Abstract

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ASSOCIATION BETWEEN SHAMMAH USE WITH PERIODONTAL DISEASE AND SHAMMAH -INDUCED LEUKOPLAKIA-LIKE LESION AMONG ADULT MALES IN DAWN VALLEY, YEMEN

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Background: The traditional type of smokeless tobacco (SLT) used in the Arabian Peninsula, especially common in Yemen is called shammah. Shammah and other risk factors play an important role in development of oral diseases.

Objectives: The present study has been undertaken to determine the prevalence of shammah use and to determine the association between shammah use with periodontal disease and oral leukoplakia-like lesions. Other associated factors with periodontal disease as well as with oral leukoplakia-like lesions were also determined.

Materials and Methods: A cross sectional study was conducted on 346 randomly selected adult males. Multi stage random sampling was used to select the study location. After completing the structured questionnaire interviews, all the participants underwent clinical exanimation for periodontal health status and oral leukoplakia-like lesions. Periodontal status was recorded using the Community Periodontal Index (CPI). Clinical features of oral leukoplakia- like lesions were characterized based on the grades of Axéll et al. (1976). Chi-square test was used for assessing significant differences in shammah status in respect to periodontal disease and oral leukoplakia like lesions. Univariable logistic regression and multivariable logistic regression were selected for assessing potential associated factors.

Results: Out of 346 male participants aged 18 years and older, 68 reported being current shammah users. The prevalence of current shammah use was 19.7% (95% CI: 15.6%, 24.2%). Chi-square test detected that significant differences exists between the study groups (i.e., never, former, and current shammah users) in respect to the presence of periodontal disease (*P*=0.001) as well as to the presence of oral leukoplakia-like lesion (*P*=0.001). Multivariable logistic regression analysis revealed that age, family income, former shammah user, current shammah user, and annual duration of shammah use were statistically associated with the presence of periodontal disease [Adjusted odds ratio (AOR)= 1.05; 95% CI: 1.03, 1.07; *P*=0.001], (AOR= 2.01; 95% CI: 1.16, 3.47; *P*=0.012), (AOR= 2.92; 95% CI: 1.20, 7.10; *P*=0.018), (AOR= 7.25; 95% CI: 3.84, 13.70; *P*=0.001), and (AOR= 2.19; 95% CI: 1.47, 3.24; *P*=0.001), respectively. The multivariable analysis also revealed that age, no formal or primary level of education, former shammah user, current shammah user, and frequency of shammah use per day were statistically associated with the presence of oral leukoplakia-like lesion (AOR= 1.03; 95% CI: 1.01, 1.06; *P*=0.006), (AOR=8.65; 95% CI: 2.81, 26.57; *P*=0.001), (AOR= 3.65; 95% CI: 1.40, 9.50; *P*=0.008), (AOR=12.99; 95% CI: 6.34, 26.59; *P*=0.001), and (AOR= 1.17; 95% CI: 1.02, 1.36; *P*=0.026), respectively.

Conclusion: The results revealed that periodontal disease and oral leukoplakia-like lesions were significantly associated with shammah use. Therefore, it is important to develop comprehensive shammah prevention programmes in Yemen.

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DETERMINATION OF TOOTH SIZE AND ARCH DIMENSION IN A PAKISTANI POPULATION: A NOVEL APPROACH UTILIZING DIGITAL MODEL

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The prime aim of this thesis is to develop the norms for tooth size, tooth size ratio (Bolton index), arch dimension, arch length and arch perimeter on subjects of Angle's class I (normal) occlusion in Pakistani population. This thesis describes the validity and reliability of digital model measurements, geomorphometrics norms of tooth size and arch dimension analysis by conventional digital caliper and digital stereomicroscope, measurement for Bolton' tooth size ratio (intermaxillary tooth size discrepancy) investigation, tooth size and intermaxillary tooth size discrepancy via circumferential tooth size measurements. In order to establish standard norms for the Pakistani population, we investigated the tooth size and arch dimension using conventional digital caliper (DC) and digital stereomicroscope (SM). The sample consisted of 128 subjects ranging in age from 18 to 24 years. Dental models of each subject for maxillary and mandibular arches were scanned via Hirox digital stereomicroscope for the fabrication of the digital models, and the tooth size and arch dimensions were measured via SM scanned digital models.

Sex differences were assessed, and interrelationships between different variables were explored within the study group. For the data obtained by SM techniques, the men had statistically significant larger arch dimensions and geomorphometrics norms of tooth size than the women ($P<0.05$). For the Bolton' tooth size ratio (intermaxillary tooth size discrepancy), the sum of anterior tooth size and overall tooth size via SM methods showed statistically significant result in relation sexual disparities ($P<0.05$). No significant sexual disparities for Bolton's anterior ratios (BAR) and Bolton's overall ratios (BOR) were observed. This study has established a new reference database of tooth size and arch dimensions via SM for first time on Pakistani population. These norms for tooth size and tooth size ratio will be helpful for clinical treatment planning in dentistry and forensic dentistry.

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EFFECT OF VEGF AND BCP ON WOUND HEALING IN CRITICAL SIZED MANDIBULAR DEFECT- IN VITRO AND IN VIVO STUDIES

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One of the most challenging problems in Oral and Maxillofacial Surgery is the repair of critical-sized bone defects in the mandible and craniofacial region that needs a solution. The aim of this study was to produce a clinically applicable model of bone tissue engineering in composite bone graft that has osteoconductive and osteoinductive properties capable of treating critical-sized bone defects in a single step surgical procedure in the rabbit's mandible, beginning with in vitro molecular phase and followed by in vivo clinical phase. Part I and II in vitro phase was conducted to evaluate the effectiveness and the potential influence of VEGF-added-BCP on both angiogenic and osteogenic gene expressions of endothelial cells (ECS) and dental stem cells (DSCs) treated with BCP extract with and without addition of VEGF respectively. The results provided critical insights into the use of BCP granules with and without addition of VEGF which is important for in vitro test before evaluating the efficacy in vivo. This was followed by performing reverse transcriptase-PCR (RT-PCR) to amplify the osteogenesis and angiogenesis-regulated genes. In part III, the in vivo study, the optimal effective concentration of VEGF in bone regeneration was established using Fibrin sealant delivery system. The in vivo clinical phase included a total of 42 male New Zealand White rabbits, aged between 5 and 6 months; giving a total of 84 mandible sides for experiment. The mandible sides were divided into 4 groups subjected to the treatment given and analysis done. 24 rabbits (48 mandible sides) were subjected to micro-CT and histological studies, while 18 rabbits (36 mandible sides) were subjected to molecular studies. Nine untreated mandible sides (designated as A) versus BCP/FS treated mandible sides (designated as B), control treatments comprising $n=9$ rabbits; and the experiment group comprising BCP/FS treated mandible sides (designated as C) versus BCP/FS + VEGF treated mandible sides (designated as D). These rabbits were sacrificed at day 14, 30, 45, and 60 after surgery for micro-computed tomography and histological analyses. For molecular analysis, same designated treatment groups were used and the rabbits were sacrificed at day 7, 14, and 21 after surgery.

Results: In part I and part II in vitro studies showed angiogenesis gene VEGF was highly expressed in ECS and DSCs by VEGF only treatment but showed some changes in combination of VEGF/BCP treatment in both cell lines. Osteogenesis genes; Bone morphogenetic proteins-2 (BMP-2), Alkaline phosphatase (ALP), Osteocalcin (OC) and Osteopontin (OPN) were shown to be positively affected by both BCP and VEGF. The BCP only treatment group induced high expression of early regulating osteogenesis genes (BMP-2 and OPN). Mineralized gene markers (ALP and OC) were however, highly expressed with VEGF/BCP treatment. Besides that, osteogenesis genes (BMP-2 and OPN) for DSCs were shown to be positively affected by both BCP only and VEGF only groups and slightly effected in VEGF/BCP group. Some genes were expressed at an earlier time interval compared to the other genes depending on the type of treatment. BCP only treatment induced high expression of initial-regulated osteogenesis genes (BMP-2 and OPN). Results in part III study shows micro-computed tomography images at week 8 after surgery demonstrating complete resorption of BCP granules and young bone formation in group D implanted with VEGF/BCP compared with other groups of study ($p<0.05$). Histological and histomorphometrical findings at week 8 after surgery (group D) showed marked new bone formation. Group D showed up-regulation of VEGF gene expression at week 1. Osteogenesis markers in group D showed higher expression than other groups at early stage of bone healing. The results suggest that the use of BCP/FS composite bone graft loaded with VEGF is ideal for local controlled release of VEGF resulting in accelerated bone formation and healing process of mandibular critical-sized bone defects and this may contribute to the scope of modern reconstructive surgery.

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CHEMICAL ANALYSIS, BIOCOMPATIBILITY AND DENTINOGENIC DIFFERENTIATION POTENTIAL OF TWO FORMULATIONS OF WHITE PORTLAND CEMENT OF DIFFERENT ORIGIN

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Advances in endodontic bio-materials are at the forefront of endodontic research. The aim of the current study was to determine and compare the chemical composition, biocompatibility and dentinogenic differentiation potential of two formulations of white Portland cement (WPC) of different origin and white mineral trioxide aggregate (WMTA). Normal and fast (addition of calcium chloride dihydrate) set formulations of WMTA, Malaysian and Egyptian WPCs (MAWPC and EGWPC) were prepared for chemical analysis. Scanning electron microscope (SEM), energy dispersive X-ray (EDX) micro-analysis and X-ray diffraction (XRD) were used for surface morphology evaluation, elemental and phase analysis, respectively. After the preparation of optimized serial dilutions, the cytotoxicity was evaluated on human periodontal ligament fibroblasts (HPLFs) and dental pulp stem cells (DPSCs) using methyl-thiazol diphenyltetrazolium (MTT) assay after 24 and 72 hours. Statistical analysis was performed using Kruskal-Wallis test (P=0.05) followed by pairwise comparisons using Mann-Whitney test. Cell attachment properties were examined on HPLFs and DPSCs under SEM using a novel technique after 24 and 72 hours. The dentinogenic differentiation of DPSCs was assessed based on the expression of BGLAP, DSPP, RUNX-2 and SPP1. After one, three, seven and 14 days of incubation, the expression was examined using real-time PCR. One way ANOVA followed by post-hoc comparisons was used for statistical analysis (P=0.05). The results showed that the surface morphology and chemical composition of both formulations demonstrated considerable variations. The elemental composition of WMTA differed from both WPCs by the presence of bismuth and absence of sulphur. Potassium was merely observed in MAWPC. Phase analysis demonstrated the presence of various chemical compounds. The cytotoxicity evaluation showed different cellular responses of HPLFs compared to DSPCs. Generally, both formulations favoured the viability of HPLFs. However, the fast set formulations demonstrated severe to moderate cytotoxicity on DPSCs at three successive concentrations. Significant differences between EGWPC and MAWPC were identified (P<0.05). The cell attachment properties of all materials were favourable, however, HPLFs attached and spread over the samples better than DPSCs. The dentinogenic differentiation potential showed fluctuating expressions at days 1, 3 and 7. However at day 14, all genes were up-regulated. Generally, fast set formulations showed higher expressions than normal set counterparts (P<0.05). In conclusion, WPC of different origin shows differences in chemical composition and biological properties. However, the biological profile of both WPC is comparable to that of WMTA. This holds promise for potential use of MAWPC and EGWPC as cost effective substitutes in clinical dentistry.

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HUMAN HERTWIG'S EPITHELIAL ROOT SHEATH CELLS IN ASSOCIATION WITH CHITOSAN SCAFFOLD AND TGFβ1 MODULATE THE CEMENTOBLASTIC DIFFERENTIATION OF STEM CELLS FROM HUMAN EXTRACTED DECIDUOUS TEETH

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Destruction of the periodontium as a result of furcal perforation may lead to loss of the tooth. egeneration of cementum is essential to allow the reconstruction of the lost attachment. Multipotent dental pulp stem cells derived from human exfoliated deciduous teeth (SHED) represent a promising cell source for tissue engineering. Hertwig's epithelial root sheath (HERS) cells have the ability to form cementum-like tissue and differentiate into cementoblast-like cells. On the other hand, transforming growth factor-β1 (TGFβ1) plays a key role in controlling cell proliferation and differentiation. Chitosan has been chosen as a scaffold in the current study because it supports cells attachment, proliferation, osteoblastic differentiation and hard tissue regeneration. To the best of our knowledge, the ability of SHED to differentiate into cementoblast-like cells has not been investigated in the dental field yet. The aim of this study was to evaluate the the ability of SHED co-cultured with HERS cells in presence of chitosan-TGFβ1 to differentiate into cementoblast-like cells. To this end, HERS cells were isolated using selective digestion method and characterized by immunofluorescence staining, flow cytometry and sqRT-PCR. Thereafter, SHED seeded on chitosan scaffold and co-cultured with HERS cells in the presence of TGFβ1. Eight groups were assigned for the downstream analysis: SHED (S), SHED+chitosan (SC), SHED+TGFβ1 (ST), SHED+chitosan+TGFβ1 (SCT), SHED+HERS (SH), SHED+HERS+chitosan (SHC), SHED+HERS+TGFβ1 (SHT), SHED+HERS+chitosan+TGFβ1 (SHCT). SHED proliferation was assessed by PrestoBlue assay. Live/dead assay was then performed and SHED attachment to chitosan scaffold was examined by scanning electron microscope (SEM). For cemento/osteogenic differentiation analysis, morphological appearance, alkaline phosphatase (ALP) activity, mineralization behaviour and gene/protein expression of cemento/osteoblast phenotype of SHED were evaluated. In addition, the inflammatory response of SHED was analysed. Results of the present study showed that HERS cells had typical epithelial-like cells morphology and expressed epithelial-like markers. SHED remained viable and attached well to the chitosan structure. HERS cells in association with chitosan-TGFβ1 significantly enhanced the proliferation and cemento/osteogenic differentiation of SHED, which was demonstrated by high ALP activity, strong mineral deposition and up-regulation of cementum/bone-related gene and protein expressions (i.e. ALP, collagen type I, bone sialoprotein, osteocalcin and cementum attachment protein). Low levels of inflammatory genes expression were detected. In conclusion, HERS cells have been successfully isolated using selective digestion method. Our co-culture system

confirmed the synergistic effect of HERS cells in a combination with chitosanTGFβ1 to induce SHED differentiation along the cemento/osteoblastic lineage; which possesses a novel therapeutic strategy for endodontic furcation perforation repair and periodontal tissue engineering.

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EFFECTS OF NON-INVASIVE LOW LEVEL LASER THERAPY, LOW INTENSITY PULSED ULTRASOUND TREATMENT AND THEIR COMBINATION ON ORTHODONTIC TOOTH MOVEMENT

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Orthodontic treatment is a process of achieving appropriate functions and esthetics by the movement of a tooth through the application of an external physical force in order to obtain physiological tissue reactions around a tooth, while avoiding the side effects of an external force. However, long-term therapy for several years is required. Accordingly, faster tooth movement without harmful effect on periodontal tissue and alveolar bone has been an important issue of interest to orthodontists and patients. Recently with the development of science, there is high attention among orthodontists to investigate the non-invasive stimulating techniques to improve orthodontic treatment. Many researchers have shown that proper use of low level laser treatment (LLLT) in orthodontic clinic can accelerate treatment, reduce appointments, and provide superior results, but there is no established regimen that all LLLT users within orthodontics are agreeing to follow. The non-invasive low intensity pulsed ultrasound (LIPUS) has also been used to stimulate bone fracture healing by improving osteogenesis, remodeling and angiogenesis. However, there are limited numbers of studies on LIPUS stimulation for orthodontic tooth movement. To the best of our knowledge, there is no other study comparing the effect of LLLT with LIPUS for orthodontic tooth movement, or comparing the stimulatory effect of the combination of LLLT with LIPUS for orthodontics. This research was aimed to study in vitro and in vivo effect of different low level laser regimes, low intensity pulsed ultrasound and the combination of both techniques on orthodontic tooth movement. For in vitro, Human fetal osteoblast cell line (hFOB) was cultured and divided into different groups; 1st group was treated with 940 nm LLLT (with power ranging between 100-300mW), 2nd group was treated with LIPUS, 3rd group was treated with combination of LLLT and LIPUS. The application of LLLT or LIPUS or combination of them was once a day for 7 days. For cell proliferation, MTT assay was used. Both alkaline phosphatase and osteocalcin activity assays were assessed for cell differentiation. RT-PCR was also performed to elucidate the osteoblasts gene expression for COL1A1, RUNX-2 and BSP. For in vivo, 6-week-old Sprague Dawley male rats were used. Orthodontic appliances were inserted. A force of 10g was applied to the molars to induce tooth movement. The rats were grouped into four groups. The 1st group was irradiated with LLLT, 2nd group was treated with LIPUS and a 3rd group was treated with combination of both LLLT and LIPUS. A 4th group was a control group. The LLLT and LIPUS were used to treat the area around the moving tooth once a day till 1, 3, 7, 14 and 21 days. To determine the amount of tooth movement, plaster models of the maxillae were made. The models were imaged and analysed. Histological examination was performed after staining with (haematoxylin and eosin) and (Alizarin red and Alcian blue) stain. RT-PCR was also performed to elucidate the gene expression of RANK, RANKL, OPG and RUNX-2 in the area of treatment. The results of in vitro showed that all treatment groups significantly increased rate in cell proliferation and differentiation compared to the control group. The LIPUS group and the 300 mW LLLT group significantly increased the amount of cell proliferation. By contrast, the combination groups showed significantly greater amount of cell differentiation and gene expression. The results of in vivo showed that the amount of tooth movement, the histological bone remodelling and the RT-PCR was significantly greater in the treatment groups than that in the control group. Among the treatment groups, the combination group was the highest and the LIPUS group was the lowest. The findings of this study suggest that LLLT and LIPUS can stimulate osteoblast cells for bone formation.

Additionally, they facilitate the velocity of tooth movement and improve the quality of bone remodelling during orthodontic tooth movement especially when they are combined together.

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MORPHOMETRIC ANALYSIS AND FABRICATION OF PROSTHETIC EAR USING CAD/CAM AND ADDITIVE MANUFACTURING TECHNOLOGIES

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Prosthetic ear rehabilitation is one of the treatments for auricular defect. Current practice by surgeon require patient to go for osseointegrated craniofacial implant surgery

for retention of the prosthetic ear. Traditionally, the wax pattern was created from the impression taken from patient and the final prosthesis is processed with silicone material. This conventional method has always been time consuming, massive work and caused discomfort to patient. Moreover the accuracy of the final prosthetic sometimes was not satisfied. Impression technique play a vital role in determining accurate reproduction of affected and unaffected ears, orientation of the ear during wax try in and fabrication of ear prostheses. Hence, the ear anthropometric data is important to determine the correct orientation and position of the prosthetic ear. This paper aims to provide morphometric data of a few standard ear parameters for Kelantanese and also describes a novel method of design and fabricating the prosthetic ear applying CAD/CAM and additive manufacturing technologies. A clinical study is done onto a patient in HUSM and comparison is made between traditional method and the new approach using computer aided technology. Study also validates the prosthetic ear obtained from both techniques with the morphometric data. The measurement technique of the final prosthetic ear to validate the result with regards to the morphometric data was done digitally using software. Morphometric study was conducted on 68 samples of normal ear for both left and right with 15 parameters measured. Data was retrieved from CT scan and convert to 3D image using soft tissue development. Mirror image technique was applied to reconstruct the missing ear, and then fabricate the 3D model of the prosthetic ear using Stereolitography (SLA) technology. The 3D model will become the master mold to produce the final prosthetic ear using vacuum casting technology.

Morphometric analysis gave the mean and standard deviation values for auricular length and width, length and height of tragus, insertion length of auricle, length and width of lobular and conchal, protusion at superaurale and tragal level as well as the inclination and symmetrical angle. While study also illustrates that there is significant different between traditional and computer aided approach. The new method shows time reduction during design and fabrication stage and also show improvement in accuracy and aesthetic appearance.

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SYNTHESIS AND EVALUATION OF SILICA PARTICLES FROM RICE HUSK FOR FABRICATION OF DENTAL NANOHYBRID COMPOSITES

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The objective of this study was to synthesize silica particles from rice husk with ideal characteristics, like spherical shape, low surface area and wide particle size distribution, for use as fillers in dental composites. The silica was leached in to a solution of NaOH to form sodium silicate. The silica was synthesized from sodium silicate solution using a simple acid precipitation technique. Several parameters were tested to obtain silica particles with ideal characteristics for use as fillers in dental composites. These included the effect of various precipitating acids and solvents, effect of dilution of sodium silicate solution, mixing speed, feed rate and various concentrations of solvents. It was possible to obtain spherical silica particles with a BET surface area of 30 m^2/g and a mean diameter of 261nm. The silica particles were modified using a coupling agent, 3–(Trimethoxysilyl) propyl methacrylate. The silica surface was successfully modified using a very simple technique and was characterized using FTIR and NMR studies. Dental composites with two different filler/matrix ratios (EC1: 40/60 and EC2: 50/50) were fabricated from the surface modified nanohybrid silica, followed by mechanical properties testing, which included flexural strength, flexural modulus, compressive strength, Vickers' hardness and surface roughness tests. Although both experimental dental composites showed promising results, EC2 exhibited better test values. EC2 showed a flexural strength of 106.6 MPa, flexural modulus of 6.2 GPA, compressive strength of 190.6 MPa, Vickers' hardness of 38.66 HV1 and surface roughness of 0.057 R_a which are comparable with a commercial dental composite. Biocompatibility of the silica particles and EC2 were tested using cytotoxicity studies on human periodontal ligament fibroblast cells using the MTT assay. The silica particles were non-cytotoxic at all the concentrations tested. The results for experimental dental composite showed that it was moderately cytotoxic only at the highest tested concentrations, which is in agreement with several other studies. The results indicate that rice husk, which is an agricultural waste, is a good and inexpensive source of silica for potential use as fillers in dental composites.

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BIOCOMPATIBILITY AND OSTEOGENESIS OF LOCALLY PRODUCED β-TRICALCIUM PHOSPHATE

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β-Tricalcium Phosphate (β-TCP) with molecular formula [β-Ca3(PO4)2] is a synthetic ceramic biomaterial that have been extensively used as a bone graft material in orthopaedic and dental field due to its biocompatibility and properties. The purpose of this study was to evaluate the biocompatibility and osteogenesis of locally produced β-TCP in-vitro using osteoblast cell line. β-TCP ceramic was locally prepared using same starting material of Ca(OH)2 and H3PO4 with Ca/P

ratio of 1.5 but synthesis by two different methods namely, hydrothermal and precipitation. The characteristics of β-TCP samples were observed using vernier caliper, Fourier Transform Infrared Spectroscope (FTIR) and Atomic Force Microscope (AFM) for its dimension, functional group and surface roughness. Osteoblast cells were cultured with extracts of β-TCP at various concentration to determine the cytotoxicity using alamar blue and MTS assay. Cell attachment and viability of osteoblast cell with β- TCP were observed using Confocal Laser Scanning Microscope (CLSM) after staining with calcein-AM and ethidium homodimer to produce fluoresecence colour. The presence of calcium deposition and extracellular matrix (ECM) protein such as Collagen 1 and fibronectin that responsible during osteogenesis were determine using alizarin red staining and western blot analysis. Chronos β-TCP as a comercial produced β-TCP was used as controls. Hydrothermal and precipitation β-TCP showed the similar characteristic in dimension, functional group and surface roughness. The results of in-vitro studies indicated the non-cytotoxicity of hydrothermal and precipitation β-TCP at the tested concentrations (6.25 mg/ml to 200 mg/ml) and images from optical microscopy showed that the cells were able to proliferate and retain its morphology after incubation with the extracts. Images from CLSM showed the viable cells had grown and able to attach on the surface of hydrothermal and precipitation β-TCP. Calcium deposition observation and ECM protein expression showed the positive osteogenesis from osteoblast cell cultured with hydrothermal and precipitation β-TCP. The results showed that the locally produced β-TCP using hydrothermal or precipitation method are non-cytotoxic and biocompatible. This biomaterials support cells attachment and cells proliferation, expressing protein related to osteogenesis and have the potential to be used as biomaterial in dental and orthopedic.

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EFFECT OF CONSUMPTION OF BEE PRODUCTS ON TELOMERE LENGTH AND LONGEVITY OF LIFE IN BEEKEEPERS

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The belief that beekeepers live longer than anyone else is present since ages and no research has been done to explore their longevity. Research has shown that telomere is associated with the longevity of life. Hence, this study aimed to investigate the telomere length in 30 male beekeepers and 30 male non-beekeepers and associate them with the longevity of life. Southern blot analysis of terminal restriction fragments (TRFs) was carried out by HinfI/RsaI digestion of human genomic DNA using TeloTAGGG Telomere Length Assay. Interestingly, the present study found that the telomere length of male beekeepers was significantly longer than those of male non-beekeepers with a *p*-value of less than 0.05, suggesting that beekeepers may have longer life compared to non-beekeepers. It was further found that the consumption of bee products for a long period and frequent consumption of bee products per day are associated with telomere length. A year increase in consuming bee products is associated with a mean increase in telomere length of 0.258 kbp. In addition, an increase in frequency of consuming bee products per day was also associated with a mean increase of 2.66 kbp in telomere length. These results suggest that bee products might play a role in telomere length maintenance.

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KERATINOCYTE DIFFERENTIATION FROM STEM CELLS OF HUMAN AMNIOTIC MEMBRANE USING CO-CULTURE SYSTEM

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Human amniotic mesenchymal stromal cells (hAMSCs) have the ability to divide, differentiate and mature to the specific types of cells as well as to renew themselves in order to regenerate other living cells. However, the differentiation of stem cells into keratinocytes still remains unexplored. Hence, this study was conducted to investigate the differentiation of keratinocytes from stem cells of human amniotic membrane using co-culture system. hAMSCs from term male neonate were isolated and characterised using stem cell markers employing Reverse Transcriptase Polymerase Chain Reaction, immunocytochemistry and flow cytometry techniques. Subsequent to that, the SRY gene expression analysis was carried out on the isolated hAMSCs at third passage to investigate whether the hAMSCs derived were from mother or male neonate. For the treatment groups using co-culture, Transwell Clear Polyester Membrane Inserts, 6-well plate were used. The human keratinocytes were seeded on the upper chamber of the plates where the hAMSCs were seeded on the lower chamber. Control groups containing hAMSCs cultured in the keratinocyte media only were seeded in 6-wells plates. The hAMSCs were harvested at day 1, 3, 7, 10, 14 and 21 and were subjected to RNA extraction. The presence of 18S and 28S bands indicated that the integrity of extracted RNA was good and suitable for One Step RT-PCR experiment. Then, the gene expression analyses of stem cell and keratinocyte markers were identified during the differentiation process. The characterisation experiments showed the presence of stem cell markers and the presence of SRY gene in hAMSCs indicated that the cells were derived from male neonate. The presence of keratinocyte gene markers KRT5, KRT14, FIL, IVL as well as AQP3 were observed on day 7 onwards after hAMSCs were co-cultured with human keratinocytes. The expression of these

markers suggested that hAMSCs successfully differentiated into keratinocyte-like cells. Comparing to the control groups, there was no expression of keratinocyte markers in the hAMSCs, even at day 21. Stem cell gene markers Nestin and NANOG were expressed until day 21. Thus, this study proves the differentiation of hAMSCs into keratinocyte-like cells using the co-culture technique.

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GENOTYPIC IDENTIFICATION OF ORAL BACTERIA USING 16S rRNA GENE IN CHILDREN WITH AND WITHOUT EARLY CHILDHOOD CARIES IN KELANTAN

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Oral microorganisms are considered as one of the primary aetiological factors in Early Childhood Caries (ECC) but they have not been completely identified. The present study aimed to identify oral bacterial genotypes in 12 caries-free children (CF) and 36 children with ECC aged 71 month and below by using 16S ribosomal RNA gene sequence. In ECC children, plaque and dentine samples were collected from intact enamel surfaces, over cavitated lesion and from dentine lesion surface. For CF children, plaque samples were collected from sound tooth surfaces at baseline and after one year follow up. The genomic DNA was extracted from all samples, subjected to 16S rRNA PCR amplification and the end products were cloned into pCR®2.1-TOPO® Vector. Five randomly selected positive clones collected from each surface were sent for sequencing. Identification of the bacterial clones was performed using BLAST search against GeneBank database. A total of 660 clones were collected from enrolled children. From ECC group, 540 clones were obtained from three different surfaces while, in CF group, 120 clones were collected from sound tooth surfaces at baseline and after one year. Several comparisons were performed between those identified oral bacteria using Pearson Chi-square test or Fisher's exact test between different categorical variables, while McNemar test was used to compare among the individual categories. A total of 39 oral bacterial genera were identified from the ECC group. At genus level, Streptococcus sp. was the most predominant bacteria among ECC group. Fusobacterium sp. is significantly higher in the intact enamel while Lactobacillus sp. is significantly higher in the dentine surface ($p < 0.05$). At species level, Fusobacterium nucleatum subsp. polymorphum was detected in the intact surface (33.3%) while Streptococcus mutans was detected over the carious lesions and dentine (33.3% and 52.7% respectively). A total 18 oral bacteria genera were identified from CF group at baseline and after one year follow up, but there were no significant differences between groups. At species level, Fusobacterium nucleatum subsp. polymorphum is found highest in the CF group. After follow up, Corynebacterium matruchotii is highest in those who remained caries free, while Porphyromonas catoniae is highest in those who developed caries. In conclusion, Streptococcus sp. is strongly associated with caries progression in children with ECC especially for Streptococcus mutans. Lactobacillus sp. is restricted to deep carious lesions. Fusobacterium sp., Leptotrichia sp., and Corynebacterium sp. may play a role in sustaining the healthy environment.

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THE EFFECT OF BIPHASIC CALCIUM PHOSPHATE AND SIMVASTATIN ON HUMAN DENTAL PULP CELLS IN VITRO ON DENTIN REGENERATION

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This study was conducted to prepare an appropriate biphasic calcium phosphate (BCP) scaffold in combination with optimal concentration of simvastatin to induce human dental pulp cells differentiation and dentin tissue regeneration. BCP scaffold of 20/80 hydroxyapatite (HA) to β-tricalcium phosphate (β-TCP) ratio with micropores < 10 µm, macropores of 300 µm, and porosity of 65 % was successfully synthesized using wet precipitation method and polyethylene microspheres as pore-creating agents. The scaffold was characterized using x-ray diffraction (XRD), fourier transform infra-red spectroscopy (FTIR), x-ray fluorescence (XRF), field emission scanning electron microscope (FESEM), linear shrinkage, and total porosity measurements. BCP sample extract was prepared and combined with four different concentrations of simvastatin (2.0, 1.5, 1.0 and 0.5 µM) for the assessment of cell viability using MTT (3-(4,5 dimethyl-thiazoyl)-2,5-diphenyl-tetrazolium bromide) assay. The alkaline phosphatase activity was assessed using alkaline phosphatase assay. The groups which showed the best cell viability and alkaline phosphatase activity were selected. They were assessed for both odontogenic differentiation potential of collagen type I alpha 1 (COL1A1), bone sialoprotein (BSP), dentin matrix protein-1 (DMP-1), dentin sialophosphoprotein (DSPP), and Runt-related transcription factor-2 (RUNX-2) genes expression analysis using reverse transcription-polymerase chain reaction (RT-PCR) and extracellular matrix mineralization detection using Alizarin Red S staining. The results showed that combination groups of BCP +1.5 µM, BCP + 1.0 µM, and BCP + 0.5 µM had higher mean cell viability index, except BCP + 2.0 µM that showed cytotoxicity. For alkaline phosphatase activity, the combination groups of BCP

+ 1.5 μM, BCP +1.0 μM, and BCP + 0.5 μM showed higher mean alkaline phosphatase activity index with BCP + 1.5 μM the highest. For the odontogenic differentiation potential, BCP + 1.5 μM combination group showed up-regulation of COL1A1, DMP-1, BSP, and DSPP genes and down-regulation of RUNX-2 gene. For the extracellular matrix mineralization, BCP + 1.5 μM combination group showed the highest ability to induce the mineral deposition. In conclusion, the combination of BCP and 1.5 μM simvastatin achieved together a preferable induction of human dental pulp cells differentiation toward dentin tissue regeneration.

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MECHANISM OF OSSEOINTEGRATION IN HYDROXYAPATITE INDUCED MURINE PRE-OSTEOBLASTS CELL LINE (MC3T3-E1) STIMULATED WITH RECOMBINANT INTERLEUKIN 6 AND/OR INTERLEUKIN 17A

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Cytokines are gaining momentum due to their possible role in enhancing osseointegration and their potential role in bone remodelling. The compatibility of the implant affects osseointegration in many ways. Therefore, extensive studies are being carried out to enhance osteoblast formation minimizing such complications. HA has been widely utilized due to its osteogenic property in current osseointegration applications. Receptor activator of NF-κB ligand (RANKL) has been shown to regulate osteoclast differentiation and its function. While osteoprotegerin (OPG) blocks the binding of RANKL thus inhibiting the differentiation of osteoclasts which then favours osteogenesis. Interleukin-6 (IL-6) and Interleukin-17 (IL-17), the key regulators of immune system have been known to play potential roles in bone remodelling. Hence, the aim of the current study was to determine the potential role of rIL-6 and rIL-17A in regulating the OPG/RANKL system of the murine osteoblast cell line (MC3T3-E1) in the presence of hydroxyapatite (HA). Cell proliferation and differentiation activity was measured by MTS and ALP assays respectively. Gene and protein expression was performed on RANKL and OPG markers using qPCR, Western blot and ELISA. In addition, SEM was carried out to observe the overall osteoblast cell attachment on the HA scaffold. This study involved the interaction of osteoblasts with cytokines alone and those seeded on HA and treated with cytokines. Analyses of MTS and ALP assays showed that osteoblasts treated with 10 ng/ml of rIL-6 or rIL-17A significantly induced proliferation and ALP activities; thus 10 ng/ml was found to be optimal for downstream experiments. Gene expression analysis showed significant up-regulation of OPG and ALP in all the treated groups (rIL-6, rIL-17A, rIL-6 + rIL-17A, HA + rIL-6, HA + rIL-17A and HA + rIL-6 + rIL-17A). In contrast, treatment of cells with rIL-6 and/or rIL-17A showed down-regulation of RANKL expression. Osteoblast cells treated with combinations of rIL-6 + rIL17A showed marked increase in OPG/RANKL ratio. Similar pattern was observed in the expression of protein in osteoblasts treated with combinations of rIL-6 + rIL-17A and rIL-17A. However, a retroactive mechanism was observed in rIL-6 as detected by Western blotting and ELISA. This pattern was further supported by the SEM analysis where rIL-6 + rIL 17A and rIL-17A were found to be perfectly attached and intercalated with HA. On the other hand, blebs and ruffles were more prominent in osteoblast treated with rIL6 compared to the other treated and control groups. Overall, the results of this study revealed that the combination of cytokines (rIL-6 + rIL-17A) show promising outcome compared to all the other treated samples. Hence, it can be concluded that the synergistic effect of rIL-17A towards IL-6 favours bone regulation. These findings suggest a new mechanism of regulation by these cytokines on the expression of OPG and RANKL, which could promote osteogenesis and diminish osteoclastogenesis with or without the presence of HA.

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