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Editorial Information

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Unravelling the Neurogenic Potential of Neurotrophic Factors Secreting Human Dental Pulp Stem Cells

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ABSTRACT

Introduction: Human dental pulp stem cells (hDPSCs) are mesenchymal stem cells (MSCs) derived from the connective tissue that resides within the dental pulp chamber. These cells originate from the neural crest lineage, exhibiting a strong neurodifferentiation profile and neuroprotective capacities. The neurotrophic factors such as Brain-Derived Neurotrophic Factor (BDNF) and Glial cell Derived Neurotrophic Factor (GDNF) are crucial for neuronal functional recovery and establishing new neuronal networks. In this study, we aim to explore the neurogenic potential of hDPSC and its therapeutic uses. **Methods:** hDPSCs were isolated from dental pulp tissue of extracted third molar using the enzymatic digestion method. hDPSCs were then propagated in culture media supplemented with a human serum substitute. Immunophenotyping and bi-lineage differentiation were carried out to characterize the isolated hDPSC. In addition, the neurotrophic factors secreted by hDPSC were analysed using a specific enzyme-linked immunosorbant assay (ELISA). **Results:** The hDPSCs were successfully isolated and expanded in vitro until passage 4. They exhibited fibroblast-like, spindle-shaped morphology in culture and showed high expression of MSC-specific surface markers (CD105, CD90, CD73 and CD29) and expressed relatively low levels of negative markers (CD45, CD14, CD34, CD79a and anti-HLA-DR). Besides that, isolated hDPSCs have shown the potential to differentiate into osteocytes and adipocytes. Furthermore, results from ELISA revealed that BDNF and GDNF were detected in high quantities. **Conclusion:** In this preliminary experiment, we have evaluated the neurotrophic factors secreted by hDPSCs in vitro culture. This study presents promising prospects in terms of the therapeutic potential of hDPSC in the prevention and treatment of neurological diseases.

Keywords: Dental Pulp Stem Cells, Neurotrophic Factors, Brain-derived Neurotrophic Factor, Glial Cell-Derived Neurotrophic Factor

Recommendations on Regulatory Requirements for Extracellular Vesicles (EV) as Therapeutic Agents

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ABSTRACT

Introduction: Extracellular vesicles (EV) are a new class of rapidly developing biological therapeutic agents that have entered the clinical trial investigation stage. To translate EV therapeutic agents to clinical settings, the product developer must demonstrate their quality, safety, and efficacy to sufficiently adhere to standards set by regulatory authorities. This clinical translation is very challenging due to the complexity and variety of EV therapeutic agents. This concept paper aims to identify the availability of specific recommendations related to EV therapeutic agents, which may facilitate establishing any regulatory guidance document. **Methods:** A literature review was performed to identify the availability of recommendations/guidance/regulatory requirements relating to the pharmaceutical development of EV therapeutic agents. **Results:** The International Society for Extracellular Vesicles (ISEV) Task Force on Regulatory Affairs and Clinical Use of EV-based Therapeutics as well as the Exosomes Committee from the International Society of Cell Therapy (ISCT) are expected to contribute to the development of EV therapeutic agents by providing regular updates on scientific progress in EV field to regulatory agencies. In addition, the Extracellular Vesicle Translation to Clinical Perspective (EVOLVE) has provided specific recommendations for manufacturing, quality control, analytics, non-clinical development, and clinical trial conduct based on current European legislation. The general categorisation of EV therapeutic agents depends on the product complexity and active substance(s). EV therapeutic agents are generally regulated as biological medicines and subcategorised to biotechnological or gene therapy products (a subclass of advanced therapy medicinal products). Some important considerations in translating EV therapeutics to clinical applications are the manufacturing process ("process is the product") and its quality attributes, including quantity, identity, purity, and biological activity. **Conclusion:** Our preliminary finding shows that current regulatory requirements and recommendation specific to EV therapeutic agents are in accordance with European legislation.

Keywords: Extracellular vesicles, Regulatory requirements, Therapuetic agents

Pre-clinical Safety Study of Wharton's Jelly-derived Mesenchymal Stem Cells Delivered via Systemic Administration

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ABSTRACT

Introduction: Mesenchymal stem cells (MSC) are unique adult cells with a large regenerative and immunomodulatory capacity with extended effects through the secretion of functional metabolites. The inconsistencies in safety results reported have prevented the clinical translation of MSC therapies. In an animal model, we conducted a study to determine the safety of Wharton's Jelly derived mesenchymal stem cell (WJ-MSC) via the intravenous route. **Methods:** This study was approved by the Animal Ethics Committee of the Faculty of Medicine, Universiti Kebangsaan Malaysia (TEC/FP/2020/YOGESWARAN/23-SEPT./1124-OCT.-2020-SEPT-2023). Healthy male Sprague Dawley rats were randomly assigned into two groups that received a single, pre-determined high-dose of WJ-MSC or saline. Physical measurements, blood analysis (serum chemistry and whole blood profile), and the signs of morbidity and mortality were observed throughout the study. Acute and sub-chronic toxicity was performed at 14 and 90 days, respectively. Upon euthanasia, necropsy and histology of primary organs (lung, liver, spleen and kidneys) were performed. **Results:** The physical measurements, blood analysis and signs of morbidity and mortality were not statistically significantly different ($P<0.05$) between the treatment and control groups. The necropsy and histopathology revealed minor pulmonary inflammation of the lungs in animals of both groups. Recovery was observed in the treatment group at both terminal stages. Regrettably, the source of inflammation was not identified. Discussion: No significant differences ($P<0.05$) were drawn from comparing the physical, biochemistry, and haematological profiles between MSC and the control group. Furthermore, microanatomical structures in inflamed alveolar and bronchial regions of lungs recovered in MSC-treated animals and improved over time. These changes were not observed in the control group even after 12 weeks. Thus, we hypothesize that the systemically infused WJ-MSC alleviated inflammatory symptoms in the lungs instead of the animals' natural recovery. **Conclusion:** The intravenous administration of WJ-MSC was safe with no adverse or side effects in the animal model.

Keywords: Mesenchymal Stem/Stromal Cell; Wharton's Jelly; Cell Transplantation; Toxicity; Rodent

Optimization of Fibrin Concentration in 3D-Printed Scaffold for Bone Angiogenesis and Osteogenesis

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ABSTRACT

Introduction: Bone regeneration involves a complex process of coupling angiogenesis and osteogenesis. Therefore, selecting scaffold material that can aid in successfully forming a blood vessel network is important in ensuring new bone tissue formation. Fibrin is a biopolymer capable of inducing angiogenesis and osteogenesis in bone regeneration owing to its unique biological properties. **Methods:** In this study, a three-dimensional (3D) printed polylactide acid (PLA) scaffold was fabricated and incorporated fibrin hydrogel. Different concentrations of fibrinogen were used to study the effect of fibrin network morphology on the properties of the scaffold. The fibrin network formed was characterized by morphology, porosity and water uptake ability. Osteoblast cells in fibrin hydrogel were incorporated into a PLA scaffold to evaluate the scaffold's biocompatibility in aiding cell growth. **Results:** As the fibrinogen concentration increased, the water uptake increased, although there was a slight decrease in porosity. More osteoblast cells were attached and spread on the fibrin network as fibrinogen concentration increased. The viability of the cells also increased with an increase in fibrinogen concentration. **Conclusion:** We can conclude that a 3D-printed PLA scaffold incorporated with fibrin hydrogel was biocompatible in aiding cell growth, with a higher concentration of fibrinogen providing better cell growth. This finding demonstrated the potential of a 3D-printed PLA scaffold incorporated with fibrin hydrogel in facilitating in-vitro vascularization through the co-culture of osteoblast and endothelial cells for future study.

Keywords: Fibrin, Bone Scaffold, Angiogenesis, Osteogenesis, Bone Regeneration

The Intra- and Extra-Telomeric Role of TRF2 in the DNA Damage Response

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ABSTRACT

Introduction: Telomere repeat binding factor 2 (TRF2) has a well-known function at the telomeres, protecting the telomere ends from being recognized as a DNA break or from unwanted recombination. This protection mechanism prevents DNA instability from mutation and subsequent severe diseases caused by the changes in DNA, such as cancer. Since TRF2 actively inhibits the DNA damage response factors from recognizing the telomere end as a DNA break, many more studies have also shown its interactions outside of the telomeres. However, very little has been discovered on the mechanisms involved in these interactions. **Methods:** A scoping review of available literature that reported on the interaction of TRF2 with the DNA Damage Response (DDR) factors or its role in DDR was conducted. **Results:** It has been well established that TRF2 actively inhibits the DDR through the ATM kinase pathway at the telomeres. However, recent findings have also reported the role of TRF2 with the DDR outside of the telomeres. It has also been reported that TRF2 may be an early response factor to extra-telomeric DNA damage. These actions may be attributed to TRF2 post-translational modification or recruitment by other DDR factors. Previous findings on its colocalization at the DNA damage site and other DDR factors outside the telomeric regions have shed light on its more extensive role in regulating and maintaining DNA stability and its clinical implications. **Conclusion:** As discussed in this review, some extra-telomeric interactions of TRF2 with DDR factors have been established. However, very few mechanisms of such interactions have been determined and explored. The investigation of the molecular mechanism in the possible interaction between TRF2 and other DDR factors, either directly or indirectly, is needed to confirm whether there is a specific role TRF2 plays in DNA damage repair if any exists.

Keywords: TRF2, DNA damage response, telomeres protection, extra-telomeric

Shelf-life and safety evaluation of the dermal fibroblast conditioned medium (DFCM) for future skin regeneration

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ABSTRACT

Introduction: Human dermal fibroblasts secreted proteins in the culture medium known as dermal fibroblast conditioned medium (DFCM) that promotes cell growth, differentiation, and tissue repair. However, these secreted proteins' shelf life and safety validation remain unknown. The aim is to evaluate the shelf-life and safety of the DFCM for skin regeneration and rejuvenation. **Methods:** Human dermal fibroblast was cultured with F12:Dulbecco's Modified Eagle medium (F12: DMEM) supplemented with 10% foetal bovine serum (FBS) until passage 3. The cells will be incubated at 37 °C in a 5% CO2 incubator for 72 h with a serum-free keratinocyte-specific medium, a growth supplement (EpiLifeTM; Gibco, USA), and the waste media will be collected as the conditioned media DFCM-KM. The DFCM-KM will be stored in different storage temperatures (room temperature, 4 °C, -20 °C and -80 °C) and storage times of (1, 3, 6, 12, 18 months). The protein profile of DFCM-KM at different storage times and temperatures will be determined via bicinchoninic acid (BCA) assay, and the effect of DFCM at different storage on the keratinocyte biological properties will be evaluated via keratinocytes attachment, proliferation, and scratch wound assay. Lastly, the stability of the DFCM-KM mixed with cream base formulation will be evaluated in terms of physical and chemical stability test, microbiological stability test and genotoxicity test. **Expected outcome:** The DFCM-KM is expected to have a shelf life of more than 6 months with storing temperature of ≤4 °C and also does not exhibit any toxic reaction towards the cells. **Summary:** By discovering the shelf-life, ideal-storing temperature and toxicity level of the DFCM-KM, we can determine whether the product is safe for usage in the healthcare industry. By determining the suitable shelf-life, we can commercialise and merchandise the product on a larger and wider scale, reducing retail waste.

Keywords: fibroblast conditioned media, shelf-life, skin regeneration

Elucidation of the Cytocompatibility of Corneal Epithelial Cells with Ovine Collagen Hydrogel

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ABSTRACT

Introduction: Corneal transplantation is a recognized surgical treatment in treating patients with corneal disorders, but unfortunately, the shortage of tissue donors remained a critical problem that limited corneal transplantation surgery. The shelf electrochemically compacted ovine tendon collagen type 1 (OTC-1) hydrogel in combination with corneal epithelial cells (CECs) represents a paradigm shift to overcome the shortage of donated corneas. This study aims to evaluate the cytocompatibility of cultivated CECs with OTC-1 hydrogel. This knowledge will address tissue shortage or delay in corneal perforation treatment. **Methods:** Collagen type 1 was extracted from the ovine's tendon by using an acid-based extraction method. This OTC-1 hydrogel was divided into 4 groups: traditional collagen hydrogel and electrochemical compacted collagen hydrogel by using dielectrophoresis (DEP) technology (Each group consists of without crosslinking and with genipin and quercetin crosslinking). The physicochemical and mechanical of OTC-1 hydrogel was evaluated (chemical compound, percentage of available elements, purity, topography and mechanical testing of OTC-1 hydrogel). Then, the isolated CECs passage 2 were seeded on OTC-1 hydrogel at a density of 10, 000 cells/cm2. The vitro-cellular viability of CECs was evaluated (CECs attachment rate, proliferation rate, viability, morphology and CECs growth profile on OTC-1 hydrogel). Microscopic observation of corneal like structure in the 3D model was also evaluated (immunocytochemical analysis and histology analysis). **Results:** The electrochemically compacted OTC-1 hydrogel crosslinked with genipin and quercetin have suitable physicochemical properties to support the attachment, viability, and proliferation of CECs. **Conclusion:** The electrochemically compacted OTC-1 hydrogel crosslinked with genipin and quercetin has suitable physicochemical properties and is more compatible with CECs than traditional OTC-1 hydrogel.

Keywords: Corneal Epithelial Cell, Collagen Hydrogel, Cyotocompatibility, Dielectrophoresis.

Morphology and characteristic of WJ-MSC expanded with different growth mediums

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ABSTRACT

Introduction: Mesenchymal stem cells (MSCs) derived from Wharton's jelly (WJ-MSCs) have many advantages, such as being easy to obtain, having immunosuppressive properties, multi-lineage differentiation potential and no ethical issues. Due to these advantages, WJ-MSCs are a promising candidate for therapeutic applications. To date, many types of mediums have been developed to expand WJ-MSCs. This study aims to compare the effects of different mediums on the proliferation, safety, and quality of WJ-MSCs for potential large-scale cell expansion. **Methods:** A total of 5 different growth mediums, i.e., DMEM-LG + 10% FBS, DMEM-LG + 10% HPL, StemMacs, MSC-brew GMP medium and Mesencult-HPL, were tested. The cells' growth kinetic was analyzed by determining the cell viability, cell yield, and population doubling time. WJ-MSCs cultured with selected growth mediums were characterised as being cultured through cell surface CD marker, tri-lineage differentiation potential, immunosuppression assay and cell cycle assay. **Results:** WJ-MSCs cultured with DMEM-LG + 10% HPL, StemMacs and MSC-brew GMP medium were smaller in size than those expanded with the other mediums. Cell yield was significantly higher and population doubling time was significantly shorter when WJ-MSCs were cultured with DMEM-LG + 10% HPL, StemMacs and MSC-brew GMP medium. Thus, only these three mediums were proceeded to WJ-MSCs characterization. The DMEM-LG + 10% HPL gave higher cell yield at lower seeding density than those expanded with StemMacs and MSC-brew GMP medium. WJ-MSCs cultured with all three mediums expressed the MSCs surface markers, able to suppress PBMC proliferation, capable of differentiating into adipogenic, chondrogenic and osteogenic lineages, and most of the cells were in G1 phase compared to S phase and G2 phase. **Conclusion:** DMEM-LG+10% HPL is the best medium for the expansion of WJ-MSCs as it can provide higher cell yield at lower passage without compromising the cell proliferation, quality and safety.

Keywords: Mesenchymal stem cell, Wharton's jelly, Growth medium, Medium optimisation

Moringa oleifera Ethanolic Leaf Extract Prevents Oxidative Stress-Induced Senescence of Mesenchymal Stem Cells

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ABSTRACT

Introduction: The senescence of mesenchymal stem cells during in vitro propagation presents a challenge in mass production for clinical trials and research activities. Oxidative stress aggravates senescence burden in vitro MSCs culture, prompting investigators to develop strategies to mitigate the effect. In this regard, the use of small-molecule inhibitors to prevent oxidative stress-induced senescence of primary cells, especially mesenchymal stem cells, is one of several investigations on this topic. Interestingly, some of the leading small molecule inhibitors with antisenescence activity are polyphenolic, proving the rationale for using *Moringa oleifera* ethanolic leaf extract with abundant polyphenols as phytomedicine with the potential ability to prevent senescence in MSCs culture. **Method:** In this study, oxidative stress was established by exposing MSCs to 3mM of doxorubicin for 2 hours, followed by its removal and subsequent treatment with graded doses of *Moringa oleifera* ethanolic leaf extract (100, 10, 1, 0.1 mg/ml) for 72 hours for downstream experiments. **Results:** Treatment with *Moringa oleifera* ethanolic leaf extract significantly improves the viability of MSCs through scavenging reactive oxygen species and fostering their re-entry into the cell cycle. Further, a decrease in the activity of senescence-associated b galactosidase was seen in *Moringa oleifera* treated groups compared to control, indicating a reduction in senescence burden. In agreement, we also observed a decrease in inflammatory cytokine secretion and apoptosis in *Moringa oleifera* treated groups. Therefore, the involvement of the antisenescence gene and antioxidant gene, FOXO3a and *nrf*-2, respectively, is proposed to be implicated as the mechanism through which the ethanolic leaf extract of *Moringa oleifera* exerts its effects. **Conclusion:** *Moringa oleifera* ethanolic leaf extract prevents oxidative stress-induced senescence by MSCs through scavenging reactive oxygen species, activation of genes involved in antioxidant enzyme expression and antiaging effects.

Keywords: *Moringa oleifera*, oxidative stress, senescence, phytomedicine, mesenchymal stem cells.

In Vitro Evaluation of Thymoquinone Incorporated Collagee Wound Dressing for Future Use in Full-Thickness Wound Healing

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ABSTRACT

Introduction: Treatment of full-thickness skin wounds is technically demanding to patients, medical care, and socio-economic environments. Delayed wound healing, infection, and sepsis are among the most challenging complications which hinder normal wound healing cascade. Thymoquinone (TQ), a hydrophobic compound, has been shown to accelerate wound healing via anti-inflammatory, anti-oxidant, and anti-bacterial properties. This study was designed to evaluate the biocompatibility of human dermal fibroblasts (HDF) towards TQ/dimethyl sulfoxide (DMSO) incorporated genipin (GNP) crosslinked collagen/gelatin/elastin (collagee) bioscaffolds (CGE_GNP). **Methods:** Briefly, GNP-crosslinked collagee bioscaffolds were prepared by lyophilisation method and characterized successfully. TQ solutions were prepared by dissolving in DMSO. The concentrations of 0.05 mg/ml and 0.1 mg/ml were chosen from dose-response and antibacterial study. Then, TQ incorporated CGE_GNP bioscaffolds were fabricated by the same method above. *In vitro* evaluation of HDF towards 0.05CGE_GNP and 0.1CGE_GNP were investigated by scanning electron microscope (SEM), Live/Dead assay, migration assay, MTT assay, cell attachment assay, and immunocytochemistry assay using anti-collagen 1, alpha-smooth muscle actin (**α**SMA), and vinculin antibodies. Non-crosslinked bioscaffolds were used as controls. **Results:** SEM micrographs and Live/Dead assay showed that 0.05CGE_GNP and 0.1CGE_GNP had good cytocompatibility. HDF migration was observed in the bioscaffolds over 7 days. The expression of Type I collagen and vinculin by immunocytochemistry analyses confirmed the HDF proliferation on the bioscaffolds. Finally, MTT assay demonstrated more than 60% viability, while cell attachment assay revealed more than 80% cell attachments. Two-way ANOVA revealed no significant differences between both TQ concentrations and no significant differences between crosslinked and non-crosslinked. **Conclusion:** The application of TQ incorporated GNP-crosslinked collagee bioscaffolds had acceptable cell attachment and biocompatibility properties comparable to the positive control. The prepared wound dressings can be further explored for future application in rapid management of full-thickness skin wounds.

Keywords: Collagee, Thymoquinone, Wound Healing, Genipin, Collagen

Inducing Neuronal Injury on NSC-34 Motor Neuron-Like Cells by Kainate Receptor Excitotoxicity

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ABSTRACT

Introduction: Glutamate toxicity is one of the mechanisms involved in motor neuron degeneration pathogenesis, such as amyotrophic lateral sclerosis and spinal cord injury. The discoveries on glutamate toxicity have mainly focused on ionotropic NMDA and AMPA receptors. However, little is known about neuronal degeneration caused by excitotoxicity of the kainate receptor. A hybrid cell line, NSC-34 is the fusion of mouse spinal cord neurons with neuroblastoma cells. It is commonly used to study the pathophysiology of motor neurons. Therefore, the aim of this study is to investigate the role of kainate receptors in mediating excitotoxicity in motor neuron-like cells, NSC-34. **Methods:** NSC-34 cells were differentiated by serum deprivation and treatment with all-trans retinoic acid. Differentiated NSC-34 cells were exposed to kainate receptor agonist, kainic acid at various concentrations. Apoptotic activity was assessed using Annexin V staining and propidium iodide. Expression of the apoptotic and injury markers were measured using quantitative polymerase chain reaction (qPCR). **Results:** Findings indicate that differentiated NSC-34 cells responded to high concentrations of kainic acid. There is an increased number of cell death as the concentration of kainic acid increased. **Conclusion:** High concentrations of kainic acid trigger excitotoxicity of kainate receptor. Therefore, this suggests that kainate-induced excitotoxicity has a potential role in motor neuron degeneration.

Keywords: NSC-34, In-vitro model, Neuronal Injury, Kainate, Glutamate excitotoxicity, Motor neuron degeneration.

Priming Wharton Jelly's Mesenchymal Stem Cells Enhance Immunosuppressive Properties and Angiogenic Potential

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ABSTRACT

Introduction: Mesenchymal stem cells (MSCs) have shown promising in cardiovascular disease over the past decade, whereby MSCs have been demonstrated to inhibit myocardial fibrosis, promote neovascularization, and induce macrophage polarization. Interestingly, different culture conditions of MSCs influence the phenotype and secretion of MSCs and subsequently alter the MSCs' functionality. Priming with cytokines or growth factors or hypoxia preconditioned has been shown to affect the gene expression and protein secretion of MSCs. Thus, this study evaluates the effects of the priming conditions for empowering the MSCs in terms of immunosuppressive properties and angiogenic potential. **Methods:** WJ-MSCs isolated from umbilical cords were primed with interferon-γ (IFN-γ) and hypoxia preconditioned. The effects of these priming conditions on the RNA expression of WJ-MSCs were evaluated. **Results:** mRNA levels of TGF-β, PGE2 and IDO were significantly increased in the IFN-γ primed WJ-MSCs group, suggesting that IFN-γ mediated the immunosuppressive properties of WJ-MSCs. Moreover, the mRNA levels of VEGF and bFGF were also significantly increased in the hypoxia preconditioned WJ-MSCs group, which suggests that the angiogenic potential of WJ-MSCs can be enhanced under hypoxic conditions. **Conclusion:** The present study suggested that IFN-γ and hypoxic preconditioned WJ-MSCs may possess greater immunosuppressive and angiogenic potential, enhancing their therapeutic efficacy in clinical translation. The preconditioned MSCs could potentially be administered to ameliorate the progression of myocardial infarction by suppressing inflammation and promoting revascularization in the peri-infarcted tissues. However, further analysis such as macrophage polarization and PBMC proliferation co-cultured with primed WJ-MSCs will be required to support this observation before conducting a proof-of-concept study in the myocardial infarction model.

Keywords: Mesenchymal stem cell, IFN-γ, hypoxia, immunosuppressive, priming

Efficacy study of allogeneic Mesenchymal Stem Cell Therapy for the treatment of Metabolic Syndrome in Animal Model

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ABSTRACT

Introduction: Recently, mesenchymal stem cells (MSCs) therapy have been proposed as a novel treatment for Metabolic Syndrome (MetS). The current study aimed to determine the efficacy of MSC IV injection in the rodent model of metabolic syndrome to alleviate the diseases associated with MetS. **Methods:** After 16 weeks of High Fat High Fructose diet, all animals were successfully induced with hypertension, hyperglycemia and hypertriglyceridemia. The animals were then intravenously administered with saline as control or pre-determined low-dose and high-dose WJ-MSCs. Throughout the 12-week study, animals were evaluated for physical measurements, blood analysis, whole-body composition and cardiovascular function. During the terminal stages, necropsy and histopathology were performed. **Results:** The hypertension, hyperglycemia and hypertriglyceridemia levels were varied among the animals. No statistically significant differences ($P<0.05$) between animals from the treatment and control groups. However, we confirmed that different sub-clusters of MetS, such as animals with diabetes, obesity or high blood pressure, were manifested, similar to our pilot study to optimize the diet-induced MetS protocol. Therefore, the data were reassessed through individual or sub-grouped conditions of MetS, which provided a more reliable interpretation of our cell therapy. The WJ-MSCs showed a strong resolution of hemodynamic indices. It had also temporarily relieved excess adiposity, osteo-degenerative factors, and sudden weight loss from chronic insulin resistance. Despite that, the histopathology of liver and lungs in WJ-MSC treated groups showed physical improvements in a dose-dependent manner. **Conclusion:** WJ-MSCs transiently preserved the health status of MetS animals, but single dosage, unchanged diet, and sedentary factors may have limited further recovery. Beyond the scope of this study, we hypothesize that implementing multiple doses or co-therapy (e.g., exercise output or caloric management) could unlock the maximum curative abilities of WJ-MSCs.

Keywords: Umbilical cord; Cell Transplantation; Metabolic Syndrome; Rodent

Development and Characterization of *In Vitro* Airway Epithelium Layer on Nasal Fibroblast Secretome Loaded Hydrogel: A Concept Paper

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ABSTRACT

Introduction: As respiratory diseases are becoming major causes of death and disabilities globally, in vitro studies on the respiratory system have become essential in drug development and toxicology studies for treatments. Epithelial cells are one of the most common models used in respiratory in vitro research; however, due to a lack of understanding of the biochemical requirements for a functional in vitro airway epithelium, they do not exhibit equivalent microphysiological reactions as native epithelial tissue. This lack of complexity in the two-dimensional (2D) cell culture, where epithelial cells are widely grown due to convenience and its low cost has become a large limitation in understanding the pathophysiology and mechanism of illness. Growth factors and extracellular matrix (ECM) proteins are required to mimic the microphysiological complex of real epithelial cells, and studies have shown that fibroblasts can secrete essential growth factors, cytokines, chemokines, and ECM proteins, thus playing an important role in addressing these biochemical issues. In addition, the cells enrich the medium by secreting or releasing proteins and small molecules that play a role in intercellular communication and have biological consequences. **Methods:** The secreted proteins in airway epithelial fibroblasts' culture media, known as secretome, could be retrieved. Furthermore, the model could be improved by using hydrogel as the base for the in vitro model to enhance its topographical cues. **Outcome:** Three dimensional (3D) in vitro culture has brought different insights into cell culture technique as it provides a more realistic model and brings sophisticated reactions such as cell to cell interaction compared to 2D cell culture. Using a hydrogel or scaffold-based model such as collagen could improve the model with cell to substrate interaction and provide ECM needed for epithelial cells such as collagen. With that in mind, this concept paper shows that these concepts can be applied to fabricate in vitro airway epithelium layer where the fibroblast secretome loaded hydrogel functions to supply the required growth factors and ECM protein needed to mimic the native epithelial tissue.

Keywords: Conditioned medium, Secretome, fibroblast, airway epithelium, in vitro model

Hybrid Gelatin-PVA Bioink for Chronic Wound Healing via 3D-Bioprinting (Extrusion-Based Approach): Characterization and Preparation

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ABSTRACT

Introduction: Wound healing is a dynamic and complicated process that initiates the immune response for tissue repair. Chronic wounds occur due to a prolonged inflammatory phase with no progress toward wound closure. Tissue engineering products facilitate wound closure, promote new tissue formation, and reduce scar formation. Extrusion-based 3D-bioprinting is an alternate strategy for fabricating tissue engineering products. The selection of appropriate biomaterials (natural and synthetic) is critical for bioink formulation. As a result, this project aimed to create and describe an in-house hybrid gelatin-polyvinyl alcohol (G-PVA) bioink crosslinked using a natural crosslinker-genipin (GNP). **Methods:** By employing 3D-bioprinting through extrusion-based technology, gelatin with different concentrations of PVA (3% and 5%) was fabricated with 0.1 % of GNP. The physicochemical analysis was performed, including swelling ratio, biodegradation, contact angle, ninhydrin assay and average pore size. The cell-bioink interaction with G-PVA was investigated using a cell proliferation test and a live/dead assay. **Results:** The G-PVA hydrogels crosslinked with GNP demonstrated excellent physicochemical properties compared to non-crosslinked hydrogels. The crosslinked hydrogels significantly demonstrated the acceptable swelling ratio (>500%), biodegradation rate (<0.03 mg/h), hydrophilicity (<90), and degree of crosslinking (>60%) and optimum average pore size (100-199 μm). In addition, G-PVA hydrogel influenced the cell biocompatibility, which successfully indicated >80% of cell viability and proliferation rate. **Conclusion:** Hybrid G-PVA hydrogels crosslinked with GNP was proven to have excellent biocompatibility properties, making them ideal bioinks for chronic wound healing.

Keywords: 3D-bioprinting, wound healing, tissue engineering, regenerative medicine, skin tissue.

The Effect of Nanohydroxyapatite Incorporated With Micro RNA 21 to Regulate Osteogenesis

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ABSTRACT

Introduction: Mesenchymal Bone is a highly specialized connective tissue with unique properties in bone regeneration. Due to its bioactivity, hydroxyapatite has been used in the osseous defect to improve osteointegration. Micro RNA 21 is endogenously expressed to regulate osteogenesis. The coupling of miR-21 and nHA is a potential intervention to harness greater and rapid healing of the host. This study will investigate the combined effect of miR 21 and nanohydroxyapatite (nHA) scaffold to promote osteogenesis by regulating the osteoblastic genes Runx2, OCN, OPN and OPG. The growth kinetics and viability of human mesenchymal stromal cells (hMSCs) upon exposure to miR 21 and nHA will be studied and hMSCs' mineralization activity. **Methods:** Wharton's Jelly cells were obtained from the umbilical cord are collected from patients undergoing Cesarean and furthered by osteoinduction to express bone cell properties. The hWJMSCc cells were characterized by tri-lineage staining and flow cytometry. The size and morphology of the biomaterial nanohydroxyapatite were characterized by dynamic light, scattering (DLS) and Field Emission Scanning Electron Microscope (FESEM). Cell viability and proliferation rate for the hWJMSCs treated with nHA and miR 21 were assessed using Presto Blue assay. The expression of bone proteins was evaluated via Western Blotting. **Results:** The exogenous miR 21 regulates bone-related gene expressions thus it plays a role in bone formation. hWJMSCs cells show no toxicity after being treated with nHA and miR 21. It also shows that these treated cells express increased bone protein expression. **Conclusion:** The combination of miR 21 with nHA can potentially have a synergistic effect on the osteogenesis of hMSCs. In future, the nHA incorporated with miR 21 to be coated on bone implants to enhance osteointegration between the implant and the host tissue.

Keywords: Micro RNA 21; Nanohydroxyapatite; Osteogenesis, RunX2, Osterix

In Vitro Modelling for Studying Schwann Cell Myelination

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ABSTRACT

Introduction: Neuronal – Schwann cell co-culture is a useful experimental model in neuroscience research. The co-culture model is more straightforward than the complex in vivo model and has a robust ethical justification that aligns with the animal ethics principles of the 3R (Replacement, Reduction, and Refinement). Establishing an in vitro myelination model using neuronal-Schwann cell co-culture can be valuable for studying myelin biology and demyelinating diseases. Thus, the study aims to develop an in vitro myelination model using neuronal – Schwann cell co-culture by inducing myelination using adenylyl cyclase agonists and ascorbic acid. **Methods:** Primary Schwann cells and dorsal root ganglia sensory neurons, isolated from Wistar rats, were co-cultured in co-culture media (DMEM D-valine containing 10% fetal bovine serum (FBS), 1% glutamax, 1% penicillin/streptomycin, 5 µM forskolin, and NGF-B 50 ng/mL) on a 24-well culture plate coated poly-L-lysine. The established co-culture was divided into two groups: the control group, which was cultured in co-culture media and the treated group, which was treated with adenylyl cyclase agonists (DMEM D-valine containing 10% FBS, 1% glutamax, 1% penicillin/streptomycin, NGF-β 50 ng/mL, 0.5 µM forskolin, and 20 µM CPT-cAMP) and 50 µg/mL ascorbic acid for 24 days to induce myelination. Both groups were analyzed using immunofluorescence techniques for detecting Schwann cell (S100), neuron cell (βIII-Tubulin), and myelin biomarkers (Krox-20 and Myelin Basic Protein (MBP)). **Results:** Immunofluorescence analysis revealed the presence of Schwann cells and neuron cells in both groups. The axon of neuron cells appeared more extended in the treated group than in the control group on Day-24. Schwann cells in the treated group exhibited a positive Krox-20 expression but a negative MBP expression, similarly observed in the control group. **Conclusion:** The study demonstrated that adenylyl cyclase agonists and ascorbic acid treatment did not induce myelination in neuronal-Schwann cell co-culture. Interestingly, after long-term culture, Schwann cells assumed a pro-myelinating phenotype even without the treatment.

Keywords: Schwann cell, Co-culture, Myelination, Krox-20, MBP

Computational Analysis of Surface Acoustic Wave (SAW) device on Collective Cell migration

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ABSTRACT

Introduction: Collective cell migration promotes tissue remodelling during development, wound healing, and cancer metastasis. Numerous studies have shown how chemical, mechanical, and electrical stimuli influence the behaviours of collective cell migration. Although an acoustic cue can be useful due to its noninvasiveness and biocompatibility, collective cell migration in response to acoustic stimulation is still poorly understood. **Methods:** In this study, a two-dimensional model comprising a Lithium Niobate ($LiNbO_3$) substrate, Aluminum based Interdigital Transducers (IDT), and Mesenchymal stem cells (MSC) and cell medium were developed using FEM analysis software COMSOL Multiphysics 5.6. Two ports of IDT with different wavelengths were modelled to generate propagating SAW and standing SAW. The formation of both SAW fields was applied to the MSC in the cell medium. The effect of maximum displacement amplitude and pressure acoustic generated by the SAW field on the cells were investigated. **Results:** We observed the local stress within cells near the cell-medium and cell-substrate interfaces. For propagating SAW, the shorter wavelength of IDTs attributed to high stress at the cell's top and bottom than the SAW device with the longer wavelength. The standing SAW occurred underneath collective cells. **Conclusion:** The results of standing SAW on cell stress at the bottom confirming that the SAW device can be useful for regulating the abnormalities in tissue remodelling activities associated with cell migration.

Keywords: Collective cell migration, Surface Acoustic Wave (SAW), Interdigital Transducer (IDT)

Fabrication And Characterization of Carrageenan/Gelatin Hydrogel For Future Use in Airway Epithelium *In Vitro* Model

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ABSTRACT

Introduction: Respiratory diseases cause a major global health impact. The airway epithelium, which acts as a frontline defence, is one of the most common targets for inhaled allergens, irritants, or microorganisms to enter the respiratory system. In tissue engineering, hydrogel is commonly used as a scaffold due to its ability to sustain a 3D structure and allow mechanical support for the cells. The combination of carrageenan (carr) and gelatine (gel) hydrogel with suitable physicochemical and mechanical properties plays a part in supporting the in vitro model formation. Thus, developing an ideal biomaterial scaffold for in vitro testing system can improve the mucosalization capability of human airway epithelial cells. The objective of the study is to optimize and characterize the fabricated hybrid hydrogel formulation for ideal physicochemical and mechanical properties. **Method:** The hydrogel was fabricated from kappa carrageenan (carr) and bovine gelatin (gel). 2% and 5% gel solutions were mixed with 2% carr solution in this study. Different ratios of carr/gel-based hydrogel were used to fabricate the hydrogel, which is carr/gel (0:10), (2:8), (4:6) and (6:4). Then, the carr/gel-based hydrogels were cross-linked with genipin, and their physicochemical and mechanical properties such as biodegradation, porosity, water contact angle, gel thickness, swelling studies, viscosity, tensile strength, and biocompatibility will be characterized. **Expected Result:** The varied concentration of gelatin and ratio of the fabricated hybrid hydrogel may be caused by different physicochemical and mechanical results. **Conclusion:** The carr/gel-based hydrogel may be a promising biomaterial scaffold for future use in the airway epithelium *in vitro* model.

Keywords: Airway epithelium, Biomaterial, Carrageenan, Gelatin

Interleukin-17A enhances osteogenic differentiation through the activation of OPG/RANKL and ERK/MAPK signalling pathways in stem cells from human exfoliated deciduous teeth

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ABSTRACT

Introduction: In tissue regenerative procedures, multipotent stem cells derived from human exfoliated deciduous teeth (SHED) represent a promising cell source. Interleukin-17A (IL-17A) have a potential role in promoting osteogenic differentiation of SHED. Herein, we investigated the stimulative effects of IL-17A on osteogenic differentiation in SHED through MAPK and OPG/RANKL signalling pathways and the possible role of miRNA-145 (miR-145) in osteogenesis of SHED. **Method:** SHED were cultured in two conditions; 1) SHED in complete alpha minimum essential medium (α-MEM); 2) SHED in complete α-MEM supplemented with osteogenic reagents and treated +/- with recombinant IL-17A. For osteogenic differentiation analysis, the expression of the miRNA-145 was quantified using real-time PCR; and the expression of specific protein markers was analyzed by Western blot. The effect on the MAPK signalling pathway was assessed by RT2 profiler PCR array and confirmed using ERK/MAPK antibodies by western blotting. **Results:** This work showed that the expression levels of several osteogenic markers such as ALP, COL1A1, RUNX2, OCN, OPN, OPG and OPG/RANKL ratio were significantly increased in IL-17A-treated SHED ($P < 0.01$). Besides, the expression of miR-145 was also up-regulated in both groups by IL-17A treatment. In addition, the result showed that IL-17A activates the MAPK signalling pathway in SHED cultured in both culture conditions by the significant upregulation of all upstream activators and downstream targets of ERK, P38 and JNK pathways. The activation of MAPK signalling was confirmed by ERK/MAPK, which was activated during the osteogenic differentiation of SHED in a time-dependent manner. **Conclusion:** These findings demonstrate that the activation of OPG/RANKL and MAPK signalling pathways and miR-145 by IL-17A is essential for osteogenic differentiation of SHED, which reiterates the significance of these intracellular signalling pathways and microRNA in regulating the osteogenic differentiation of SHED; which is an alternative cell source for bone tissue engineering.

Keywords: Interleukin-17A, MAPK signalling pathway, OPG/RANKL, Osteogenic differentiation, Stem cell

Bilayered Woven Cellulose-Collagen Bioscaffold as Acellular Skin Substitute for Future Use in Diabetic Ulcer Treatment

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ABSTRACT

Introduction: Wound re-epithelialisation is one of the crucial phases in wound healing. The mechanism is dynamic and well-orchestrated, yet it is a complicated process. The hallmark of wound healing is to promote wound regeneration in lesser time without the invasion of skin pathogens into the injury site. This study aimed to develop bilayered woven cellulose-collagen (WCC) bioscaffolds as acellular skin substitutes for future use in chronic wounds. **Methods:** The bioscaffolds were prepared by layering the woven cellulose onto the ovine tendon collagen type I (OTC-I). The WCCs were then posted cross-linked with 0.1% of genipin (GNP) as a natural crosslinking agent. The physicochemical characteristics of bioscaffolds were evaluated through Ninhydrin assay for crosslinking degree, water retention ability, biodegradation rate, wettability (hydrophilic or hydrophobic), resilience, compression, water vapour transmission rate, X-ray Diffraction study (XRD) and Fourier Transform Infrared Spectrophotometry (FTIR). Meanwhile, live and dead assays determined the interaction between cells to bioscaffolds. **Results:** The concentration of free amines in crosslinked and non-crosslinked groups were 0.3 mg/ml and 0.7mg/ml, respectively. The water retention capacity was more than 2000% in crosslinked groups than in non-crosslinked groups, while hydrophilicity was lesser than 900 in both groups. Crosslinked groups were able to return to their original states and resist to extra pressure than the non-crosslinked groups. Besides that, crosslinked groups have a good biodegradation rate (0.014g/hour) compared to non-crosslinked groups (0.023g/hour). Furthermore, the water evaporation rate for crosslinked groups was higher (1200g/m2h) than for the non-crosslinked groups (800g/m2h) after 24 hours. XRD results showed an intensity peak for bilayer groups at 220. FTIR for each peptide group (Amide I, II and III) corresponded to collagen type I, while peaks 2922cm-1, 1624cm-1 & 1422cm-1 corresponded to carboxymethyl cellulose. Both crosslinked and non-crosslinked groups demonstrated cell viability of more than 90%. **Conclusion:** The present study portrayed that WCC bioscaffold possesses good physicochemical properties and is a potential acellular skin substitute for treating diabetic wounds.

Keywords: Diabetes, Wound regeneration, Skin pathogens, Skin substitute, Woven cellulose-collagen

Multifunctionalized Human Collagen Type I Vs. Ovine Collagen Type I Biomatrix for Skin Burn: A Comparative Study

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ABSTRACT

Introduction: Burn injuries are under-appreciated traumatic injuries associated with substantial morbidity and mortality. It affects, particularly severe burns, various functions of the skin and treating them has always been a challenge for humans. Considering the variety of roles played by collagen in lots of tissues, health care and medicine were revolutionized in recent years by developing novel biomaterials to mimic the complex fibrillar architecture that would function as cell scaffolding to replace native collagen-based ECM. This study aimed to extract and develop a novel human collagen type I biomatrix incorporated with quercetin versus ovine collagen type I biomatrix. **Method:** Briefly, collagen type I will be extracted from redundant human skin and ovine tendons using chemical-based extraction, mixed with quercetin, and fabricated via freeze-dry technology. Besides, the physicochemical, cellular compatibility, toxicity, growth profile, and migration will be evaluated. For quercetin efficiency, it will be analyzed by DPPH assay. **Results:** The results demonstrated that the human collagen type I (HC.I) scaffold had better physicochemical and mechanical properties compared to the counterpart scaffold, which is incorporated with quercetin (HC.I-Q), ovine collagen type I (OTC.I) scaffold and (OTC.I-Q). However, all groups showed physicochemical properties within the accepted level. In addition, all groups showed no toxic effect on cells as it promoted optimum cell attachment and proliferation of human dermal fibroblasts (HDF). Furthermore, the antioxidant properties were obvious in the groups incorporated with quercetin (HC.I-Q & OTC.I-Q). **Conclusion:** The findings of this study suggested that both HC.I with/out quercetin, cell-free scaffolds could be promising for the rapid treatment of skin burns.

Keywords: Skin Burn, Multi-functional biomaterials, Human collagen type I, Ovine collagen Type I, Quercetin

Development of a gel formulation with human umbilical cord mesenchymal stem cell-derived exosomes (UC-MSC-Exo) for cosmetic applications

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ABSTRACT

Introduction: Functional cosmetics, which involves the collaboration of stem cell technology with cosmetics, is an emerging trend in the cosmetic industry. Various studies indicated that umbilical cord-derived mesenchymal stem cell (UC-MSC) conditioned media contained various growth factors and exosomes that promote wound closure and re-epithelialization. However, it is still unclear if the UC-MSC-derived exosomes can be used as a cosmetic ingredient to enhance skin rejuvenation. Therefore, the current study investigates UC-MSC-Exo effectiveness in stimulating skin ECM synthesis and formulated as a gel formulation for cosmetic applications. **Methods:** Exosomes will be isolated from UC-MSCs and characterized. Then, the cytotoxicity of exosomes on human skin fibroblasts will be determined through MTT assay and cell growth rate will be determined via trypan blue exclusion assay. The efficacy of UC-MSC-Exo in promoting extracellular matrix (ECM) protein will be investigated through real time-polymerase chain reaction (RT-PCR). Then, the penetration of UC-MSC-Exo into the skin will be determined using fluorescent microscopy. UC-MSC-Exo gel formulation will be developed, and the stability of the formulation will be tested. Functional analysis of UC-MSC-Exo formulation for animal skin permeation and promoting extracellular matrix protein synthesis of human skin fibroblasts will be investigated using fluorescent microscopy and RT-PCR. **Expected Results:** UC-MSC-Exo will stimulate the ECM protein synthesis by dermal fibroblasts. The optimal concentration of UC-MSC-Exo will be used to prepare as a formulation in the gel form for cosmetic applications. UC-MSC-Exo-based formulation shall be safe, stable and effective in stimulating ECM protein synthesis. **Conclusion:** UC-MSC-Exo is expected to stimulate skin ECM synthesis and formulated as a formulation for cosmetic applications. UC-MSC-Exo may have the potential to be used as a cosmetic ingredient in cosmeceuticals to promote skin rejuvenation.

Keywords: Exosome, Mesenchymal Stem Cell, ECM synthesis, Skin rejuvenation, Cosmetic.

Fabrication of Genipin-crosslinked Gelatine Hydrogel for Glottic Insufficiency

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ABSTRACT

Introduction: Glottic insufficiency is characterized by an inability of the vocal fold to close completely during phonation leading to aspiration and phonation issues. Injectable biomaterials are preferred because it is easy, cheap and time-saving. However, current biomaterials used to augment the vocal fold have limitations such as inflammation and degradation. Therefore, better biomaterial with long term effects should be introduced. This study aims to optimize the best genipin-crosslinked gelatine hydrogel with the best physicomechanical properties for glottic insufficiency. **Methods:** Different percentages of gelatine and genipin ranging from 6% to 10% and 0.1% to 0.5% were fabricated. A total of 15 sets was tested with gelation time. After optimizing the gelation time, a total of 9 sets were further characterized using biodegradation, swelling ratio, crosslinking index, contact angle, porosity and water vapour transmission rate (WVTR). **Results:** With 6, 8, 10% of gelatine, 0.3% to 0.5% of genipin were able to achieve gelation time below 20 minutes at 37°C. For the biodegradation and swelling ratio test, 0.4% and 0.5% genipin of 6% and 8% gelatine exhibited less swelling and degradation. In addition, 6% and 8% gelatine with 0.5% genipin had higher crosslinking than 0.4% genipin. The optimized hydrogel sets had hydrophilic properties (<90°), 50 to 60% porosity and acceptable WVTR. **Conclusion:** The optimized 6% and 8% gelatine with 0.4% and 0.5% genipin had acceptable physicomechanical properties. Further characterization using pore size, biocompatibility and cellular response tests should be elucidated to strengthen the application of the gelatine hydrogel for vocal fold augmentation.

Keywords: Glottic Insufficiency; Gelatine; Vocal Fold; Physicomechanical; Injectable Hydrogel

Potential of Small Extracellular Vesicles in Articular Cartilage Regeneration

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ABSTRACT

Introduction: Osteoarthritis (OA) is the most prevalent form of degenerative joint disease, affecting joint functionality and quality of life. The current treatments of OA mainly focus on symptom control and improving joint function. However, it remains a huge challenge to cure or ameliorate OA progression. There is promising evidence showing that cells promote tissue repair mainly via paracrine secretion, including small extracellular vesicles (sEVs). This systematic review investigated the in vivo evidence of sEVs on cartilage regeneration. **Methods:** A literature search was conducted on the PubMed and Scopus databases using the keywords of (exosome OR extracellular vesicle) AND (cartilage OR osteoarthritis OR osteochondral). The Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) risk of bias tool was used to assess the methodological quality of the studies. **Results:** A total of 29 studies with low or unclear risk of bias were included in the review. Intra-articular injection of sEVs into small animal models reduced joint pain and restored joint function with improved joint macroscopic, histological and biochemical features without showing severe immune responses. The modification of the cargo of sEVs further enhanced the therapeutic efficacy. **Conclusion:** The in vivo findings indicated that small extracellular vesicles promote cartilage repair and regeneration without adverse effects.

Keywords: Small extracellular vesicle; exosome; osteoarthritis; cartilage; chondrocyte

Elucidating the multi-approach treatments at different targeted stages of bone healing progression

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ABSTRACT

Introduction: Bone fracture is quite a common incident in every age range. Over the years, various studies and trials have been conducted to identify effective treatments in enhancing the complex and intricate process. Researchers have recently diverted the scope of bone healing treatment toward a tissue engineering loop by merging the method with biomaterials. The primary purpose of this narrative review is to elucidate the potential treatment of bone healing progression by focusing on several targeted stages. The complex bone healing progression involves many growth factors, cytokines, differentiation of cytoblasts, chondroblast, and collagen-rich fibrocartilaginous network before the bone remodelling stages. **Results:** Recombinant human bone morphogenetic proteins-2 (rhBMP-2) are one of the beneficial treatments for bone healing at the early inflammatory stage. Additionally, some articles discovered that the modulation of the Wnt/β-catenin signalling pathway is one of the most critical signalling pathways involved in fracture repair by osteoblast differentiation and osteoblastic matrix production. Besides, collagen is proven effective in wound care management thus, there is a potential application in bone healing progression additionally, some articles have shown that the use of TG2-modified COL I provides a promising new scaffold for promoting bone healing. Both Wnt/β-catenin signalling pathway and TG2-modified COL I are involved in the repair stage. **Conclusion**: This review summarizes that mentioned potential treatment can have a promising effect on bone regeneration and repair.

Keywords: Bone healing, rhBMP, Wnt signaling, Collagen

Study on the characterisation of gelatin-cellulose-chitosan bioscaffold as a potential wound dressing

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ABSTRACT

Introduction: Management of chronic wounds remains challenging even in the 21st century. Conventional wound dressing is ineffective, while skin grafting may fail. Biopolymers that mimic the skin's extracellular matrix are known to promote wound healing, and the mixing of different biopolymers can overcome each other's shortcomings. This study aims to fabricate a natural-based composite bioscaffold using gelatin, cellulose and chitosan as a skin substitute. **Methods:** Gelatin 5% w/v stock was prepared by dissolving gelatin in distilled water at 37°C. Chitosan 2% w/v stock was prepared by dissolving chitosan in 1% v/v acetic acid at room temperature. The stocks were then mixed at the ratio of gelatin: chitosan at 10:0, 7:3, 5:5, 3:7 and 0:10 with the addition of 0.5% w/v nanocrystalline cellulose (NCC) and crosslinked with 0.1% w/v genipin. The mixtures were magnetically stirred for 30 minutes at 37°C, then transferred to silicon moulds, pre-frozen at -80 °C for 6 hours and lyophilized for 24 h. The physicochemical characterisations include swelling percentage, porosity, contact angle, in-vitro biodegradation behaviour, water vapour transmission rate (WVTR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and Fourier transform infrared spectrophotometry (FTIR). **Results:** The highest swelling percentage, porosity and WVTR were demonstrated in the composite bioscaffold of the ratio 0:10, followed by 3:7, 5:5, 7:3 and 10:0. The opposite trend was observed in the biodegradation rate and contact angle. SEM revealed that the composite of ratio 3:7 showed interconnected pores with optimal pore size ranging from 76.05 to 176.5 µm with EDX identified the main elements were carbon (51.5%), followed by oxygen (40.8%) and nitrogen (8.0%). FTIR saw the presence of all functional groups of gelatin, chitosan, and nanocellulose. **Conclusion:** Overall, gelatin-cellulose-chitosan composite bioscaffolds present as the potential skin substitutes in wound healing with the ratio of 3:7 showed to be promising.

Keywords: Wound healing, Scaffold, Tissue engineering

Reusing Umbilical Cord Tissue Sample for Explant Extraction and Isolation of Wharton's Jelly Mesenchymal Stem Cells: A Qualitative Study

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ABSTRACT

Introduction: Wharton's Jelly derived mesenchymal stem cells (WJMSC) have been a preferred source for stem cell studies for their non-invasive and ethical source and high proliferation ability. Several methods have been developed to isolate WJMSC, and currently, the main method that has been used is explant culture and enzymatic dissociation method. However, the tissues cannot be used after the enzymatic dissociation and to date, the tissues are discarded after single explant culture. Thus, this study would explore the possibility of reusing the tissue from the first explant culture into several explant cultures to optimise a single tissue sample usage. **Methods:** Wharton's Jelly was pinched and cut off from a cut open umbilical cord section into about 2 mm to 5 mm size and seeded into a T25 flask. After 3 days of culture, the tissue fragments were removed, washed and seeded onto a new flask. This step was repeated 5 more times. The morphology of the cells was observed under a microscope, and photos of them were taken. **Results:** From the microscopic observation taken after 6 days, we found that the tissue fragments were still viable for seeding even after reseeding 5 times with little to no contamination or losing senescence. This method would help maximise the number of cells isolated and expanded from one tissue sample. Further studies on this method should be done to validate the characteristics of the WJMSC expanded mesenchymal stem cells.

Keywords: Wharton's Jelly Mesenchymal Stem Cells, Explant Culture, Umbilical Cord

Biocompatibility Study of Human Hair Waste on Wharton's Jelly-derived Mesenchymal Stem Cells

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ABSTRACT

Introduction: Natural biomaterials are increasingly popular among researchers in supporting and enhancing the growth of various cells. Interestingly, human hair has shown significant value as a biomaterial. However, most studies utilized its derivatives, mainly keratin, compared to the whole hair fibre. Moreover, carbonized biomaterials have been reported to affect the development of various stem cells positively. Thus, the current study has explored the biocompatibility effect of raw and pyrolyzed human hair samples on Wharton's Jelly derived-mesenchymal stem cells (WJ-MSCs). **Methods:** Carbonized human hair was obtained through the pyrolysis method. Then, the structural morphology and elemental analysis of raw and pyrolyzed hair samples were analyzed under scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). Next, the WJ-MSCs were treated with a different group of human hair samples. Further, the biocompatibility and cytotoxicity effects of the human hair samples on WJ-MSCs were assessed after 24h and 48h incubation time. **Results:** The carbon content of pyrolyzed hair increased compared to raw hair. After 48 h incubation time, the treated cells showed an increased viability percentage of WJ-MSCs. Cell viability on 2 mg of raw hair (2RH) sample shows the highest compared to others. Furthermore, the optical image shows WJ-MSCs internalized carbon particles. **Conclusion:** The present study showed that the whole hair fibre and carbonized hair do not cause significant cytotoxicity on WJ-MSCs. Thus, the human hair fibre and carbonized hair could be considered potential biomaterials in inducing the development of WJ-MSCs.

Keywords: Human hair, Wharton's Jelly-derived Mesenchymal Stem Cells, Natural Biomaterials, Carbon, Biocompatibility

Generation of induced pluripotent stem cells by lentiviral reprogramming with six transcription factors

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ABSTRACT

Introduction: Induced pluripotent stem cell (iPSC)-based models using patient-derived samples are useful resources for studying disease mechanisms in-vitro, particularly in personalized medicine. iPSCs can be differentiated into any cell type in the body and be utilized for disease modelling and therapeutic drug screening. iPSCs can be generated using a combination of key transcription factors; OCT4, SOX2, NANOG, LIN28, KLF4 and C-MYC. This study examined the efficiency of generating iPSCs from three human dermal fibroblasts by integrative lentivirus (LV) reprogramming using 6 transcription factors. **Methods:** Human dermal fibroblasts were transduced with LV encoding transcription factors OSKMNL. Cells grown on inactivated fibroblast feeder layer in an embryonic stem (ES) cell medium for 20-27 days were subjected to daily morphological analysis. In a feeder-free culture system, ES-like cell colonies were picked and expanded by clonal isolation and further characterized by morphology and immunophenotyping for pluripotency and differential potential by embryoid body formation. **Results:** Transduction with LV cocktail of 6 transcription factors produced ES-like cell colonies. Morphological changes in transduced cells were seen as early as 9 days. ES-like colonies from both systems could expand and maintain ES-like cell morphology. ES-like colonies expressed pluripotent nuclear markers OCT4, SOX2 and NANOG, and surface markers TRA-1-81, TRA-1-61 and SSEA4. Expression of >90% of pluripotent markers SSEA4 and TRA-1-81 and <5% differentiation marker SSEA1 was identified by flow cytometry. IPSCs generated were able to form embryoid bodies and spontaneously differentiate into cells expressing markers of ectoderm (AFP), mesoderm (SMA) and endoderm (BIII-tubulin). **Conclusion:** We have successfully generated iPSCs using LV reprogramming of human dermal fibroblasts with a combination of 6 transcription factors. Generated iPSC-lines expressed pluripotency markers with minimal differentiation markers and can differentiate into cells from three embryological lineages. Future applications of the iPSC-cell lines include disease modelling and targeted therapeutics for inherited metabolic and genetic diseases.

Keywords: Induced pluripotent stem cells, Lentivirus, Transcription factor, Reprogramming

Musculoskeletal Anti-Frailty Effects of Wharton's Jelly Mesenchymal Stem Cell and its Derived Small Extracellular Vesicles Transplantation in Aging Mice

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ABSTRACT

Introduction: Projected life expectancy continues to grow worldwide due to the advancement of new treatments and technologies leading to the rapid growth of the geriatric population. Thus, age-associated diseases, especially the musculoskeletal system, are becoming more common. Loss of bone (osteoporosis) and muscle (sarcopenia) mass are conditions whose prevalence is increasing because of the change in population distribution in the world toward an older mean age. Currently, there is no treatment to prevent and reverse age-related musculoskeletal frailty. Wharton's jelly mesenchymal stem cell (WJMSC) and its derived small extracellular vesicles (sEV) transplantation is a promising approach to attenuate age-related musculoskeletal frailty. **Methods:** 18 months old C57BL/6J male mice were randomly allotted into 3 groups: Group 1 (intravenous injection with 0.9% saline); Group 2 (intravenous injection of 25 million WJMSCs/kg body weight); Group 3 (intravenous injection of 100 µg WJMSC derived sEV in 100µL saline). 2 months old young mice injected intravenously with 0.9% saline were used as controls. The effects of WJMSC and its derived sEV transplantation on body composition and physical function, skeletal muscle fibre and mitochondrial function, and the bone density of ageing mice were assessed. **Results:** The body composition and physical function, skeletal muscle fibre and mitochondrial function, and bone density of ageing mice were expected to improve after WJMSC and its derived sEV. **Conclusion:** WJMSC and its derived sEV transplantation may potentially become a promising regenerative therapy for age-related musculoskeletal frailty.

Keywords: mesenchymal stem cells; ageing frailty; musculoskeletal system; bone; muscle

Evaluation of an antioxidant-hydrogel system as a dressing for chronic wounds

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ABSTRACT

Introduction: Chronic wounds represent a high risk of morbidity and mortality since effective therapies for their treatment are still lacking. Particularly, hydrogels are useful as support and protection materials for cutaneous wound healing processes. Furthermore, astaxanthin is one of the most powerful antioxidants, it effectively protects the cell membrane from free radicals and other oxidizing substances. The free radicals play an important role in wound closure time and the quality and aesthetics of the newly formed tissue. **Methods:** Polyethylene glycol (PEG; Mw= 2000, 4000 and 8000 g/mol) hydrogels were synthesized by free radical photopolymerization. Then, astaxanthin (antioxidant) was encapsulated into hydrogels and experimentally evaluated release kinetics. Physicochemical characterization (FTRI, DSC, phase-contrast microcopy and swelling behaviour at 5,20 and 37°C) was carried out. Additionally, hemocompatibility tests in human peripheric blood cells were performed. **Results:** The hydrogel was successfully prepared for the controlled release of astaxanthin. The physicochemical characterization confirmed the formation of the hydrogel system and the incorporation of the antioxidant. The micrographs revealed the porous structure of the polymeric material, however, interconnected pores were not found on the microscale. The highest swelling was reported for the hydrogel prepared with PEG 2000 g/mol (≈1400 %). The hemolysis assay, platelet aggregation and morphology of mononuclear cells demonstrated that the hydrogel-antioxidant system was hemocompatible with blood cells. **Conclusion:** The present study showed that the antioxidant-hydrogel system has good physicochemical properties and is hemocompatible. Thus, the polyethene glycol-astaxanthin hydrogel can be a good material and may be suitable for the treatment of chronic wounds

Keywords: Hydrogel, Antioxidant, Skin tissue engineering

Biodegradable Cross-linked Composite Hydrogels based on Natural Components and Si-βTCP Enriched with Icariin for Osteochondral Regeneration

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ABSTRACT

Introduction: Developing a single monophasic scaffold that can regenerate osteochondral defects becomes a challenge because it requires repairing the damaged articular cartilage, the underlying subchondral bone, and the interface between these tissues. The current study has developed variants of biodegradable cross-linked composite hydrogel based on gelatin, polysaccharidic components (chondroitin-4-sulphate and hyaluronic acid), in a ratio of 2:0.08:0.02 (w/w/w) and mixed with Si-β tricalcium phosphate (Si-βTCP), in two ratios of 1:1 and 2:1 (w/w). Both composite hydrogel variants were cross-linked with (N, N-(3-dimethylaminopropyl)-N-ethyl carbodiimide (EDC) and enriched with Icariin (ICA). **Methods:** The composite hydrogels were characterized for their physicochemical properties such as the enzymatic biodegradation (type I collagenase), the swelling capacity, the degree of crosslinking (TNBS assay) and morphology (SEM). The cytocompatibility was evaluated by analyses of cell viability and cell cycle (flow cytometry), cell proliferation (Neutral Red assay) and cell adhesion to composite hydrogels (SEM) using NCTC clone L929 cell line. **Results:** The final results have shown that both cross-linked composite hydrogel variants enriched with Ica presented optimal physicochemical and structural properties as a scaffold for osteochondral healing. The cells were capable to spreading and proliferating on the surface of composite hydrogels. Also, our data did not reveal any toxicity of composite hydrogels in the NCTC cell line within the tested range of concentrations (10 – 50 mg/mL). **Conclusion:** The present study showed that the designed biodegradable cross-linked composites enriched with Si and Ica are recommended for further testing as natural temporary scaffolds, allowing cell migration and synthesis of new extracellular matrix within osteochondral defects.

Keywords: Composites, Gelatin, Icariin, Osteochondral defect

The Effectiveness of Cord Blood-Platelet Lysate and Peripheral Blood-Platelet Lysate for *In Vitro* Oral Wound Healing

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ABSTRACT

Introduction: Platelet plays an important role during wound healing as it is rich in growth factors such as platelet-derived growth factor. It was observed that Cord Blood-Platelet Lysate (CB-PL), harvested from human umbilical cord blood, has similar efficacy to Peripheral Blood-Platelet Lysate (PB-PL) in initiating the cell growth and differentiation which makes it a unique alternative to implementing in oral ulceration healing. This research study compares the effectiveness of cord blood platelet lysate gel (CB-PLG) and peripheral blood-platelet lysate gel (PB-PLG) in promoting the healing of oral ulcers. **Methods:** Normal oral mucosal fibroblasts were taken during minor oral surgery procedures and underwent a cell culture process. Cord blood was collected through the in utero method, while the peripheral blood was collected from the healthy donors. The blood underwent double centrifugation to obtain the platelet-rich plasma and was analysed for platelet count. The platelet was subjected to three freeze and thaw cycles to produce PL. In order to determine the optimal concentration of CB-PLG and PB-PLG, Alamar Blue Assay was used to examine the cell proliferation of human oral mucosal fibroblasts. Subsequently, a real-time polymerase chain reaction determined the vimentin gene expression in the fibroblasts of CB-PLG and PB-PLG groups. **Conclusion:** It is postulated that CB-PL is as effective as PB-PL and can be used as an alternative to promote the healing of oral ulcers.

Keywords: Cord blood, Peripheral blood, Platelet lysate, Oral ulcer, Wound healing

Fabrication of a nerve conduit with piezoelectric properties containing curcumin in gellan substrate for nerve tissue repair

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ABSTRACT

Introduction: A neural conduit with the piezoelectric behaviour and drug delivery potential would provide the desired microenvironment for peripheral nerve regeneration. Piezoelectric structures with the self-electrical are preferred structures to manage electrical demands and drug release at the target zone in the case of nerve tissue engineering. **Methods:** Bi-layered core-shell neural conduit with the piezoelectric behaviour was synthesized using electrospinning of Poly L-Lactic acid (PLLA) in the outer layer, polycaprolactone (PCL)- Barium Titanate in the inner layer. The pre-gelation method injected the in situ gel (Gellan) into the core structure containing curcumin loaded in sodium alginate nanoparticles. The characterization methods were SEM, FTIR, XRD, DLS, piezoelectric, swelling, gelation time, and drug release. **Results:** In different concentrations of PCL polymer, 10 (%w/v), the proper morphology of fibres was achieved. PCL nanofibres containing (BaTiO3) and PLA showed smooth fibres without any specific beads. SEM analysis shows the proper fibre distribution. XRD spectra indicate the increased crystalline phase compared to the pure one. Synthesized nanofibres indicate piezoelectric properties and Alginate nanoparticles in SEM and DLS tests with spherical morphology and an average particle size of 80 nm. In the drug delivery aspect, a curcumin release profile of 3 days in the dialysis bag and 7 days in the case of nanoparticles loading in the gel was detected. **Conclusion:** Our obtained results illustrate that core-shell conduit filled with Gellan containing curcumin-loaded sodium alginate nanoparticles provides a suitable substrate for peripheral nerve regeneration.

Keywords: Nerve, Tissue Engineering, Nanofibres, Gellan, Conduit

Comparison between human platelet lysate and foetal bovine serum for the expansion of primary human cells

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ABSTRACT

Introduction: Foetal bovine serum (FBS) is widely used to culture primary human cells as it is rich in biological factors that are crucial in maintaining cell survival and stimulating cell proliferation. However, limitations such as the risk of zoonotic pathogen transmission and animal protein contamination have rendered FBS not ideal for expanding cells for clinical applications. Thus, we performed experiments to determine the possibility of substituting FBS with human platelet lysate (HPL) prepared from expired human platelet concentrates for xeno-free expansion of primary human cells, i.e., Wharton's jelly derived mesenchymal stem cells (WJ-MSCs) and chondrocytes. **Methods:** WJ-MSCs and chondrocytes were cultured with HPL and FBS up to passage 3. **Results:** Results showed that WJ-MSCs and chondrocytes cultured with HPL achieved a higher yield with shorter population doubling time than those expanded with FBS. Morphologically, WJ-MSCs and chondrocytes cultured with HPL appeared smaller in size than those expanded with FBS. The WJ-MSCs cultured with HPL showed similar trilineage differentiation potential and expression of CD markers as those expanded with FBS. As for chondrocytes, both HPL and FBS cannot prevent the dedifferentiation of chondrocytes. **Conclusion:** HPL can be used to expand WJ-MSCs and chondrocytes, especially those intended for clinical use.

Keywords: Mesenchymal stem cell, chondrocyte, human platelet lysate, foetal bovine serum, cell expansion

Hybrid Quercetin-Gelastin Injectable hydrogel as Provisional Bio-Template for Future Application: *In Vitro* Evaluation

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ABSTRACT

Introduction: Instantaneous treatment for cutaneous injury is a realistic approach to improve the rate of healing and minimize the risk of complications. Functionalized biomaterials have been proven to be a potential strategy to embark the chronic skin wound management e.g. diabetic ulcers. This study aimed to evaluate the effectiveness of gelatin-elastin (Gelastin) injectable hydrogel incorporated with quercetin (QC) to promote wound closure and newly-formed skin. **Methods:** Briefly, gelatin hydrogel was pre-mixed homogenously with QC followed by the crosslinking with genipin (GNP). Hence the MTT Cell Toxicity assay of the fabricated hydrogels was observed, followed by the physicochemical profile via the swelling ratio, enzymatic invitro biodegradation, and water vapour transmission rate (WVTR). Whereas, for cell-bioscaffold interaction, various assays such as live and dead assay, MTT Cell Viability assay, and Cell Migration have been utilized. **Results:** The crosslinked hybrid biomatrix demonstrated WVTR demonstrated >1500 g/m^{-2} h^{-1}, an optimal moisture content for cell proliferation and regular function. Furthermore, cell-scaffold interaction shows cell viability average of above 90% for live and dead assay, a consistent increase from day 1 to day 7 (85% to <150%) for the MTT Cell Viability assay, and cell successfully migrate through from day 0 to day 7 show great biocompatibility. **Conclusion:** The outcomes convey synergistic functions as a rapid acellular treatment, it is about time to explore the cell-free treatment focus on smart natural biomaterial.

Keywords: Injectable hydrogel, Quercetin, wound healing, biomaterial, acellular

Minidystrophin$^{\Delta H2\text{-}R19}$ Improves Akt Expression and P70 S6 Kinase Activation During Dystrophin-Deficient Myoblasts Differentiation

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ABSTRACT

Introduction: The gene encoding dystrophin is one of the largest genes and results in a rod-shaped dystrophin protein responsible for connecting the cytoskeleton to the extracellular matrix. The absence of full-length dystrophin in Duchenne Muscular Dystrophy (DMD) has previously been shown to cause impaired PTEN/PI3K/Akt. Therefore, the restoration of this protein is hoped to compensate for these effects. However, full-length dystrophin restoration is extremely difficult due to the large size of the dystrophin protein, and consequently, mini-dystrophin has been utilised. **Methods:** In this study, stable minidystrophin-eGFP tagged transfected myoblasts were used. Both C2C12 (non-dystrophic) and dfd13 (dystrophin-deficient) myoblasts were transfected with pCR3.1 eGFP-mini dystrophin (pMDysE) and differentiated for 10 days. **Results:** It is shown that dfd13 myoblasts failed to achieve terminal differentiation when minidystrophin dfd13 myoblasts were introduced. Immunoblot analyses of MyHC and MF-20 were expressed late compared to C2C12-eGFP myoblasts. In dfd13-minidystrophin myoblasts, PTEN expression was found to be reduced upon differentiation. Akt was highly activated at the undifferentiated stage, while p70S6K showed activation upon differentiation. Surprisingly, p70S6K showed significantly more massive activation in dfd13-minidystrophin myoblasts than dfd13-eGFP myoblasts. Although the total expression reduced, it remained active in dfd13-minidystrophin myoblasts upon differentiation. **Conclusion:** Minidystrophin$^{\Delta H2\text{-}R19}$ does not increase differentiation in dfd13 but increases protein synthesis activation in dystrophin-deficient myoblasts.

Keywords: Minidystrophin; Duchene Muscular Dystrophy; myoblast; Akt signalling

A Human Osteoarthritis Cartilage Explant Model For BMSCs Conjugated Antibody Micromass Delivery

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ABSTRACT

Introduction: Human osteoarthritis (OA)-mimicking in vitro models is crucial for pathophysiological studies and evaluating novel treatment approaches such as cartilage tissue engineering. The construction and characterisation of a human OA osteochondral explant culture are presented. **Methods:** Extracted joint tissues from total knee replacement surgeries were sliced into various small and standardised osteochondral plugs for OA explant culturing at different oxygen tensions. The explant cultures were thoroughly described before co-culture with antibody-conjugated MSCs micromass. We evaluated the potency of antibody-conjugated MSCs micromass co-cultured in an OA-mimicking environment for up to 45 minutes and one week. **Results:** We discovered that the OA explant culture cells remained viable for extended periods while preserving the typical chondrogenic and unique OA phenotypes. Our preliminary data showed a significant increase in the binding capacity of antibody-conjugated MSCs micromass to the hyaline cartilage explants ex vivo compared to the MSCs alone (control). The increased fluorescence intensity in antibody-conjugated MSC micromass compared to MSCs alone suggests that our sample of interest is bound higher to the affected site. **Conclusion:** Nevertheless, a 3D quantitative assessment and further investigations involving in vivo models are warranted in the future to prove the binding efficacy and effectiveness of the construct in cartilage regeneration.

Keywords: Cross-linking, Cartilage, Tissue engineering, Stem cell, Scaffolds, Bone marrow