#### **Abstracts**

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

## A THREE DIMENSIONAL COMPUTED TOMOGRAPHY (3D-CT) STUDY OF MAXILLARY SINUS IN MALAYS

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Introduction: The development pattern of the maxillary sinus varies widely according to the individual and the age. Even in the same person, both maxillary sinuses may develop in a different ways. Understanding the maxillary sinus size in different ages is important for many clinicians. This information can be used in the future as a baseline reference data for diagnosis and management of maxillary sinus abnormalities. 3D-CT offers the ability of observing maxillary sinus from several viewing angles with interactive and rapid repositioning of the 3D images.

Objective: To study the size of the maxillary sinus which includes linear and volume measurements in Malays at specifically selected age categories.

**Methods:** Three dimensional computed tomography (3D-CT) images of 144 Malays (288 maxillary sinuses) with no clinical evidence of craniofacial and maxillary sinus abnormalities were selected. The subjects aged from 0.4 to 30 years (Mean = 13.7, SD = 8.53 years), underwent the CT scanning using General Electric (GE) Light Speed Plus CT Scanner System (Wisconsin, USA) at the Radiology department, HUSM for reasons other than research purposes. The initial 2D formats were then reconstructed into 3D utilising OsiriX V3.7 (Geneva, Switzerland) software. Linear dimensions and volume of maxillary sinus were measured for each subject in different age categories and gender.

Results: The maxillary sinus increased in sizes and volume from birth to 30 years of age. Females demonstrated to have significantly larger maxillary sinus width (P = 0.02), height (P = 0.04) and depth (P < 0.01) than males in o to 6 years age category. The male's maxillary sinus width and height were significantly larger than females in 7 to 12 (P < 0.01) and 21 to 30 (P = 0.02) years age categories. Moreover, maxillary sinus depth were found to be significantly larger in males than females in 21 to 30 years age category (P < 0.01). Males also exhibited significantly larger maxillary sinus volume than females in 7 to 12 (P < 0.01) and 21 to 30 (P < 0.01) years age categories. No significant different (P < 0.05) between the right and left maxillary sinus in most age categories. Significant correlations were detected between the maxillary sinus, nasal cavity and maxilla from the age o to 12 years. No significant correlations were observed beyond the age 12 years.

**Conclusion:** The maxillary sinus size showed sexual dimorphism at most age categories. Gender consideration should be taken into account in clinical assessment and procedures which may affect the maxillary sinus.

Supervisor: Dr Asilah Yusof Co-Supervisor: Associate Professor Dr Rozita Hassan

EVALUATION OF SHEAR BOND STRENGTH OF RESIN MODIFIED GLASS IONOMER CEMENT AND ENAMEL SURFACE CONDITION WITH DIFFERENT SURFACE PREPARATION: IN VITRO STUDY

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**Introduction:** Brackets in orthodontic needs a long lasting durability under the influence of different stresses; therefore, it must possess a clinically acceptable shear bond strength (SBS) to withstand orthodontic movement force, as well as additional forces.

**Objective:** To evaluate the shear bond strength of orthodontic bracket bonding with RMGIC indifferent enamel surface preparation. In addition, the adhesive remnant index (ARI) after orthodontic bracket debonding and the enamel surface roughness after removal of bonding residues were studied

Methods: One hundred forty four (144) teeth extracted due to orthodontic and/or periodontal problems were collected and divided randomly into four groups, with four different surface preparations. Group I (control group): 37% Phosphoric acid; Group II (control group): sandblasting; Group III (experimental group): sodium hypochlorite and 37% phosphoric acid; and Group IV (experimental group): sodium hypochlorite and sandblasting. Orthodontic bracket bonding were done by RMGIC. Instron machine was used to measure the shear bond strength in Mega Pascal (MPa). After bracket debonding for all groups, the adhesive remnant index of the remnant adhesive was calculated, the enamel surface roughness after cleanup with tungsten carbide bur in low speed hand piece and sandblasting, 27 µm particle size with 80 per square inch (Psi) was measured, and surface morphology was observed using a scanning electron microscope.

**Result:** The results showed that the mean values of SBS for groups I, II, III, and IV were 13.86, 9.08, 17, and 9.63 MPa, respectively. The mean percentages (%) of the

adhesive remnant on the tooth surface were 11.16, 2.06, 20.66, and 3.73. The median and inter quartile Range (IQR) of the surface roughness after cleanup with TCB and SB represented by Ra (arithmetical average roughness height) for all groups were as follows: Group1: TCB were 0.65 (0.53), 0.85 (0.55), 4.41 (2.85), and SB were 0.70 (0.58), 0.90 (0.65), 5.00 (3.20), respectively. Group 2: TCB were 0.70 (0.53), 0.90 (0.63), 5.0 (3.20), and SB 0.85 (0.48), 1.05 (0.55), 5.45 (3.00), respectively. Group 3: TCB were 0.70 (0.11), 0.91 (0.19), 4.85 (1.92), and SB were 0.75 (0.28), 1.00 (0.28), 5.65 (2.65), respectively. Group 4: TCB were 0.70 (0.10), 0.95 (0.17), 5.70 (2.13), and SB 0.80 (0.20), 1.00 (0.10), 5.50 (0.30), respectively. Scanning electron microscope (SD) revealed no difference in morphology between all groups.

Conclusion: Applying 5.25% NaOCL to the enamel surface eliminates the organic elements, allowing the acid etchant to penetrate more effectively into the enamel, creating type 1 and 2 etching patterns. The increased bonding strength allows the orthodontist to use fluoride- releasing RMGICs as bonding adhesivesto possibly protect the enamel from developing white spot lesion, a major iatrogenic effect of orthodontic treatment. Combining the clinical aims and experience with the best available evidence should be an important goal of every clinician. In conclusion, the addition of NaOCL prior to acid etch and orthodontic bracket bonding using RMGIC increased the shear bond strength as well as utilising fluoride releasing effect, with higher ARI.

Supervisor: Dr Mohammad Khursheed Alam Co-Supervisor: Associate Professor Dr Rozita Hassan

#### DENTAL CARIES AND SALIVARY PARAMETERS OF DOWN SYNDROME CHILDREN IN KOTA BHARU, KELANTAN

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**Introduction:** Down syndrome (DS) is a genetic disorder caused by alteration of chromosome 21 with a range of 1: 800 to 1:1000 live births. Several studies showed that children with DS have low caries experience due to better salivary parameters, however the findings are inconclusive.

**Objectives:** This study aimed to determine the prevalence of dental caries and caries experience in DS and normal children. It also aimed to determine and compare the salivary pH, salivary buffering capacity and salivary immunoglobulin A (SIgA) between these groups of children. The factors associated with caries experience in DS and normal children were also determined.

Methods: A comparative cross sectional study was

conducted on 73 DS children and randomly selected 73 normal children aged 7-12 years old. The DS children were recruited from special classes in normal primary schools, Pusat Pemulihan Dalam Komuniti and private centers whereas normal children were taken from the same primary schools. The children were asked not to eat an hour before the procedure. Un-stimulated saliva samples were collected from 8:30 am to 10:30 am from all children. The salivary pH and salivary buffering capacity were determined at the same time using pH and buffer test strips according to manufacturer's protocol. Thirty two saliva samples were randomly selected from each group and were stored under -80 °C. SIgA concentration was then tested using commercially available enzyme immunoassay kit, ELISA. Clinical oral examination was done after the saliva collection procedures using disposable probe and mouth mirror. The caries experience was charted using the index of decay-filledteeth (dft) for deciduous dentition and Decay-Missing-Filled Teeth (DMFT) for permanent dentition.

Results: Among the DS children, caries prevalence of deciduous dentition (67%) was higher compared to normal children (62%). While, the caries prevalence of permanent dentition was higher among normal children (38%) compared to DS children (27%). Salivary parameters such as salivary pH, salivary buffering capacity and SIgA, were significantly lower among the DS compared to normal children (P < 0.001). Among DS children, only age showed positive association with DMFT score (P = 0.016) i.e. as age increases by one year, caries experience increase by 0.22. Regarding the normal children, age (P < 0.001) and salivary buffering capacity (P = 0.008)showed negative association with dft, i.e. as age increase by one year, caries experience decreases by 0.88 and if salivary buffering capacity increases by one unit the caries experience score decreases by 0.30. Age showed positive association with DMFT (P = 0.016) among normal children, a one year increase in age will increase the caries experience by 0.51.

Conclusions: Higher caries prevalence in deciduous dentition was found in DS, while for caries prevalence of the permanent dentition showed the opposite trend. All salivary parameters were lower among DS children compared to normal children. Among normal children, caries experience in deciduous dentition decreases with increase in age and salivary buffering capacity. The caries experience in permanent dentition showed to increase with increase in age among both DS and normal children. Further research is required to include more risk factors as caries is a multifactorial disease.

Supervisor: Associate Professor Dr Azizah Yusoff Co-Supervisor: Associate Professor Dr Normastura Abd. Rahman Dr Wan Majdiah Wan Mohamad

IMMUNOGENICITY OF SYNTHETIC HYBRID PROTEIN NEURAMINIDASE OF MALAYSIAN INFLUENZA A IN BALB/c MICE

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Introduction: Vaccination against H5N1 seems to be an appropriate approach as primary method for preventing H5N1 and the severe complications related to the disease. Neuraminidase (NA) protein is primarily immunogenic and elicits immune response. Introduction of the recombinant synthetic protein as vaccine may induce humoral and cellular immune responses at systemic level.

**Objective:** In this study, we investigated the humoral and cellular immune response in mice after intraperitoneal administration of NA synthetic protein in the presence and absence of adjuvant.

Methods: BALB/c mice were immunised with 5 μg, 50 μg and 100μg of NA protein in the presence and absence of adjuvant via intraperitoneal administration followed by two booster doses at three weeks interval. Serum was collected pre- and post- immunization before administering booster doses and was subjected for ELISA analyses. The mice were sacrificed by cervical dislocation 3 weeks after final immunization. Peritoneal washout was used for macrophage count using CD 14- FITC via flow cytometer. Splenocytes were used for cell subsets analysis and cultured for proliferation assay and cytokine assay (IFN-γ and IL-4).

**Results:** The study showed that intraperitoneal administration of synthetic protein in the presence of adjuvant, stimulated humoral and cellular immune responses. Additionally, the presence of adjuvant elicited the Th1 and Th2 profile, suggesting promotion of humoral and cell-mediated immune response. The immunisation of protein alone induced low antibody response and Th1- type immune response.

**Conclusion:** The results obtained from this study indicate that the protein NA is immunogenic and is promising for the future development of pandemic influenza vaccine.

Supervisor: Associate Professor Dr Azizah Yusoff Co-Supervisor: Associate Professor Dr Normastura Abd Rahman Dr Wan Majdiah Wan Mohamad

THE EFFECTIVENESS OF THE TREATMENT WITH REVERSE TWIN BLOCK AND REVERSE PULL FACE MASK APPLIANCES FOR CLASS III MALOCCLUSION: A RANDOMISED CLINICAL TRIAL

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Introduction: The effects of functional appliances on craniofacial morphology of Class III malocclusion among

Malay children in Malaysia remains a puzzle.

**Objective:** A randomized clinical trial was undertaken to evaluate the effectiveness of treatment with reverse twin block and reverse pull face mask appliances for Class III malocclusion among males and females Malay children in the early and late mixed dentition stages.

Methods: A total of 97 Class III malocclusion Malay children aged from 8 to 9 and 10 to 11 years were enrolled into two treatment groups. No dropout was recorded. 49 children were treated with reverse twin block appliance and 48 children received reverse pull face mask appliance for a period of eight months therapy. Lateral cephalometric radiographs and upper and lower impressions for dental study models were taken and questionnaires interview was done before and after treatment. Multiple regression analysis was used to evaluate the relationship between age and sex on the skeletal change, dental arches change and perception of the patient after treatment with reverse twin block and reverse pull face mask appliances. Also, multiple regression analysis was used to compare the skeletal changes, dental arches change and perception of the patient after treatment with reverse twin block and reverse pull face mask appliances. The level of significance was set at 0.05

Result: Results showed that there is no relationship between age and sex affecting the dentoskeletal changes for both appliances. However, reverse pull face mask appliance had induced more significant dentoskeletal changes than reverse twin block appliance in this group of study among Malay children. Assessment of post-treatment study models showed that both appliances changed the maxillary and mandibular dental arch shape but do not affect the inter-teeth distances. However, only the reverse pull face mask appliance showed increased upper dental arch length. All patients showed significant satisfaction following treatment with reverse pull face mask appliance when compared with reverse twin block appliance. There were no differences found between the treatment responses in early or late mixed dentition stages in both males and females in both treatment groups.

**Conclusion:** In conclusion, reverse pull face mask appliance induced significant dentoskeletal changes in the treatment of Class III malocclusion when compared with reverse twin block appliance.

Supervisor: Dr Mohd. Fadhli Khamis Co-Supervisor: Professor Dato' Dr Ab Rani Samsudin Associate Professor Dr Noor Hayati Abdul Razak Dr Norehan Mokhtar

SYNTHESIS OF SILICA NANOPARTICLES AND THEIR SURFACE MODIFICATION FOR FABRICATION OF HYBRID DENTAL NANOCOMPOSITES

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**Objectives:** The aim of this study was to synthesis silica nanoparticles fillers for fabrication of hybrid dental nanocomposites and to evaluate their properties, including surface roughness and mechanical properties. Hybrid of three different sizes of silica nanoparticles ranging from 10 to 100 nm were synthesised via a sol-gel method using three different concentrations of ammonia. Surface modification of the silica nanoparticles was carried out using  $\gamma$ -methacryloxypropyltrim ethoxysilane ( $\gamma$ -MPS) as a coupling agent via co-condensation and one-pot method.

**Methods:** The silica surface was modified in order to improve interfacial adhesion between nanofiller and the matrix. The characterisations of unmodified and modified fillers were carried out using Transmission Electron Microscope (TEM), Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric analysis (TGA), BET analysis, Nuclear Magnetic Resonance (NMR) and scanning electron microscopy equipped with energy dispersive X-ray spectroscopy (SEM-EDX).

**Result:** The hybrid dental nanocomposites with filler content of 40 wt% containing a combination of different sizes (19.6 [SD 3.3], 27.6 [SD 2.3] and 64.6 [SD 8.2] nm for unmodified filler, and 16.4 (SD 5.5), 38.1 (SD 8.8) and 70.9 (SD 15.3) nm for modified filler via one-pot method), were fabricated. The hardness, flexural strength and flexural modulus were evaluated. The hybrid dental nanocomposites reinforced by modified filler (E-Po.2) showed higher flexural strength, flexural modulus, and hardness than those unmodified filler. The hybrid dental nanocomposites fabricated with 1:2:1 (16.4; 38.1; 70.9 nm) filler weight ratio have shown the best mechanical properties.

**Conclusion:** Based on the results obtained, the modified silica nanoparticles are a promising material for the fabrication of tooth-filling nanocomposites.

Supervisor: Profesor Ismail Ab. Rahman Co-Supervisor: Dr Dasmawati Mohamad

#### A PILOT STUDY OF USM TISSUE BANK PRODUCED AN-ORGANIC BOVINE BONE GRAFT POST MAXILLARY SINUS AUGMENTATION USING CONE BEAM COMPUTED TOMOGRAPHY

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*Introduction:* An-organic bovine bone graft is a xenograft with the potential of bone formation.

Objective: The aim of this study was to evaluate the

bone density using cone beam computed tomography scans around functional endosseous implant in the region of both augmented maxillary sinus with the an-organic bovine bone graft and the alveolar bone over which the graft was placed to provide space for the implants.

Methods: Sterile freeze dried bovine bone graft (OsteoLEMB) produced by National Tissue Bank, Universiti Sains Malaysia was used for 1-stage implant placement with maxillary sinus augmentation in a total of 19 subjects with 19 implants. The age of all subjects ranged between 40−60 years with a mean age 51 (SD 4.70). After a period of one and a half year, all subjects underwent a follow up Cone beam computed tomography scan using Planmeca Promax 3D ®Cone beam computed tomography scanner at the Radiology department, Hospital Universiti Sains Malaysia. The collected data was then analysed to evaluate bone density in Hounsfield Units using Planmeca Romexis™ Imaging Software 2.2 ®which is specialised accompanying software of the cone beam computed tomography machine.

**Results:** There was bone formation at the site of the augmented sinus. A significant increase (P < 0.005) in bone density was reported at the augmented site compared to the bone density of the existing alveolar bone. Statistically bone formation at the augmented site was independent of age, gender and diameter of the implant used. In the existing alveolar bone males showed a statistically significant (P < 0.005) higher density than female subjects.

**Conclusion:** Our results suggest that an-organic bovine bone graft is a viable osteoconductive grafting material when used for the procedure of maxillary sinus augmentation and Cone beam Computed Tomography may be a useful tool for determining the in vivo bone density.

Supervisor: Dr Shaifulizan Ab. Rahman Co-Supervisor: Dr Zaihan Ariffin Dr Mohammad Khursheed Alam

# ISOLATION, CHARACTERIZATION AND CYTOTOXIC EFFECTS OF SEA CUCUMBER (Bohadschia bivittata) COLLAGEN ON HUMAN PERIODONTAL LIGAMENT FIBROBLASTS CELL LINE

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Introduction: About two-third of sea cucumber wall protein is composed of highly insoluble collagen fibres. Collagen has been found to have promising role in tissue healing and regeneration. A human periodontal ligament (HPDL) is known to be a reservoir for the tissues of the periodontium and subjected to trauma/diseases.

Objective: The aim of this study was to isolate and

to characterise pepsin-solubilised collagen (PSC) from the body wall of sea cucumber, *B. bivittata* and also to determine and compare the cytotoxic effects of non gamma and gamma irradiated PSC on HPDLFs cell line.

Methods: Body wall of B. bivittata were cut into small pieces followed by washing with distilled water and then replaced with 4 mM ethylenediaminetetraacetic acid (EDTA), 0.1 M Tris-HCl, pH 8.0, and stirred for 3 days to get precipitated crude collagen fibrils. Disaggregated insoluble crude collagen fibrils were treated with 0.1 M Sodium hydroxide (NaOH), followed by 0.5 M acetic acid containing porcine pepsin to get PSC. The ultra-structures were analyzed by using Quanta<sup>™</sup> Scanning Electron Microscope (SEM), Netherlands, under 130 Pa pressure, at a working distance of 6-10 mm at 4.0 to 5.0 kV from magnification 5,000  $\times$  to 100 000 ×. Sodium dodecyl sulfate electrophoresis (SDS-PAGE) was performed by using a discontinuous Tris-HCl/glycine buffer system with 7.5% resolving gel and 4% stacking gel. PSC was added in alpha modification essential medium (α-MEM) and incubated for 5 days at 37oC. At day 4, the HPDLFs cells were cultured in 96-well plates for one day at 37oC and 5% Carbon dioxide (CO2), and consequently the extract was added into the wells at seven serial dilutions for 3 days. Then, 15 µl of 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added for 4 hours, and the readings were recorded using an ELISA reader at 570 nm and 600 nm for the test and reference wavelengths, respectively.

PSC was successfully isolated by using pepsin-solubilised collagen method, with 65% yield. SEM analysis of crude collagen displayed a regularly repeated striated pattern, whereas, PSC displayed non-banded sheet like fibers with twisted configuration. According to the electrophoretic pattern, PSC was identified as type I collagen, with the molecular composition of  $(\alpha 1)^2$  and  $(\alpha 2)^1$  chains of approximately 138 kDa each. The MTT assay showed a significant increase in the cell viability values indicating that PSC is non-cytotoxic. Cell viability values of HPDLFs cell line by non gamma irradiated PSC was significantly higher than gamma irradiated PSC at 50%, 25%, 12.5% and 6.25% concentrations (P < 0.05).

**Conclusion:** In conclusion, the results indicated that PSC extract from B. bivittata did not induce cytotoxicity to HPDLFs. As high as 65% of PSC was successfully isolated from 100 g of B. bivittata body wall pieces and classified as type I collagen. The potential use of the collagen from the body wall of *B. bivittata*, as an alternative to mammalian collagen in healing and regeneration procedures need to be further explored.

Supervisor: Associate Professor Dr Erry Mochamad Arief Co-Supervisor: Associate Professor Dr Azizah Yusoff Associate Professor Dr Suzina Sheikh Abdul Hamid