskeletal problems and remains a challenge to treat owing to the poor intrinsic reparative ability of tendon tissues. Mesenchymal stem cells (MSC) therapy is a promising alternative strategy to augment tendon repair due to its proliferative and multilineage differentiation potential. Hypoxic conditioning of MSC with the activation and stabilisation of hypoxia inducible factor-1 alpha (HIF-1α), have been shown to enhance their tenogenic differentiation capacity. To understand the cellular and molecular mechanisms involved, the effects of Roxadustat, a specific hypoxia mimetic mediator, on the proliferation and tenogenic differentiation of adipose derived mesenchymal stromal cells (ADMSCs) were examined. **Materials and Methods:** Isolated ADMSCs were expanded and characterised. Microscopic imaging, cell proliferation and cell viability assay were used to evaluate the cytotoxic effects of Roxadustat and HIF-1α inhibitor (CAY10585). HIF-1α level was measured using ELISA assay. The optimum concentration of Roxadustat and CAY10585 were used to induce the tenogenic differentiation of the cells. The experimental cells were analysed using total collagen assay, collagen immunocytochemistry staining and tenogenic gene markers expression. **Results:** The isolated ADMSCs fulfilled the minimal criteria of MSCs. Based on cell proliferation assay, 12.5 μM Roxadustat for 24h pre-conditioned and 3.5 μM CAY10585 gave minimal effective inhibitory effects to the cells. 12.5 μM Roxadustat shows the highest expression of HIF-1α with significant reduction when the cells further treated with CAY10585. Roxadustat pre-conditioning up-regulate the expression of Collagen 1 and 3 that involved in matrix remodelling. The expression of transcription factor

Scleraxis, Tenascin C and Collagen 3 are mediated by HIF-1α. **Conclusion:** HIF-1α induced by Roxadustat pre-conditioning, can influence the cell proliferation and triggers tenogenic differentiation of ADMSCs. This study may provide a valuable fundamental information for the Roxadustat pre-conditioned ADMSCs application on pre-clinical *in vivo* model in tendon regeneration.

Keywords: Breast cancer metastasis; cellular traction force; drug testing readout; MCF-7; MDA-MB-231

Acknowledgement: We thank the Early Career Researcher (ECR) Grant Scheme 2022 (ECR-000043) supported by the School of Pharmacy (SOP), Monash University, Malaysia (SOP/SRG-ECR/04/2022) for providing the research funding for this study.

A009

A Co-Culture Model for Studying Amelioration of Microglia-Mediated Neurotoxicity

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ABSTRACT

Introduction: Microglia are protective neuroinflammatory cells that releases reactive oxygen species (ROS), nitric oxide (NO) and cytokines to remove damaging molecules, but in the process may harm healthy neurons. Previous work showed mesenchymal stem cells can reduce microglial inflammatory responses. To prove whether this ameliorates neuronal damage, *in vitro* microglia-induced neurotoxicity models (MINT) have been developed; however, several caveats were unaccounted for. **Materials and methods:** We develop a MINT using BV2 microglia and N2a neuroblastoma cells, IFNγ, LPS, serum-deprivation (SD) and retinoic acid analogue, EC23, and characterize with Griess, DCFDA, MTS and LDH assay, live imaging, beta-tubulin III (Tuj1) immunocytochemistry, NeuronJ, AnnexinV/PI flow cytometry and ApoTox Glo assay. BV2 cells were stimulated with IFNγ, LPS and IFNγ+LPS, in co-stimulation and priming protocols. Co-stimulation 200 ng/ml LPS + 2.5 ng/ml IFNγ was chosen based on intracellular ROS and NO production. N2a cells were differentiated with SD to 0.5% and 0.1% FBS ± 1 µM EC23. **Results:** Tuj1-staining and NeuronJ revealed 0.5% FBS-treated group had significantly longer total neurite length (79.47±57.14 µm) compared to undifferentiated cells (56.63±26.57 µm) and EC23-treated cells (75.42±61.15 µm) ($p<0.05$). 0.5% FBS and EC23-treated cells had significantly longer primary neurites (84.15±58.04 µm and 83.07±64.75 µm, respectively) compared to undifferentiated cells (58.31±26.72 µm). Live imaging showed neurite retractions in LPS and co-stimulated BV2 supernatant at 24 hours while Tuj1 photomicrographs indicate reduced cell number and length. **Discussion:** Limitations discovered were that N2a cells die in the process of differentiation, making supernatant-based assays such as MTS and LDH results not representative of neurotoxicity, and live imaging revealed N2a neurite extension is a reversable process, unlike post-mitotic neuron formation. Future works are to

determine extent of apoptosis and necrosis. **Conclusion:** Overall, in supernatant transfer MINT model with BV2 and N2a cells, neurotoxicity is best demonstrated by live imaging, parameters of neurite length and counts.

Keywords: BV2; in vitro; neuroinflammation; neurite; N2a

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A010

Prediction and Expression of Microrna Targeting Il-17a in The Chondrocyte and Synovial Fibroblasts Isolated from Osteoarthritic Knee

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ABSTRACT

Introduction: Osteoarthritis (OA) is a prevalent degenerative joint disorder associated with pain and disability. Dysregulation of microRNAs (miRNAs) and inflammation process play significant roles in OA pathogenesis, with interleukin-17A (IL-17A) being a key pro-inflammatory cytokine implicated in cartilage degradation and synovial inflammation. Understanding the regulation of IL-17A signaling in chondrocytes and synovial fibroblasts is crucial for unraveling the underlying disease mechanisms. This study aimed to predict and determine the expression level of miRNAs targeting IL-17A in chondrocytes and synovial fibroblasts isolated from osteoarthritic knee joints. **Materials and Methods:** Bioinformatics tools, including TargetScan, miRWalk, and miRDB, were employed to identify potential miRNAs targeting IL-17A. The miRNA with the highest and the lowest predicted binding affinity were selected. DIANA-mirPATH analysis web server was used to identify potential pathways that could be targeted by selected miRNAs. The expression levels of these miRNAs were assessed via quantitative polymerase chain reaction (qPCR) in isolated chondrocytes and synovial fibroblasts from osteoarthritis patients. CT value was calculated in the study. **Result and discussion:** Hsa-mir-1913 exhibited the highest predicted binding affinity to IL-17A mRNA, while hsa-mir-514a-5p showed lower binding affinity. These miRNAs were used as a comparative measure to validate the accuracy of the results obtained from the bioinformatics tools. Target prediction of the expressed miRNAs identified different inflammation-linked pathways that are consistent with previous studies. The qPCR analysis demonstrated that hsa-mir-1913 showed consistent upregulation in chondrocyte and synovium fibroblast samples (14.36 ± 0.72; 14.12 ± 0.69). On the other hand, hsa-mir-514a-5p (-4.65 ± 0.45; -5.05 ± 0.60) exhibited consistent downregulation in both samples.

This implies that hsa-mir-1913 is expressed abundantly in the chondrocyte and synovium fibroblast samples, leading to the detection of higher mRNA levels with fewer amplification cycles (lower CT values) and stronger fluorescence signals. It is essential to acknowledge that this study represents a preliminary investigation into miRNAs that potentially regulate IL-17A in cartilage and synovial fibroblasts of osteoarthritis patients. Further research is needed to fully understand the specific mechanisms and functional consequences of these miRNAs in the pathogenesis of osteoarthritis. **Conclusion:** This study successfully predicted and detected the expression of miRNA targeting IL-17A in chondrocytes and synovial fibroblasts isolated from osteoarthritic knee joints. The identification of miRNA targeting IL-17A may highlight the intricate role of miRNAs in modulating the inflammatory and degenerative process associated with OA and open new avenues for future research and development of targeted therapies for OA.

Keywords: Chondrocytes and synovial fibroblasts; interleukin-17A; microRNAs; osteoarthritis

A011

The Effects of HIF-1α Accumulation Induced by Hypoxia-Mimicking Agent on the Apoptosis of Human Chondrocyte from Osteoarthritic Cartilage *In Vitro*

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ABSTRACT

Osteoarthritis (OA) stands as the most common chronic joint disease, imposing a significant socioeconomic burden. Despite this, no treatment has proven entirely successful in effectively stopping or reversing the degradation of cartilage, which is the key pathological characteristic of OA. Chondrocytes, the sole resident cells in cartilage, are responsible for producing extracellular matrix (ECM) proteins to maintain the structure and integrity of cartilage. A continuous loss of chondrocytes through apoptosis is believed to contribute to cartilage deterioration. This creates a vicious cycle, where the progression of one factor exacerbates the other, further increasing the severity of OA. A transcription factor referred to as hypoxia inducible factor 1α (HIF-1α) is rising as an attractive target for OA treatment owing to the hypoxic nature of cartilage. In this study, we investigated the effects of HIF-1α accumulation induced by hypoxia-mimicking agent, Roxadustat, in modulating chondrocyte apoptosis. Human chondrocytes were isolated from the cartilage of OA patients. A cell death model was established using an oxidative stress inducer, tert-Butyl hydroperoxide (TBHP) to mimic chondrocyte apoptosis. HIF-1α protein induced by Roxadustat was quantified using ELISA assay. The effects of HIF-1α protein accumulation on chondrocytes were analysed based on cell morphology, cell proliferation, cell viability, and cell apoptosis, using microscopic assessment, alamarBlue® assay, trypan blue cell counting and Annexin V-Propidium Iodide assay, respectively. Our results showed that HIF-1α accumulation in chondrocytes was highest when treated with 20 μM of Roxadustat for 12 hours. Pre-treatment with Roxadustat protected chondrocytes from apoptosis, as evidenced by the significant

reduction in the number of early apoptotic cells. In conclusion, chondrocyte apoptosis induced by oxidative stress inducers such as TBHP could be inhibited by HIF-1α accumulation. The findings in this study serve to provide fundamental insights into the role of HIF-1α in chondrocyte survival in cartilage tissues.

Keywords: Cartilage; cell death; chondrocytes; hypoxia; oxidative stress

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A013

The Effects of Pro-Inflammatory Cytokines on Tenogenic Differentiation of Human Mesenchymal Stromal Cells Subjected to Cyclic Tensile Loading

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ABSTRACT

Currently, mesenchymal stromal cells (MSCs) are recognized as an ideal cell source for tendon regeneration due to immunomodulatory properties, mechanosensitivity and ability to differentiate into tenocytes. During inflammation, pro-inflammatory cytokines stimulate catabolic changes in tendon cell matrix. The inflammatory niche plays a key role in triggering the reparative and immunomodulatory functions of MSCs. However, the role of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) on tenogenesis of mechanically stimulated MSCs have not been elucidated. A study was thus conducted to investigate the effect of pro-inflammatory cytokines on the morphology, proliferation, collagen, and catabolic protein expression, and tenogenesis of MSCs *in vitro*. MSCs were isolated, expanded, and characterised. Isolated MSCs were stimulated with optimum concentration of IL-1β (6 ng/mL) and TNF-α (8 ng/mL), respectively, with exposure to cyclic tensile loading (1Hz+8%) for 24 and 48 hours *in vitro*. Analyses include cell proliferation by alamarBlue® assay, immunochemistry staining (collagen 1&3, laminin and fibronectin), tenogenic gene expression (Scleraxis, Tenomodulin, Tenascin C, Collagen1&3, Decorin and Fibronectin), and catabolic protein expression (MMP-13). The IL-1β or TNF-α stimulated MSCs showed no significant reduction in cell proliferation rate but slight decrement in the cell density, with significant increase in MMP-13 expression. Collagen, laminin and fibronectin immunostaining expression were inhibited, and tenogenic gene expression were downregulated after treated with the pro-inflammatory cytokines. However, exposure to cyclic loading resulted in significant reduction of MMP-13 expression while enhanced collagen, laminin

and fibronectin expression. Significant upregulation of tenogenic gene expression were also shown. Significant increase were observed on cell proliferation rate. This study demonstrated that cell proliferation rate, collagen, laminin, fibronectin, catabolic protein and tenogenic gene expression of MSCs are affected by IL-1β and TNF-α can be modulated by mechanical stimulation. This preliminary finding provides the fundamental understanding of the potential use of mechanical loading in improving immunomodulatory behaviour of MSCs.

Keywords: Cyclic tensile loading; MSCs; pro-inflammatory cytokines; tenogenic differentiation

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A014

The Potential of Small Extracellular Vesicles Derived from Mesenchymal Stromal Cells and Cartilage Tissues on Articular Cartilage Regeneration

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ABSTRACT

Osteoarthritis (OA) is the most common chronic joint disorder that affects joint function and patient's quality of life. OA is mainly characterized by the degeneration of articular cartilage. Due to the poor intrinsic repair capacity of the cartilage tissue, the current treatments for OA are focused on symptom management, and joint replacement surgery has been the usual surgical intervention for patients with severe OA. Stem cell therapy has emerged as a potential approach to promote cartilage regeneration in recent decades. Recently, the therapeutic effects of stem cells are found to be attributed mainly to their paracrine secretion, particularly extracellular vehicles (EVs). Small EVs (sEVs) were harvested from umbilical cord-mesenchymal stromal cells (MSCs) and cartilage tissues via the ultrafiltration technique. The sEVs were characterized, and the cellular uptake of sEVs by OA chondrocytes was determined. Its effects on proliferation, migration, and gene expression of OA chondrocytes were also evaluated. sEVs derived from cartilage tissues showed cellular uptake by OA chondrocytes. OA chondrocytes cultured with MSC-sEVs exhibited a higher proliferation rate, while cartilage-sEVs demonstrated a greater effect on up-regulating the chondrocyte-associated genes. sEVs from both sources

did not influence the migration of OA chondrocytes. sEVs from both sources demonstrated potential in promoting cartilage repair, however, through different mechanisms, i.e., MSC-sEVs promoted chondrocytes proliferation and cartilage-sEVs modulate the chondrogenic properties of OA chondrocytes.

Keywords: Chondrocyte; exosome; extracellular vesicle; osteoarthritis

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A015

Biofabrication of the Hybrid Gelatin-PVA Bioinks for Potential Chronic Wound Healing Via 3d-Bioprinting Technology: In Vitro Study

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ABSTRACT

Introduction: Skin graft is one of the standard procedures that clinically available for chronic wound. However, one of the major limitations of skin graft treatment is insufficient of donor site for larger wound size. **Materials and Methods:**3D-Bioprinting is an alternative approach for the development of tissue engineering products. The selection of suitable biomaterials (natural and synthetic) is the essential key for the bioinks development. Therefore, this study aimed to develop a biocompatible in-house hybrid gelatin-PVA (G-PVA) bioinks crosslinked with natural crosslinker-genipin (GNP). Gelatin with different concentrations of PVA (3% and 5%) was fabricated with 0.1% genipin by using 3D-bioprinting, respectively. **Results:** The G-PVA hydrogels crosslinked with GNP proved to be outstanding in biocompatibility testing to promote cellular interaction with an excellent surface roughness >60 ± 0.3 nm, >85 ± 3.54% of cell viability and proliferation rate, >600 ± 35.3 μm of migration rate, maintained HDFs morphology with the expression of collagen type I, and 30% of alpha-smooth muscle actin. **Discussion:** Hydrogels exhibit considerable potential as a category of skin substitute owing to their porous, biodegradable, and capacity for the integration of growth factors. Hybrid G-PVA hydrogels crosslinked with GNP was proven to have an excellent biocompatibility property required as a potential bioinks for chronic wound healing. **Conclusion:** The 3D-bioprinted hydrogels thus demonstrated outstanding qualities and satisfied for suitable medical applications to replace skin grafts.

Keywords: 3D-bioprinting; bioinks; gelatin-PVA; hydrogels; wound healing

Acknowledgement: The study was funded by Ministry of Higher Education (MoHE) under the Fundamental Research Grant Scheme (FRGS), grant code: FRGS/1/2020/STG05/UKM/02/7.

A017

Unlocking the Comestic Potential of Human Umbilical Cord Mesenchymal Stem Cell-Derived Small Extracellular Vesicles (UC-MSC-sEV)

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ABSTRACT

The application of stem cell technology to the cosmetic industry has brought about breakthrough advancement, leading to the rise of functional cosmetics. Recent research has revealed that extracellular vesicles found in umbilical cord-derived mesenchymal stem cells (UC-MSCs) conditioned media have been shown to have positive effects on skin rejuvenation, re-epithelialization, and wound healing. However, more research is needed to fully comprehend the specific cosmetic effects of small extracellular vesicles (UC-MSC-sEV) derived from umbilical cord MSCs on the skin, particularly about their anti-scarring and skin-rejuvenation capabilities. Hence, the present study aims to investigate the impacts of UC-MSC-sEV in promoting proliferation, migration, extracellular matrix (ECM) synthesis and antioxidant enzyme expression in human dermal fibroblast. Isolation and characterization of small extracellular vesicles (sEV) derived from UC-MSCs were carried out and their impact on the proliferation and migration rate of human dermal fibroblasts (HDFs) were assessed. Besides, gene expression analysis and Superoxide Dismutase (SOD) assay were conducted on HDFs that co-cultured with different UC-MSC-sEV concentration to evaluate their role in modulating ECM protein synthesis and antioxidant enzyme expression. The findings indicated that higher concentrations of UC-MSC-sEV significantly promote the proliferation of HDF. However, the migration rate did not show significant difference among different treatment groups. Furthermore, the presence of UC-MSC-sEV did not affect the expression of Collagen 1 and Collagen 3 in HDFs, while increasing concentration of UC-MSC-sEV did increase the gene expressions of fibronectin, matrix metallopeptidase (MMP) 1, and MMP3. Besides, sEV did not influenced the

antioxidant enzyme activity in HDFs. These results highlight that UC-MSC-sEV can promote HDF proliferation, modulate ECM gene expression, suggesting that UC-MSC-sEV might be incorporated in cosmetic formulations to achieve the effect of anti-scarring properties or skin rejuvenation in the cosmeceutical sector.

Keywords: Anti-scarring; cosmetic; extracellular vesicle; mesenchymal stem cell; skin rejuvenation

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P002

Tunable Blending of Polycaprolactone and Polyethylene Oxide for Enhanced Properties of Electrospun Nanofibrous Scaffolds for Tissueengineering Applications

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ABSTRACT

Introduction: Electrospinning has utilized a diverse array of natural and synthetic polymers to produce nanofibers for tissue engineering purposes. Synthetic biodegradable polymers offer distinct advantages in terms of controllable nanofibrous morphology and processability compared to natural polymers. **Materials and methods:** This study explores the blending of Polycaprolactone (PCL) with Polyethylene oxide (PEO) at various ratios (90/10, 80/20, 70/30, 60/40) via an electrospinning technique. The dissolution of PCL and PEO mixtures in acetic and formic acids (7:3 ratio) allows for the adjustment of hydrolytic stability and mechanical properties of the resulting fibrous mats. **Results:** The findings demonstrate that the inclusion of PEO in PCL scaffolds enhances the properties of PCL and facilitates the creation of scaffolds with improved hydrophilicity and mechanical strength. Neat PCL exhibits a tensile strength of 4.88 MPa, while the tensile strength increases to 6.73 MPa for PCL/PEO 90/10 blends. Atomic Force Microscopy analysis confirms an increase of roughness in the blended scaffolds in the PCL-PEO 90-10 ratio. **Conclusion:** Despite previous reports on the immiscibility of PCL and PEO, the electrospinning process successfully produces nanofibers with mixed PCL/PEO blends, as confirmed by SEM analysis. Overall, this study highlights the potential of PCL/PEO blend scaffolds for tissue engineering applications.

Keywords: Bone tissue engineering; electrospinning; fibrous scaffolds; polycaprolactone (PCL); Polyethylene oxide (PEO)

P003/A018

Biofabrication of the Multifunctionalized Human Collagen Type I Versus Ovine Collagen Type I Bioscaffolds for Potential Skin Burn Healing: Comparative Study

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ABSTRACT

Introduction: Burn injuries cause significant morbidity and mortality, challenging skin function restoration. Recent advances in biomaterials, particularly collagen-based scaffolds, offer promising solutions to mimic the native extracellular matrix and facilitate skin healing in severe burns. **Materials and methods:** The study aimed to develop novel human collagen type I biomatrix with quercetin compared to ovine collagen type I biomatrix. Collagen extraction from human skin and ovine tendons, mixed with quercetin, and freeze drying technology will be used. Evaluation includes physicochemical properties, cellular compatibility, toxicity, growth profile, migration, and quercetin efficiency using DPPH assay. **Results:** The study compared different scaffold types for potential skin burn healing, including human collagen type I (HC.I), human collagen type I with quercetin (HC.I-Q), ovine collagen type I (OTC.I), and ovine collagen type I with quercetin (OTC.I-Q). The results indicated that HC. I scaffold had superior physicochemical and mechanical properties compared to the other counterparts. However, all groups still met acceptable levels of physicochemical properties. Moreover, all scaffold types showed no toxic effects on cells and supported the attachment and proliferation of human dermal fibroblasts (HDF). The groups incorporated with quercetin (HC.I-Q & OTC.I-Q) exhibited antioxidant properties. Based on the findings, both HC.I with or without quercetin were considered promising for the rapid treatment of skin burns. **Discussion:** human collagen bioscaffolds is a promising skin substitutes due to their porous, biodegradable nature, and cellular compatibility. **Conclusion:** The Human collagen biomaterial demonstrated outstanding qualities and satisfied for suitable medical applications to treat skin burn injuries.

Keywords: Human collagen type I; multifunctional biomaterials; ovine collagen type i; quercetin; skin burn

P004

Elucidating the Effects of Baicalein-Enriched Fraction Preconditioning on Neural Stem Cell Gene Expression

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ABSTRACT

Introduction: The P The regeneration capacity of tissue in the central nervous system is limited, as a consequence, lifelong disabilities are often associated with ischemic stroke injury. Currently, stem cell-based regenerative therapy is emerging as a favorable alternative treatment to enhance neurological recovery from ischemic stroke. Neural stem cells (NSCs) have been reported to enhance neuroplasticity and neural reorganization, nourish angiogenesis, and regenerate endogenous cells by differentiating into mature neural cell types. However, the therapeutic efficiency of these cells are limited by their low survival rates after transplanted into ischemic stroke brain. In this study, the therapeutic potential of NSCs was enhanced using a neuroprotective natural product known as baicalein-enriched fraction (BEF) extracted from *Oroxylum indicum* leaves. It is an attractive option due to its potential to increase cell viability, survival and proliferation of NSCs. Firstly, the BEF was characterized using phytochemical analysis, Fourier transform infrared spectrophotometer (FTIR) and high-performance liquid chromatography (HPLC) assays. Subsequently, the BEF was used to treat the NSCs and the effects of BEF on NSCs gene expression were investigated using real-time PCR. The phytochemical analysis revealed the presence of phenol, tannins, phlobatannin, flavonoid, saponin, quinines, and glycosides. FTIR spectroscopy identified phenol, hydroxyl and carbonyl as the major functional groups while HPLC analysis revealed that the extract contained 29% of baicalein. Moreover, the NSCs treated with 3.125 µg/ml of BEF for 48 hours showed significant upregulated of superoxide dismutase (SOD2, 3.38-fold) and angiopoietin (ANGPT1, 6.24-fold) genes, compared to non-treated NSCs. In conclusion, this study showed significant upregulation of anti-oxidant and anegiogenesis gene expression in the BEF-treated NSCs and suggested the potential neuroprotective effects of BEF extracted from *O. indicum* where it can serve as basis data for the future development of alternative treatment for ischemic stroke disease.

P009

The Effect of *Piper sarmentosum* Aqueous Extract on Human Osteoarthritic Chondrocytes via Differential Gene Expression Yi

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ABSTRACT

Introduction: Osteoarthritis (OA) is a degenerative joint condition which may leading to physical impairment. Current available treatment approaches only aim to relief pain temporary and may result in negative side effects. Hence, it may be interesting to explore alternative treatments like phytomedicine. In this study, the effects of *Piper sarmentosum* (PS), a medicinal plant showing anti-inflammatory and antioxidant effects which traditionally used to relief joint pain has been determine to evaluate its effects on human osteoarthritic chondrocytes. **Materials and methods:** Human chondrocytes isolated from articular cartilage of OA patients were cultured in media supplemented with different concentration of PS aqueous extract for 3 days. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was done to determine the cytotoxicity of different PS concentration. Real-time polymerase chain reaction (PCR) was used to determine the effect of PS on anabolic and catabolic differential gene expression. **Results:** The chondrocytes maintained their morphology as culturing in media supplemented with different PS concentration. The OA chondrocytes showed increase in expression of anabolic genes including aggrecan core protein (ACP), collagen type II and SOX9 and reduce in catabolic genes (COX2 and iNOS) expression. Moreover, the PCR results showed no significant difference in gene expression of inflammatory cytokines (interleukin 6, matrix metalloproteinase 1 and 13). However, an augmentation in gene expression was observed. **Discussion and Conclusion:** These results indicated that the PS aqueous extract demonstrated promote anabolic gene expression of chondrocytes and inhibiting catabolic genes that impede extracellular matrix synthesis. This suggested PS aqueous extract may hold potential as a treatment for OA. However, further study are needed to to elucidate the specific pathways involved.

Keywords: Antioxidant; chondrocytes; osteoarthritis; *Piper sarmentosum*

Acknowledgement: This research was funded by Universiti Kebangsaan Malaysia (UKM).

P010

Understanding the Mode of Non-Integrative Reprogramming Strategies in Establishment of Human Induced Pluripotent Stem Cells (HIPSCS)

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ABSTRACT

Currently, mesenchymal stromal cells (MSCs) are recognized as an ideal cell source for tendon **Introduction:** The ability to reprogram primary cells into pluripotent stem cells holds great promise in the area of regenerative medicine and disease modelling. However, current practice of using viral vectors for cell reprogramming results in integration of the genes into host genome that can have unintended consequences including dysregulated cell growth and altered cell functions. Hence, non-integrating viral and episomal vectors are predicted to have a safer profile and highly desirable for clinical applications. Hence, we propose to embark the study to investigate the mode of reprogramming of non-integrated vectors encoding for the transcription factors (Oct4, Sox2, Nanog, Lin28, L-Myc, Klf4 and SV40LT) in establishing of human induced pluripotent stem cells (hiPSCs) from CD34+ primary cells. **Materials and methods:** On the first part of preliminary study, comparison between integrating (LV) and non – integrating (IDLV) lentivirus produced via standard calcium phosphate co-precipitation transfection method will be done and transduction efficiency was studied using both the IDLV (ID-SFFV-GFP) and their wild-type counterparts (integrase-proficient SFFV-GFP). GFP expression was analyzed by fluorescence microscope and FACS analysis. Second part of study will incorporate CD34+ primary cells derived iPSCs via episomal non – integrating episomal vectors. PBMCs from healthy donors will be used to isolate CD34+ cells using MACS cells separation and further electroporated with a cocktail of integrase-free episomal reprogramming vectors. Pluripotency of these iPSC-like

colonies are then examined. **Results:** Our preliminary study that the number of the GFP-positive cells in ID-SFFV-GFP-transduced U937 cells decreased rapidly over time. The percentage of GFP-positive cells decreased from ~50 % to almost 0, up to 10 days post-transduction. In wild type SFFV-GFP-transduced cells, GFP expression is remained consistently at about 100%. **Conclusion:** The iPSCs derived using this strategy can potentially be differentiated into any cell type and transplanted for treatment purposes without the risk of unknown genomic alteration.

Keywords: CD34+; episomal; iPSC; lentiviral; non - integrated reprogramming

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P011

Establishing Downregulated Pax7 Expression in DMD Skeletal Myoblasts: An Optimization on Transfection Approaches on Sensitive Primary Cells

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ABSTRACT

Introduction: The Paired box 7 (Pax7) protein is well-defined in its role of regulating myogenesis, proliferation, differentiation, and repair in both embryonic and adult skeletal muscle cells. In the context of Duchenne Muscular Dystrophy (DMD), Pax7 signaling patterns are not well understood and require extensive study. DMD is a progressive muscle wasting disease arising from a genetic mutation of the DMD gene resulting in compromised cytoskeletal integrity. It was previously reported that Pax7 is upregulated in DMD. However, primary cells are known to be sensitive and often a challenge to transfect. In this study, we aim to optimize transfection parameters best suited for DMD primary skeletal muscle cells (DMD SkM) to establish a downregulated Pax7 DMD model. **Materials and Methods:** Pax7 downregulation is achieved via transfecting Pax7 shRNA to induce gene silencing on DMD SkM with Lipofectamine® LTX. We tested the following parameters: Lipofectamine LTX volume (2.0 L, 2.5 L, 3.0 L, and 5.0 L); transfection time (24h, and 48h); transfectant selection time (24h, and 48h); seeding density (2.0 x 10^5cells/cm2, and 5.0 x 10^5cells/cm2); and splitting ratio (1:3, and 1:2). Transfectants were subjected to antibiotic selection for 48 hours. Transfection efficiency was examined via immunostaining Pax7-positive cells. Simultaneously, a GFP reporter gene in a scrambled shRNA control was also transfected to identify approximate shRNA uptake based on the transfection method. **Results and Discussion:** The results have shown that transfection was optimized at a seeding density of 5.0 x 10^5cells/cm2 assisted with 2.5 L of Lipofectamine LTX for 48 hours, and transfectant selection for 24 hours. Immunostaining analysis demonstrates a three-fold downregulation of Pax7 expression (19.71%) as compared to the non-transfected control (69.48%). **Conclusion:** We have fine-tuned transfectant parameters for DMD SkM which will help further studies in DMD.

Keywords: Duchenne muscular dystrophy; Pax7; primary cells; transfection

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T003

Identification of Key Signalling Pathways Associated with Collagen Type 1-Induced Osteogenic Differentiation of Dental Pulp Stem Cells

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ABSTRACT

Dental pulp stem cells (DPSC) showed an extensive ability to differentiate into bone cells, suggesting potential application in bone regenerative therapy. The osteogenic differentiation of DPSC is regulated by specific signalling molecules and multiple activated intracellular networks. Collagen type 1 (Col1) has been proposed as a suitable material for bone tissue engineering, with excellent evidence of osteoinductivity and osteoconductivity. However, the key signalling pathway associated with Col1-induced osteoblast proliferation and differentiation of stem cells remains inadequately understood. The present study assessed the osteogenic potential of DPSC via Col1 induction and analysed the effect of pathway inhibition assay on the expression of genes and proteins of osteogenic signalling. Briefly, DPSCs were seeded on different concentrations of Col1/Matrigel™, and osteogenic capacity were examined by alizarin red staining and RT-PCR to ascertain the optimal differentiation capacity. Next, DPSC were cultured on Col1 (2 mg/ml) and treated with pathway inhibitors targeting: P13K/AKT, Smad and ERK. The gene and protein expression levels were analysed via qRT-PCR and Western blot analysis. Comparatively, Col1 successfully stimulate differentiation of DPSC into bone cells, as efficient as osteogenic supplementation. Results of qRT PCR suggested that all three investigated pathways involved with Col1-induced osteogenic differentiation, as observed in the fluctuating trend of osteogenic gene markers (Dlx5, Osx, OPN and OCN) upon treatment of inhibitors. The data gathered from protein analysis suggest the potential role of PI3K/AKT as the predominant signalling pathway in Col1-induced osteogenesis of DPSC. Results also indicated possible crosstalk of PI3K/AKT with other signalling pathways during the differentiation process. In depth

knowledge on the role of PI3K/AKT pathway in Col-1 osteoinduction may provide a better understanding on stem cells biology, which can be manipulated to design an efficient cell-based scaffold for bone regeneration.

Keywords: bone regeneration; dental stem cells; protein; scaffold; signalling pathway

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T004/A006

Plasma-Polymerised Antibacterial Carvone Coated Ovine Collagen Type I (OTC-I) Biomatrices for Future Use in Diabetic Wound Care

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ABSTRACT

Diabetic ulcers (DU) are a common complication that accelerates morbidity and increases the cost of wound care management. Total contact-cast is the standard gold treatment for DUs. However, it causes iatrogenic complications associated with increased mortality, such as delayed wound healing, severe infection, and secondary bacteraemia. Alternative treatments showed ineffective improvements in healing rates and infection susceptibility. Current treatments are also expensive and require multi-step processing. Thus, research into rapid, cost-effective, accessible, and efficient wound care is critical for DU treatment. Therefore, this study aimed to develop an acellular, ready-to-use ovine collagen type I (OTC-I) bioscaffold with an antibacterial coating for the immediate treatment of diabetic wounds. Carvone, from spearmint, is used as the antibacterial chemical via plasma polymerisation, which deposits monomers into polymers with active functional groups. Fourier transform infrared spectrometry (FTIR) demonstrated successful collagen extraction of ovine tendon from the presence of collagen type I (Col-I) chains. The 3D scaffolds of OTC-I were lyophilised and crosslinked with genipin (GNP) or dehydrothermal treatment (DHT), followed by carvone plasma polymerisation (ppCar). The physicochemical, biomechanical, biodegradation, antibacterial, angiogenesis, and biocompatibility properties of scaffolds were analysed. OTC-I crosslinked with GNP and underwent ppCar (GNPppCar) was found to have suitable physical (porosity ≥ 90% with pore size ≥ 100 μm) and superior tensile (Young's modulus; 10.64 $\pm$ 7.91 MPa) properties. Furthermore, ppCar prevented bacterial growth (reduced ≥ 60% of *E.*

coli and *S. aureus*) and supported angiogenesis from the positive tubule formation of HUVEC cells. The cell attachment, proliferation, and migration of human dermal fibroblasts (HDF) in were enhanced, reinforced by the western blot of PI3K/AKT and FAK pathway. Finally, skin wounds on type I diabetic C57BL/6 mice treated with GNPppCar healed faster (≤ 19 days) than the control. GNPppCar has potential to effectively provide rapid treatment for DU.

Keywords: Antibacterial; biomatrix; collagen; crosslinking; plasma polymerisation

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T006

The Effect of Nanohydroxyapetite Incorporated with Micro RNA 21 to Regulate Osteogenesis

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ABSTRACT

Hydroxyapatite due to its bioactivity is widely used in osseous defects to improve osteointegration of bone implants. MicroRNA-21 (miR-21) are endogenously expressed to regulate osteogenesis. Coupling of miR-21 and nanohydroxyapatite (nHA) can be a potential intervention to promote greater and rapid healing of bone fracture. **Objective** of the study is to investigates the combined effect of miR-21 and nHA scaffold in promoting osteogenesis by regulating the osteoblastic genes Runt-related transcription factor-2, osteocalcin, osteopontin and osteoprotegerin. **Methodology** concise of evaluating the growth kinetics and viability of human mesenchymal stromal cells (hMSCs) and mineralization activities upon exposure to miR-21 and nHA were studied. hMSCS cells were obtained from Wharton's Jelly (WJ) of umbilical cord from patients who underwent Cesarean procedure. hWJMSCs were further cultured in osteoinductive media to induce osteogenic differentiation. The hWJMSCc cells were characterized by their tri-lineage differentiation capacity and surface markers expression measured using special staining and flow cytometry respectively. The size and morphology of the nanohydroxyapatite were characterized by dynamic light scattering and field emission scanning electron microscopy. The incorporation of miR-21 onto nHA was verified using confocal imaging and quantified using flow cytometry. A dose curve response was generated for hWJMSCs treated with different concentration of nHA+miR-21 and assessed using Presto Blue assay. Expression of bone markers was evaluated via Western blotting and PCR. **Results** shows that exogenous miR-21 upregulates bone related gene expressions and osteogenic proteins production confirming its role in osteogenesis. hWJMSCs cells shows no toxicity after treatment with nHA, miR-21 and nHA+miR-21. Significant increase in alkaline phosphatase was detected in hWJMSCs treated with nHA+ miR-21 compared with nHA or miR-21 alone. **Hypothesis** posits that there is a potential synergistic effect on the osteogenesis of

human mesenchymal stem cells (hMSCs) through the combination of miR-21 and nHA

Keywords: Bone resorption; bone homeostasis; exogenous *miR21;* microRNA 21; osteogenesis

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T007

Natural Biologic for the Treatment of Osteoarthritic Chondrocytes

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ABSTRACT

It is estimated that more than 500 million people of the global population were living with knee osteoarthritis. In Malaysia, 9.3% of adult Malaysians complained of knee pain, swelling, and problems of moving the joints. At present, there is no cure for osteoarthritis. Articular cartilage are lack of blood vessel and nerve supply. Once damaged, they are difficult to repair. An adjuvant therapy based on platelet-derived extracellular vesicles (PEVs), is a natural biologic in which concentrated with bioactive proteins to assist cartilage repair. PEVs is a subset of platelet-rich plasma (PRP) and nanosized, allows efficient uptake into cells. However, PEVs have rarely been studied, yet it is unclear which bioactive proteins directly affect the regeneration of damaged cartilage under various knee osteoarthritis (OA) conditions. Platelet-derived EVs were isolated from healthy donors and subjected to morpho-functional analysis for characterization. The OA chondrocytes *in vitro* model was created by pre-stimulating human chondrocytes with interleukin-1 beta (IL-1β) to mimic OA environment and subsequently the culture was supplemented with PEVs. The major bioactive proteins were analyzed using high-resolution liquid chromatography-tandem mass spectrometry. Characterization of PEVs demonstrated a highly heterogenous size and number, with particle size range of 80-500nm. *In vitro*, PEVs are found to stimulate chondrocytes proliferation. Pre-stimulation with IL-1β induced distinct shrinkage of chondrocytes indicating the stress condition of the chondrocytes. The condition is reversed by PEVs and further promote chondrocytes proliferation. Proteomic analysis identified 594 proteins and these proteins are closely related to cell proliferation. In conclusion, PEVs diminished inflammatory IL-1β mediated effects on human OA chondrocytes by modulating the protein profiles in which related to promoting chondrocytes proliferation.

Keywords: Chondrocyte proliferation; osteoarthritis; platelet-derived extracellular vesicles; proteomic

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T009

From Grafting to Printing

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ABSTRACT

Introduction: Chronic wounds can be treated temporarily or permanently with skin substitutes. The selection of skin substitutes is dependent on the severity of the wounds. Generally, skin grafts (SGs) are occasionally limited by a larger wound size and it has a poor survival rate due to immunological rejection during a painful process. **Materials and Methods:** Thus, considering the current global donor shortage for SGs, 3D-bioprinting technology emerged as a potential alternative to replace the SGs treatment. 3D bioprinting yields scaffold fabrications with the combination of biomaterials and cells to form bioinks. The use of biodegradable polymers such as natural and synthetic polymers display notable benefits for the skin to regenerate and the scaffold will degrade at an appropriate time. **Results:** Bioprinted skin with 3D-bioprinting technology is an excellent method for regenerating damaged tissues and accelerating the healing process, as specific cells and active compounds that promote healing can be incorporated to provide a patient-specific treatment. **Discussion:** Hydrogels exhibit considerable potential as a category of skin substitute owing to their porous, biodegradable, and capacity for the integration of growth factors. The hydrogel facilitates the provision of moisture, hence facilitating the painless removal of necrotic and infected tissue, stimulating the formation of granulation tissue, and promoting the achievement of full wound healing. Due to their high-water content, these materials possess limited absorbency, rendering them suitable for managing wounds characterised by mild to moderate exudate levels. **Conclusion:** The 3D-bioprinted hydrogels thus demonstrated outstanding qualities and satisfied for suitable medical applications to replace skin grafts.

Keywords: 3D-bioprinting; bioinks; hydrogels; skin-grafts; wound healing

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T010

Hydroxytyrosol Prevents Intimal Hyperplasia in Vascular *Ex Vivo* Sapehnopus Vein Organ Culture Model

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ABSTRACT

Introduction: Intimal hyperplasia (IH) that lead to luminal vascular thickening, occurs due to excessive proliferation and migration of smooth muscle cells (SMC). IH development decreases the long-term patency of vascular graft post coronary artery bypass grafting (CABG) procedure and threatens percutaneous coronary intervention (PCI) long term outcome too. The use of antiproliferative drugs such as paclitaxel, that successfully inhibit SMC proliferation, unfortunately, inhibits reendothelisation. Reactive oxygen species (ROS) imbalances that further worsen endothelial inflammation and vascular remodelling. Hence, utilising Hydroxytyrosol, an antioxidant extracted from olive which is well studied for its anti-inflammatory and antiproliferative effects potentially circumvents IH development. The hypothesis is tested through *in vitro* effect of different HT doses that potentially promote endothelial cells (EC) but inhibit SMC proliferation. EC and SMC were isolated from surplus saphenous veins post-CABG procedures. HT's half-maximal inhibitory concentration (logIC50) on platelet derived growth factor (PDGF)-induced SMC determined through an MTT assay is 2.59. HT at a concentration of 40 M to 320 μM significantly decreased PDGF-induced SMC proliferation, migration, cell cycle and chemotaxis effect *in vitro*. HT was also found to downregulate the PDGF-induced PI3K/AKT pathway and upregulated NRF2/HO-1 antioxidant pathway. Conversely, up to 50 μM of HT preserves EC viability, proliferation, and migration ability via activating the AKT and NRF2/HO-1 pathway. ROS production in TNF-α

stimulated-EC was decreased with HT. Concurrently, IH *ex vivo* model was set up by culturing saphenous vein rings with and without HT treatment for up to 21 days. A significant increase in cellular proliferation was detected using the EdU proliferation assay from day 5 to day 21 at *ex vivo*. HT significantly suppressed the proliferation of SMC in the *ex vivo* model by inhibiting the AKT pathway. These findings support the therapeutic potential of HT in preventing intimal hyperplasia and reendothelisation of vascular grafts.

Keywords: Endothelial cells; hydroxytyrosol; intimal hyperplasia; smooth muscle cells

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T011

Synergistic Effects of Multifunctionalized-Collagen Type I Biomatrix for Future Use in Treatment of Skin Burn: Ovine Tendon vs Human Skin Resources

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ABSTRACT

Burn is a traumatic injury accompanied by oxidative stress, bacterial infection and inflammatory response frequently delay the healing process. Hence, this study aimed to compare human versus ovine collagen as promising biomaterials for skin burn. The study was done by using six biological replicates to extract human collagen type I (HCol.I) from redundant human skin and ovine collagen type I (OCol.I) from tendons, fabricated in sponge form followed by physicochemical characterization and biocompatibility towards primary human dermal fibroblasts (HDF). Quercetin (1 mg/ml), a natural antioxidant drug, was incorporated with human (HCol.I+Q) and ovine (OCol.I+Q) collagen. Genipin (0.1%) w/v, as a natural crosslinker, has been mixed as follows: (HCol.I_GNP), (OCol.I_GNP), (HCol.I+Q_GNP) and (OCol.I+Q_GNP). Additionally, a novel PVA-Gelatin formula with ratio (2:1) incorporated with graphene oxide silver nanoparticles (AgGO) (0.1 mg/ml) was fabricated as a sprayable antibacterial wound dressing and tested on *Pseudomonas aeruginosa* and *Bacillus cereus*. The results of physicochemical characterizations for swelling ratio, compression and resilience, water contact angle, and water vapor transmission rate revealed that both biomaterials had the standard characteristics of ideal sponge bioscaffolds. Low rate of biodegradation was assessed enzymatically. The crosslinking degree was determined by Ninhydrin assay with P value (>0.0001) between all groups. The antioxidant properties of Quercetin were detected by using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay. The percentage of free radical scavenging activity was between 80-90 ± 2% for all Quercetin groups. HCol.I groups investigated better cytotoxicity analysis compared to the OCol.I groups. The cell-scaffold interaction showed good cell proliferation and migration in HCol.I groups against OCol.I groups. The immunogenicity study showed that HCol.I groups had favorable inflammatory response compared to OCol.I groups. The formula of PVA-Gelatin spray has been developed to be compatible with AgGO

with no cytotoxicity and antigenicity response. Our results present a comprehensive comparison of collagen-based biomaterials developed for skin burn regeneration.

A012

OCT4 and *BMP4* Expressions in Human Adipose-Derived Stem Cells from Subcutaneous and Visceral Fat

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ABSTRACT

Adipose derived stem cells (ADSC), type of mesenchymal stem cell (MSC) found in the vascular stroma of human fat tissue, have been proposed as a potential alternative to human donor corneal tissue. Octamer-binding transcription factor 4 (OCT4) regulates pluripotency in ADSC, while Bone morphogenetic protein 4 (BMP4) signalling is essential for the differentiation of ADSC towards ectodermal lineages. In this study, the expression of BMP4 and OCT4 in primary ADSCs from different adipose tissue depots were assessed. Subcutaneous and visceral adipose tissue were harvested from the abdomen of 6 female (mean age 35 $\pm$ 7.1 years, range 28-47 years) that underwent caesarean section or laparotomy at the Obstetrics and Gynaecology department Hospital Canselor Tuanku Muhriz. The subcutaneous and visceral adipose tissues were processed and ADSCs were isolated. The cells were then seeded at 4000 cells/cm^{2}until confluence and multiplied up to passage 4 (P4). The expression of *BMP4* and *OCT4* were assessed at P4 via qPCR and immunohistochemistry and compared to ADSC cell line as a control group. Generally, the expression of *OCT4* and *BMP4* was higher in primary ADSC from both subcutaneous and visceral locations compared to the control. The *OCT4* and *BMP4* expression in visceral and subcutaneous ADSCs showed a different pattern. Majority (67% and 80%) of the visceral ADSCs expressed higher OCT4 and BMP4 respectively. These findings suggest a potential correlation between the potential differentiation capacity and the location of the ADSCs, which can be explained by the underlying regulatory pathways interplay between pluripotency and ectodermal differentiation.

Keywords: *ADSC*; *BMP4; OCT4*; subcutaneous; visceral