Abstracts

Abstracts of Theses Approved for the MSc at the School of Dental Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia

THE STUDY OF DENTAL PULP STEM CELLS FOLLOWING RESTORATIVE PROCEDURES ON DECIDUOUS MOLARS

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Introduction: The biological examination of pulp injury, repair events, and the response of dental pulp stem cells to dental restorative materials are of paramount importance for the understanding of the post-operative development and the use of these repair activities in restorative treatment.

Objectives: The objectives of this study were to compare the stem cells proliferative activity using colorimetric proliferation reagent alamarBlue™, and to evaluate their response by comparing the tertiary dentin thickness (TDT) formation following restorative procedures on the teeth using haematoxylin and eosin staining for histological section and microscopic viewing using the Image-Pro Express software.

Methods: Healthy paediatric dental patients aged 9 to 11 years were selected; these patients attended the Paediatric Dental Clinic, School of Dental Sciences, Universiti Sains Malaysia Health Campus, for dental treatment, with carious teeth on deciduous molars without pulpal exposure. The patients' teeth were divided into 2 groups: Group 1 comprised of 29 teeth filled with glass ionomer cement (GIC) alone, and Group 2 comprised of 29 teeth lined with $Ca(OH)_2$ cement and filled with GIC. The teeth were reviewed for up to 6 months before being extracted according to standardised procedures based on the selected criteria.

Results: A total of 34 teeth were extracted (17 teeth per group). In each study group, 7 teeth were assigned for stem cell culture and 10 teeth were subjected to histological sections. The results showed that stem cells from human extracted deciduous teeth (SHED) were positive for surface antigenic markers CD105 and CD166 following immunocytochemistry test. The proliferative activity (median, IQR) for Group 1 (38, 19) was significantly less ($P > 0.05$) compared with in Group 2 (41, 19). However, the TDT area (median, IQR) in Group 2 (0.15, 0.03) was significantly higher ($P < 0.001$) compared with in Group 1 (0.05, 0.01).

Conclusion: The pulp of deciduous teeth contained stem cell population, which responded well and maintained proliferative activity following restorative procedures using $Ca(OH)_2$ cement and GIC. However, these materials created different responses of SHED in tertiary dentin deposition, where the reactionary dentin deposition appeared to be more under restorations lined with $Ca(OH)_2$. Thus $Ca(OH)_2$ cement has high credibility to be used in deep cavities for pulp protection.

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IN VITRO CYTOTOXICITY EVALUATION OF PROCESSED NATURAL CORAL ON HUMAN FIBROBLAST AND OSTEOBLAST CELL LINES

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Introduction: The development of bone-graft substitutes has evolved from the understanding of how autografts and allografts are used intra-operatively and how they are remodelled by the body after transplantation. The research on natural coral as a bone substitute has been reported in many experimental studies. Natural coral has been proven to be biocompatible and biodegradable. All newly developed biomaterials must fulfil stringent criteria laid out by government authorities and international agencies, before receiving approval for clinical application. Standard cell-based cytotoxicity assay are performed in vitro and high-risk materials are removed at early stage.

Objective: The aim of this study was to evaluate the in vitro cytotoxicity of the locally produced (Universiti Sains Malaysia Health Campus, Malaysia), processed natural coral (PNC) from the *Porites* species in terms of proliferation, apoptosis study, and cell attachment, by using human osteoblast (HOS) and fibroblast (MRC-5) cell lines.

Methods: The in vitro cytotoxicity of PNC was evaluated using test on extract and direct contact as per ISO 10993-5 (1999). HOS cells were used to study the magnitude of proliferation when exposed to the extraction medium of 2 different particle sizes of PNC: granules (0.5–1.0 mm) and powder (1–50 μm). The proliferation of HOS cells was analysed using tetrazolium-based colourimetric assay (MTT) and neutral red assay (NR). The material is considered toxic if the proliferation rate was less than 50%. For the apoptosis study, HOS cells were exposed to the extraction medium of PNC powder at a concentration of 200 mg/mL for 48 and 72 hours. Then, the cell pellet (1×10^6 cells) was resuspended in Annexin-V-FLOUS labelling solution and subjected to flow

cytometric analysis. Proliferation study via direct contact of PNC disc was carried out using HOS and MRC-5 cell lines. Both cell lines were exposed to the PNC material at 1, 24, 72, and 168 hours and analysed using NR assay; the medium was never changed through out the incubation periods. The material is considered toxic if the proliferation rate was less than 50%. For the cell attachment study, MRC-5 cells and HOS cells were cultured on the PNC discs and rubber latex (positive control) and observed under inverted microscope after 72 hours of incubation period. For the Scanning Electron Microscopic analysis, HOS cells were cultured on the PNC disc and thermanox plastic (negative control) and observed after 72 hours of incubation period.

Results: Proliferation study of HOS cells on the extraction of PNC granules and powder showed that both particles were not cytotoxic. Also, both PNC granules and powder did not induce any cytotoxicity, as was evident from their proliferation rate, which was above 50%. The flow cytometry analysis showed that the viable cell percentage for the HOS cells was high and the apoptotic cell percentage was low, indicating that PNC did not cause a remarkable damage to the cells. The results of the magnitude of proliferation of HOS and MRC-5 cells when exposed to the PNC material at different incubation periods using NR assay indicated that there was no cytotoxicity until an incubation period of 72 hours. The cell attachment study showed that both MRC-5 and HOS cells were attached on the edge of the PNC disc, which later grew into the pores of the PNC disc.

Conclusion: All the above results show that the locally produced PNC material by Universiti Sains Malaysia Health Campus is non-cytotoxic and favours the growth of HOS and MRC-5 cells.

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