

Osteoconductive Property of Hydroxyapatite from Egg Shell as a Novel Bone Substitute

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Objectives: To evaluate the osteoconductive property and healing of locally synthetic hydroxyapatite from egg shell in extracted tooth socket. **Materials and methods:** Animal ethic approval was obtained from UiTM Care Ref No: 25/2013. Toxicity of hydroxyapatite from egg shell was tested on fibroblast and osteoblast cells line. Upper central incisors of thirty six adult male Sprague Dawley rats were extracted. Animals were divided into four groups; sockets of group 1(control) were left empty, group 2 filled with commercially available hydroxyapatite, group 3 with locally synthetic hydroxyapatite from egg shell, while group 4 with locally synthetic hydroxyapatite from egg shell and platelet-rich plasma . All sockets were sutured with resorbable dental suture. Animals were scarified, two, three and four weeks after the extractions. Serial sections were obtained and stained with hematoxylin and eosin, immunohistochemistry using osteocalcin as marker and Masson's Trichrome staining. Blood samples were taken before and after experiment for biochemical analysis. All statistical analysis done using SPSS version 20. **Results:** Hydroxyapatite from egg shell showed no toxicity on fibroblasts and osteoblasts cell lines. There were no changes in biochemical blood parameters of experimental groups compared to control group. Histological examination on all staining demonstrated that sockets treated with locally synthetic hydroxyapatite from egg shell combined with platelet-rich plasma has high expression of osteocalcin compared to other groups which indicate high osteoconductive activity. Fibroblast and collagen fibres were more dominant in experimental groups than the control. **Conclusion:** Locally synthetic hydroxyapatite from egg shell combined with platelet-rich plasma showed significant increase of bone formation by osteoconductive activity.

Effect of Low Pressure Condition on Shear Bond Strength of Veneer-Coping Interface

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Introduction: Regularly, Air Force pilots, paratroopers and elite teams of Malaysian Armed Forces are exposed to intense condition during parachuting and skydiving trainings. However, concerns arise if there is any influence during these activities on fixed restorations that were prescribed to them especially on pressure changes. The aim of this study is to evaluate the influence of hypobaric pressure on the shear bond strength of veneering ceramic to zirconia and metal. **Methods:** Twenty specimens were prepared according to manufacturer's instruction for each zirconia and metal group. Each group was further divided into different barometric pressures (n=10): control and hypobaric subgroups. Corresponding veneering ceramic (4 mm X 4.5 mm) was fabricated on zirconia and metal specimens (14 mm X 4 mm). The specimens were then pre-treated with hypobaric condition in simulated hypobaric chamber. Shear bond strength test was carried out using semicircular knife-edge jig at 0.5 mm/min crosshead speed. Microscopic examinations were done for evaluation of failure modes of specimens. **Results:** The zirconia group in hypobaric condition showed the highest mean shear bond strength (26.76 ± 7.37 MPa). Meanwhile, metal group in hypobaric condition showed the lowest mean shear bond strength (17.02 ± 6.08 MPa). One-way ANOVA showed that there was significant difference in shear bond strength between groups (Tukey's test, $p < 0.05$). Microscopic examination showed mixed adhesive/cohesive failure for most of the specimens. **Conclusion:** Hypobaric pressure influenced the shear bond strength of veneer-coping interface of zirconia and metal.

A Rapid and Reliable Method of Intracranial Volume Measurement for Pre- and Post-Operative Decompressive Craniectomy

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Introduction: Intracranial volume (ICV) is important in the management of patients underwent decompressive craniectomy (DC) surgery. **Objective:** The objective of this study was to validate ICV measurement using Cavalieri method based on computed tomography (CT) images for clinical purposes. **Methods:** A cross-sectional study of 55 CT head scans for pre- and post-operative DC patients were retrieved from September 2009 until December 2012. Ethical approval of the study was obtained from the Research and Ethics Committee, Universiti Sains Malaysia. CT images with a slice thickness of 1 mm and a matrix of 512x512 pixels each were analysed using open-source MITK 3M3 software. The ICV was measured by segmenting every slice of the CT images, and compared with estimated ICV calculated using 1-in-10 sampling strategy based on Cavalieri method. An independent *t* test was conducted to compare ICV measurements between two different methods. The calculation using Cavalieri method was repeated three times for reliability analysis using intraclass correlations coefficient (ICC). Bland-Altman plot was used to measure agreement between the methods for both pre- and post-operative ICV measurements. **Results:** The mean ICV (\pm SD) were 1341.1 ml \pm 122.1(manual) and 1344.11 ml \pm 122.6(Cavalieri) for pre-operative CT data. The mean ICV (\pm SD) were 1396.4 ml \pm 132.4(manual) and 1400.53 ml \pm 132.1(Cavalieri) for post-operative CT data. No significant difference was found in ICV measurement using the manual and the Cavalieri methods ($p = .983$ for pre-op, and $p = .960$ for post-op). The intrarater ICC showed a significant correlation; ICC = 1.00. Bland-Altman plot showed good agreement between the manual and the Cavalieri methods. **Conclusion:** The Cavalieri method with 1-in-10 sampling strategy gave comparable results in estimating ICV compared to the manual segmentation. Thus, this method could be used in clinical settings for rapid, reliable and repeatable ICV estimations.

Clustering of Maxillary Dental Arches in Relation to Stock Impression Tray Design

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Objectives: The objective of this study was to group Malaysian dental arches into clusters of shapes and sizes by applying the agglomerative hierarchical clustering (AHC) method, with the intention of designing stock impression trays **Methods:** Maxillary casts of 70 fully dentate subjects were studied. Eighteen variables, related to 5 teeth and the hamular notches in each cast were measured. The lengths and widths were measured using Mitutoyo digimatic callipers, and palate depths were measured using Mitutoyo digimatic indicator. Values of the 18 variables were subjected to normality tests, and the AHC method was applied to establish clusters of dental arches. **Results:** Dental arches in the study were grouped into 3 clusters. Twenty casts (28.6%) were in Cluster I, 24 (34.3%) in Cluster II and 26 (37.1%) in Cluster III. The descriptive statistics (mean and standard deviation in mm) for total arch length, width at canine region, width at first molar region and the greatest palate depth of each cluster respectively are as follows: Cluster I (64.21±4.92, 35.69±1.97, 56.66±1.33 and 21.45±2.38), Cluster II (65.93±5.08, 35.40±0.97, 53.13±1.24 and 20.06±1.48) and Cluster III (64.50±5.36, 33.99±1.36, 51.15±1.86 and 20.81±1.70). Three types of palate shape were found in the arches: deep (50%), moderate (30%) and shallow (20%). All these palate shapes were found in each cluster. **Conclusions:** It may be possible that three arch shapes and sizes are adequate for the design of impression trays for the sample studied. However, a set of different palate depths may be required to accommodate the different palate depths that exist in each cluster.

**Local Responses Following Systemic Treatment with Gelam Honey
(*Melaleuca Cajuputi*) in Experimentally-Induced Periodontitis Rats.**

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Introduction: Periodontitis is one of the most common dental diseases. It is well known as an inflammatory condition involving the supporting tissues of a tooth that can cause tooth loss. Previous researchers have shown that Gelam honey has an anti-inflammatory effect and potential for treating inflammatory condition. The aim of the study was to determine the local effect of Gelam honey on the inflammatory mediators namely IL-1 β in periodontitis Sprague-Dawley rats. **Methods:** Two rats for baseline group, 28 were randomly assigned into 4 groups: NLS (non-ligated given saline), NLH (non-ligated given Gelam honey, 3g/kg body weight), LS (periodontitis given saline) and LH (periodontitis given Gelam honey, 3g/kg body weight). Periodontitis was induced by ligating black silk suture 5/0 around lower left first molar. On day 15, rats were sacrificed. The tissue samples were observed and analysed histologically and immunohistochemically at the gingivomucosal and interdental area. The changes of the inflammatory cells were expressed as mean value and were analysed by using one-way ANOVA while in the immunohistochemistry analysis, the value were expressed as mean percentage and were analysed by using non-parametric test of Kruskal-Wallis. **Results:** The LS group showed significantly higher number of inflammatory cells (p=0.008, interdental area and p=0.001, gingivomucosal area). The LS group also exhibited significant higher level of immunohistochemically reaction of IL-1 β for both interdental (p=0.001) and gingivomucosal (p=0.002) area than other groups. A p-value less than 0.05 was considered statistically significant in this research (p<0.05). However, no significant difference between both observed areas. Gelam honey was found to be able to reduce the level of IL-1 β in periodontitis rats with a total reduction of 81.27%. **Conclusion:** Gelam honey able to reduce IL-1 β locally in an experimental periodontitis and has the potential for treating periodontal disease in human.

Expression of Growth Factors Upon Exposure of Dental Pulp Stem Cells to Dycal® Eluded Solution

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Objectives: The objectives of this study were to isolate, expand & characterize dental pulp stem cells (DPSC) from permanent teeth of healthy donors, and to identify the expression of growth factors when DPSC is exposed to Dycal® eluded solution.

Materials & methods: Samples were collected from sound, permanent teeth (age between 18-40 years old), with informed consent from patients who came for dental extraction at Faculty of Dentistry, University of Malaya. (Ethics approval: DFRD 1203/0014(P)). The isolation, culturing methods, growth kinetics, flowcytometry analysis and multilineage differentiation of DPSC were carried out as described by Govindasamy *et al.*, (2010). Dycal® was moulded into a cylinder shape, and were immersed and incubated in culture medium for 2,5 and 8 days at 37⁰C. Each sample obtained was then diluted with fresh culture media to produce Dycal® of 10%, 30%, 50% and 100% concentration [adopted from Hirschman *et al.*, (2012)]. Then, Dycal® eluded solutions were exposed to DPSC for 24 hours. Following this, cells were observed and counted using Trypan blue dye exclusion. This step was repeated three times for each culture media of different Dycal® concentration. DPSC not exposed to Dycal® eluded solution were used as control. Dycal concentration with the most consistent number of viable cells was chosen followed by RNA isolation. The cells were then loaded in RT² Profiler PCR array. Results were analyzed using Sequence Detection System (SDS) v1.4 software. **Results:** DPSCs were successfully isolated, expanded and characterized in the present study. Cell count at 2 days with 50% concentration of Dycal® eluded solution was chosen as test group as this concentration produced the most consistent number of viable cells (SD ± 4.975). Upon exposure, a total of 38% genes were up regulated whereas, a total of 61% genes were down regulated. The upregulated genes expressed were 12 functional and 14 canonical pathways. **Conclusion:** Exposure of DPSC to Dycal® eluded solution resulted in expression of certain group of growth factors.

Ex-Vivo Comparison of Moisture-Tolerant and Conventional Resin-Based Pit and Fissure Sealants on Different Surface Preparations

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Introduction: The traditional resin-based sealant is the choice of material for pit and fissure sealing. However, the drawback of this standard traditional resin-based sealant is its moisture sensitivity. Recent development of hydrophilic sealant with wetness-tolerant chemistry was intended to overcome this problem. **Objective:** This study aimed to compare the microleakage and penetration ability between wetness-tolerant (Embrace WetBond[®]) and conventional resin-based sealant (Clinpro[®]) under three different enamel surface preparations. **Methods:** One hundred and twenty extracted, caries free premolars were cleaned and randomly divided into six groups of equal numbers. In Group 1 and 2, teeth were prepared according to Clinpro[®] and Embrace WetBond[®] manufacturers' instructions respectively. In Group 3(Clinpro[®]) and 4(Embrace WetBond[®]), teeth were exposed to artificial saliva prior to sealant placement. Meanwhile, in group 5 (Clinpro[®]) and group 6 (Embrace WetBond[®]), bur preparation to the teeth were done. All the teeth were stored in artificial saliva for 1 week at 37°C and then subjected to thermocycling. Nail polish was used to coat the unsealed surface before immersion in 4% methylene blue. Each tooth was embedded into acrylic resin before sectioning at two places, producing 4 sections per teeth. Microleakage and unfilled surface areas were then measured using a 3D optical measurement device (Alicona Infinite Focus). **Results:** A significant difference in the microleakage between the two sealants was only noted in the bur prepared enamel surfaces, where Clinpro[®] sealant showed more microleakage than Embrace WetBond[®] (p<0.05). No significant differences were noted between the two sealants in terms of penetration ability with the 3 different enamel surface preparations. **Conclusion:** Both sealants are effective for pit and fissure sealing, however Embrace WetBond[®] is advocated in circumstances where enameloplasty with bur is required.

Directing the Cementoblastic Differentiation of SHED by HERS cells in Association with Chitosan Scaffold and TGF β ₁

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Introduction: Endodontic perforation located in the furcation of teeth remains both an endodontic and a periodontal problem. It is considered the second greatest cause of endodontic treatment failure which is occurred by either iatrogenic or pathologic causes. Destruction of the periodontium leads to the loss of the tooth if not treated properly. Regeneration of cementum is a very essential event which allows the reattachment of the periodontal ligament to the alveolar bone and root. Human hertwig's epithelial root sheath (HERS) cells have the ability to form cementum-like tissue and to differentiate into cementoblast-like cells. Stem cells from human exfoliated deciduous teeth (SHED) have intrinsic regenerative capacity, distinctive proliferation, and multipotential differentiation capabilities. However, their ability to differentiate into cementoblast-like cells has not been studied yet. **Objectives:** The aim of this study was to evaluate the synergistic effects of HERS cells and chitosan/TGF β ₁ on SHED proliferation and cemento/osteogenic differentiation. **Methods:** HERS cells were isolated and characterized then co-cultured with SHED with/without chitosan/TGF β ₁. Cell proliferation was assessed by prestoblue and cell attachment to chitosan scaffold was examined by SEM. Thereafter, alkaline phosphatase activity was quantified and mineralization assay was evaluated. Real time-PCR and western blot was performed. **Results:** Our results showed that HERS cells in association with chitosan-TGF β ₁ enhanced proliferation and cemento/osteogenic differentiation of SHED, which was demonstrated by high ALP activity, strong mineral deposition, and the up-regulation of cementum/bone-related gene/protein expression (ALP, COL I, BSP, OCN, and CAP). **Conclusions:** Our novel co-culture system confirmed the synergistic effect of HERS cells in a combination with chitosan-TGF β ₁ to stimulate the differentiation of SHED along the cementoblastic lineage; which possesses a novel therapeutic strategy for endodontic perforation and periodontitis.