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A003

Citrate-Modified Bacterial Cellulose for Biomedical Applications

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ABSTRACT

Microbial attachment to medical devices such as implants and drug delivery systems limits their efficiency and functionality. Citrate-based biopolymers have attracted researchers' attention due to their unique biocompatibility, antimicrobial, and antioxidant potentials. A novel citrate-based bacterial cellulose (BC) film was developed by chemical cross-linking with citric acid. The modified BC's (MBC) properties were characterized by FTIR, XRD, SEM, SR, TGA and tensile analysis. The MBC films displayed an excellent radical scavenging activity when tested on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and inhibits bacterial growth when tested on *E. coli, P. aeruginosa, S. aureus,* and *E. faecalis* using disc diffusion technique (DDT) and minimum inhibitory concentration (MIC). The MBC film reported here can be relevant in biomedical applications such as wound dressings and tissue regeneration. Similarly, the approach used here can be applied for the quantification of antibiotics on biocide-releasing polymers.

Keywords: bacterial cellulose, citric acid, cross-linking, biomedical application, citrate-based biopolymers

A004

Electrospun Nanofiber for Drug Delivery Applications

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ABSTRACT

Electrospinning is a generally used approach to construct nanofibers of broad range of morphologies. This technique is commonly a very effective and economically feasible method as it is competent to fabricate flexible and scalable nanofibers from a generous variety of raw materials. Electrospun natural and synthetics polymeric nanofibers have recently demonstrated to be a bright method to construct matrices used for drug delivery system. Nanofibers that are designed through electrospinning for drug release purpose is commonly constructed of simple structures. The fabrication of nanofibers through electrospinning process is somehow creating matrices with nanoscale fibers structure, large surface area to volume ratio, and a high porosity with a small pore size. The large surface area to volume ratio of the nanofibers can aid with processes for instance cell binding and multiplication, drug loading as well as mass transfer processes. In addition, drug delivery system that uses nanofibers can be used to deliver particular drugs to specific locations and at specific time to ensure the production of desired therapeutic results. By means, electrospun nanofibers is the drug carrier in drug delivery system as drug is encapsulated within it. Research in the field of nanofibers loaded drugs are primarily categorized into two: first, the preparation and characterization of nanofibers loaded drugs and second, therapeutic applications examination. Drugs that are commonly used in the electrospun nanofibers can be divided into three major groups in which they are antibiotics and antimicrobial agents, anti-inflammatory agents as well as vitamins with medicinal applications.

Keywords: electrospinning, nanofiber, polymer, drug delivery system, drug carrier

A005

Challenges and Opportunities on the Role of Liposomes in Tissue Engineering

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ABSTRACT

Tissue engineering effectively is a technique for encouraging the regeneration of tissues in the body. To create biological organisms, this technique could include combining established types of cells with engineered biomaterial scaffolds. Indeed, Cells, scaffolds, and bioactive factors substantially are the three most major general components of the tissue. In comparison to notably other types of carrier systems, liposomes have quite several benefits. They are derived from natural sources, such as lipids, and can be conveniently synthesized, which is one of their great benefits. Whenever liposomes are paired with biomaterial scaffolds, their therapeutic ability is remarkably moderately enhanced. By allowing improved delivery ecological tolerance and efficacy, these configurations resolve the divergent requirements of treatments. Nevertheless, these were discovered which liposomes were collected in tissues like the liver and spleen and purged through macrophages (the immune system), with clearing being linked to the liposomes' density and structure. Liposomes typically have pretty several benefits as a drug delivery system, including (1) targeted delivery through specifically increased conductivity and persistence in the cancer tissue, (2) superior release of drug management, (3) reduced drug side effects, and (4) enhanced drug stability, which mainly makes them helpful in distributing medicines, vaccinations, and generally other products. Liposomes, on the other hand, have disadvantages that in general restrict their particularly medicinal uses, including (1) complications distributing liquid formulations, (2) lack of kind of physical characteristics, and (3) failure to sustain drug doses in the specific environment. Liposomes have several drawbacks, including inferior chemical stability and solubility, a substantially limited circulating half-life, sensitivity to lipid hydrolysis and oxidation, higher processing prices, and sterilization challenges. Although engineered Liposomes composite Scaffolds are being utilized in Tissue Engineering and Regenerative Medicine, unmodified Scaffolds have restricted potential to establish tissue reconstruction and illness treatment. In general, focuses

on the strengths of liposomes and scaffolds, it is fair to assume that incorporating liposomes and Scaffolds will support drugs retain specific doses in tissues for a long time, as well as the combination structures could create additional features.

Keywords: liposome, regenerative medicine, biomaterial scaffolds , drug delivery, cancer tissue, immune system

A006

Potential Use of Stingless Bee Honey for Wound Healing

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ABSTRACT

Honey produced by stingless bees is known with various names such as Meliponine honey, stingless bee honey (SBH), pot-honey, and also Kelulut honey (in Malaysia). Kelulut honey is multi-floral honey produced by stingless bees, and it gets its name from the bees that produce it, which are known locally as Kelulut. Kelulut honey is gaining popularity among Malaysians due to its distinct flavor and distinct sour taste. It is a valuable bee product that ancient people attributed with medicinal properties. Currently, the demand for stingless bee products such as honey, propolis, and the hive is increasing. Many scientific studies have shown that Kelulut honey has anti-inflammatory, antibacterial, antioxidant, anti-aging, anti-cancer, anti- ulcer, wound healing, antidiabetic, treatment of eye disease, and fertility effects. When compared to Tualang, Gelam, Pineapple, and Borneo honey, kelulut honey has the highest total phenolic content. As a result, the potential use of stingless bee honey for wound healing applications is currently being investigated.

Keywords: stingless bee honey, phenolic content, antibacterial, antioxidant, wound healing

A007

Polymer Composite Electrospun Nanofibers

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ABSTRACT

Polymeric nanofibers are fabricated by various techniques including phase separation, template synthesis, drawing, self-assembly, and electrospinning. Among these techniques, electrospinning is the simplest, most economical, scalable, and flexible technique. Electrospinning uses an electrically charged jet of a polymer solution to produce continuous nanofibers. It has the potential to fabricate nanostructures with outstanding characteristics such as high surface-to-volume ratio, ease of functionalization, and inter/intra fibrous porosity. This electrodynamic technique has attracted the interest of not only academics but also gained immense attention on an industrial scale. A wide range of both natural and synthetic polymers, as well as their blends, with the suspensions of nanoparticles could be electrospun to fabricate composite nanofibers (CNFs) with scalable physical, mechanical and biological characteristics. CNFs have many appealing properties, such as large surface area, improved mechanical properties, material design versatility, tuneable functional properties, durability, wettability, and cellular compatibility. These properties encouraged and broaden their use in a variety of biomedical applications (as drug delivery, tissue engineering, stem cell treatment, cancer therapy, and wound healing) as compare to monophasic nanofibers. Although multiple complications like multiphase compatibility, phase separation, different degradation rates, and surface immobilization are associated with CNFs. However, by choosing suitable natural and synthetic materials, solvents and optimized parameters, CNFs with desired characteristics could be obtained by electrospinning. Furthermore, CNFs could be functionalized by adopting numerous surface functionalization strategies. These advanced nanofibers could find could further expand the application of CNFs biomedical engineering, particularly in tissue engineering.

Keywords: nanofibers, electrospinning, tissue engineering, biopolymers, nanostructures, biomedical applications

A008

3D Bioprinting: Are We Up to The Challenges?

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ABSTRACT

3D printing of porous scaffolds for bone implant or craniomaxillofacial reconstruction should be clearly differentiated from 3D bioprinting modalities. The former method does not use any cells for printing or often introduce cells post printing, if any. The materials used to fabricate the scaffold or fixation is normally from that of metals or hard plastics. 3D bioprinting on the other hand is a technology for fabrication of living tissue construct using biological cells (cell lines, stem cells), biomaterials (crosslinkable polymers) and biomolecules (growth factors) that allow precise placement. This revolutionized approach enables the manufacture of organoids, tissues and organs without the use of traditional tissue engineered scaffold (scaffoldless) thus the term organ printing. Near term in vitro applications of 3D bioprinting include drug discovery, cosmetic testing and personalized medicine. In vivo potential applications for clinical use such as in situ on wound-site treatment using allogenic skin cell and small-diameter blood vessel construct. However printing fully functioning organs remains immature at the present moment. The most significant challenges of 3D bioprinting include vascularization, biomaterials or bioinks, cell source, ethical issues and awareness in integrating the technology. The aforementioned challenges are currently being rigorously explored leading to rapid advancement.

Keywords: 3D bioprinting, tissue engineering, challenges

A010

Safety Study of Allogeneic Mesenchymal Stem Cell Therapy in Animal Model

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ABSTRACT

Mesenchymal stem cells are described as multipotent cells adept in mass proliferation, secretion of bioactive metabolites and ability to differentiate into an array of specialized tissue. The desirable traits for clinical application of MSC encompasses their wide regenerative potential, immunomodulatory properties and biocompatibility of allogeneic grafts to hosts. However, the barriers preventing successful clinical translation is the establishment of a safe and effective dose, a uniformed delivery method and an effective protocol for isolation and expansion of cells. In this study, the safety of high dose Wharton's Jelly-derived mesenchymal stem cells (WJ-MSC) via intra-venous (IV) route in an animal model was determined. Fifteen Sprague-Dawley rats were randomized into a treatment group (n = 9) of high dose WJ-MSC ($10x10^6$ cells/kg B.W.) or a control group (n = 6) of equal volume of saline. Physical parameters and blood analysis (whole blood profile and serum biochemistry) were performed in periods of Week 0, 2, 4, 8 and 12. During Week 2, three rodent subjects from WJ-MSC group were euthanized for acute toxicity study. The remaining subjects were maintained until Week 12 for sub-chronic toxicity evaluation. The post-euthanasia assays performed included pathological observation and histological staining of target metabolic organs. The physical measurements and blood profile was reported unaffected by the intervention of WJ-MSC. Similarly, the post-mortem evaluation presented no significant comparisons

between the control group to acute and sub-chronic phases. The serum biochemistry was unaffected, except for alanine phosphatase (ALP) and lactose dehydrogenase (LDH). The plotted results expressed abnormal ($P<0.05$) fluctuations throughout Week 0 to 12. Despite that, there were minimal-to-absent variations between the two groups for the aforementioned tests. Thus, inferencing that the cell therapy had no correlation to these outcomes. In conclusion, the safety of high dose WJ-MSCs paired with an IV route was successfully determined in an animal model.

Keywords: mesenchymal stem cell, wharton's jelly, cell transplantation, toxicity, rodent

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A012

Quantification of Gelation Time of Biomaterials for Vocal Fold Injection in Glottic Insufficiency: A Preliminary Study

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ABSTRACT

Glottic insufficiency is characterized by incomplete closure of the vocal fold. This condition needs urgent treatment as it influences social functionality and increases the risk of aspiration pneumonia, thus reduces quality of life. Recent studies suggested that vocal fold injection is a promising treatment as it is least invasive and it can be performed in-office, without the need of general anaesthesia. However, current injectable biomaterials such as hyaluronic acid (HA) has drawbacks such as expensive cost, reduced half-life and need of reinjections. The objective of the current study is to develop an injectable biomaterial by using genipin or carbodiimide (EDC)-crosslinked gelatine embedded with Wharton Jelly Mesenchymal Stem Cell (WJMSC) to provide long term regenerative effect. In this preliminary study, optimization of gelation time was the first parameter to be quantified. This is because it is the most fundamental characteristic to ensure that the biomaterial will continue to provide the physical effect of medialization, and is not washed out after injection. In this study, gelation time was studied using vial inverted method. Heating process and concentration of gelatin and crosslinkers were optimized to achieve desired gelation time (less than 3 minutes). Combination of sets (gelatin and crosslinkers) were tested, ranging 5% to 15% gelatine, 0.3 to 0.5% genipin and 0.1% to 0.3% EDC. Total of six different combination sets of 10%, 15% gelatin and crosslinkers: 0.3 and 0.5% of genipin, 0.15% to 0.3% of EDC were able to achieve gelation time less than 3 minutes. The heating range were 60°C to 69°C for gelatin-genipin while 44°C to 50°C for gelatine-EDC sets. Therefore, we suggest to use these six sets of hydrogel formulation to conduct further characterizations such as cell viability, viscosity, crosslinking index, swelling ratio, pore size analysis and elemental analysis to further quantify its suitability as biomaterial in vocal fold injection.

Keywords: vocal fold augmentation, glottic insufficiency, characterization, development, material

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A013

Biofabrication and Characterization of Gelatin-Genipin Bioink via 3D Bioprinting for Wound Dressings Applications

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ABSTRACT

Three-dimensional bioprinting is one of the most promising approaches in the developing field of regenerative medicine. Bioinks made of different compatible biomaterials loaded living cells and growth factors are deposited layer by layer to create complex, highly layered structures that mimic the nature of the human tissues. However, many biomaterials engaged in wound dressing fabrication undergo fastbiodegradation post-implantation and delayed cell migration, resulting in significant tissue regenerationfailure, especially in large wounds. This study aimed to evaluate different concentrations of gelatin (10%, 12.5% and 15%) crosslinked genipin as a bioink via 3D-bioprinting technology to form skin constructs through extrusion method. The construct was printed at a temperature of 24°C using a temperature-controlled in-house bioprinter to further analyze; physicochemical properties and cell- scaffold interaction. The construct showed excellent shape fidelity and shape recovery (~100%), high swelling ratios (more than 200%), and interesting rheological properties. The biodegradation assay showed that the constructs presented low degradation rates (0.01 mg/h) and maintained significantly slow weight loss. Moreover, the skin constructs loaded human dermal fibroblasts (HDFs) showed high cell viability (>90%). This study proves that skin constructs made of crosslinked gelatine have great potential to be used as wound dressings, supporting materials, and drug delivery systems.

Keywords: 3D bio-printing, biomaterial, bioink

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A015

An Efficient Lentiviral System and Optimized Transduction for Gene Transfer in Human Fibroblasts

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ABSTRACT

Lentiviral vectors are one of the most used transfer vehicles for delivery of genetic material to target cells. Continuous improvements for the safety and efficiency of lentiviruses have led to the commercialization of a 4th generation lentiviral packaging system. The 4th generation lentiviral system contains 5 separate packaging plasmids as opposed to 3 packaging plasmids in more widely used 3rd generation lentiviral system, presumed to offer higher safety during lentiviral production. However, the separation of packaging plasmids may cause reduction in transfection efficiency. To obtain the best method for lentiviral production, we investigated the compatibility and performance of 4th generation lentiviral packaging against our standard 3rd generation system used in our laboratory with the use of a 3rd generation GFP-expressing plasmid to transfect and produce infective lentiviral particles. Subsequently, we used the produced lentiviruses carrying GFP gene from the 4th generation system and examined the optimal parameters for transduction and gene expression in our target cell of interest. Lentiviruses encoding a marker gene, GFP were packaged in LentiX 293T either using TakaraBio 4th generation lentiviral packaging kit supplied with Xfect Transfection reagent or 3rd generation lentivirus packaging consisting of pMDLg-pRRE, pRSV-rev and pMD2-VSVG by standard calcium phosphate co-precipitation. Transfection efficiency and subsequent number of viable vector particles was determined by fluorescent microscopy and FACS analysis in LentiX 293T cells. Viral containing supernatant was concentrated and transduced into BJ neonatal foreskin fibroblasts for 24 hours. Expression of GFP was measured by flowcytometry at 72 hours post transduction unless otherwise stated. Multiplicity of infection (MOI), optimal time to analyse GFP expression and effect of polycationic molecule use in transduction were studied

to obtain optimal transduction in our target cells. The 4th generation lentiviral packaging used produced 94% GFP-expressing cells compared to 73% using 3rd generation lentiviral packaging. The relative mean fluorescence index (MFI) of the expressed GFP was also increase by 55% by using the 4th generation system. Viral titre obtained following viral supernatant concentration expressed as transducing units per millilitre (TU/ml) was increased 5.7 times using the 4th generation system. Increasing efficiency of transduction measured by percentage of GFP-expressing cells was obtained in BJ cells between MOI of 1 to 10. Transduction rate reached maximum of 80% at MOI 10. GFP expression increased 10 times when transduced cells were analysed at 72 hours compared to 48 hours. The addition of Polybrene during transduction showed improved transduction rate at lower concentrations over the use of DEAE-dextran. However, at 8ug/ml, both polycationic molecules showed similar transduction rate. Conclusion, the use of commercial 4th generation lentiviral packaging improved the transfection rate of GFP in LentiX 293T cells subsequently resulting in a higher viral titre. Efficiency of transduction in human fibroblasts was affected by the volume of virus used, the duration for gene expression and the use of polycationic adjuvants. This data will lay a foundation for further experiment for reprograming of fibroblast cells into iPSC.

Keywords: lentivirus, human fibroblast, gene transfer

A016

Preliminary Report of Platelet-Rich Plasma Versus Prolotherapy for Treatment of Rotator Cuff Tendinopathy

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ABSTRACT

Rotator cuff tendinopathy is one of the most common shoulder pains seen in clinical practise. The pathophysiology of the conditions is multifactorial from intrinsic such as degenerative tendon to extrinsic cause for example compression to the subacromial space. This results in challenging in the treatment. Studies suggest treatment of the injured tendon may restore the overall function of the tendon. Regenerative treatment with platelet rich plasma and prolotherapy is the options for this condition, which shown good outcome for other tendon pathologies. This randomized clinical trial was done in Sports Medicine Clinic, University Malaya Medical Centre from January 2020 to July 2021. Sixty patients diagnosed with rotator cuff tendinopathy with pain more than 3 months were included. They were randomized into PRP and prolotherapy group. Y cell Biokit system were used for PRP. For prolotherapy dextrose 50% in the concentration of 16.5% were used. All patients underwent similar study protocol with single dose injection under ultrasound guidance into the lesion except the intervention was different. Follow up were done at 3,6,12 and 24 weeks which include pain score, range of movement (ROM), and ultrasound changes. In this report, 30 patients completed 6 months follow up. All outcome showed improvement compared to pretreatment. However, for prolotherapy the pain reduction and improvement in ROM only can be seen after 6 weeks or 3 months. No significant ultrasound changes seen. Three cases reported subacromial bursitis after prolotherapy injection. PRP showed better outcome at earlier time point compared to prolotherapy. However, both studies showed some improvement compared to pretreatment which may indicate the effectiveness of PRP and prolotherapy. Adverse effect was less in PRP compared to prolotherapy. PRP and prolotherapy is a safe treatment option for chronic shoulder pain related to rotator cuff tendinopathy.

Keywords: Regenerative, shoulder, PRP, prolotherapy

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A017

The Development of a Nanofiber Scaffold to Expedite Wound Healing

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ABSTRACT

Recently, electrospun nanofiber scaffolds are gaining enormous attention as wound dressing due to its structural similarities with extracellular matrix and ability to promote cellular proliferation and migration. Extracellular calcium has been proved to influence the healing process of injuries and could be used as a novel therapy for skin wound healing. However, a better understanding of its effect, together with a system to obtain a controlled release is needed. In this study, we examined whether a nanofiber scaffold incorporated with calcium may promote wound healing on human fibroblasts and keratinocytes in-vitro. Cells were cultured in the presence of medium containing different calcium concentrations, ranging from 0.0675 to 1.08 mM Ca^{2+}. A concentration of 0.27 to 0.33 mM of $CaCl_2$ was found to have better morphological distinction, confluence and viability, proliferation and migration rate in both human dermal fibroblasts and keratinocytes in comparison to the other concentrations tested. ECM gene expression study on fibroblasts showed that the expressions of Col I, Col III, FN, and Elastin decreased as the concentrations of calcium reduced. Immunohistochemical staining will be performed on keratinocytes to examine the levels of CK10 and CK14 markers. Interestingly, the results we have obtained thus far showed a dose-dependent behaviour. Later, media conditioned with a specific calcium concentration will be electrospun with PLGA to optimize electrospinning parameters. SEM, FTIR and EDX imaging will be performed on the nanofiber scaffold to understand the physical properties such as fiber morphology, diameter, and alignment. The release of incorporated calcium from the nanofiber scaffold will be evaluated using a Calcium Colorimetric Assay kit. Lastly, keratinocytes and fibroblasts will be seeded on the nanofibrous scaffold to determine its biocompatibility. In summary, this study aims to prove that calcium incorporated nanofiber scaffolds are biostimulators that can be applied when developing dressings for chronic wound healing.

Keywords: calcium, bio-material, nanofiber

A018

Fabrication and Characterisation of Ovine Collagen Type-I (OTC-I) Incorporated with Carvone via Plasma Polymerisation

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ABSTRACT

Emerging research for chronic wound disease involves fabricating ready-to-use biomaterial that provides rapid treatment as well as prevents infections. Carvone can potentially enhance the antibacterial, wound healing and skin regeneration properties of the scaffold for better alternative therapy for chronic wounds. This study focuses on evaluating antibacterial essential oil (R)-(-)-Carvone from the mint incorporated ovine collagen type I (OTC-I) biomatrix followed by different crosslinking methods. The OTC-I is crosslinked through chemical and physical-based approaches of natural plant-based genipin and dehydrothermal treatment (DHT), respectively. Furthermore, carvone was plasma polymerised onto freeze-dried OTC-I biomatrix to form a thin layer composite. The composite biomatrix will be fabricated and evaluated *in vitro* using human skin cells for physicochemical, antibacterial, angiogenesis and cytocompatibility characterisation. Plasma polymerised carvone deposition on OTC-I with both crosslinkers show strong potential for wound healing due to effective antibacterial against *E. coli* and *S. aureus* and angiogenic effect on HUVEC cells. Further research is needed in vivo as well as additional parameters, however, study outcome conveys synergistic functions of acellular treatments, advancing future therapeutic use for DUs using tissue-engineered skin substitutes.

Keywords: wound healing, collagen scaffold, chronic wound, antibacterial, carvone

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A019

Electrically Stimulated Polystyrene Particle Paradigm Towards HEK Cell Migration by Dielectrophoresis for Chronic Wound Curative

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ABSTRACT

Diabetes affects approximately 170 million people globally, with the number expected to double by 2030. In the developed countries, foot ulcers are the leading cause of hospitalization for people with diabetes and major morbidity associated with diabetes, often resulting in pain, suffering, and a poor quality of life for patients. Impaired wound healing is a common and serious problem among diabetic patients. Electrical stimulation (ES) via Dielectrophoresis (DEP) was found to be successful in improving healing rates in diabetic patients with open ulcers. DEP-based cell manipulation methods have several benefits, including low cell viability effects, limited interaction with the cellular environment, label-free and inexpensive. This research used DEP force (F_{DEP}) to manipulate 15 μm polystyrene particles to predict the migration capability of human epidermal keratinocytes (HEK) cells in a highly conductive medium. A numerical modeling method, MyDEP, was used to predict the interpretation of CMF of polystyrene particles and HEK cells. DEP experiments on 15 m polystyrene particles suspended in 0.15 M NaCl suspending medium and HEK cells suspended in Epilife conductive medium respectively were carried out in a tapered electrode using a non-uniform electric field. Negative DEP (N_{DEP}) was observed for 15 μm polystyrene particles and HEK cells at frequencies ranging from 50 kHz to 25 MHz. The estimated experimental data also support the numerical analysis. Therefore, we conducted experiments with polystyrene particles in a highly conductive medium that mimics the migration conditions of HEK cells. The N_{DEP} force is used to migrate target cells in a high conductivity medium. Applying an appropriate electric field frequency to HEK cells in random locations allows them to align at the desired target, which speeds up the healing process.

Keywords: chronic wound curative, dielectrophoresis, electrical stimulation, human epidermal keratinocyte, polystyrene particle

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A020

Dielectrophoresis as an Adjunctive Technique for Fibroblast Cell Migration to Expedite Wound Healing

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ABSTRACT

According to epidemiological studies, the number of patients with diabetes increased from 30 million cases in 1985, 177 million in 2000, 285 million in 2010, about 366 million people worldwide suffering from diabetes in 2011 and the number is estimated to grow to 552 million by 2030. Diabetic patient suffers from impaired or delayed wound healing due to few factors leading to development of chronic wound. It has been proposed that external application of an electrical potential at the wound site mimics the endogenous bioelectric at the wound site to promote wound healing. In this study, we use dielectrophoretic force (F_{DEP}) as an alternative strategy to drive human dermal fibroblast (HDF) cells to expedite the rate of wound healing. This paper describes a velocity spectrum method for studying cell DEP spectra without relying on the simulation of the cell trajectories. In this work, the velocity of the HDF cells were analyse at 10 V_{pp} between 100 kHz and 500 kHz using tapered microarray electrode with 80 μm gap. The findings show that the proposed technique has the potential to drive cell to the targeted area to expedite wound healing. However, there is a high standard deviation compared to the mean value of the velocity of fibroblast cell at every frequency. This high standard deviation indicates that the dielectrophoretic force (F_{DEP}) generated by 10 V_{pp} AC potential is insufficient to significantly manipulate and migrate the cell between the 80 μm electrode gap. Since dielectrophoretic force is directly proportional to the gradient of electric field, $\nabla|\vec{E}|$, there is a need to enhance the electric field gradient. This can also be achieved by conducting single-cell analysis.

Keywords: dielectrophoresis, fibroblast cell, wound healing

A021

The Post-Crosslinking Effects of Genipin as a Natural Crosslinker Towards Collagen-Gelatin-Elastin Bioscaffold Fabrication

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ABSTRACT

The low mechanical strength, fast degradation rate and thermally unstable lead to the unfavorable of collagen, gelatin or elastin scaffold in wound healing. These drawbacks contributed to slow healing and high rate of post-implantation failure. Thus, this study aimed to characterize the effects of genipin (GNP) on collagen-gelatin-elastin (CollaGee) bioscaffold. The fabricated CollaGee bioscaffold with different ratios were divided into non-crosslinked and post-crosslinked with GNP (w/v; 0.1%). The gross appearance and physicochemical evaluation via scanning electron Microscopy (SEM), biodegradation rate, resilience, swelling ratio, porosity, energy dispersive x-ray (EDX), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), and Young's modulus were further evaluated. The post-crosslinked with ratio 4:1:5 (415PO), 4.5:0.5:10 (4510PO) and non-crosslinked with ratio 4:1:5 (415_NC), 4.5:0.5:10 (4510_NC) were compared. Gross appearance described post-crosslinked and non-crosslinked as light blue and white, respectively. SEM revealed heterogeneous porous structure in all groups with water retention capacity more than 1500%. Post-crosslinked groups unraveled 4 to 5 days complete degradation, resilience of more than 60%, denaturation temperature within the range of 80°C to 100°C, Young's modulus between 1.25 to 1.67 Gpa and 70% to 80% porosity. FTIR spectra of non-crosslinked groups demonstrated absorbance peaks for Amide A due to the NH stretching (3317 cm^{-1}), amide I (1631 cm^{-1}), amide II (1550 cm^{-1}), and amide III (1328 cm^{-1}). No major shifting observed after crosslinking with GNP. XRD spectra at 2θ = 20-30° confirmed the amorphous nature in all crosslinked and non-crosslinked groups. These findings suggested that GNP crosslinking enhances microstructure stability and mechanical characteristics of CollaGee bioscaffold.

Keywords: collagen, gelatin, elastin, genipin, biomaterial

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A022

In Vitro Cytotoxicity Analysis of Bioceramic Root Canal Sealers on Human Gingival Fibroblast Cells

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ABSTRACT

Cytotoxicity analysis of various bioceramic root canal sealers are not well understood due to the limited scientific data. Previous research focused on comparing the conventional and bioceramic root canal sealers but there was no comparison between the latter. The aim of this study is to evaluate the cytotoxicity analysis of bioceramic root canal sealers and to propose the material that is less cytotoxic for clinical purposes. Bioceramic root canal sealers, such as GuttaFlow Bioseal, MTA Fillapex, CeraSeal Bioceramic root canal sealer and iRoot SP root canal sealer were mixed according to the manufacturers' instructions and placed into a sterilised cylindrical silicone mold with 5 mm diameter and 3 mm thickness. Fourier transform infrared spectroscopy was used to analyse changes in the quality and consistency of all the bioceramic root canal sealers. Human gingival fibroblast cells were cultivated and exposed to material extracts for 24 h, 48 h and 72 h in a 5% CO_2 humidified incubator at 37°C. The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay was conducted to determine cell viability at each incubation period and compared between all the bioceramic root canal sealers. The cell viability was qualitatively graded as severe (<30%), moderate (30% - 60%), mild (60% - 90%), or non-cytotoxic (>90%). The data were analysed using Statistical Package for Social Sciences, version 25.0. GuttaFlow Bioseal showed less cytotoxicity followed by CeraSeal Bioceramic root canal sealer, iRoot SP root canal sealer, and MTA Fillapex. Clinicians should select bioceramic root canal sealers that have low cytotoxic effects during root canal treatment procedure.

Keywords: bioceramic root canal sealers, endodontics, cell viability,

biocompatibility, human gingival fibroblast cell

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A023

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

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ABSTRACT

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 invading of skin pathogens into the injury site. This study aimed to develop a bilayered woven cellulose-collagen (WCC) bioscaffold as an acellular skin substitute for future use in chronic wounds. The bioscaffold was prepared by layering the woven cellulose onto the ovine tendon collagen type I (OTC-I). The WCC was then post cross-linked with 0.1% 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 and water vapour transmission rate. The results demonstrated less concentration of free amine group in cross-linked bioscaffold which was 0.3 mg/ml than in non-crosslinked group (0.7 mg/ml) , water retention capacity > 2000% in cross linked group than in non-crosslinked group, the biodegradation rate was 0.0007 g/hour for crosslinked group as compared to non-crosslinked group (0.014 g/hour), the hydrophilicity < 90° in both cross linked and non-cross linked groups, ability of the cross linked bioscaffold to return to its original state was more than 80% in cross linked group compared to non-crosslinked group which was 50%, resist extra pressure after applied load was lesser than 20% in crosslinked group than in non-crosslinked group which was 50% and water being evaporated from cross linked group after 24 hours was 1200 g/m^{2}h than in non-crosslinked group which was 800 g/m^{2}h. In conclusion, these results portrayed that WCC bioscaffold as a potential acellular skin substitute for treating diabetic wounds in future.

Keywords: diabetes, wound regeneration, skin pathogens, skin substitute, woven cellulose-collagen

A024

Dielectrophoresis Microelectrode Fabrication for Extracellular Vesicles Application

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ABSTRACT

In this millennial, many efforts have been done to enhance the research to cater the clinical laboratory issues. Lengthy procedures were the main issues nowadays. Dielectrophoresis (DEP) has been introduced and proven to solve these problems; extracellular vesicles (EVs) are in need to be assisted in their isolation process. In this work, MyDEP software has been used to predict the outcome of DEP forces towards EVs; to observe the working frequency for positive DEP, negative DEP and the crossover frequency. On the other hand, COMSOL Multiphysics software provides the insight on how the electric field in the DEP mechanism works on the microelectrode. The DEP microelectrodes have been fabricated using simple and clean methods to manipulate the polystyrene (PS) beads to observe the ability of these new microelectrodes on producing DEP force. Results shown the microelectrode able to produce positive dielectrophoresis (PDEP) at 10 V peak to peak (Vp-p) with 100 kHz as the input frequency. This result has been in tally with the simulation results by using MyDEP and COMSOL Multiphysics software, thus, these microelectrodes will extend their usage for EVs samples in the near future.

Keywords: Dielectrophoresis, Extracellular Vesicles, Microelectrode, Microfabrication

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A025

Deposition of Silk Fibroin Derived Protein Via Atmospheric Plasma Torch

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ABSTRACT

Natural polymers, such as silk fibroin, have been widely used in biomedical filed, especially in the realization of bioactive coating. Various applications require from the coating not only biological properties, but also good mechanical and chemical stability, strong adhesion to underlying surface, with processability of the deposition method. In this study, silk fibroin aerosol solution was injected into atmospheric plasma torch, as a precursor gas, followed with the deposition on glass, Polyethylene terephthalate (PET), poly(dimethylsiloxane) (PDMS), and Ti alloy (Ti6Al4V). High-resolution images of deposited coatings were obtained using filed emission electron microscopy (FESEM). Surface topography was investigated by atomic force microscopy (AFM). The deposition of silk fibroin coatings was confirmed by Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). The stability of the films was observed in phosphate-buffered saline (PBS) solution (pH 7.4) for 2 weeks at 37°C, followed by treatment in sonication bath for 10 min. Adhesion strength was evaluated by peeling test using adhesive tape. Plasma-assisted deposition combined advantages of plasma process and natural polymers and consisted of the combination of two processes: plasma modification of the substrate and formation of the coatings. Covalent bonding with enhanced electrostatic interaction provided outstanding adhesion between substrate and coating. Moreover, due to crosslinking reaction and modification of the polymer during deposition excellent stability was observed. This method provided uniform and homogeneous deposition of silk fibroin coatings on metals with complex geometry, glass with smooth surface, and flexible and stretchable materials, such as PET and PDMS. The deposition was carried out at room temperature and required no pre-treatment or post-treatment procedures, reducing the time and complexity of the process. The processability and reproducibility of the plasma deposition process can promote this method for industrial biomedical applications, such as bioelectronics, medical devices, and implants.

Keywords: Silk fibroin, plasma deposition, thin films, coatings

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A026

Oxygen Donor Microparticles to Prevent Hypoxia in Cell-Laden Scaffolds

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ABSTRACT

In the last decade research in tissue engineering has grown exponentially to overcome the issues associated with treating tissue and organ loss by transplant or prosthetics. One of the latest challenges of tissue engineering is the construction of cell laden scaffolds, consisting in cells encapsulated in hydrogels engineered to sustain their survival and send appropriate biological cues for the meant application. A common problem to overcome in big cell laden constructs (>200 μm) is oxygen deficiency in the core resulting in cell death. This work aims at tackling hypoxia in cell laden scaffolds, by introducing oxygen donor particles. The system studied consisted in alginate hydrogels, physically crosslinked with Ca^{2+}, and containing CaO_2 as a solid oxygen donor. CaO_2 releases oxygen when reacting with water, after the production of hydrogen peroxide as an intermediate. CaO_2 is widely used in various fields, including the biomedical one, in which it is FDA approved for dentistry applications. In this work of research CaO_2 was synthesized in the form of microparticles with a PEG coating to favor dispersion in alginate and uniformity of oxygen release. The particles produced were characterized by XRD and SEM before and after sterilization by γ-ray irradiation. Alginate hydrogels with various particle contents were tested in terms of rheological properties, oxygen release capability and cell viability. The particles were demonstrated able to release oxygen for at least 3 days in alginate hydrogels and to cause cytotoxicity in a dose dependent manner above the 0.7% w/w (on alginic acid content). During rheology the particles also showed to degrade alginate and decrease its storage modulus by oxidation, which should help degradation *in vivo* in mammals. Future work is still needed to optimize this system, which showed to be a promising way to tackle hypoxia.

Keywords: hypoxia, oxygen donors, oxygenated alginate hydrogels, cell laden scaffolds, calcium peroxide

A027

Comsol Characterization and Optimization of The Dielectrophoresis Microelectrodes Gap for High-Efficiency Manipulation of *Enterobacter aerogenes*

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ABSTRACT

This paper is discussing the Finite Element Method (FEM) as numerical analysis to estimate the capability of characterization and optimization Dielectrophoresis (DEP) microelectrode critical gap for manipulation *Enterobacter aerogenes* ATCC 51697 *(E. aerogenes)* pathogen in suspended medium. Meanwhile this study is focused on enhancing the rate of manipulation *EA* pathogen with redesign DEP electrode gaps from previous design 80 µm to 20 µm. The COMSOL Multiphysics software were successfully conducted the 2D electric field intensity and *E. aerogenes* particles trajectory simulations at region of interest (ROI) of DEP microelectrode. The electric field intensity of 80 µm gap DEP microelectrode were around 0.48 x 10^5 V/m to 8.5 x 10^5 V/m and their *E. aerogenes* particles trajectory have velocities around 2 x 10^{-5} m/s to 1.85 x 10^{-4} m/s, while for 20 µm gap microelectrode were 0.12 x 10^6 V/m to 2.11 x 10^6 V/m and 0.05 m/s to 0.5 m/s respectively. This preliminary analysis was indicated by reducing 80 µm to 20 µm microelectrode gap can raise the intensity of electric field strength and *E. aerogenes* particles velocities, thus it can make the high-efficiency of manipulation.

Keywords: dielectrophoresis (DEP), *Enterobacter aerogenes* (EA), critical gap & particle trajectory

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A028

Temperature-triggered Drug (Sea Buckthorn Oil) Releasing Patch for Wound Healing

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ABSTRACT

Encapsulation of lipophilic substances provides an indispensable tool for the formulation of pharmaceutical and tissue-engineered products, improving the handling and storage of oils and lipophilic drugs. Nowadays, microparticulate drug delivery systems are an interesting and promising option when developing a dermal/transdermal controlled release system and bioactive wound dressings. In this work, a thermo-responsive drug delivery system for lipophilic substances was developed for wound healing applications. It permits the release of substances only when in contact with the skin and allows its storage at room temperature. Sea Buckthorn oil, a natural oil beneficial for skin diseases and wound healing, was considered as a model. The emulsions have been formulated after the evaluation of the HLB value of the oil, which was found to be equal to 8. Surfactants were selected in order to guarantee a long-term emulsion stability and to reduce the potential toxicity. For this purpose, a non-ionic surfactant/co-surfactant mixture (Lemon essential oil/Capryol90) was selected, and its cytotoxicity was analyzed by LDH assay test. Gelatin-based emulsions were characterized by rheology and DSC to assess the temperature triggered transition from gel to liquid phase, optical microscopy for the particle size evaluation and in vitro tests to study the cytotoxicity by LDH assay. The cell migration ability was investigated by a scratch assay test in order to simulate the wound healing capability of the mixtures. The thermo-responsive formulation was then loaded in a multilayer reservoir patch designed for wound healing, based on alginate. The oil release was monitored by UV-VIS spectroscopy. The patch offers advantages in terms of versatility, since it can be filled with any gelatin based emulsion/drug depending on the application considered, i.e. cosmetics, wound healing, pharmaceutical and tissue engineering.

Keywords: wound healing, sea buckthorn oil, lipophilic drugs, biomaterials

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A029

Anticancer Effects of Combined Retinoic Acid and Ginger Extract on Hela Cervical Cancer Cells

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ABSTRACT

Cervical cancer is the second most commonly diagnosed cancer in developing countries with high recurrence rate mainly due to the existence of drug-resistant cancer stem cells (CSCs) subpopulation within the tumor. This study was aimed at identifying the anticancer effects of combined Retinoic acid (RA)-ginger extract (GE) treatment on HeLa cells representing an aggressive form of adenocarcinoma of the cervix. The Cell-titre-glo, CyQuant Proliferation, Caspase 3/7 enzyme activity and cell invasion assays were conducted to examine the anticancer effects of RA, GE and RA-GE treatments on HeLa cells viability, proliferation, ability to induce apoptosis and metastatic capacity respectively. In addition, the RA-GE interaction was also studied via the Isobologram-combination index analysis. The effect of RA (5-50μM), GE (25-200μM) and combined RA-GE using RA (5-50μM) and GE (IC_{50}:100μM) were tested on HeLa cells. The inhibition percentage of cell viability and reduction in proliferative capacity of Hela cells resulted in a dose dependent manner with $RA_{(5\text{-}50uM)}$ (8.11% - 68.14%; 80.51% - 25.91%), $GE_{(25\text{-}200uM)}$ (13.59% – 76.49%; 80.06% - 22.67%) and combined $RA_{(5\text{-}50uM)}$-$GE_{(IC5)}$ (54.67% - 96.76%; 43.23 - 3.42%) treatments respectively. In addition, the combined RA-GE_{IC50} also exhibited a clear synergistic effect (CI: 1.20-0.26) on HeLa cells. Next, the combined RA-GE_{IC50} treatment exhibited apoptosis induction via increased Caspase-3/7 activity in HeLa cells from as low as 6 μM RA (1.73%) compared to untreated cells (normalized to 1%) as well as HeLa cells treated with solo RA (6 μM:1.04%) and GE (IC_{50}:1.56%). In contrast to increased apoptotic induction, the combined RA_{IC50}- GE_{IC50} treatment on HeLa cells significantly reduced the metastatic capacity to 36.78%. In conclusion, these findings altogether suggest that the combined RA-GE treatment is a potential therapeutic strategy for cervical adenocarcinoma.

Keywords: ginger extract, retinoic acid, cervical cancer, apoptosis

A031

A Study of The Impact of Using Edible Bird's Nest (EBN) for Serum-Free Cell Culture on Human Mesenchymal Stem Cells

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Edible bird's nest (EBN) which is produced by the hardened saliva of cave-dwelling birds was used by the ancient Chinese due to many health benefits. EBN is mainly composed of different glycoproteins and carbohydrates including sialic acid, glucosamine, and galactosamine. Several studies suggested that EBN has many biological effects including enhancing immunity, protection of dopaminergic neurons, inhibition of influenza viral infections, enhancing cartilage regeneration, and so on. The presence of EBN in the cell culture medium is also found to promote cellular proliferation of rabbit corneal cells and human adipose-derived stem cells. This study aims to evaluate the impact of adding EBN into the serum-free medium for bone marrow-derived human mesenchymal stem cells (hMSCs). hMSCs were cultured in the serum-free medium with different concentrations of EBN (0.05%, 0.2%, 0,4% and 1%) up to 14 days. Metabolic activity and proliferation were performed by AlamarBlue assay and PicoGreen assay, respectively, to check the general cell behavior under different conditions. Immune staining of α5 and β1 integrins were observed by confocal image analysis to evaluate the cell morphology and the activation of receptors over time. To characterize the stemness of the cells cultured with EBN, several markers for stem cells (positive: CD73, CD90, CD105; negative: CD14, CD34, CD45) were selected and the expression levels were measured by real-time PCR. The results showed that only certain concentrations of EBN (0.2% and 0.4%) could stimulate the increasing metabolic activity and cell number, while 1% EBN had a negative impact on cell behavior. The immunostaining showed that the morphology of cells changed over time with the presence of EBN. 0.2% and 0.4%EBN could also activate more expression of α5β1 integrins. The gene expression showed that the cells treated with 0.2% and 0.4% EBN accelerate the differentiation of hMSCs and 0.05% EBN could keep the cells remaining the stemness compared with other groups.

A032

Generation and Characterization of Amniotic Fluid Stem Cell (AFSC) Line-derived Neural Stem Cells (NSCS)

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ABSTRACT

Neural stem cells (NSCs) serves as a high prospective cell for neuro transplantation as one of the treatments for neurogenerative diseases (ND). The inaccessibility of the brain sourced NSCs has limit its application and leads to the idea of generating NSCs from non-brain sources. Amniotic fluid stem cells (AFSCs) are neurogenic and may be a potential non-brain source of NSCs. Due to its accessibility and therapeutic potential, the isolation of AFSCs from full-term gestation has garnered a lot of attention, including as the potential non-brain source for NSCs. This study aims to evaluate the proof of concept on potential of full-term rat amniotic fluid stem cell line (R3) to generate NSCs and the ability of the R3-derived NSCs to form neurospheres and differentiate into neurons. R3 was cultured in a culture containing GMEM supplemented with 10% foetal bovine serum (FBS), other essential supplements and 20 ng/ml Leukemia inhibitory factor (LIF) prior to trans-differentiation into NSCs through monolayer differentiation assay in NSC culture medium for two days. The generation of NSCs was then confirmed by immunocytochemistry analysis on the expression of NSC protein markers (Sox1, Nestin). R3-derived NSCs were further tested on its ability to form multicellular aggregates- neurospheres by plating NSCs in uncoated bacteriological grade dish in the NSC medium for 3 days. The diameter and the ability of the neurospheres to form neurons were examined using the cell Sens Standard computer software and immunocytochemistry for neuronal markers expression (Tuj1, MAP2), respectively. R3 have transdifferentiated into NSCs upon induction with NSC medium and expressed NSC markers (Sox1 and Nestin). The NSC properties were further confirmed with the formation of neurospheres with diameter of 100-150 μm, and the differentiation of neurons expressing (Tuj1, MAP2) marks the success in generating NSCs from R3. Thus, this finding signified the ability of AFSCs from full-term amniotic fluid as a potential non-brain source of NSCs.

Keywords: stem cell, neural stem cell, amniotic fluid stem cell, full-term rat amniotic fluid stem cell, neurosphere

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A033

Effect of Enzymatic Fragmentation on Silk Fibroin Properties

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ABSTRACT

Silk fibroin, derived from silkworms, is a natural polymer with remarkable properties. It gained much interest in the biomedical field, due to its ease of processability and modification, tunable mechanical properties, biodegradability, cell friendly behavior. Fibroin has been reported in several regenerative medicine applications such as for bone, ligament, blood vessels, nervus system regeneration, among others. Silk fibroin particular secondary structure can rearrange into different structures according to the treatment performed. Degumming time and conditions induce some degradation in the fibroin final molecular weight, that is not predictable and controllable. However, according to the final application, a controlled tunability of silk molecular weight and secondary structure rearrangement might be required. For this reason, here we proposed an enzymatic silk fibroin fragmentation process which can selectively cleave silk fibroin in precise sites along its sequence, and in a controlled manner. Collagenase type G was used at different concentrations to fragment fibroin, and the derived materials with different fragmentation levels were analyzed in solution and as cast films, stabilized via both water-vapor annealing and via methanol. DLS, GPC, TNBS, FTIR, DSC, TGA, and NMR were performed to investigate the properties of the fragmented materials compared to the untreated silk. We demonstrated that molecular weight can be precisely decreased by varying the enzyme concentration, and that the cleavage at the peptide bond generates free terminal NH_2 groups, that are suitable for tailored conjugations. The fragmented fibroins have been proved to be stable in water up to 7 days. In this preliminary work the selective fragmentation was demonstrated to be a useful tool for preparing fibroin-like shorter aminoacidic sequences, tailoring their secondary structure, with interesting perspectives for applications in the biomedical field.

Keywords: silk fibroin, fragmentation, tunability, collagenase G

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A034

Piezoelectricity of a Bio-inspired Multifunctionalized Silk Fibroin

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ABSTRACT

The piezoelectricity of the bio-inspired multi-functionalized silk fibroin (BMS) (described in our previous work) was evaluated by means of a modified multimeter connected to a compression machine. The BMS solution was exposed to an electric field generated by a potential of 24 V or 220 V in direct or alternating current up to the BMS hydrogel formation. The presence of collagen type IV and laminin peptides conjugated to the silk fibroin increases the potential of the SF-based hydrogel, mainly, due to the collagen piezoelectricity expressed after shear deformation. The acquisition of a voltage in the range of 8,5 +/- 3,5 V during the BMS hydrogel compression opens the possibility to apply the system as a scaffold for the spinal cord regeneration in order to help the cell-cell interaction and neural stem cells differentiation into neurons. Indeed, the excitatory postsynaptic potential is around 12 mV, as well as the voltage measured after hyperpolarization (AHP) is around 5.4 mV in native conditions. The mechanical deformation of the BMS hydrogel might be obtainable by the movement of the neck or the back, generating the potential directly inside the spine.

Keywords: hydrogel, piezoelectricity, silk fibroin, BMS

A035

Micro-computed Tomography to Investigate the Bone Microstructure in Mice with Ovariectomy-induced Osteoporosis

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ABSTRACT

Micro-Computed Tomography (μCT) is a state-of-the-art technique to investigate bone microstructure. In the present study, we have examined the effect of ovariectomy on bone microstructure, so that the role of ovariectomy (ovx) may be assessed when the influence of estrogenis removed. Ovariectomy were performed to 3 out of 6 mice to represent osteoporosis in human. After 10 weeks, the mice were euthanised and the femurs were dissected. Then, the femurs were dried prior to μCT scanning after stored in formalin buffered 10% overnight and subsequently immersed in saline. The femurs were imaged with μCT Skyscan 1172 (Bruker μCT, Kontich, Belgium); voxel size 8 μm, 20.5 min scan and analysed using CT-analyser (CTAn) for bone quantification. Micro-ct analysis demonstrate 13.7% reduction in bone volume (BV/TV) and increase bone separation (0.2%) can be observed in osteoporotic mice. The same trend can be found for cortical thickness at distal region. Trabecular thickness did not show substantial variation in the two cases. This study enabled non-destructive μCT analysis of trabecular and cortical bones in the investigation of mineralised bone quantity. Under an idealised situation where estrogen is present, the amount of bone is more than the ovx mice, suggesting that the estrogen contribute to the underlying mechanism of bone regulation in adult bone. When the bone turnover is not regulated, the amount of space in the bone increased. This seems to suggest that the bone is more porous and this is inevitable in osteoporosis adult bone. Substantial trabecular bone reduction occurred at proximal femur region, particularly in ovx mice is reliably detected by the μCT. This finding may be useful in the studies of osteoporosis bone and other related orthopaedic treatment strategies, asestrogen presence has an important role in the

bone regulation process.

Keywords: Micro-Computed Tomography (μCT), ovariectomy, bone morphology, osteoporosis, bone effects

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This study was supported by FRGS-RACER 5/3 (096/2019) and GPK 5/3 (241/2020) Research Grants.

A036

The Effect of Different Concentration of Aqueous Extracts of *Labisia pumila* in Preventing Osteoporosis and Improvement of Dermal Elasticity in Polycystic Ovary Syndrome Rats

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ABSTRACT

Labisia pumila (LP) a Malaysian herb thought to have phytoestrogenic effects in rats with PCOS. In this study we investigate the effects of LP aqueous extracts on bone composition, bone biomarkers and metabolic disorder in female rats fed continuously with high fat diet to induce PCOS. The administration of *Labisia pumila* treatment in PCOS rats for 90 days will ameliorate the adverse effect of osteoporosis by reducing the inflammatory cytokine and improving the dermal elasticity of PCOS rats. On the 90th day of the development of PCOS model rats fed with high fat diet and after the vaginal smear analysis indicating a prolonged estrus cycle of more than 2 weeks, all PCOS rats were divided into 4 groups which consisted of control, placebo (water), LP25 (LP 25 mg/kg) and LP50 (LP 50 mg/kg) respectively. All PCOS continue to receive the formulated high fat diet and control animals continued to received normal chow and water ad libitum. Vehicle and treatments, which were given orally by using stomach gavaging needle size 16 gauge straight and curve retrolingual administration. The rats were sacrificed at the end of the trial phase and organs, tissues and blood samples were harvested for multiple assays and analysis listed below. The level of estradiol was significantly increased in LP25 and LP50 as compared with placebo. The inflammatory cytokine of C-reactive protein and TNF-α were significantly decreased in LP25 and LP50. Treatment of LP extract might reduce the inflammatory cytokine related to the formation of osteoporosis and loss of bone mass, increase the insulin sensitivity and reduce of osteoporosis in PCOS patients. The phytoestrogenic of LP all of the above significant positive results are proven in lowering osteoporosis and metabolic disorder in PCOS rats.

Keywords: *Labisia pumila*; PCOS; osteoporosis; skin elasticity

Abbreviations: LP, *Labisia pumila*; PCOS, polycystic ovary syndrome; TNF-α, tumor necrosis alpha; DHT, dihydrotestosterone; HFD, high fat diet; UPM, Universiti Putra Malaysia; BSEM, bone scanning electron microscope; SEM, scanning electron microscope; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkanline phosphate.

A038

Angiogenic Potential of Fiber-coated Human Amniotic Membrane Scaffold for Ischemic Tissue Repair

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ABSTRACT

We previously fabricated EF-HAM, a composite human amniotic membrane (HAM) scaffold coated with aligned poly lactic-co-glycolic acid (PLGA) electrospun fibers (EF), thus producing a mechanically competent tissue engineered scaffold with cell guiding ability. At present, we aimed to evaluate the angiogenic potential of skeletal muscle cell-seeded EF-HAM on endothelial cells via paracrine secretion for future use in ischemic tissue repair. 20% PLGA 50:50 polymer was electrospun on decellularized HAM for 3 mins, 5 mins or 7 mins to create composite EF-HAM scaffolds with varying fiber thicknesses. Skeletal myoblast-enriched cells were later seeded on HAM and EF-HAM scaffolds at 20,000 cells/cm^2 until reaching confluence and the conditioned media (CM) from the tissue constructs were collected in serum-free culture medium after 72 hours. The concentration of selected angiogenic factors in each CM were determined using multiplex assay and subsequently tested on HUVECs for cell proliferation, migration and tubule formation. CM collected from skeletal muscle cell-seeded HAM and EF-HAM scaffolds contained significantly elevated concentrations of pro-angiogenic factors including angiogenin, IL-8, FGF basic, PlGF and VEGF-A compared to Plain CM, which was conditioned medium collected from skeletal muscle cells seeded on 2D plain surface. At Day 5, the viability of HUVECs grown in the presence of CMs derived from EF-HAM 3 min, 5 min and 7 min scaffolds were significantly higher than those grown in Plain CM. HUVEC growth rates in all groups were significantly higher when compared to Non-CM control. A significant increase in the migration capacity of HUVECs and higher tubule formation potential on Matrigel were observed when endothelial cells were co-cultured with HAM- and EF-HAM-derived CMs in comparison to Non-CM control. However, only EF-HAM 7 min CM could induce higher migration capacity in HUVECs and form more elaborate capillary-like network on Matrigel against Plain CM. Skeletal muscle cell-seeded EF-HAM scaffolds can promote tissue vascularization through paracrine secretion of potent angiogenic mediators. In summary, EF-HAM composite scaffold design holds promising application in the regeneration of ischemic tissue in the

future.

Keywords: angiogenesis, electrospinning, amniotic membrane, myoblast, paracrine

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A039

In Vitro Modelling for Studying Schwann Cell Myelination

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ABSTRACT

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 become a valuable tool for studying myelin biology and demyelinating diseases. 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. Sensory neurons and Schwann cells were dissociated from Wistar rat and co-cultured in a co-culture medium on a 24-well culture plate. The established co-culture was treated with adenylyl cyclase agonists (forskolin and CPT-cAMP) and ascorbic acid for 24 days to induce myelination. The induced co-culture was then analyzed using immunofluorescence and fluorescent microscopy techniques for myelin biomarkers (Krox-20 and Myelin Basic Protein (MBP)). Immunofluorescence analysis revealed that Schwann cells align the axon of neurons in the control group and myelination induce group. Schwann cells in the treated group also exhibited a positive Krox-20 expression but a negative MBP expression which was similarly observed in the control group. Using low dose forskolin in culture media possibly sufficient to promote Krox-20 but not MBP expression, as evidenced of CPT-cAMP incapable to induce myelination. However, cell-to-cell interactions between neuron cells and Schwann cells also might able to trigger pro-myelinating signal in Schwann cells, which suggests the existence of axonal signals that leads to the activation of adenylyl cyclase to promote Schwann cell myelination. The study demonstrated that adenylyl cyclase agonists and ascorbic acid treatment did not induce myelination in neuronal-Schwann cell co-culture. Interestingly, Schwann cells assumed a pro-myelinating phenotype after long-term culture even without the treatment.

Keywords: schwann cell, co-culture, myelination, Krox-20, MBP

Acknowledgement: FRGS grant and UniKL MESTECH

P001

Elucidating the Correlation between Estrogen and Wnt4 in Vagina Epithelial Cells: An Epithelial-Mesenchymal Transition (EMT) Event in Vagina Agenesis

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ABSTRACT

Young women with vaginal agenesis (no vagina) usually present with primary amenorrhoea (no menses). However, those with functioning uterus but no vagina, will experience cyclical abdominal pain due to accumulation of blood in the uterus (haematometra) hence require laparoscopic drainage for symptomatic relief. Mainstay of treatment will be a two-steps procedure: creation of vagina (neovagina) and later anastomosis of uterus with the newly created vagina. This allows menstrual outflow and coital function. Several techniques had been described for neovagina using different material such as peritoneum, amnion, buccal mucosa, allogenic epidermal sheets and Interceed to line the vaginal canal. Recently, autologous vaginal mucosa had been shown to have promising result. Hence, we aim to elucidate the correlation between estrogen and Wnt4 signaling in vagina epithelial cells (VECs). Vaginal mucosa tissue will be harvested from the hysterectomy specimens of non-menopausal patients and will be differential trypsinized. The cells will be cultured and characterised via MTT assay, flow cytometry, western blot and immunofluorescence techniques. In this study, two vagina epithelial models will be established for correlation studies: 1) Active Wnt4-V5 vagina epithelial cells and 2) Wnt4-shRNA-eGFP vagina epithelial cell line (VK2) models will be carried out using Lipofectamine® LTX and PLUSTM Reagents. Both will be subjected to transfectants validation by immunofluorescence and western blot techniques. These cells will be induced with estrogen and optimization will be conducted for determining the optimal growth conditions of the cells. Downstream proteins of Wnt signalling such as β-catenin and LEF-1 and epithelial mesenchymal transition (EMT) markers, such as E-cadherin, vimentin and SNAIL/SLUG will be analysed via western blot technique. The findings from this study will give new insights into the

mechanisms between estrogen and Wnt4 in regulating vagina epithelialisation to ensure better surgical outcome

Keywords: Epithelial-mesenchymal transition, estrogen, vaginal agenesis, Wnt4

P002

Coronary Artery Bypass Grafting (CABG) Grafts: Human Saphenous Veins (HSV) Comprehensive Histopathologic Extracellular Matrix (ECM) Study

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ABSTRACT

Human saphenous vein (hSV), are the most used graft covering around 70-75% of coronary artery bypass grafting (CABG). Unfortunately, SV graft patency are recognized to be poor with 50% graft failure observed at 5 years after CABG bypass. Extracellular matrix (ECM) provides the structural and mechanical support as well as an anchor for cells. We hypothesized that the saphenous vein used as a graft in CABG are diseased hence impact the long-term patency of the graft in coronary position. Hence, the present study aims to evaluates endothelial cell (EC) lumen coverage and several ECM components i.e., collagen, elastin and glycosaminoglycans (GAGs) in surplus hSV collected from CABG surgery. Surplus hSV from CABG surgery at Hospital Canselor Tuanku Mukhriz (HCTM) were collected directly from operation theatre and transported to the lab to be fixed and processed. The tissue was then embedded, sectioned and stained. The lumen cell's nucleus, collagen content, elastic fibres, as well as glycoprotein were quantified via histological sections stained with Hematoxylin and Eosin (H&E), Picrosirius Red (PSR), Elastin Van Geison (EVG) and Alcian blue respectively. All observation with regards to ECM in the vein samples was quantified using ImageJ and data are presented as mean ± standard error of mean of at least N = 6. The saphenous vein comprehensive histopathologic ECM study was done. The EC coverage was around 2.2 ± 0.3 % of the lumen surface. The collagen content was observed using polarized light microscopy shows slightly higher thin collagen fibres with 0.09 ± 0.01% as compared to 0.06 ± 0.01% thick fibres. Elastin was observed to constitute 0.18 ± 0.04% of the vein ECM with 0.1 ± 0.01% was observed to be GAGs. The saphenous veins endothelial layers were mostly absent maybe due to damage during extraction before implantation. The observation of ECM content serves as a baseline ECM content for comparison with suggested treatment groups in future ex vivo graft improvement studies.

Keywords: CABG, saphenous veins, extracellular matrix, histological

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P003

The Effect of Injectable Hybrid Gelastin Hydrogel for Wound Healing: Quercetin vs Epigalocatechin Gallate

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ABSTRACT

Immediate treatment for full-thickness skin 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. This study aimed to evaluate the effectiveness of gelatin-elastin (Gelastin) injectable hydrogel incorporated with either quercetin (QC) or epigalocatechin gallate (EGCG) to promote wound healing and skin regeneration. Briefly, gelatin hydrogel is pre-mixed with QC/EGCG followed by the addition of genipin (GNP) as a natural crosslinker. The gross morphology of the non-crosslink and crosslinked hydrogel was observed, followed by the physicochemical properties; via the mechanical profile, swelling ratio, enzymatic biodegradation crosslinking degree, WVTR followed by cellular compatibility through live & dead assay. The crosslinked biomatrix demonstrated better mechanical strength (compressed <15% and resilience 100%), swelling ratio (500 ± 10%), degradation rate (<0.002 mg/hour) and crosslinking degree (>0.5 mg/ml free amine group) in comparison to the non-crosslinked hybrid biomatrix. In addition, WVTR demonstrated >1500 g/m^2 h, an optimal moisture content for cell proliferation and regular function supported by live and dead results with excellent cell viability for EGCG and QC hydrogel. In conclusion, the EGCG and quercetin incorporated hydrogels provide the optimum outcomes to be used as a provisional biotemplate of skin tissue.

Keywords: wound healing, full-thickness skin wound, quercetin, epigalocatechin gallate, injectable hydrogel

P004

Physicochemical and Mechanical Properties of Hybrid Gelatin- PVA Bioinks for Future in Vitro 3D Skin Model Development

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ABSTRACT

The 3D in vitro model is an alternative approach to skin safety testing for pharmaceutical and cosmeceutical applications. Prolongation and stability are key successes of 3D model development from natural-based materials. Thus, the combination of gelatin and polyvinyl alcohol (PVA) as a natural and synthetic polymer, respectively, could provide a better improvement of physicochemical and mechanical strength. This study aimed to develop in-house hybrid gelatin-PVA (G-PVA) bioinks and determine the physicochemical and mechanical properties of the printed hybrid G-PVA hydrogels crosslinked with the genipin (GNP) as a natural crosslinker. The hybrid gelatin/gelatin:PVA hydrogels were fabricated with 0.1% of genipin as crosslinker by using the conventional 3D-bioprinting approach. The physicochemical analysis tests including swelling ratio, biodegradation, wettability, water vapour transmission rate and crosslinking degree were evaluated. The mechanical strength via compression and resilience was also measured to evaluate its ability to withstand pressure. The gelatin-PVA hydrogels crosslinked with genipin demonstrated excellent physicochemical properties compared to non-crosslinked hydrogels. The crosslinked hydrogels significantly show the optimum swelling ratio (>300%), biodegradation (<10 mg/h), hydrophilicity (<90°), water vapour transmission rate (>1500 g/m^2 h^{-1}). Besides, the crosslinked hydrogels also significantly indicate the improvement of mechanical strength (>70%) after a combination of gelatin with 5% PVA and 0.1% of genipin compared to non-crosslinked and crosslinked gelatin hydrogels. Hybrid gelatin-PVA hydrogels crosslinked with genipin proven to have excellent physicochemical and mechanical properties as a bioink for 3D in vitro skin model.

Keywords: 3D-bioprinting, skin, bioink, gelatin, PVA

P005

Incorporation of Hybrid Graphene Oxide-Silver Nanoparticle-nanocellulose on Bilayer Gelatin/ Collagen Scaffold

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Single-layer biomaterials have shown impressive results throughout the years in skin tissue engineering. However, single-layer scaffolds commonly have low mechanical strength and degraded faster prior to tissue regeneration. Besides, a secondary infection could delay the normal wound healing and ultimately causing post-implantation failure. Therefore, this study aimed to fabricate and characterize a bilayer scaffold containing of collagen sponge and functionalized-nanocellulose (CH) hydrogel. The ovine tendon collagen type-I (OTC-I) sponge was used as the bottom layer of biomatrix to facilitate cell proliferation and migration, while the functionalized CH at the top layer acts as a barrier enhanced with antibacterial capacity. Hybrid graphene oxide and silver nanoparticles were incorporated in the CH to maximise the antibacterial effect. Various tests were done to investigate the physico-chemical properties of fabricated scaffold that includes the water absorption ability, the degree of crosslinking, enzymatic biodegradation, surface wettability, porosity, homogeneity, water vapor transmission rate and FTIR. Additionally, the mechanical strength of bilayer scaffold was determined using tensile strength analysis. The bilayer scaffold exhibited favorable results for wound healing applications demonstrated acceptable swelling ability (1347.01 $\pm$ 471.31%), highly porous microstructure (>50%), and good wettability (48.10 $\pm$ 1.86°). Moreover, the fabricated bilayer scaffold showed great mechanical properties as the ultimate tensile strength is 3.71 $\pm$ 0.02 MPa. Thus, the fabricated bilayer scaffold can be a potential candidate for the wound healing application.

Keywords: biomaterial, skin tissue engineering, nanotechnologies, collagen, cellulose

P006

Dual-layer Hybrid Biomatrix Incorporated with Elastin for Diabetic Ulcer

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ABSTRACT

Diabetic foot ulcer is one of the most common complications of diabetes associated with neuropathy or peripheral arterial disease at lower limb. Amputation most likely the final resort if it is not treated properly and no progress. The gold standard of treatment for diabetic ulcer is by total contact cast (TTC). However, TCC application has been led to iatrogenic complications, such as new ulcer information, blisters, abrasions, infection and ulcer. This study is aimed to fabricate and characterize a bilayered hybrid biomatrix; film (gelatin mixed with PVA) and sponge (collagen) for the treatment of diabetic foot ulcer. The film (top layer) consists of halal gelatin (IFANCA certified) produced from buffalo bones mixed with polyvinyl alcohol incorporated with elastin from chicken act as a bioactive component that potentially reduce skin contraction. Additionally, the collagen sponge (bottom layer) sourced from ovine tendon. The formulation and the fabrication method of the bilayer biomatrix was optimized by mixing 4% of gelatin and 4% of PVA and incorporated with 0.25 mg/mL of elastin, followed by crosslinking with genipin. Then, the fabricated collagen sponge was put on top of semi-polymerized film solution and dried for few days. The gross appearance was observed via physicochemical properties: identification of elemental composition (EDX), surface topography (SEM), chemical interactions (FTIR), water vapor permeability, hydrophilicity, crosslinking degree and dose response for elastin. EDX demonstrated 62.23% of carbon, 30.16% of oxygen and 7.47% of nitrogen with no significance different indicating homogeneously prepared. Next, the SEM showed the optimum pore size for the biomatrix for cell migration around 80-300 micrometers. The FTIR result described the detection at certain peaks which shows the interaction between chemicals comparing to the control group. Other than that, the biomatrix proven to have an optimum water

vapor permeability and a good crosslinking degree. Lastly, for the determination of concentration of elastin in the scaffold, a cytotoxicity test for elastin was done and showed a good cell viability at 0.25 mg/mL for maximum effect as a potential bioactive. In conclusion, the fabricated bilayer biomatrix crosslinked with genipin presented high potential as a cutaneous skin template for diabetic foot ulcer and skin tissue engineering.

Keyword: bilayer, biomaterial, wound healing

P007

Fabrication of Hybrid Nanocollagen-Gelatin Thermoresponsive Hydrogel for Skin Tissue Engineering

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ABSTRACT

Recently, the use of advanced nanotechnology primarily from collagen biomaterial is greatly advancing. It is a well-established natural polymer extensively used in tissue engineering applications. Nanocollagen could be a suitable carrier for any potential drugs or growth factors for deep skin tissue injury. Hence, the study aimed to fabricate and characterise the hybrid nanocollagen-gelatin hydrogel (NGH) skin tissue engineering. Briefly, nanocollagen was fabricated at different grinding times of 2, 4, 6, 8 and 10 minutes via cryogenic milling approach, followed by mixing with graphene oxide (GO). The fabricated nanocollagen was encapsulated with gelatin hydrogel crosslinked with 0.1% (w/v) genipin. It has been characterised using Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), energy dispersive x-ray (EDX) spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), water vapor transmission rate (WVTR), mechanical profile and particle size evaluation. SEM and TEM results show nanocollagen ground in the time range of 6 minutes display particle sizes of less than 100 nm. The NGH is expected to improve the mechanical strength with a controlled release of encapsulated nanocollagen, forming a wet microenvironment that aids in wound healing. FTIR demonstrated its chemical structure containing Amide A, B and I, II, III even after nano production. The major elements present are expected to be carbon, oxygen and nitrogen with various percentage. The NGH demonstrated mostly the amorphous structure resembling its native collagen. Therefore, the fabricated NGH has a high potential to expedite skin wound healing.

Keywords: nanocollagen, gelatin hydrogel, graphene oxide, wound healing

P008

Generation of Functional Insulin-Producing Cells Using mRNA-Based Genetic Reprogramming for Pancreatic Beta-cell Regeneration in Type I Diabetes Mellitus (T1DM)

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ABSTRACT

Despite major advances in healthcare management, type 1 diabetes mellitus (T1DM) which is characterized by the loss of pancreatic β-cells, remains associated with substantial premature mortality. The capacity for self-renewal and differentiation of human mesenchymal stem cells (huMSCs) makes them a potential new source for generation of functional pancreaticislet cells for treating T1DM. The field of gene therapy has seen an increasing trend of utilizingsynthetic mRNA as a new class of therapeutic option. However, the usage of mRNA-based genetic reprogramming technology approach to differentiate huMSCs into insulin-producing cells (IPCs) is still new and have not yet been sufficiently elucidated. Therefore, the present study sought to investigate the potential differentiation of huMSCs into functional IPCs using synthetic mRNA transcription factors co-expressing four different transcription factors (Pdx1,Ngn3, MafA and Pax4). These transcription factors are highly specific and plays an important role in driving β-cell development, reprogramming and proliferation. The coding sequences ofhuman Pdx1 (ID: 3651), Ngn3 (ID: 50674), MafA (ID: 389692) and Pax4 (ID: 5078) was retrieved from NCBI. These sequences were cloned into a vector containing the T7 promoter for *in vitro* transcription. To ensure stable mRNA, two important synthesis procedures were included: 5′ capping and 3′ tailing. Early results indicate successful production of recombinant plasmid confirmed via visualization of electrophoresis gel. Recombinant plasmid is also sequenced to confirm correct insert orientation via Sanger sequencing. The methodology described here will facilitate the development of mRNA-driven differentiation strategies for generating functional IPCs widely applicable to disease modeling and cell replacement therapy for T1DM. Future *in vitro* and *in vivo* validation of the potency of the differentiated functional IPCs deserve attention for future bench-top testing.

Keywords: Diabetes Mellitus; synthetic mRNA; cell reprogramming; transcription factor.

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P009

Generation of High-Clinical Grade Induced Pluripotent Stem Cells (iPSCs) through Cell Reprogramming Strategy

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ABSTRACT

Pluripotent stem cells are cells that can self-renew and divide into 3 primary germ layers of an early embryo which therefore can become any cells of an adult body except the extra embryonic tissues such as placenta. Induced pluripotent stems cells (iPSCs) are generated in lab after reprogramming the somatic cells unlike embryonic stem cells (ESCs) that derived from inner cell mass of embryo. The advantage of iPSC over ESCs is the avoidance of immune rejection as they can be autologous and also no ethical issues can arise. In 2006, Takahashi and Yamanaka used ectopic expression of a select number of transcription factors to convert adult mouse fibroblasts to iPSCs, demonstrating direct reprogramming of somatic cells to pluripotency. They also tried with the human fibroblast cells while other teams were checking this technology with various transcription factors such as Nanog and Lin28. Thus, direct reprogramming in human cells was achieved, making a significant contribution to the field of regenerative medicine. However, iPSC generation must be concerning its drawback such as the genomic integration of transgenes that leads to insertional mutagenesis and inefficiency at generating high-clinical grade iPSCs. Hence, several variables must be considered in order to reproducibly obtain iPSCs. In this concept map, we will discuss the factors need to be considered or modified step by step to generate successful iPSCs that are beneficial and higher safety profile in clinical settings such as the (1) choice of factors used to reprogram cells; (2) the delivery method of these factors; (3) the target cell type; (4) the parameters of factor expression, (5) the culture conditions of iPSCs; and the (6) techniques in identifying the reprogrammed cells and finally the assays used to verify pluripotency. Successfully generated ipsc colonies will have the bona fide ipsc morphology and characterization.

Keywords: Ipsc; cell reprogramming; safe clinical application; high-clinical grade iPSCs

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P011

Hydroxytyrosol in Combating Intimal Hyperplasia Formation: In Vitro Model

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ABSTRACT

Intimal hyperplasia is a response of a vessel begins with endothelial injury or inflammation. The formation of intimal hyperplasia involves both endothelial cells (EC) and smooth muscle cells (SMC) where EC damage will trigger tissue adaptive repair and remodelling which involves excessive SMC proliferation. IH gradually decreases the patency of bypass graft post Coronary artery bypass graft surgery (CABG) and Percutaneous Coronary Intervention (PCI) procedures. Therefore, antiproliferative drugs such as paclitaxel and sirolimus are administered to control the over proliferation of SMCs. However, these drugs show the same effect on endothelial cells and impedes the reendothelisation. Hydroxytyrosol (HT) is a polyphenol extracted from olive plant. Its effect on vascular EC and SMC proliferation and migration has not been extensively studied. Our objectives are to study the effect of HT in EC proliferation and SMC inhibition in vitro prior to its application in an ex vivo IH model. EC and SMC were collected from surplus saphenous vein of patients undergoing CABG surgery at Hospital Canselor Tuanku Mukhriz (HCTM), Malaysia. The effect of HT on cellular cytotoxicity were carried out using MTT assay. VEGFR2 protein expression were further validated via western blots analysis. We found that 80 μM of HT significantly increases the proliferation of EC. The maximal effective concentration (EC50) is 78.1 μM ($p<0.05$). VEGFR2 protein that are responsible for proliferation and angiogenesis were highly expressed in HT-treated EC. The half maximal inhibitory concentration (IC50) of HT-treated SMC is 300 μM. A concentration of 80 μM up to 320 μM of HT potentially decreases the proliferation of SMC. HT improves EC proliferation whilst inhibit SMC proliferation in vitro at a concentration dependent manner. Therefore, we hypothesise these preliminary finding will serve as good reference and enable us to study future HT application in an ex vivo IH model.

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

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P012

Cryopreservation Effect on Mesenchymal Stem Cells Characteristic Isolated from Wharton's Jelly

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ABSTRACT

The cryopreservation workflow is most challenging in the standardized preparation of cell therapy products based on methods used, such as adapted controls of the work environment, quality control, reagents, and equipment. This study's key role was to determine the mesenchymal stem cells (MSCs) characteristics after the cryo revival with various cell recovery periods, mainly to provide the cells with the time needed for recovery before being used in cell-based products after cryo-storage. Here, we aimed to determine the effect of cryopreservation on the stability of MSC characteristic, comparing fresh cell and different post-thaw cell recovery time period after cryopreservation. The MSCs were derived from the human Wharton's Jelly umbilical cord (hWJ-MSCs) (n = 4). The cells were revived in culture after thawing between 0-h (Cryo-revived cell: CRC 0-h), 24 hours (CRC 24-h) and 7 days (CRC 7-days) and evaluated by cell viability, doubling time, morphology, trilineage differentiation potential, growth kinetics, and MSC surface marker expression analysis. The cell viability of the CRC-0h group was 90%, while the CRC 24-h group showed 80-85% viability. However, cell attachment results showed that CRC 24-h had a notably high attachment rate. All the CRC groups showed expression of CD90, CD73, or CD44 that meet the minimum requirements as per the ISCT standards, while CD105 expression was significantly reduced in the CRC groups and lower than the minimum requirement. According to analysis, the cryo-revived cells need at least 24 hours of revival period to improve the cell attachment. In this study, we established that the revival period after cryopreservation significantly enhances cell quality. Based on the results, we suggest that at least 24-hours is necessary to improve the quality of MSC so that it is adequate for cell therapy use.

Keywords: mesenchymal stem cells, cryopreservation, cryo revived cell, post-thawed recovery period

P013

The Effect of Nanohydroxyapatite Incoperated with Micro RNA 21 to Regulate Osteogenesis

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Bone is highly specialized connective tissue with unique property in bone regeneration. Hydroxyapatite due to its bioactivity has been used in osseous defect to improve osteointegration. Micro RNA 21 are endogenously expressed to regulate osteogenesis. Coupling of miR-21 and nHA is a potential intervention to harness greater and rapid healing of the host. Wharton's Jelly cells were obtained from umbilical cord are collected from patients undergoing Cesarean sections at Hospital Canselor Tuanku Mukhriz (HCTM), Malaysia. hWJMSCs are further osteoinduced with osteoinduction media to express bone cells properties. The hWJMSCc cells were characterized by tri-lineage staining and flow cytometry. The size and morphology of the biomaterial nanohydroxyapatite was characterized by dynamic light, scattering (DLS) and Field Emission Scanning Electron Microscope (FESEM). Dose curve response and proliferation rate for the hWJMSCs treated with nHA and miR 21 was assessed by using Presto Blue assay. The expression of bone proteins was evaluated via Western Blotting. The tri lineage staining confirmed the hWJMSC is multipotency cell. The flow results expressed immunopositively for markers which characterize MSCs. The size of nHA ranging from 42 nm - 97 nm and spherical structure has been observed under the FESEM. The DLS showed the nHA ranged from 10 nm to 100 nm. The dose curve response demonstrated the range of nHA 50 µg/ml between 250 µg/ml shows no toxicity and increased proliferation rate. The concentration of 20 nM to 50 nM of miR 21 evaluated in hWJMSCs show no toxicity. The western blots results showed that hWJMSCs exposed to the miR 21 incorporated with nHA in hWJMSCs expresses bone related protein RUNX2 , Osterix and Osteocalcin. hWJMSCs cells shows no toxicity after treated with nHA and miR 21. It also shows these treated cells express bone proteins.

Keywords: Micro RNA 21, Nanohydroxyapatite, Osteogenesis

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P014

Dielectrophoresis: Repulsion and Attraction of Selective Albumin Manipulation in Artificial Kidney Application

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ABSTRACT

Dielectrophoresis can be defined as the induced force on a particle when it is being placed in an electric field gradient was first discovered by Herbert Pohl in 1970. The aim of this research is to visualize protein manipulation using dielectrophoresis (DEP). The quantification of DEP is done by using an electrochemical technique known as cyclic voltammetry (CV) since albumin is non-visible without any fluorescent probe or dye. The principles of DEP were generated by electric field on a tapered DEP microelectrodes. However, the visualization of DEP response (pDEP, nDEP and f_{xo}) is not possible because of the limitation of the apparatus. Thus, a method of electrical quantification known as CV technique is used. The detection of BSA using CV method is successful. As concentration of BSA increases, the peak current obtained from voltammogram decreases. The peak current obtained from CV voltammograms can be an indicator of DEP response as it correlates to the adsorption of the protein onto the electrodes. The principle of CV were analysed using different concentration of albumin on a screen-printed carbon electrode. Using preliminary data from both DEP and CV methods as a future prospect for the integration of both techniques to do electrical quantification of DEP forces. The integration of both methods could give rise to a new technique with precision to be implemented into the dialyzers used in renal haemodialysis treatment for manipulation and sensing of protein albumin.

Keywords: dielectrophoresis, protein, albumin, renal kidney disease, artificial kidney

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P016

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

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ABSTRACT

To date, corneal transplantation is a gold standard in treating patients with corneal disorders, but unfortunately, the shortage of tissue donors remained a critical problem. The presence of on-the-shelf electrochemically-compacted ovine tendon collagen type-1 (OTC-1) hydrogel combined 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. Knowledge of the CECs culture's biocompatibility over this biomaterial will address tissue shortage or delay in corneal perforation treatment. Collagen type 1 is extracted from the ovine's tendon by using acid based extraction method and neutralized with sodium hydroxide. This OTC-1 hydrogel is divided into 4 groups: traditional collagen hydrogel (without crosslinking and with crosslinking) and electrochemical-compacted OTC-1 hydrogel (without crosslinking and with crosslinking). For electrochemical compacted OTC-1, collagen in the OTC-1 solution will be aligned by using dielectrophoresis (DEP) technology. For crosslinked group then will be crosslinked by using genipin and quercetin. The physicochemical and mechanical of OTC-1 hydrogel is evaluated (The chemical compound, percentage of available elements, purity, topography and mechanical testing of OTC-1 hydrogel). The CECs isolated from the human redundant corneal rim will be seeded on OTC-1 hydrogel at a density of 10, 000 cells/cm^2. The vitro cellular viability of CECs is also evaluated, which are CECs attachment rate, proliferation rate, viability, morphology and CECs growth profile on OTC-1 hydrogel. Microscopic observation of CECs on OTC-1

hydrogel is also evaluated, which is immunocytochemical and histology analysis. The electrochemically compacted OTC-1 hydrogel crosslinked with genipin and quercetin should high mechanical strength, support the attachment, viability, proliferation and maintain normal morphology of CECs compared with traditional OTC-1 hydrogel. The electrochemically compacted OTC-1 hydrogel crosslinked with genipin and quercetin increases the mechanical strength and biocompatibility with CECs compared with traditional OTC-1 hydrogel.

Keywords: collagen hydrogel, corneal epithelial cells, biocompatibility

P017

Neuroenhancement Effect of Raw Extract of *Centella asiatica* (RECA) on Transdifferentiation of Full-term Human Amniotic Fluid Stem Cells (hAFSCs) into Neural Stem Cells (NSCs)

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ABSTRACT

Neural stem cells (NSCs) have been demonstrated to serve as a good source for neuro-transplantation as the means to treat neurodegenerative diseases (ND), a group of heterogenous disorders, mainly caused by neuronal dysfunction and progressive neuronal cell death. However, due to limited source of brain NSCs, treatment via neuro-transplantation has become very challenging. Thus, finding a new source of NSCs from non-brain sources and inducer of NSCs transdifferentiation is crucial. This study aims to evaluate the neuroenhancement effect of raw extract of *Centella asiatica* (RECA), which has been consumed traditionally as memory tonic, on transdifferentiation potential of full-term human amniotic fluid stem cells (hAFSCs), a highly potent stem cells, into NSCs. hAFSCs were cultured in culture medium (AmnioMAX-II complete medium) prior to MTT assay for dosage determination of RECA. In this study, hAFSCs were treated with RECA at concentration of 10 µg/mL and with 5 µM of dibutyryl camp (dBcAMP) as positive control and subjected to undergo transdifferentiation using monolayer adherent culture technique. The transdifferentiation of hAFSCs into NSCs was evaluated based on the morphology of NSCs and the expression of NSCs specific markers (Nestin, and SOX1) through immunocytochemistry. The generation of NSCs was confirmed by the ability of the cells to form neurospheres, the multicellular aggregates of NSCs in low attachment plate through neurosphere assay. At post-treatment of RECA, greater expression of NSC specific markers and higher number of neurospheres (with a diameter of 100-150 µm) from hAFSCs as compared to the untreated group were observed. Generation of neurospheres with appropriate size is crucial for adequate supply

of nutrient and metabolite for viability and differentiation of neural progenitor cells. Thus, this finding highly suggests the prospective application of RECA as a potential NSCs inducer, which is useful for therapeutic application. Besides, this study signifies the capabilities of non-brain sourced stem cell, from fluid that is merely discarded to transdifferentiate into brain cells.

Keywords: stem cell, amniotic fluid stem cell, neural stem cell, Centella asiatica, transdifferentiation, neuroenhancement

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P018

Establishment of a Three-Dimensional Organotypic Hyperglycaemic Wound Model

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ABSTRACT

Three dimensional (3D) organotypic skin model has been established for cosmetics or drug testing. It involves primary fibroblasts and keratinocytes derived from healthy human skin as the cellular component and collagen as the acellular dermal matrix. To model diabetic foot ulcer (DFU), DFU-derived fibroblasts have been previously deployed. However, it is hindered by limited cell availability and ethical concern. Attempt has also been made to maintain wound model with chronic wound fluid, but not in a hyperglycaemic condition. In this current proposed project, we aim to establish a 3D hyperglycaemic wound model, in which primary human fibroblasts and keratinocytes are cultured under hyperglycaemic condition, to address the effect of hyperglycaemia on wound healing. Dermal layer will be established by culturing fibroblasts and ovine type I collagen (Col-I) in culture inserts. Keratinocytes will then be cultured on top of the polymerized dermal layer and allowed to proliferate. The culture inserts will be lifted up to air-liquid interface for keratinocytes differentiation and stratification. The skin model will be maintained under normoglycaemic, hyperglycaemic and osmotic control. Upon maturation of the skin model, wound induction will be performed using biopsy punch. The quality of the skin model will be evaluated by determining the expression of cytokeratin-14, involucrin and Col-I using immunofluorescence staining. Haematoxylin and eosin (H&E) staining will be performed and wound gap comparison will be carried out to investigate the re-epithelialization process of the hyperglycaemic wound model and its normal counterpart. Glycation markers advanced glycation end products (AGE) and its receptor (RAGE) will be evaluated using immunohistochemistry staining. The 3D hyperglycaemic wound model will exhibit a delay in re-epithelialization with upregulated expression of AGE and RAGE compared to the normal wound model. This established hyperglycaemic wound model may potentially serve as a 3D model

that better resemble hyperglycaemic wound.

Keywords: 3D skin model, 3D wound model, hyperglycaemic wound healing, fibroblasts, keratinocytes

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P019

Antioxidant and Wound Healing Properties of *Mitragyna speciosa* Methanol Extract in Murine Fibroblast 3T3-L1 Cell Line

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ABSTRACT

Prolonged exposure to free radicals can lead to oxidative stress which has detrimental effects on cell integrity and survival. Persistent oxidative stress is among the most leading factors for the development of various illnesses such as inflammatory disease, diabetes, and cancer. Plant-based medications have been widely used as an alternative treatment of many diseases due to the extensive range of pharmacological properties exhibited by phytochemicals in plants including their ability to scavenge free radicals. *Mitragyna speciosa (M. speciosa*) or commonly known as kratom is an indigenous plant that can mostly be found in Thailand and the northern part of Malaysia. The plant leaf is consumed by natives to treat diarrhea, fever, diabetes, and hypertension. However, the scientific evidence on anti-oxidative and wound healing properties of *M. speciosa* is lacking. Thus, this study intended to explore the antioxidant properties of *M. speciosa* methanol extract (MSME) and its effects on wound healing in 3T3-L1 murine fibroblast cells. The antioxidant content and scavenging activity of MSME were determined by total phenolic content (TPC) and total flavonoid content (TFC) as well as 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Cytotoxicity of MSME on 3T3-L1 cells was determined by MTS assay. The same method was also used to determine the anti-oxidative effect of MSME against oxidative stress in H_2O_2-induced 3T3-L1 cells. A linear scratch was created on the monolayer of 3T3-L1 cell culture to investigate the ability of MSME in accelerating cell migration and wound closure. The viability of 3T3-L1 cells was not affected when treated with low to medium concentrations (25, 50, 100, and 250 µg/ml) of MSME compared to high concentration (500 µg/ml), which caused cytotoxicity and cell death. The TPC of MSME (0.1 mg/ml = 85.85 $\pm$ 8.25 mg GAE/g extract; 1 mg/ml = 167.43 $\pm$ 13.50 mg GAE/g extract; 10 mg/ml = 408.94 $\pm$ 7.17 mg GAE/g extract) was lower than pterostilbene, the positive control

drug (76.37 ± 2.75; 230.52 ± 10.92; 835.44 ± 6.84 mg GAE/g extract). Conversely, the TFC of MSME (0.1 mg/ml = 32.17 ± 27.92 mg QE/g extract; 1 mg/ml = 347.72 ± 15.97 mg QE/g extract; 10 mg/ml = 739.81 ± 5.56 mg QE/g extract) was slightly higher than pterostilbene (ND; 212.73 ± 17.92; 700.50 ± 3.47 mg QE/g extract). The DPPH MSME showed comparatively similar antioxidant scavenging activity (IC_{50}=4.34 µg/ml) with pterostilbene (IC_{50}=4.393 µg/ml). However, ABTS MSME showed lower antioxidant scavenging activity (IC_{50}=4.26 µg/ml) than pterostilbene (IC_{50}=1.556 µg/ml). MSME protects 3T3-L1 cells from oxidative stress when treated simultaneously with H_2O_2 or pre-treated for 24h prior to exposure with H_2O_2 in a concentration-dependent manner. However, high concentrations of MSME (250 and 500 µg/ml) decreased cell survival as similarly shown in treatment with MSME alone. Conversely, MSME only shows minimal protection to 3T3-L1 cells from oxidative stress when treated after 3h of exposure to H_2O_2. In addition, MSME also able to accelerate the migration of cells and induce wound closure. *M. speciosa* may have a potential bioactive compound that possesses anti-oxidative and wound healing properties. However, a high concentration of MSME could cause toxicity effect on the 3T3-L1 cells.

Keywords: *Mitragyna speciosa*, free radicals, oxidative stress, antioxidant, wound healing

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P020

Optimization of Enzymatic Denudation Protocol of Human Umbilical Artery

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ABSTRACT

Coronary artery bypass graft (CABG) is a surgical procedure performed to treat coronary artery disease in order to restore blood flow to the myocardium. However, more than 50% of vein graft used in CABG resulted in vein graft failure within 10 years post-operation. The graft failure is initiated by damage to the endothelium, resulting in the narrowing of the vessel graft. It was thought that EC layer acts as an inert layer of the vessel as it remains quiescence under normal condition to regulate vascular homeostasis. However, damage to the endothelium proves the complex role it plays as the activation of the monolayer, triggers a series of inflammatory responses that results in intimal layer thickening, intimal hyperplasia (IH) due to migration of smooth muscle cells. Therefore, understanding the downstream responses following EC injury and consequence EC repair mechanism requires the need of a denuded vessel model. This study aims to establish a denuded human umbilical artery using gentle enzymatic denudation method. Human umbilical artery was subjected for gentle denudation protocol using TrypLE Select enzyme. The denudation time and temperature were manipulated to obtain total denudation of the artery. The denuded artery was fixed and stained with Haematoxylin & Eosin (H&E) to determine the percent of EC coverage following the denudation protocol. Result shows denudation protocol at 37°C manages to remove more EC as compared to room temperature. However, no significance difference was observed between the two temperatures. Denudation protocol for 5 mins at 37°C and for 10 mins at 37°C managed to remove the most EC (80% EC removal). In conclusion, 5-10 mins denudation time in 37°C incubator manage to remove almost all EC layer in the umbilical artery. Having a denuded artery model could help further study to understand the potential of re-endothelialization and can have a significant impact

on the discovery of alternative vessel graft for CABG procedure.

Keywords: endothelial denudation, denuded artery, umbilical artery

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P021

Unravelling the Schwann Cells Differentiation Methods on Mesenchymal Stem Cells

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Over the years, researchers had been doing lots of research regarding nerve injury treatment and artificial tissue treatment continues to be considered the most efficient way in solving the problem. But creating an entire tissue is not easy and specific cells such as Schwann cells needed to be precisely cultured. Many differentiations method of Schwann cells had been introduced but there is no optimizing research that can prove regarding their efficiency among the methods. Wharton's Jelly mesenchymal stem cells (WJMSCs) and Dental pulp stem cells (DPSCs) are two types of stem cells that were widely used in the differentiating neural lineage. These stem cells had higher rate of replication and most readily available tissue to get. Few methods of differentiation of Schwann cell had been introduced by using WJMSCs and DPSCs. One of the common methods are the chemical induction method which usually come with the present of epidermal growth factor (EGF), sonic hedgehog protein, nerve growth factor and with specific condition media. In addition, methods of blocking of SMAD signalling using few inhibitors also quite efficient in differentiating Schwann cells as it generates highly expandable and reduce cost. Furthermore, another work has demonstrated that using *Centella asiatica* can also promote the differentiation of the neural lineage. However, even though *Centella asiatica* is able to induce the neural differentiation, the anti-proliferative effect that were expressed by it can be either reduce or inhibit the proliferation rate at higher concentration. Among the previous research papers, using the chemical induction method were the most reliable and efficient in inducing good outcome. Utilizing the best method in differentiating Schwann cells can help other researcher to have a proper validated procedure in creating artificial tissue. Just as importantly, it will provide better references toward future study especially in cell culture that involve neural induction.

Keywords: schwann cells, mesenchymal stem cells, neural induction, SMAD inhibitor, *Centella asiatica*

P022

Optimisation of Human Wharton's Jelly Mesenchymal Stem Cells Culture in Xeno-Free Condition

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ABSTRACT

Mesenchymal stem cells (MSCs) have been used in cell transplantation and tissue engineering for their self-renewal and multipotency capabilities. The conventional stem cell expansion normally performed on a two-dimension surfaces such as culture flasks under xeno-based supplementation. In this study, alternative supplements (Human AB Serum, Human Platelet Lysate) were used to identify the potential of xeno-free culture environment in MSCs proliferation for further up-scaling in future. The expanded cells were characterised according to the minimum criteria listed by International Society for Cell and Gene Therapy (ISCT). The cell yield, immunophenotyping, and cell plasticity have been compared against those cultured in standard fetal bovine serum supplemented medium (FBS). The MSCs cultured in human platelet lysate supplemented medium exhibit highest cell yield with comparable cell characteristic aforementioned to the control. However, lower viability was observed compared to MSCs cultured in media supplemented with human serum and FBS. In immunophenotyping and cell plasticity, cells cultured in xeno-free condition did express positive MSCs positive markers (CD90, CD105, CD73, and CD44) and able to differentiate into osteocytes, adipocytes, and chondrocytes. The results of current study demonstrate the feasibility of scale-up production of MSCs under xeno-free environment. It also highlights the potential of xeno-free culture condition in future MSCs up-scaling production for tissue engineering application prior to clinical used.

Keywords: mesenchymal stem cell, microcarrier, three-dimensional culture, upscale production

P023

Elucidating the Effects of CX3CL1 on the Re-Endothelialisation of Decellularised Human Umbilical Arteries

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ABSTRACT

Cardiovascular diseases represent an ever-growing health and socioeconomical burden around the globe. Recent advancements in tissue engineering techniques have allowed for the development of alternative decellularised tissue-engineered vascular graft for use in coronary vascular bypass grafting (CABG); which is the "gold standard" treatment option for patients with multivessel coronary artery disease. Unfortunately, decellularised grafts have seen limited clinical successes to date due to premature graft failure resulting from the absence of a sufficient and/or functional endothelial lining. Multiple previous studies have suggested that the bio-functionalisation of graft luminal surfaces through the application of biologically active coatings can promote endothelial repopulation, thus circumventing the aforementioned issues. CX3CL1 represents a coating of interest in this study due to its pro-chemotactic and pro-angiogenic properties. The luminal surface of decellularised human umbilical arteries (hUA) will be coated with CX3CL1, which is hypothesised to promote the recruitment and adhesion of circulating peripheral blood monocytes via the interaction between CX3CL1 and its specific receptor CX3CR1 expressed by monocytes. Monocytes have been previously shown to be capable of expressing an endothelial phenotype, which would aid in the repopulation of endothelial cells on the decellularised hUA graft, thus, allowing for the creation of functional alternative off-the-shelf vascular grafts for use in CABG.

Keywords: decellularize, artery, coating, graft, cvd

P024

Elucidation of miRNA Profile in Small Extracellular Vesicles of Different Cell Sources and Its Relationship With Cartilage Regeneration

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ABSTRACT

Osteoarthritis (OA) is one of the most common chronic diseases that cause joint pain and stiffness, joint deformity, and subsequently loss of joint function. It was the 15th highest contributor to global disability in terms of years lived with disability (YLDs) in 2019. The current OA treatments mainly focusing on pain control and to improve the quality of life, but unable to cure or ameliorate OA progression. Regenerative medicine is a potential approach to treat OA by inducing cartilage regeneration. Lately, there are promising evidence showed that stem cell secrete paracrine factors in the form of extracellular vesicles to promote tissue repair. Small extracellular vesicles (sEVs), including exosomes are crucial messengers in intercellular communication via transfer of bioactive lipids, proteins and RNAs. Micro-RNAs of sEVs play an important role in cartilage homeostasis and modulating OA progression and regeneration. The sEV biological functions and miRNA profile have been reported to be different depending on the cell type origin. Specific miRNAs have been found to play vital role in cartilage regeneration Thus, it is important to profile the miRNA cargo of sEVs secreted by different cell types and examine their efficacy in promoting cartilage regeneration *in vitro* and *in vivo*.

Keywords: small extracellular vesicles, osteoarthritis, cartilage, chondrocytes, microRNA

P025

Gelatin Hydrogel as Immediate Filler for Skin Wound Ulcer

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ABSTRACT

The current treatments for a deep irregular skin wound have several issues, including long production time and high-end price. Thus, there is a space to produce a smart scaffold that offers quick healing and a low price. In this research, a polymerised gelipin (genipin-crosslinked gelatin) hydrogel (GNP_GH) has been fabricated as a potential biodegradable filler for a deep irregular skin wound. Briefly, GNP_GH bioscaffolds were becoming gel within three minutes at room temperature (22-24°C). The characteristics of GNP_GH bioscaffolds were tested accordingly, followed by the biocompatibility evaluation via trypan blue assay. All GNP_GH formulations revealed viscosity values within the range of 0.01 to 1MPa.s, which represented them as flowable materials. Amongst GNP_GH groups, 0.1%GNP_GH10% displayed the highest injectability (97.3 $\pm$ 0.6%). Cross-section photographs illustrated interconnected porous structures for all GNP_GH groups. The elementary study presented no major changes upon GNP modification. Trypan blue assay demonstrated that dermal fibroblasts and epidermal keratinocytes were attached at the top of GNP_GH. In conclusion, the aforementioned findings indicated that gelipin hydrogels were optimum to be used in the future as a rapid treatment for skin wounds.

Keywords: gelatin, hydrogel, skin, wound

P026

Elucidating the Effects of Wharton's Jelly-Derived Mesenchymal Stem Cell and its Extracellular Vesicle Transplantation on the Immune System in Ageing Mice

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ABSTRACT

Aging precipitates 'frailty', which is the cumulative deterioration of anatomical and physiological functions leading to illnesses with higher morbidity and mortality. The impact of age on frailty is manifested into immunosenescence and inflammaging. Both conditions are synchronously related and can be presented as the dysregulation of the immune function causing a persistent subclinical inflammatory state. This results in a constantly elevated inflammatory parameters in the elderly when compared to their younger counterparts. Mesenchymal stem cell (MSC) is a potential approach for the maintenance of healthy aging. Although MSCs do not differentiate into immune cells, they can form a supporting microenvironmental niche for haematopoietic stem cells (HSCs) to differentiate into immune cells. MSC also exerts immunomodulation through paracrine signalling, which may be attributed to the release of its extracellular vesicles (EVs). Literature has shown that MSC has been promising in reducing the inflammatory markers in disease models. However, there are inadequate data on the immunomodulatory effects of MSC and MSC-EVs in old age. Thus, we investigate the immunomodulatory effects of MSC and its derived EVs in the ageing mice model. The main objective of this study is to evaluate the immunomodulatory effects of MSC and MSC-EV transplantation using an ageing mice model. We postulating that MSCs and MSC-EVs can subside systemic inflammation of ageing mice.

Keywords: mesenchymal stem cell, frailty, immunosenescence, inflammaging, extracellular vesicle

P027

Elucidating the Effects of Mesenchymal Stem Cell and its Derived Extracellular Vesicle Transplantation on Cognitive Function of Ageing Mice

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ABSTRACT

The climbing population of elderly individuals in the world has initiated numerous studies to understand the ageing process to improve and to ensure the quality of aged life. Ageing is associated with chronic diseases, particularly degenerative diseases. The decline of cognitive functions with age is a process known as cognitive ageing. We postulated that the declined of cognitive functions in ageing could be due to the increased oxidative stress in aged brain which then increased the permeability of blood brain barrier. Altered intercellular communication and cellular senescence in the brain further increase the oxidative stress damage to the brain. Numerous studies have demonstrated the ameliorative effects of mesenchymal stem cells in pathological conditions. However, there is no study elucidate and compare the effect between WJMSC and WJMSC-EV on aged cognitive functions. In this concept presentation, we postulate how mesenchymal stem cell (MSC) and its derived extracellular vesicle (MSC-EV) may stop the viscous cycle of oxidative stress induced damage and reserve cognitive function via the restoration of the blood brain barrier integrity via MSC or MSC-EV mediated immunomodulation and the rescue of senescence neurons via direct cell-to-cell contact or secreted vesicles or factors. A future study to determine the mechanism of action of MSC and MSC-EV in reserving the ageing-related cognitive changes through Morris Water Maze, electrophysiological study and biochemical and micro and ultrastructural evaluation of the brain hippocampus and blood brain barrier is recommended. The comparison of the efficacy and therapeutic effect of MSC versus EVs derived from the same batch of MSCs will elucidate the advantage of cell versus cell-free approaches.

Keywords: cognitive function, ageing, mesenchymal stem cells, extracellular vesicle, blood brain barrier

P028

Synthesis and Characterization of Silver Nanoparticles via Biologically Reduction of Silver Nitrate ($AGNO_3$) using Turmeric Aqueous Extract

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ABSTRACT

Silver nanoparticles (AgNPs), a potential candidate as an antimicrobial agent against broad spectrum of microorganisms and has been extensively used in drug delivery system, disease treatment and biomedical devices. In this work, AgNPs were synthesized via biologically reduction of silver nitrate ($AgNO_3$) using turmeric aqueous extract as an alternative to conventional chemical reduction process. The reduction process was done by addition of turmeric aqueous extract (5% (v/v)) dropwise into 10 mM of silver nitrate aqueous solution and followed by magnetic stirring for 24 hours. A colour changes in reactant solution was observed from colourless to dark yellow which indicated AgNPs formation. The reduction process can be analyzed via its maximum absorption peak at around 405 to 427 nm by using UV-vis spectrometer surface plasmon resonance (SPR) of AgNP. The effect of several parameters on AgNPs synthesis was investigated by varying concentration of turmeric aqueous extract, volume of turmeric aqueous extract and concentration of $AgNO_3$ at one time. It was found that as the level of parameters were increased, the AgNPs synthesis also increased until reached maximum level due to reactants limitation. The AgNPs has been successfully obtained using this simple synthesis route and is expected to have a great potential to inhibit bacterial growth.

Keywords: silver nanoparticles, antimicrobial agent, biological synthesis, nanomaterial

P029

Efficacy of Umbilical Cord Mesenchymal Stem Cell Derived Extracellular Vesicles in Treatment of Atopic Dermatitis

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ABSTRACT

Topic dermatitis is an inflammatory, non-contagious disease of multifactorial origin, showing an increasing prevalence worldwide. Current treatment is mainly symptomatic in absence of curative options. Pathogenesis of AD is complex but is characterized by skin barrier defects and immune dysregulation. Small extracellular vesicles are a subclass of extracellular vesicles produced by almost all types of cells as means of intercellular communication. Recent studies have shown sEVs derived from mesenchymal stem cells to possess immunomodulatory and regenerative properties resembling their parent cells. In the present study, we are examining the therapeutic properties of sEVs derived from umbilical cord mesenchymal cells on atopic dermatitis. MSCs will be isolated from umbilical cords, culture-expanded, and characterized following ISCT guidelines. This will be followed by isolation and characterization of sEVs according to MISEV18 guidelines. For potency testing, the project encompasses two parts, in vitro and in vivo. For the in vitro part, the immunomodulatory and pro-regenerative potential of sEVs will be tested on peripheral blood mononuclear cells, keratinocytes, and fibroblasts. For in vivo, Nc/Nga mice, which are genetically modified models of atopic dermatitis, will be used to test the therapeutic effects of sEVs. This will be achieved by thoroughly examining skin structure, ceramides, and inflammatory markers.

Keywords: atopic dermatitis, extracellular vesicles, exosomes, mesenchymal stem cells

P030

Scaling Up of Wharton's Jelly-Derived Mesenchymal Stem Cells (WJ-MSCs) For Clinical Applications

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ABSTRACT

Mesenchymal stem cells (MSCs) can be isolated from many different tissue sources, including Wharton's jelly of the umbilical cord. Wharton's jelly MSCs (WJ-MSCs) are abundant, easy to isolate and have multilineage differentiation potential and immunosuppressive property. Due to these advantages, WJ-MSCs have huge clinical potential for the treatment of a myriad of diseases. For treatment purposes, WJ-MSCs need to be expanded in large-scale to meet the huge cell number needed per patient. This study was conducted to optimize the culture condition and large-scale expansion of WJ-MSCs using CellSTACK-2 chamber. WJ-MSCs were isolated and cultured with three medium which were DMEM-LG+10% HPL, StemMACS™ and MSC-Brew GMP up until passage 3. Subsequently, characterization of WJ-MSCs cultured using different mediums were performed through surface marker analysis, tri-lineage differentiation potential, immunosuppresion assay and cell cycle assay. DMEM-LG+10% HPL was identified as the best culture medium and used for WJ-MSCs upscaling. Once more, up scaled WJ-MSCs were characterize. WJ-MSCs cultured with DMEM-LG+10% HPL, StemMACS™, MSC-Brew GMP and upscaled WJ-MSCs expressed the MSC surface markers, able to suppress PBMC proliferation, capable of differentiate into adipogenic, chondrogenic and osteogenic lineages, and most of the cells were in G1 phase compared to S and G2 phase. As a conclusion, DMEM-LG+10% HPL is effective for large-scale expansion of WJ-MSCs as the cells maintain the MSC characteristics, i.e., express surface markers that fulfill the ISCT guideline, able to differentiate into multiple lineages, and capable to suppress PBMC proliferation.

Keywords: wharton's jelly mesenchymal stem cells (WJ-MSCs), up scaling, WJ-MSCs characterisation

P031

Synergistic Effects of Multifunctionalised Human Collagen Type-I Biomatrix for Skin Burn

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ABSTRACT

Many wound management care protocols have been developed to enhance wound healing after burns with the primordial aim to restore the functions of the skin side by side to provide a better esthetic outcome. The primary drawback of current skin tissue engineering products is commonly degraded faster post-transplantation, giving a point of weakness in the tissue regeneration field. Furthermore, it could cause delays in the wound healing process and increasing susceptibility to bacterial infection. Due to increasing microbial resistance against antibiotics, developing an alternative antimicrobial from different sources such as silver or gold could reduce the severity. This study aimed to fabricate and characterize human collagen type-I biomatrix incorporated with quercetin and externally sprayed with Ag-GO layer as an antibacterial effect. Briefly, collagen type-I will be extracted from human redundant skin using chemical-based extraction, mixed with quercetin and fabricated via freeze-dry approach. In addition, the fabricated biomatrix will crosslink with genipin for 6 hours. Besides, the evaluation of physicochemical, mechanical, cellular compatibility, toxicity and growth profile and migration will be performed. The antibacterial properties of Ag-GO will be tested in gram-negative and gram-positive bacteria by using disk diffusion assay. For quercetin efficiency will be analysed by ROS assay. Therefore, the potential of Ag-GO combination as an antibacterial in this study to be sprayed on the fabricated collagen biomatrix, crosslinked with genipin/quercetin biomaterials crosslinkers, will be explored.

Keywords: skin burn, multi-functional biomaterials, collagen sponge bioscaffold, quercetin, graphene oxide-nano silver (GO-Ag).

P032

Fabrication of Metal-Ceramic Based Scaffolds and Its Regulation on Stem Cell Behaviors and Osteointegration in Animal Models

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ABSTRACT

Tissue engineering involves the use of cells, growth factors and scaffolds to develop a biological substitute to improve tissue function. The scaffold acts as a mechanical support for cells but is not specifically designed to interact with desired cell populations; yet the initial interaction between cells and the scaffold is very important and will determine the success or failure of the engineered device. Titanium-ceramic compounds enables many novel approaches in modelling, design and fabrication of complex materials with enhanced functionality to improve cell–matrix interactions. The key is its bioactivity that serves as an important feature to interface design, simulation, and tissue fabrication. This study was conducted to investigate the affinity of mesenchymal stem cells towards both titanium-hydroxyapatite and titanium wollastonite. Surface characteristics through scanning electron microscopy show that the composite materials exhibit rough surfaces as well as good cell attachment. This has been also confirmed using atomic force microscopy to give a quantitative assessment of the materials concerned. The analysis shows that the material exhibited a rough surface in due to the presence of the wollastonite present within the composite and this has given the cells an opportunity to attach and proliferate well as cells tend to react better to rough surfaces as compared to smooth ones. Exposure of mesenchymal stem cells to the titanium wollastonite shows no cytotoxic effect and significant proliferation 7 days after seeding. In addition, data on the mesenchymal stem cell attachment onto the material was also brought to light showing positive attachment through the live and dead stain study. Micro CT data done from a rabbit study via femoral implantation at three points shows new bone formation after 2 months of implantation primarily

on the titanium hydroxyapatite and titanium wollastonite with good integration grip between the implant and the femurs.

Keywords: titanium, wollastonite, bioceramic, mesenchymal stem cells

P033

The Increased Binding Capacity of MSC on the Ex-Vivo Osteochondral Model of OA via Coll-2 Antibody Conjugation

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Human osteoarthritis (OA)-mimicking in vitro models are crucial for pathophysiological studies and the evaluation of novel treatment approaches such as cartilage tissue engineering. The construction and characterisation of a human OA osteochondral explant culture are presented. 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. We discovered that cells in the OA explant culture remained viable for extended periods while preserving the typical chondrogenic and the 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 increase in fluorescence intensity in antibody-conjugated MSC micromass compared to MSCs alone suggests that our sample of interest is bound higher to the affected site. 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