UNIVERSITI SAINS MALAYSIA

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*

FINAL REPORT OF SHORT TERM RESEARCH PROJECT

Sila kemukakan laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/Pengarah/Ketua Jabatan kepada Pejabat Pelantar Penyelidikan

1. Nama Ketua Penyelidik: Dr Siti Noor Fazliah Mohd Noor
Name of Research Leader

☐ Profesor Madya/ *Assoc. Prof.* ☐ Dr./ *Dr.* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. Pusat Tanggungjawab (PTJ): PP Sains Pergigian
School/Department

TERIMA
23 OCT 2008
PEJABAT PENYELIDIKAN KLINIKAL
UNIVERSITI SAINS MALAYSIA
KAMPUS KESIHATAN

3. Nama Penyelidik Bersama: Dr TP Kannan
Name of Co-Researcher

4. Tajuk Projek:
Title of Project

Evaluation and response of dental stem cells in pulp tissue following pulp capping using CaOH and GIC or restorative procedure using GIC for primary molars

5. Ringkasan Penilaian/*Summary of Assessment*:	Tidak Mencukupi *Inadequate* 1 2	Boleh Diterima *Acceptable* 3	Sangat Baik *Very Good* 4 5
i) Pencapaian objektif projek: *Achievement of project objectives*	☐ ☐	☐	☐ ☑
ii) Kualiti output: *Quality of outputs*	☐ ☐	☐	☐ ☑
iii) Kualiti impak: *Quality of impacts*	☐ ☐	☑	☐ ☐
iv) Pemindahan teknologi/potensi pengkomersialan: *Technology transfer/commercialization potential*	☐ ☐	☐	☐ ☑
v) Kualiti dan usahasama : *Quality and intensity of collaboration*	☐ ☐	☑	☐ ☐
vi) Penilaian kepentingan secara keseluruhan: *Overall assessment of benefits*	☐ ☐	☐	☐ ☑

6. **Abstrak Penyelidikan**
(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris**. Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Abstract of Research
(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English).
This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Attached as Appendix 1

7. **Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.**
[Sila gunakan kertas berasingan]
Applicant are required to prepare a Comprehensive Technical Report explaning the project.
(This report must be appended separately)

Attached as Appendix 2

Senaraikan kunci yang merminkan penyelidikan anda:
List the key words that reflects your research:

Bahasa Malaysia	*Bahasa Inggeris*
Simen Kaca Ionomer	Glass Ionomer Cement
Kalsium hidroxida	Calcium hydroxide
Sel stem dari gigi	Stem cells from tooth

8. **Output dan Faedah Projek**
Output and Benefits of Project

(a) * Penerbitan Jurnal
Publication of Journals
(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbit/diserahkan)
(State type, title, author/ed... publication, year and where it has been published/submitted)

1. Type: Clinical research **(Appendix 3)**
Title: Proliferative activity and response of Stem Cells from Human Exfoliated Deciduous Teeth to dental materials
Authors: Lutfi AN, Fazliah SN, Kannan TP, Saidi J, Jamaruddin MA.
Journal: Stem Cells Development (Impact factor 3.07)
Status: Manuscript submitted, under review

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

This study had enabled us to save on the tooth from being discarded and cause environmental problems. The procedure of isolating the stem cells from dental pulp allowed us to save these stem cells so that it can be use for possible source of replacing damaged tissue since it has been shown that these cells are capable of differentiating into many cells types if induced.

The high proliferative activity shown by these cells had proven that it capable of yielding high cells numbers and can provide source for tissue engineering.

These cells also prove useful for future used as the main cells for biocompatibility testing of dental materials.

* Sila berikan salinan/*Kindly provide copies*

(c) **Latihan Sumber Manusia**
Training in Human Resources

i) Pelajar Sarjana:
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)
Name: Alaa Nassier Lutfi
Degree: MSc
Title: Evaluation and response of dental stem cells in pulp tissue following pulp capping using CaOH and GIC or restorative procedure using GIC for primary molars
Status: Thesis submitted, awaiting viva voce

ii) Lain-lain:
Others

1. Siti Noor Fazliah Mohd Noor, Thirumulu Ponnuraj Kannan, Alaa Nassier Lutfi (2007). SHED proliferation following restorative procedures. 1st International Bone and Dental Technology Symposium. 12-13th November 2007. Grand Mercure Fortune, Bangkok, Thailand. (Poster Presentation)

2. AN Lutfi, SNF Mohd Noor, S Jaafar, TP Kannan (2008). Dental Materials Effect on SHED Following Pulp Capping. 7th Scientific Meeting and 9th Annual General Meting of IADR Malaysian Section. 23 February 2008. Universiti Teknologi Mara (UITM). (Oral Presentation)

3. AN Lutfi, SNF Mohd Noor, S Jaafar, TP Kannan (2008). SHED proliferative activity response to dentistry Materials. 12th International Conference of Asia Pacific association of Surgical Tissue Banks. 2-6 June 2008. Renaissance Kuala Lumpur. (Oral Presentation)

9. **Peralatan yang Telah Dibeli:**
Equipment that has been purchased

Pembelian Alatan Di bawah Vot 35000 bagi 'Elipar Freelight 11 230v GB' (telah pun direkodkan oleh pihak Bendahari USM)
No akaun: 304/PPSG/6131527

Tandatangan Penyelidik
Signature of Researcher

27/7/08
Tarikh
Date

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

Penyelidikan ini telah dijalankan dengan jayanya dengan penerbitan beberapa kertas penyelidikan dan keluaran beberapa pelajar siswazah.

PROFESSOR ZULKIFLI AHMAD
Professor of Community Medicine
Deputy Dean
(Research & Postgraduate Studies)
School of Dental Sciences
USM Health Campus
16150 Kubang Kerian,
Kelantan.

22.10.2008

TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN
PUSAT PENGAJIAN/PUSAT
Signature of Chairman
[Research Committee of School/Centre]

Tarikh
Date

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN GERAN USM(304)
PENYATA PERBELANJAAN SEHINGGA 31 OGOS 2008

Jumlah Geran:	RM	19,997.00	Ketua Projek:	DR SITI NOOR FAZILAH MOHD NOOR
Peruntukan 2007 (Tahun 1)	RM	10,820.00	Tajuk Projek:	Evaluation and Response of Dental Stem Cells in Pulp Tissue Following Pulp Capping using CaOH and GIC for Restorative Procedure using GIC for Primary Molars
Peruntukan 2008 (Tahun 2)	RM	9,177.00		
Peruntukan 2009 (Tahun 3)	RM	0.00	Tempoh:	01 Mei 07 - 30 Apr 09
			No.Akaun:	304/PPSG/6131527

Kwg	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan T'kumpul Hingga Tahun Lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
304	11000	PPSG	6131527		-	-	-	-	-	-	-
304	14000	PPSG	6131527		-	-	-	-	-	-	-
304	15000	PPSG	6131527		-	-	-	-	-	-	-
304	21000	PPSG	6131527		4,670.00	1,417.00	3,253.00	-	61.80	61.80	3,191.20
304	22000	PPSG	6131527		-	-	-	-		-	-
304	23000	PPSG	6131527		300.00	-	300.00	-		-	300.00
304	24000	PPSG	6131527		-	-	-	-		-	-
304	25000	PPSG	6131527		-	-	-	-		-	-
304	26000	PPSG	6131527		-	-	-	-		-	-
304	27000	PPSG	6131527		14,027.00	10,586.90	3,440.10	-	-742.90	(742.90)	4,183.00
304	28000	PPSG	6131527		-	-	-	-		-	-
304	29000	PPSG	6131527		1,000.00	3,505.41	(2,505.41)	-	190.00	190.00	(2,695.41)
304	32000	PPSG	6131527		-	-	-	-		-	-
304	35000	PPSG	6131527		-	-	-	-	4,600.00	4,600.00	(4,600.00)
					19,997.00	15,509.31	4,487.69	-	4,108.90	4,108.90	378.79

Appendix 1

PENILAIAN DAN RESPON SEL-SEL STEM SELEPAS PROSEDUR PELAPIKAN PULPA MENGGUNAKAN $Ca(OH)_2$ DAN GIC ATAU PROSEDUR RESTORASI MENGGUNAKAN GIC UNTUK GIGI MOLAR DESIDUS

ABSTRAK

Pemeriksaan biologi untuk kecederaan pulpa, memperbaiki kerosakan dan respon sel-sel stem pulpa gigi terhadap bahan-bahan yang digunakan untuk restorasi adalah amat penting bukan hanya ketika memahami perkembangan gigi selepas pembedahan tetapi juga ketika menjalankan aktiviti-aktiviti pembaikan untuk menyempurnakan rawatan restorasi. Struktur gigi selalunya terdedah kepada bahan-bahan pergigian yang berbeza dan gigi desidus, seperti yang diketahui adalah sumber yang kaya dengan sel-sel stem. Oleh itu adalah amat penting untuk menilai respon sel-sel stem pulpa gigi desidus terhadap bahan-bahan pergigian yang biasa digunakan pada pesakit pergigian pediatrik. Bahan-bahan yang biasa digunakan ialah simen Ionomer Kaca (GIC) dan simen Pelapik Kalsium Hidroksida $Ca(OH)_2$. Tujuan kajian ini dijalankan adalah untuk memastikan kewujudan sel-sel stem pada gigi susu yang telah dicabut, dengan menggunakan kaedah imunositokimia, dan juga untuk menilai aktiviti pembiakan sel-sel stem serta respon sel-sel ini dengan membandingkan pembentukan Ketebalan Dentin Tersier (TDT) selepas prosedur restorasi gigi dilakukan. Pesakit-pesakit pergigian pediatrik yang sihat yang datang ke klinik pergigian pediatrik untuk rawatan gigi di Pusat Pengajian Sains Pergigian, Kampus Kesihatan, Universiti Sains Malaysia, berumur antara 9 hingga 11 tahun serta mempunyai karies pada gigi molar desidus pulpanya tidak terdedah telah dipilih sebagai responden. Para responden telah dibahagikan kepada dua kumpulan: untuk Kumpulan 1, gigi pesakit telah ditampal dengan hanya simen Ionomer Kaca (kapsul Fuji IXGP, GC, Jepun) dan

untuk Kumpulan 2, gigi pesakit telah dilapik dengan simen Kalsium Hidroksida (Dycal ®, Dentsply, Jerman) dan kemudiannya ditampal dengan simen Ionomer Kaca (kapsul Fuji IXGP, GC, Jepun). Gigi-gigi tersebut telah dikaji selama 6 bulan sebelum dicabut menggunakan kriteria yang telah ditentukan dan juga mengikut prosedur piawai. Kewujudan sel-sel stem pada gigi susu telah dipastikan oleh imunositokimia dengan menggunakan petunjuk permukaan antigen CD 105 dan CD 166. Reagen proliferasi kolometrik alamarBlue™ telah digunakan untuk menilai aktiviti pembiakan untuk responden Kumpulan 1 dan Kumpulan 2. Respon sel-sel stem pulpa gigi selepas prosedur restorasi telah dinilai menggunakan pewarna H&E untuk bahagian histologi yang kemudiannya dilihat di bawah mikroskop dengan menggunakan sofwer 'image pro' (Media cypernetics. Inc., USA). SHED telah didapati positif untuk CD 105 dan CD 166. Aktiviti proliferasi untuk kedua-dua kumpulan 1 dan 2 menunjukkan sedikit perbezaan tetapi secara statistiknya tidak signifikan. Walau bagaimanapun, kawasan ketebalan dentin tersier dari kumpulan 2 adalah lebih signifikan dari kumpulan 1. Pulpa gigi desidus mengandungi populasi sel stem dan selepas prosedur penutupan pulpa menggunakan simen Kalsium Hidroksida (Dycal ®, Dentsply, Jerman) dan simen Ionomer Kaca (kapsul Fuji IXGP, GC, Jepun) dilakukan, sel-sel tersebut menunjukkan respon yang baik serta dapat mengekalkan aktiviti proliferasi mereka. Walau bagaimanapun, bahan-bahan ini mewujudkan respon SHED yang berbeza pada pembentukan dentin tersier dan pembentukan dentin reaksioner pula adalah lebih pada pemulihan gigi yang dilapik Kalsium Hidroksida $Ca(OH)_2$. Oleh itu, untuk melindungi pulpa, simen kalsium hidroksida mempunyai kredibiliti yang lebih baik ketika merawat pesakit yang mempunyai karies dengan kaviti dalam pada gigi desidus.

Appendix I

EVALUATION AND RESPONSE OF DENTAL PULP STEM CELLS FOLLOWING PULP CAPPING PROCEDURE USING CALCIUM HYDROXIDE CEMENT AND GIC ~~OR RESTORATIVE PROCEDURE USING GIC IN PRIMARY MOLARS~~

ABSTRACT

The biological examination of pulp injury, repair events and the response of dental pulp stem cells to dental restorative materials is of paramount importance for understanding the post operative development and the use of these repair activities to accomplish restorative treatment. The structure of the tooth is almost always exposed to different dental materials and the deciduous teeth are known to be a rich source of stem cells. Therefore, it is important to evaluate the response of dental pulp stem cells to the commonly used dental materials in pediatric dentistry such as Glass Ionomer Cement (GIC) and Calcium hydroxide $Ca(OH)_2$ lining cement. The aims of this study were to confirm the presence of stem cells in deciduous extracted teeth by immunocytochemistry, and evaluate the stem cells proliferative activity and their response by comparing the Tertiary Dentin Thickness (TDT) formation following restorative procedures on the teeth. Healthy pediatric dental patients aged between 9 to 11 years old with caries teeth on primary molars without pulpal exposure who attended the pediatric dental clinic for dental treatment in School of Dental Sciences, Health Campus, Universiti Sains Malaysia, were selected. The teeth were divided into two groups: for *Group 1* the teeth were filled with GIC (Fuji IXGP capsule, GC, Japan) alone and in *Group 2,* the teeth were lined with $Ca(OH)_2$ cement (Dycal ®, Dentsply, Germany) and filled with GIC (Fuji IXGP capsule, GC, Japan). The teeth were reviewed for up to 6 months before being extracted according to the selected criteria and under standardized normal procedures. The presence of stem cells in deciduous teeth was

confirmed by immunocytochemistry using surface antigenic markers CD105 and CD166. Colorimetric proliferation reagent alamarBlue™ was used to evaluate the proliferative activity for *Group 1* and *Group 2*. The dental pulp stem cells response following restorative procedures was evaluated using Haematoxyline and Eosin (H&E) staining for histological section under a microscope using image pro software (Media cypernetics. Inc., USA). Stem cells from human extracted deciduous teeth were found positive for CD105 and CD166. The proliferative activity for both *Group 1* and *Group 2* showed slight difference, but it was not statistically significant. However, the TDT areas in *Group 2* were significantly more than *Group 1*. The pulp of deciduous teeth contains stem cell population and following pulp capping procedure using $Ca(OH)_2$ cement (Dycal®, Dentsply, Germany) and GIC (Fuji IXGP capsule, GC, Japan), responded well and maintained their proliferative activity. However, these materials create different response of STEM 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.

Technical Report: MATERIALS AND METHODS

Study design: This is a combined clinical and experimental study; the study was carried out at Pediatric Dental Clinic and in the Craniofacial Science Laboratory, School of Dental Sciences, Health Campus, Universiti Sains Malaysia.

Sampling frame: The sampling frame consisted of 50 patients who received treatment in 10 months duration from January 2007 to October 2007 at the Pediatric Dental Clinic, School of Dental Sciences, Health Campus, Universiti Sains Malaysia.

Patient inclusion and exclusion criteria: Healthy patients aged between nine to eleven years old, with mixed dentition stage were included in the study. However, patients with medical history (hearing, speech, congenital cardiac disease, poor healing potential) that contraindicate the treatment procedure were excluded from the study.

Teeth inclusion and exclusion criteria for restorative treatment: Primary molar teeth with minor class I cavities or caries with no pulp exposure were included in the study. Patients with dental history (acute odontogenic infection, tenderness to percussion, mobile tooth) that contraindicate the treatment were excluded from the study.

Teeth inclusion and exclusion criteria for extraction procedure: Teeth with restoration continuous with existing anatomical form, no explorer catch, no visual evidence of marginal discoloration, no recurrent caries and surface texture similar to polished enamel and showing signs of exfoliation were included for extraction procedure. However, teeth with spontaneous or continuous pain, soft tissue swelling and sinus formation were excluded from the study.

Ethical consideration: The study was approved by Human Ethical Committee of Universiti Sains Malaysia Health campus Ref: USM/ PPSP®/ Ethics Com. /2006 182.3(3), FWA Reg. No: 00007718, IRB Reg. No: 00004494, dated 11th January 2007.

Consent form: Prior to the treatment, the parent/guardian was informed about the study and the consent form data sheet in Bahasa Malaysia and English version was filled and signed by the parent/guardian.

Data collection

Restorative procedure: The tooth was isolated before restoration placement; the isolation was achieved by placing rubber dam, cotton wool rolls and saliva ejector (Fig. 1). Class I occlusal cavity was accessed by round diamond bur in high speed rotational drill with water coolant. Then the caries was removed by using round stainless steel bur in slow speed rotational drill with water coolant (Fig. 2). All carious dentine was removed until a layer of sound, non-carious dentine remained. Then the cavity was washed using water spray and dried by dry tips or gently air dried (Murray *et al.*, 2002h). GC cavity conditioner (GC Corporation, Tokyo, Japan) was applied for ten seconds and then washed out by water spray. Later, gently dried by dry tips or air dried. Then the prepared cavities were divided into two groups.

Fig. 1: Rubber dam isolation

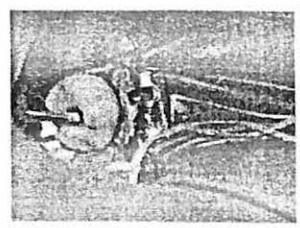


Fig. 2: Caries removal using round stainless steel bur

For *Group 1*, prepared cavity was filled with glass ionomer cement (Fuji IXGP capsule, GC, Japan). The capsule was activated and mixed for 10 seconds. Then it was dispensed directly into the cavity within 10 seconds. Condenser was used to adapt the material to the cavity walls and after 6 minutes from the mixing time, the morphology of the restoration was adjusted with sharp hand instrument, or by abrasive stones in a slow hand piece under water spray. The occlusion was checked by articulating paper and high spots were removed and excessive material was finished by polishing using abrasive stone in a slow hand piece under water spray. After occlusion and articulation were

checked, GC Fuji COAT LC was applied and light cured for 10 seconds (GC Fuji IXGP Application Technique) (Fig. 3).

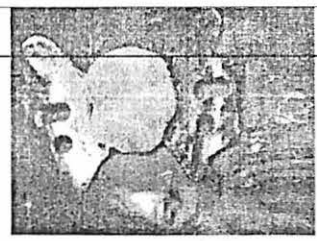


Fig. 3: Final Fuji IXGP restoration

However, in *Group 2*, the floor of prepared cavity as mentioned above was lined with a thin layer of $Ca(OH)_2$ (Dycal ®, Dentsply, Germany) (Fig. 4). Then the GIC capsule (Fuji IXGP capsule, GC, Japan) was activated and mixed for 10 seconds. Then it was dispensed directly into the cavity within 10 seconds. Condenser was used to adapt the material to the cavity walls and after 6 minutes from the mixing time, the morphology of the restoration was adjusted with sharp hand instrument, or by abrasive stones in a slow hand piece under water spray. The occlusion was checked by articulating paper and high spots were removed and excessive material was finished by polishing using abrasive stone in a slow hand piece under water spray. After occlusion and articulation were checked, GC Fuji COAT LC was applied and light cured for 10 seconds (GC Fuji IXGP Application Technique) (Fig. 5).

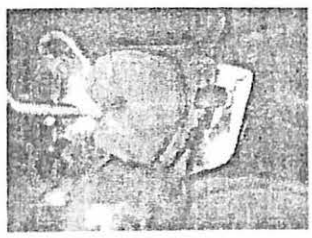


Fig. 4: Application of Dycal

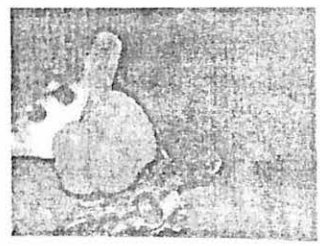


Fig. 5: Final restoration

Follow up: Patients were reviewed by the same operator once every month for providing oral hygiene care, checking and maintenance of the restorations. If treatment failed, clinical reasons were documented in the data form sheet and each case was treated according to the clinical situation.

Extraction procedure: Topical anesthesia gel was applied which contain benzocaine 18%, tetracaine hydrochloride 2% (ZAP™, Germiphene, China). After that, local anesthesia Xylocaine 2% (ASTRA™, Astra, UK) was deposited at extraction site. Then, atraumatic extraction procedure was used to extract the tooth by the standard pediatric molar forceps (MEDESY™, Italy). Post operative instructions and analgesics (paracetamol) were given to the patients. Extracted teeth were cleaned and washed immediately with normal saline and divided into two groups according to the objectives of the study.

Isolation and culture of SHED: After washing the extracted tooth with normal saline, it was transferred into a container with 20 ml of transport medium containing Hank's Balance Salt Solution (HBSS) (Gibco™, Invitrogen, USA), supplemented with 1% Penicillin-Streptomycin (Gibco™, Invitrogen, USA) and 5% Fetal Bovine Serum (FBS) (Gibco™, Invitrogen, USA). The gingival and periodontal tissue was scraped off from the tooth surface. The tooth surface was cleaned with iodine and 70% ethanol, after which it was washed five times with phosphate-buffered saline (PBS). Hard tissue material cutter (Exakt 300, Exakt Apparatebau GMBH & Co. KG, Germany) was used to cut around enamel-cementum junction to separate enamel and root to expose the pulp tissue in the pulp chamber (Fig. 6).

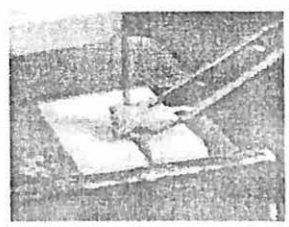


Fig. 6: Cutting around enamel-cementum junction

The sectioned tooth was then briefly immersed in 70% ethanol (Merck, Germany) followed by Dulbecco's Phosphate Buffered Saline (DPBS) (Gibco™, Invitrogen, USA). Then the sterile tooth was placed in a beaker containing fresh DPBS (Gibco™, Invitrogen, USA), and the extraction of the dental pulp was done in a class II safety biocabinet (Delta Series, Labconco, USA). The pulp tissue was picked up with sterile tissue forceps and small or medium size barb roach and minced.

The minced pulp tissue was then digested using digestion solution containing 3mg/ml of Collagenase Type 1 (Gibco™, Invitrogen, Germany) and 4mg/ml of Dispase (Gibco™, Invitrogen, Germany) and this process was performed in a CO_2 incubator (Thermo Forma, USA) at 37° C with 5% (CO_2) for one hour.

After digestion, 5 ml of culture medium containing alpha Modified Eagle's Medium (α MEM) (BioWhittaker™, Cambrex, USA) supplemented with 10% fetal calf serum (FCS) (Gibco), 100 μ*M* L-ascorbic acid 2-phosphate (Stem cell Technologies, Canada), 2 m*M* L-glutamine (Gibco™, Invitrogen, Japan) and penicillin (100 U/ml), and streptomycin (100 mg/ml) (Gibco™, Invitrogen, Germany) was added and later it was centrifuged (Hettichzentrifugen, Germany) at 1200 rpm for 10 minutes. The cell pellet was suspended in culture medium and the cells were passed through a 70-μm pore size strainer to obtain single cell suspension.

Then the cells were seeded into T- 25 culture flask (Nunc, Denmark) with 5 ml of culture medium (Fig. 7) and incubated in CO_2 incubator (Thermo Forma, USA) at 37° C with 5% CO_2. After 24 hours, non-adherent cells were removed. The adherent cells were washed vigorously twice with PBS and were shaken to remove adherent debris and fresh complete medium was then added. The observation of cells was made every day to check the culture condition. The medium was changed every 3 days until the fibroblast like cells in the flask reached confluence (Liu *et al.*, 2006).

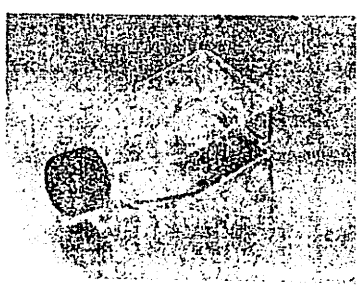


Fig. 7: Cells seeded into T- 25 culture flask

Characterization of SHED using immunocytochemistry: Immunostaining was performed by biotin-strepavidin-horseradish peroxidase (HRP) complexed antibodies to detect primary antibody. SHED, HMSCs which served as positive control and breast cancer cells which served as negative control, were subcultured into two four-chamber slides (Nunc, Denmark) with density of 4×10^4 cells per well. After two days, the cells were fixed in absolute cold methanol (Merck, Germany) and incubated at 4° C for 20 minutes. Blocking reagent was then added for 5 minutes and then the cells were incubated with one primary antibodies in each four-well chamber slides either with primary antibodies of monoclonal mouse anti-human endoglin (CD105) (Chemicon, USA) with dilution 1:25 or with primary antibodies of monoclonal mouse anti human (CD166) (DakoCytomation, Denmark) with dilution 1:50, over night. Primary antibodies were then detected using Chemicon IHC Select™ secondary detection system (Chemicon, USA), in which the samples were subsequently incubated with purified mouse secondary antibodies and strepavidin for 10 minutes, respectively according to manufacturer's protocol.

Proliferation assessment of SHED: After SHED were grown until confluence, approximately 30,000 cells from each study group were subcultured in 24-well plate with 2 ml of culture medium. The cells were attached to the 24-well plate after 24 hours. Then the culture medium in the 24-well plate was changed and incubated in CO_2 incubator (Thermo Forma, USA) at 37° C with 5% CO_2 for 4 hours. After that, 10% of metabolic indicator dye, alamarBlue (Biosource International, Inc, USA) was added to each well with cells as well as to medium culture without cells which served as the negative control. 100 µl of solution from each well plate was then taken and added to 96- well plate and absorbance of the media was measured spectrophotometrically using ELISA reader (Dynatech, DK5il) at a wavelength of 570nm and 600nm at 0 minute, 60 minutes, 120 minutes and 180 minutes. Subsequently, the medium was changed every day for 7 days. The processes of alamarBlue staining and absorbance readings were repeated as done before, every day. In monitoring alamarBlue reduction spectrophotometrically, reduction is expressed as a percentage (% Reduced) at $\lambda 1 = 570$ nm and $\lambda 2 = 600$ nm by the following formula (adapted from BioSource, USA).

Histological sections: After the tooth was extracted from the patient, it was immediately washed with normal saline and transferred into a container with 20 ml of neutral buffered formalin 10% solution for 24 hours. Then the tooth was demineralized in a sodium formic acid solution (3.4 % sodium formiate HCOONa in 15% formic acid) (Merck, Germany) for 21 days until fully decalcified and then processed through a series of graded alcohol and xylene. Finally, the tooth was embedded in paraffin wax for histological examination (Fig. 8).

Fig. 8: Tooth embedded in paraffin wax for sectioning

Sections were cut at 6µm thick using a microtome (MICROM, Germany) and stained with Hematoxylin and eosin (Murray *et al.*, 2003a) (Fig. 9). Tertiary dentin deposition at the pulp site related to the cavity floor (Duque *et al.*, 2006) and remaining dentin thickness between the base of the prepared tooth and the odontoblastic surface (Murray *et al.*, 2000b) were analyzed using light microscope (Zeiss, Germany) fitted with a video camera (Cybernetics, USA). The video images at 10x magnification were loaded into a computer in which the tertiary dentin thickness area and remaining dentin thickness were measured in each slide using Image Pro Express software (Media cypernetics. Inc., USA).

Fig. 9: Hematoxylin and eosin stained slide

Dr Siti Noor Fazliah

From: Dr Tp Kannan [tpkannan@kb.usm.my]
Sent: Tuesday, July 15, 2008 5:13 PM
To: Dr Siti Noor Fazliah
Subject: Fw: Stem Cells and Development - Manuscript ID SCD-2008-0177

----- Original Message -----
From: <aetkin@liebertpub.com>
To: <tpkannan@kb.usm.my>; <tpkkannan@hotmail.com>
Cc: <dr_alutfi@hotmail.com>; <tpkannan@kb.usm.my>; <tpkkannan@hotmail.com>; <fazliah@kb.usm.my>; <saidi@kb.usm.my>
Sent: Saturday, June 28, 2008 5:10 PM
Subject: Stem Cells and Development - Manuscript ID SCD-2008-0177

> 28-Jun-2008
>
> Dear Dr. THIRUMULU:
>
> Your manuscript entitled "Proliferative Activity of Stem Cells from Human Extracted Deciduous Teeth in Response to Treatment with Dental Materials" has been successfully submitted online and is presently being given full consideration for publication in Stem Cells and Development.
>
> Your manuscript ID is SCD-2008-0177.
>
> Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to Manuscript Central at http://mc.manuscriptcentral.com/scd and edit your user information as appropriate.
>
> You can also view the status of your manuscript at any time by checking your Author Center after logging in to http://mc.manuscriptcentral.com/scd
> .
>
> ATTENTION AUTHORS:
>
> Liebert Instant Online (LION)
>
> To enable the release of new scientific findings as quickly as possible, the Journal has adopted a policy of prepublishing all manuscripts in unedited format shortly after acceptance. The papers will have undergone full peer review but will not have been copyedited, typeset, or proofread by the authors.
>
> Beginning March 3, 2008, newly accepted manuscripts will be the first to appear online as a part of Liebert Instant Online (LION). It is anticipated that all our authors will be excited to take advantage of this accelerated publication service.
>
> Following its appearance on LION, the paper will progress through the normal publishing process, including author correction of galley proofs and online publication of the final edited and typeset manuscript ahead of print.
>
> Thank you for submitting your manuscript to Stem Cells and Development.
>
> Sincerely,
> Stem Cells and Development Editorial Office
>

Proliferative Activity of Stem Cells from Human Extracted Deciduous Teeth in Response to Treatment with Dental Materials

Alaa Nasser Lutfi[1], Thirumulu Ponnuraj Kannan[1*], Siti Noor Fazliah Mohd Noor[1], Saidi Jaafar[1],

[1]School of Dental Sciences, Universiti Sains Malaysia, Health Campus
16150 Kubang Kerian, Kelantan, Malaysia

* Corresponding author:

Dr. Thirumulu Ponnuraj Kannan
School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia
Telephone: +609 7663684
Fax: +609 764 2026
Email: tpkannan@kb.usm.my

ABSTRACT

Stem cells are undifferentiated cells that have the capacity for self- renewal, which have been discovered in many adult tissues, including teeth. The objective was to compare the proliferative activity of stem cells between teeth filled with glass ionomer cement (GIC, Fuji IXGP) alone and teeth lined with calcium hydroxide cement (Dycal ®) and filled with GIC, by using colorimetric cell proliferation reagent, alamarBlue (AB). Healthy patients aged between 9 to 11 years with carious primary molars without pulp exposure were selected. Patients were divided into *Group 1*, who had their teeth restored with Glass Ionomer Cement (GIC) and *Group 2*, who had their teeth, lined using Dycal® and restored with GIC. Their teeth were reviewed for up to 180 days before being extracted. Laboratory procedures were carried out according to standardized protocol for dental pulp stem cells' isolation and cultured. Immunocytochemistry confirmation was done using mesenchymal stem cell (MSC) markers, CD105 and CD166. The proliferative activity of the Stem cells from Human Extracted Deciduous Teeth (SHED) from the two groups were determined using alamarBlue™ assay. The results showed that SHED was found positive for the two markers, CD105 and CD166. Proliferative activity of SHED showed no difference between *Group 1* and *Group 2*. Hence, it can be concluded that the use of calcium hydroxide or GIC as a lining material in indirect pulp capping procedure has the same effect on SHED which have responded favourably to the restorative treatments.

INTRODUCTION

The goals of restorative therapy are to restore teeth to a state of health, function, esthetic appearance and to prevent the recurrence of caries [1]. The management of the child dental patient is geared towards the preservation of both the primary and permanent dentition. This is because the primary dentition is important for stimulating the development of the dental arches; maintaining normal occlusion relationship and play a role in speech development [2]. The requirements for a restorative material in the primary dentition are different from those in the permanent dentition and a material which is ideal for one may not be ideal for the other. Primary teeth in terms of human life span are only temporary having a maximum normal life of 8-9 years. Consequently, restorations will last for a limited time in function in the oral environment [3,4]. It is well documented that the use of adhesive materials in children allows a less destructive cavity preparation and smaller restoration. This subsequently reduces treatment time and may limit the use of local analgesia. The advantages of using adhesive restorative materials such as glass ionomer cement (GIC), resin modified GICs and polyacid modified composite resins lie in some of their properties, like liberation of long term fluoride release and the ability to be recharged by exposure to fluoride solutions and gels [5]; simplicity and a good cost effectiveness ratio [6,7]. Besides, there is no formation of initial or recurrent carious lesions in cavity walls restored with GIC adhesive materials [8]. This might be attributed to fluoride released by these materials, which is incorporated by the enamel and dentine, increasing their resistance to demineralization from bacterial attack [9]. Moreover, guidelines on pulp therapy for primary and young permanent teeth suggested in some cases such as deep cavities is that a protective base material must be placed on the pulpal surface of the cavity preparation in order to cover exposed dentin tubules and act as a protective barrier between the restorative material or lining cement and the pulp of the tooth [10].

Commonly used protective bases are calcium hydroxide [$Ca(OH)_2$] and GICs, which possess suitable physical and biological properties [10]. Calcium hydroxide liners are often placed in deep cavities under restorative materials to protect the pulpal tissues from chemical insults. Calcium hydroxide has been used for many years as a dental base or pulp capping material due to its ability to stimulate dentinal bridge formation with direct pulp contact and serve as protective barrier for pulp tissue not only by blocking patent dentinal tubules, but also by neutralizing the attack of inorganic acids and leached products from certain cements. This, leads to the widely use of this material in dental practice [11].

Tissue-engineering therapies offer exciting treatment possibilities in restorative dentistry and one of these new therapies is using stem cell, which offers tremendous potential for addressing human diseases, that to date have been difficult or impossible to be treated effectively. Among the conditions cited are Alzheimer's disease, cancer, lack of saliva for chewing and speaking, diabetes and many others [12]. Furthermore, stem cells have been identified in the pulp of human primary teeth. These cells have been designated as SHED (Stem Cells from Human Exfoliated Deciduous teeth), and have been found to divide continuously and differentiate into a variety of other cells types, including nerves, fat, and tooth-generating cells [13]. In addition, deciduous teeth may provide an ideal source of stem cells, which can proliferate and differentiate into dentin-forming odontoblasts [14,15]. Damaged odontoblasts can be replaced by newly generated populations of odontoblasts derived from stem cells from pulp. Following physiological stimulation or injury, such as caries and operative procedures, stem cells in pulp may be mobilized to proliferate and differentiate into odontoblasts by morphogens released from the surrounding dentin matrix [16]. Tissue engineering with the triad of dental pulp progenitor/stem cells, morphogens, and scaffolds may provide a useful alternative method for pulp-capping and root canal treatment [17]. It is necessary to evaluate the biological changes, which take place in teeth such as tooth pulp reactions to the restorative procedure as well as tooth pulp reactions to restorative materials in order to optimize current treatment and stimulate regenerative processes to accomplish the therapy by tissue engineering [18]. However, to our knowledge, there is limited data currently available regarding the biological response of stem cells to various dental restorative materials such as GIC and lining materials such as $Ca(OH)_2$. Therefore, this study is directed towards confirming the presence of stem cells in extracted deciduous teeth by using surface antigenic markers and to compare the response of SHED by measuring its proliferative activity in relation to the placement of dental material that have direct contact with the floor of the prepared cavity.

MATERIALS AND METHODS

Selection of patients

Fifty healthy patients aged between 9 to 11 years, with primary molar teeth having minor class I cavities or caries with no pulp exposure were included in the study. Prior to the treatment, the parent/guardian was informed about the study and the consent form data sheet was filled and signed by the parent/guardian.

The study was approved by Human Ethical Committee of Universiti Sains Malaysia Health Campus Ref: USM/ PPSP®/ Ethics Com. /2006 182.3(3), FWA Reg. No: 00007718, IRB Reg. No: 00004494, dated 11th January 2007.

Restorative procedure

Briefly, the restorative procedure was performed after isolation of tooth using rubber dam, cotton wool rolls and saliva ejector. Class I occlusal cavity was assessed by round diamond bur in high speed rotational drill with water coolant. Then, the caries was removed by using round stainless steel bur in slow speed rotational drill with water coolant. All carious dentine was removed until a layer of sound, non carious dentine remained. Then the cavity was washed using water spray and dried. GC cavity conditioner (GC Corporation, Tokyo, Japan) was applied for ten seconds and then washed out by water spray and dried. The prepared cavities were divided into two groups. For *Group 1*, the prepared cavity was filled with GIC (Fuji IXGP capsule, GC, Japan) following the manufacturer instructions (GC Fuji IXGP Application Technique). However, in *Group 2*, the floor of prepared cavity was lined with a thin layer of $Ca(OH)_2$ (Dycal ®, Dentsply, Germany), and filled with GIC (Fuji IXGP capsule, GC, Japan) following the manufacturer instructions. Patients were reviewed by the same operator once every month for providing oral hygiene care, checking and maintenance of the restorations. If the treatment failed, clinical reasons were documented in the data sheet and each case was treated according to the clinical situation. Extraction procedure was made under local anesthesia and performed only for teeth with alpha scale restoration according to U.S Health Public Service's (USPHS) that exhibited mobility and signs of exfoliation after six months of time lapse.

Isolation and culture

After washing the extracted tooth with normal saline, it was transferred into a container with 20 ml of transport medium containing Hank's Balanced Salt Solution (HBSS) (Gibco™, Invitrogen, USA), supplemented with 1% Penicillin-Streptomycin (Gibco™, Invitrogen, USA) and 5% Fetal Bovine Serum (FBS) (Gibco™, Invitrogen, USA). The gingival and periodontal tissue was scraped off from the tooth surface. The tooth surface was cleaned with iodine and 70% ethanol, and then was washed five times with phosphate-buffered saline (PBS). Hard tissue material cutter (Exakt 300, Exakt Apparatebau GMBH & Co. KG, Germany) was used to cut around enamel-cementum junction to separate enamel and root to expose the pulp tissue in the pulp chamber. The sectioned tooth was then briefly

immersed in 70% ethanol (Merck, Germany) followed by immersion in Dulbecco's Phosphate Buffered Saline (DPBS) (GibcoTM, Invitrogen, USA). Then the sterile tooth was placed in a beaker containing fresh DPBS (GibcoTM, Invitrogen, USA), and the extraction of the dental pulp was done in a class II safety biocabinet (Delta Series, Labconco, USA). The pulp tissue was picked up with sterile tissue forceps and small or medium size barb roach and minced. The minced pulp tissue was then digested using digestion solution containing 3mg/ml of Collagenase Type 1 (GibcoTM, Invitrogen, Germany) and 4mg/ml of Dispase (GibcoTM, Invitrogen, Germany) and this process was performed in a CO_2 incubator (Thermo Forma, USA) at 37° C with 5% (CO_2) for one hour. After digestion, 5 ml of culture medium containing alpha Modified Eagle's Medium (α MEM) (BioWhittakerTM, Cambrex, USA) supplemented with 10% fetal calf serum (FCS) (Gibco), 100 µM L-ascorbic acid 2-phosphate (Stem cell Technologies, Canada), 2 mM L-glutamine (GibcoTM, Invitrogen, Japan) and penicillin (100 U/ml), and streptomycin (100 mg/ml) (GibcoTM, Invitrogen, Germany) was added and later was centrifuged (Hettichzentrifugen, Germany) at 1200 rpm for 10 minutes. The cell pellet was suspended in culture medium and the cells were passed through a 70-µm pore size strainer to obtain single cell suspension. Then, the cells were seeded into T- 25 culture flask (Nunc, Denmark) with 5 ml of culture medium and incubated in CO_2 incubator (Thermo Forma, USA) at 37° C with 5% CO_2. After 24 hours, non-adherent cells were removed. The adherent cells were washed vigorously twice with PBS and were shaken to remove adherent debris and fresh complete medium was then added. The cells were observed every day to check for the culture condition and the medium was changed every 3 days until the fibroblast like cells in the flask reached confluence.

Characterization of SHED using immunocytochemistry

Immunostaining was performed using biotin-strepavidin-horseradish peroxidase (HRP) complexed antibodies to detect the primary antibody. SHED and HMSCs (positive control) and breast cancer cells (negative control), were sub-cultured into two four-chamber slides (Nunc, Denmark) with density of 4×10^4 cells per well. After two days, the cells were fixed in absolute cold methanol (Merck, Germany) and incubated at 4°C for 20 minutes. Blocking reagent was then added for 5 minutes and then the cells were incubated with one primary antibody in each four-well chamber slides either with primary antibodies of monoclonal mouse anti-human endoglin (CD105) (Chemicon, USA) with dilution 1:25 or with primary antibodies of monoclonal mouse anti human (CD166) (DakoCytomation, Denmark) with dilution 1:50, over night.

Primary antibodies were then detected using Chemicon IHC Select™ secondary detection system (Chemicon, USA), in which the samples were subsequently incubated with purified mouse secondary antibodies and strepavidin for 10 minutes, respectively, according to manufacturer's protocol.

Proliferation assessment of SHED

After SHED was grown until confluence, they were sub-cultured in 24-well plate with 2 ml of culture medium. The cells were found to be attached to the 24-well plate after 24 hours. Then, the culture medium in the 24-well plate was changed and incubated in a CO_2 incubator (Thermo Forma, USA) at 37° C with 5% CO_2 for 4 hours. After that, 10% of metabolic indicator dye, alamarBlue (Biosource International, Inc, USA) was added to each well containing cells as well as to the wells containing only medium without cells (negative control). 100 µl of solution from each well plate was then taken and added to 96- well plate and the absorbance of the media was measured spectrophotometrically using ELISA reader (Tecan, DKSH, Germany) at a wavelength of 570nm and 600nm at 0 minute, 60, 120 and 180 minutes. Subsequently, the medium was changed every day for 7 days. The processes of alamarBlue staining and absorbance readings were repeated as done before, every day. In monitoring alamarBlue reduction spectrophotometrically, reduction is expressed as a percentage (% Reduced) at 570 nm and 600 nm (BioSource, USA).

RESULTS AND DISCUSSION

The presence of SHED was confirmed using immunocytochemistry procedure for the cultured stem cells that had been removed from the dental pulp of extracted deciduous tooth. The characterization of SHED, HMSCs, which were used as positive control and breast cancer cells which were used as negative control, were done using mouse anti-human CD166 monoclonal antibody and mouse anti-human CD105 monoclonal antibody. Then, goat anti-mouse and anti-rabbit Immunoglobulin G (IgG) as a secondary antibody (Chemicon, USA) were added and the expression of the cell surface markers were then detected by using Immunoperoxidase DAB secondary detection system (Chemicon, USA).

The results showed the presence of brownish colour which indicated positive reactivity for CD105 (Fig. 1) and CD166 (Fig. 2) primary antibodies on SHED and HMSCs. However, there was no colour expression on the breast cancer cells. Here, in the present study, the characterization of SHED was demonstrated by immunocytochemistry staining as this procedure has many advantages over other biochemical tests, such as stability of the

staining, sensitivity and the clear cytomorphological details that can be obtained. Also, it is easy to use, cost effective and needs low technology [19]. To characterize cell surface marker, the Cluster of Differentiation prefix CD was used to identify specific cell membrane molecule that are expressed on cells. Antigenic surface marker of the SHED in this study has been detected by immunocytochemistry using CD105 and CD166.

CD105 is also known as Human Endoglin (ancillary TGF-beta receptor). Endoglin is a transmembrane glycoprotein expressed by vascular endothelial cells and also expressed by activated macrophages. Endoglin expression is up regulated on activated endothelium in tissues undergoing angiogenesis, such as in tumors, or in cases of wound healing or dermal inflammation [20,21]. Meanwhile, CD166 (Activated Leukocyte Cell Adhesion Molecule (ALCAM)), is a member of the Ig superfamily and is expressed primarily in the spleen, placenta, and liver (weakly) and on activated T-cells, B-cells, and other cells including thymic epithelial cells, fibroblasts, keratinocytes and neurons. CD166 is preferentially expressed in highly metastatic melanoma cell lines [22].

In this study, SHED was found to be positive for both antibodies against human antigens CD105 and CD166, and displayed mesenchymal stem cell phenotypic profile due to their expression of the markers CD105 and CD166. Similar results have been obtained from many researchers in the same field, where MSCs isolated from placenta/umbilical cord cells population were also positive for CD54, CD29, CD73, CD13, CD44, CD105 and CD166. These results suggested that immunophenotypical and morphological profiles of these cells are the same as those of MSCs isolated from bone marrow [23,24,25]. Moreover, CD13, CD29, CD44, CD73 and CD105 were expressed in human Dental Pulp Stem Cells (DPSCs), when compared to HMSCs [26,27]. It was also found that CD29, CD44, CD105, CD106 and CD166 were positive surface marker antigens for Periodontal Ligament Stem Cells (PDLSCs) [28,29].

Assessment of proliferation is a fundamental measurement that can be made with cells in culture. Determination of proliferative activity enables researchers to optimize cell culture conditions, quantitate the activity of cell growth factors including cytokines, facilitate the discovery of new therapeutic agents, assess the efficacy of therapeutic agents, assess the toxicity of environmental pollutants, assess cell mediated toxicity and quantitate apoptosis (alamarBlue™, Biosource, USA). In this study, proliferation assessment offer demonstration of the efficacy of dental materials that have been used. Several methods are available for proliferation assessment; by measuring DNA synthesis such as quantitation of ^{3}H-thymidine incorporation, quantitation of 5-bromodeoxyuridine (BrdU) incorporation and monitoring

DNA quantity with the fluorescent dye, Hoechst 33258 or by monitoring the quantitation reduction of tetrazolium salt reduction or quantitation of alamarBlue reduction (alamarBlue™, Biosource, USA) in the culture environment.

Colorimetric alamarBlue assay is designed to measure the proliferation of various human and animal cell lines, bacteria and fungi. This assay is simple to perform, since it is water soluble, thus eliminating the washing/fixing and extraction steps required in other commonly used cell proliferation assays. Moreover, it is stable in culture medium, non-toxic and does not alter the viability of cells cultured for various times as monitored by Trypan Blue exclusion [30]. Therefore, the cells under study can be returned to the culture or used for other purposes. It has also been found that the cultured cells activity such as hybridoma cells to secrete antibody cells does not interfere with alamarBlue as well as cell growth and doubling found to be similar as non-alamarBlue exposed cells [30].

In stem cell research, there is no standard method for investigating the proliferation activity. Though, several methods are available, they are limited by complexity of the tests and expensive reagents or equipments, such as bromodeoxyuridine which are used to measure the proliferative activity of SHED [31], and WST-1 colorimetric cell proliferation reagent, which has been used to demonstrate the proliferation activity of dental pulp stem cells and PDLSCs [29]. Recently, alamarBlue has been used to assess the proliferative activity of the SHED [32]. Indirect pulp capping procedure was adopted in order to compare the proliferative activity of dental pulp stem cells between the materials that have been used in this study. The statistical analysis of the alamarBlue reduction percentage between SHED without restorative material (control), SHED obtained from teeth restored with GIC alone (*Group 1*) and SHED obtained from teeth lined with $Ca(OH)_2$ cement and restored with GIC (*Group 2*) were not significant (Table 1). Strong evidence suggests the presence of resting progenitor or stem cells in dental pulp [13,31]. In restorative dentistry, dentinal repair occurs through the activity of specialized cells called odontoblasts. Shallow or mild injury to the pulp stimulates the secretory activity of the odontoblasts to elaborate reactionary dentin, while in deep cavities or severe injury to the pulp, may lead to partial or total destruction of the odontoblasts layer. These conditions attract cells to injury site and differentiate into odontoblast-like cells that can replace the necrotic odontoblasts and secrete a reparative dentin matrix [33]. Moreover, in the case of exposed cavities, a reparative dentinogenesis response was observed, while in non exposed cavities, a reactionary dentinogenesis response was stimulated [34].

Study of pulpal injury and dentin repair activity with regard to the variables of cavity cutting, restoration materials and patients, showed that the cavity remaining dentin thickness (RDT) is considered the most important variable to influence pulp activity and plays a central role in determining the extent of pulp injury. Reductions in RDT increasingly sensitize the pulp to cavity preparation injury and the possible cytotoxicity of dental materials. However, maximizing the RDT could have beneficial effect to limit pulp tissue destruction with buffering properties of dentin which could modify the possible cytotoxic properties of dental materials [35]. In very deep cavities, RDT between 0.25-0.01 mm or in pulp exposure cavities, dental pulp stem cells proliferate, migrate to the site of injury and differentiate to form odontoblastoid cells. These odontoblastoid cells replace the severely damaged odontoblasts that have been reduced 100% and secrete reparative dentin, which is considered to be the most visible repair response to pulp injury [18].

From the histological samples that have been used in this study, the mean RDT were 0.3mm in *Group 1* and 0.31mm in *Group 2*, in which reactionary dentin formation takes place due to survival of odontoblasts and the buffering activity of the remaining dentin protects the pulp tissues from the injuries that may be associated with the cavity preparation procedure and chemical irritant of restorative materials that have been used in this study

Stem cells are thought to reside in a specific area of each tissue where they may remain quiescent until they are activated by disease or tissue injury [36]. Proliferation, migration and differentiation activities of the dental pulp stem cells seems to be inactive, when the there is no pulp exposure or there is no severe loss of odontoblast cells related to increased dentin loss and decrease the cavity preparation RDT [18]. This theory was approved by a study which was directed to investigate the activation and migration of stem cells in response to pulpal injury. It has been found that proliferation, migration and differentiation of dental pulp stem cells take place when there is pulpal injury and not dentin injury [37].

The indirect pulp capping that have been used with RDT were preserved to avoid pulp exposure and to protect the pulp tissues from chemical and mechanical irritants [38,39]. Guidelines have been followed to make sure the cavity preparation procedure was atraumatic to the underlying tissues [40,41]. The restorative materials that have been used on the pulp-dentin complex tissues have good biocompatibility and physical properties [42,43]. These could be the reasons that reflect the similarity between these materials in proliferation activity, in which signals for induction of proliferation, migration and differentiation of the

new generation of odontoblast-like cells were prohibited, since the injury was at dentinal level and did not reach the pulpal level.

Limitations of the study

Radiograph assessment was not provided during this study due to ethical considerations. However, to overcome the lack of this valuable tool for the purpose of diagnosis, follow up at 1, 3 and 6 months were useful to detect any early postoperative complications by evaluation of the patient, mobility and restoration material condition in clinical sitting.

It was not possible to distinguish whether the presence of tertiary dentin that was secreted in response to variety of stimuli in this study was either reactionary or reparative in nature. The tertiary dentin (reactionary dentin) was seen in the present study, which is in agreement with the previous studies [37,44].

ACKNOWLEDGEMENT

This work was funded by Universiti Sains Malaysia Short Term Grant (No. 304/PPSG/6131527) and Scientific Advancement Grant Allocation from Akademi Sains Malaysia (No. 304/PPSG/6153006A118).

REFERENCES

1. Lutz FU, I Krejci and M Besek. (1997). Operative dentistry: the missing clinical standards. Pract Periodontics Aesthet Dent 9: 541-548.
2. Welbury R and AG Walton. (1999). Continued apexogenesis of immature permanent incisors following trauma. *Br Dent J* 187: 643-644.
3. Knibbs PJ, CG Plant and GJ Pearson. (1986). The use of a glass ionomer cement to restore Class III cavities. Restorative Dent 2: 42, 45-48.
4. Roeters JJ, F Frankenmolen, RC Burgersdijk and TC Peters. (1998). Clinical evaluation of Dyract in primary molars: 3-year results. Am J Dent 11: 143-148.
5. Bilgin Z and N Ozalp. (1998). Fluoride release from three different types of glass ionomer cements after exposure to NaF solution and APF gel. J Clin Pediatr Dent 22: 237-241.
6. Frencken JE, Y Songpaisan, P Phantumvanit and T Pilot. (1994). An atraumatic restorative treatment (ART) technique: evaluation after one year. Int Dent J 44: 460-464.
7. Songpaisan Y, D Bratthall, P Phantumvanit and Y Somridhivej. (1995). Effects of glass ionomer cement, resin-based pit and fissure sealant and HF applications on occlusal caries in a developing country field trial. Community Dent Oral Epidemiol 23: 25-29.
8. Mount GJ. (1995). Some physical and biological properties of glass ionomer cement. Int Dent J 45: 135-140.
9. Wandera A. (1998). In vitro enamel effects of a resin-modified glass ionomer: fluoride uptake and resistance to demineralization. Pediatr Dent 20: 411-417.
10. American Academy of Pediatirc Dentistry. (2005). Guideline on pulp therapy for primary and young permanent teeth. Pediatr Dent 27: 130-134.
11. Stanley HR and CH Pameijer. (1997). Dentistry's friend: calcium hydroxide. Oper Dent 22: 1-3.
12. Daley GQ. (2004). Missed opportunities in embryonic stem-cell research. N Engl J Med 351: 627-628.
13. Miura M, S Gronthos, M Zhao, B Lu, LW Fisher, PG Robey and S Shi. (2003). SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA 100: 5807-5812.

14. Nakashima M. (1994). Induction of dentin formation on canine amputated pulp by recombinant human bone morphogenetic proteins (BMP)-2 and -4. J Dent Res 73: 1515-1522.

15. Gronthos S, J Brahim, W Li, L W Fisher, N Cherman, A Boyde, P DenBesten, PG Robey and S Shi. (2002). Stem cell properties of human dental pulp stem cells. J Dent Res 81: 531-535.

16. Tziafas D, A J Smith & H Lesot. (2000). Designing new treatment strategies in vital pulp therapy. *J Dent,* **28**(2), 77-92.

17. Nakashima M and AH Reddi. (2003). The application of bone morphogenetic proteins to dental tissue engineering. Nat Biotechnol 21: 1025-1032.

18. Murray PE, LJ Windsor, TW Smyth, AA Hafez and CF Cox. (2002). Analysis of pulpal reactions to restorative procedures, materials, pulp capping, and future therapies. Crit Rev Oral Biol Med 13: 509-520.

19. Leong SY. (1996) Principles and practice of medical laboratory science. Vol. 1, Basic histotechnology, Churchill Livingstone, New York.

20. Conley BA, JD Smith, M Guerrero, C Bernabeu and CP Vary. (2000). Endoglin, a TGF-beta receptor-associated protein, is expressed by smooth muscle cells in human atherosclerotic plaques. Atherosclerosis 153: 323-335.

21. Ma X, M Labinaz, J Goldstein, H Miller, WJ Keon, M Letarte and E O'Brien. (2000). Endoglin is overexpressed after arterial injury and is required for transforming growth factor-beta-induced inhibition of smooth muscle cell migration. Arterioscler Thromb Vasc Biol 20: 2546-2552.

22. Van kempen LC, JJ Oord, GN Muijen, UH Weidle, HP Bloemers and GW Swart. (2000). Activated leukocyte cell adhesion molecule/CD166, a marker of tumor progression in primary malignant melanoma of the skin. Am J Pathol 156: 769-774.

23. Kadivar M, S Khatami, Y Mortazavi, M Soleimani, M Taghikhani and MA Shokrgozar. (2005). Isolation, culture and characterization of Postnatal Human Umbilical vein-derived Mesenchymal Stem Cells. DARU 13: 170-176.

24. Yen BL, HI Huang, CC Chien, HY Jui, BS Ko, M Yao, CT Shun, ML Yen, MC Lee and YC Chen. (2005). Isolation of multipotent cells from human term placenta. Stem Cells 23: 3-9.

25. Sato Y, H Araki, J Kato, K Nakamura, Y Kawano, M Kobune, T Sato, K Miyanishi, T Takayama, M Takahashi, R Takimoto, S Iyama, T Matsunaga, S Ohtani, A Matsuura, H Hamada and Y Niitsu. (2005). Human mesenchymal stem cells xenografted directly

to rat liver are differentiated into human hepatocytes without fusion. Blood 106: 756-763.

26. Yamada Y, A Fujimoto, A Ito, R Yoshimi and M Ueda. (2006). Cluster analysis and gene expression profiles: a cDNA microarray system-based comparison between human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs) for tissue engineering cell therapy. Biomaterials 27: 3766-3781.
27. Bowen A, A English, E Jones, S Wood, J Kirkham and XB Yang. (2006). Isolation and preliminary characterisation of stem cells from human dental pulp. European cells and materials 11: 58.
28. Kramer PR, S Nares, SF Kramer, D Grogan and M Kaiser. (2004). Mesenchymal stem cells acquire characteristics of cells in the periodontal ligament in vitro. J Dent Res 83: 27-34.
29. Gronthos S, K Mrozik, S Shi and P M Bartold. (2006). Ovine periodontal ligament stem cells: isolation, characterization, and differentiation potential. Calcif Tissue Int 79: 310-317.
30. Ahmed SA, M Gogal and JE Walsh (1994). A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H]thymidine incorporation assay. J Immunol Methods 170: 211-224.
31. Gronthos S, M Mankani, J Brahim, PG Robey and S Shi. (2000). Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA 97: 13625-13630.
32. Noor SNFM, TP Kannan, AN Lutfi. (2007) SHED Proliferation Following Restorative Procedures. 1st International Bone and Dental Technology Symposium, Thailand, 132.
33. Murray PE, AA Hafez, AJ Smith and CF Cox. (2002). Bacterial microleakage and pulp inflammation associated with various restorative materials. Dent Mater 18: 470-478.
34. Smith AJ [illegible] H Lesot. (2001). Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? Crit Rev Oral Biol Med 12: 425-437.
35. Murray PE, I About, PJ Lumley, JC Franquin, M Remusat and AJ Smith. (2000). Human odontoblast cell numbers after dental injury. J Dent 28: 277-285.
36. Casagrande L, LG Mattuella, FB Araujo and J Eduardo. (2006). Stem cells in dental practice: perspectives in conservative pulp therapies. J Clin Pediatr Dent 31: 25-27.

37. Tecles O, P Laurent, S Zygouritsas, AS Burger, J Camps, J Dejou and I About. (2005). Activation of human dental pulp progenitor/stem cells in response to odontoblast injury. Arch Oral Biol 50: 103-108.

38. Murray PE, AA Hafez, LJ Windsor, AJ Smith and CF Cox. (2002). Comparison of pulp responses following restoration of exposed and non-exposed cavities. J Dent 30: 213-222.

39. Murray PE, PJ Lumley and AJ Smith. (2002). Preserving the vital pulp in operative dentistry: 3. Thickness of remaining cavity dentine as a key mediator of pulpal injury and repair responses. Dent Update 29: 172-178.

40. Murray PE, PJ Lumley, AA Hafez, CF Cox and AJ Smith. (2002). Preserving the vital pulp in operative dentistry: 4. Factors influencing successful pulp capping. Dent Update 29: 225-233.

41. Murray PE, PJ Lumley and AJ Smith. (2002). Preserving the vital pulp in operative dentistry: 2. Guidelines for successful restoration of unexposed dentinal lesions. Dent Update 29: 127-134.

42. Six N, JJ Lasfargues and M Goldberg. (2000). In vivo study of the pulp reaction to Fuji IX, a glass ionomer cement. J Dent 28: 413-422.

43. Alexandra MQ, S Assed, MR Leonardo, PN Filho and LA Silva. (2005). MTA and calcium hydroxide for pulp capping. J Appl Oral Sci 13: 126-130.

44. Smith AJ, N Cassidy, H Perry, C Begue-Kirn, JV Ruch and H Lesot. (1995). Reactionary dentinogenesis. Int J Dev Biol 39: 273-280.

List of tables

Table 1: The percentage of alamarBlue® reduction between the control and tested groups

Variable	Groups	*N*	Median (IQR)	κ^2 statistic (df)[a]	*p* value[a]
alamarBlue® reduction %	*Control*	7	44 (24)	1.26 (2)	0.532
	Group 1	7	38 (19)		
	Group 2	7	41 (19)		

A Kruskal-wallis test

List of Figures

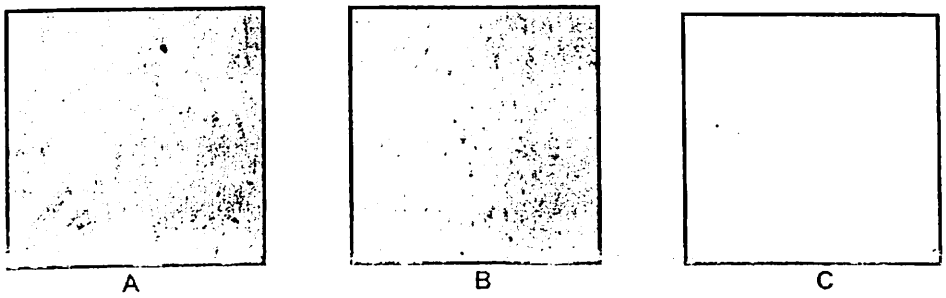


Figure 1: Characterization of SHED by immunocytochemistry staining using CD105 antibody viewed at 100x. The CD105 expressions on SHED (A) and HMSC (B) - Positive controls. No expression detected on breast cancer cells (C) - Negative control.

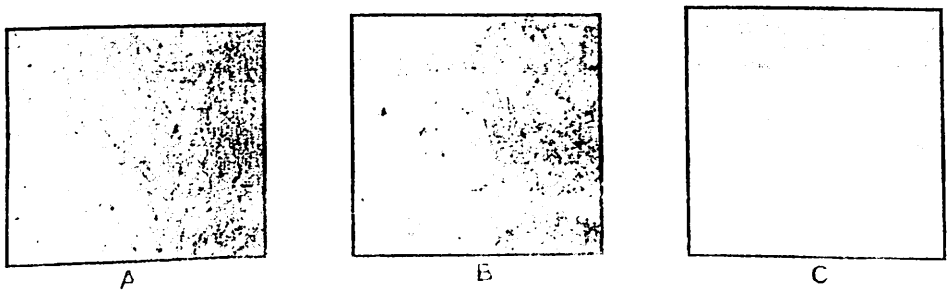


Figure 2: Characterization of SHED by immunocytochemistry staining using CD166 antibody viewed at 100x. The CD166 expressions on SHED (A) and HMSC (B) - Positive controls. No expression detected on breast cancer cells (C) - negative control.

UNIVERSITI SAINS MALAYSIA
INSTITUT PENGAJIAN SISWAZAH • INSTITUTE OF GRADUATE STUDIES
11800 USM • Pulau Pinang • Malaysia • Tel:604-653 3888 • ext. 2941/2944/2946/2948 • Faks:604-653 2931•
dean_ips@usm.my • www.ips.usm.my

Our Ref : P-HSM0042
Date : 21 Dec 2005

ALAA N.A. LUTFI
P.O.BOX 164
00971 AJMAN
UNITED ARAB EMIRATES

Dear,sir

Change of Topic And Field

I am pleased to inform you that the University has approved your application for the change of topic and field as follows:-

New Field : **PAEDIATRIC DENTISTRY**

New Topic: **PULP THERAPHY IN PRIMARY TEETH**

Thank you.

Yours sincerely,

(MOHD ZULKARNAIN MOHAMAD TAJULARIFFIN)
for Dean

c.c. Dean, School of Dental Sciences

(MS) DR. SITI NOOR FAZLIAH MOHD NOOR
(CS) PROF. AB. RANI SAMSUDIN
(CS2) DR. KARIMA AKOOL MENKHI AL-SALIHI

MZMT/rs
c.topic

UNIVERSITI SAINS MALAYSIA
INSTITUT PENGAJIAN SISWAZAH • INSTITUTE OF GRADUATE STUDIES
11800 USM · Pulau Pinang · Malaysia · Tel:604-653 3888 · ext.2941/2944/2946/2948 · Faks:604-653 2931·
dean_ips@usm.my · www.ips.usm.my

Our Ref : P-HSM0042

Date : 09 January 2008

Alaa Nasser Lutfi
School Of Dental Sciences
16150 Kubang Kerian
Kelantan

Dear Sir,

Appointment As Two Co- Supervisor

I am pleased to inform you that the University has appointed DR. SAIDI JAAFAR as your co - supervisor (3) and DR. TP KANNAN as your co-supervisor (4) for your candidature effective date 27 December 2007.

Thank you

Yours sincerely ,

(MOHD ZULKARNAIN MOHAMAD TAJULARIFFIN)
Assistant Registrar
c.c Dean
Telephone: 04-6532936
E-mail: mzulkarnain@notes.usm.my

c.c. Dean, School of Dental Sciences

(MS) : Dr. Siti Noor Fazliah Binti Mohd Noor

(CS1): Dr. Karima Akool Menkhi Al Salihi

(CS2): Professor Ab Rani Bin Samsudin

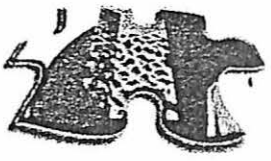
(CS3): Dr. Saidi Jaafar
(enclosed is the letter of appointment for supervisor)

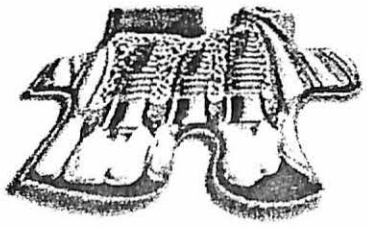
(CS4): Dr. Tp Kannan
(enclosed is the letter of appointment for supervisor)

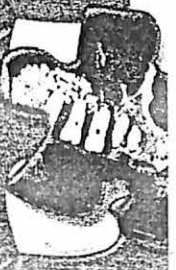
MZMT/rs

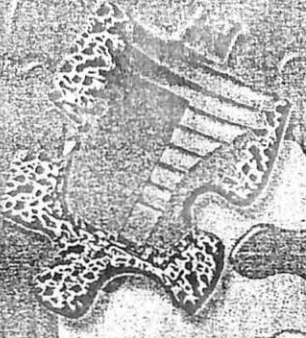
1st International Bone and Dental Technology Symposium 2007

www.bone2007.com









Grand Mercure Fortune

Bangkok, Thailand

November 12 -13, 2007

BONE SYMPOSIUM

P-24

SHED Proliferation Following Restorative Procedures

Siti Noor Fazliah Mohd Noor, Thirumulu Ponnuraj Kannan, Alaa Nassier Lutfi
School of Dental Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia
fazliah@kck.usm.my

PURPOSE: To assess the proliferative activity of stem cells from human extracted deciduous teeth following pulp capping and restorative procedures.

METHODS: It is a clinical and experimental study. Healthy patients aged 8-10 years old with carious teeth on primary molars without pulpal exposure were selected. The teeth were divided into two groups: for Group 1 the teeth were restored with Glass Ionomer Cement (GIC) and for Group 2 the teeth were restored using Calcium Hydroxide (Dycal®) as cavity liner and followed with GIC in a clinical setting. The teeth were reviewed for up to 90 days before being extracted according to the selected criteria and under standardized procedures. Within 24 hours, the tooth was cut at the cemento-enamel junction using hard material cutter. The sectioned tooth was briefly immersed in 75% ethanol, soaked in Phosphate Buffer Saline. The pulp was separated from the crown and then digested in a solution of 3 mg/ml collagenase type I (Worthington Biochem, Freehold, NJ) and 4 mg/ml dispase (Boehringer Mannheim) for 1 h at 37°C. Single-cell suspensions were obtained by passing the cells through a 70-μm strainer (Falcon) and cultured with alpha modification of Eagle's medium supplemented with 20% Fetal Bovine Serum, 100 μM L-ascorbic acid 2-phosphate, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin). The cells were incubated at 37°C in 5% CO2. The culture was observed daily under microscope. Immunohistochemistry confirmation was done using CD105 and CD166. The proliferative activity of the stem cells from human extracted deciduous teeth from the two groups were determined using alamarBlue™ kit.

RESULTS: The cells changed its shape from rounded to fibroblastic-like. Tooth pulp-derived progenitor cells were found positive for CD 105 and CD 166 (mesenchymal lineage markers), which were consistent with the finding for Human Mesenchymal Stem Cells (HMSC). Proliferative activity of SHED was higher for Group 2 as compared to Group 1.

CONCLUSION: A clonogenic cell population was isolated from dental pulp tissues and has high proliferate activity. SHED have responded favourably to restorative treatment and are able to withstand the changes following restorative procedures thus making them a possible source for tissue engineering in the future.

This study was supported by the Universiti Sains Malaysia Grant (304/PPSG/6131527)

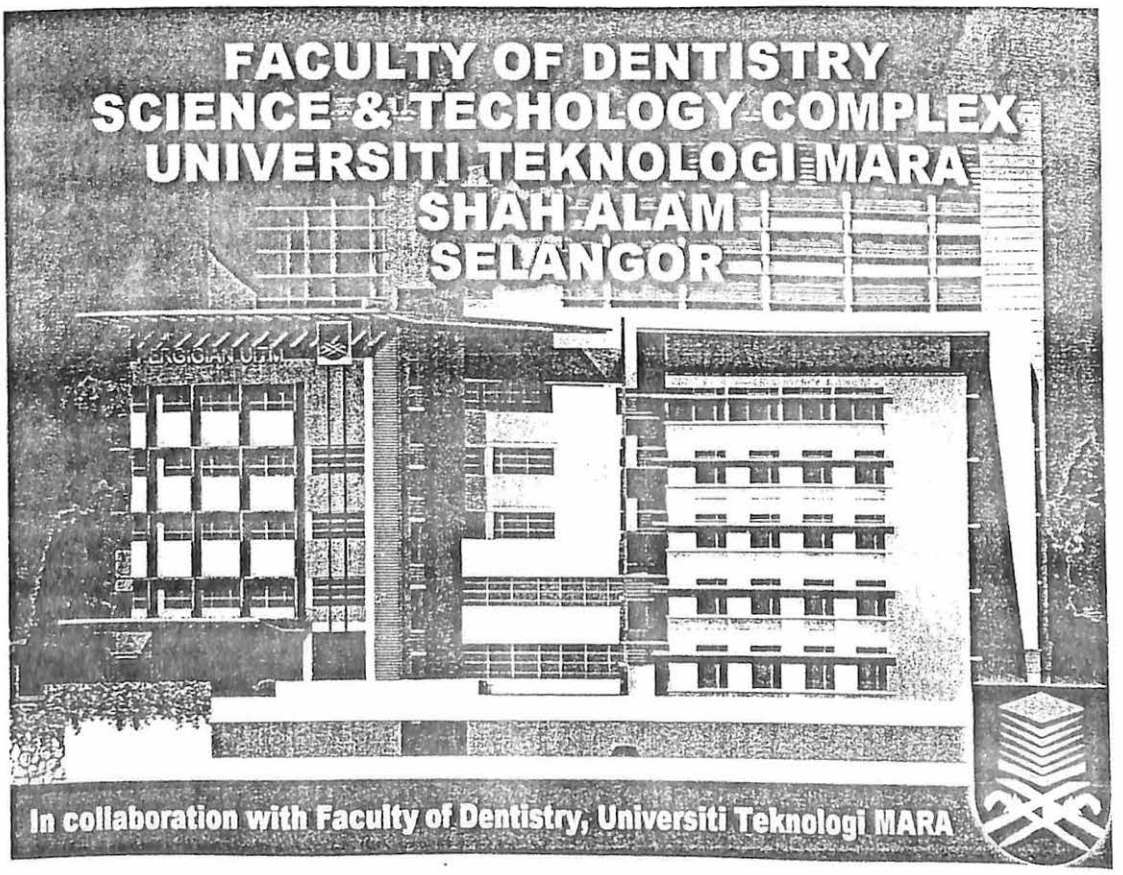
REFERENCES:

1. M Miura, S Gronthos, M Zhao, Bai Lu, LW Fisher, P Gehron Robey, and S Shi. "SHED: Stem cells from human exfoliated deciduous teeth." *Proc Natl Acad Sci,* vol. 100, pp. 5807-5812, 2003.
2. MW Lee, JS, MS Yang, JS Park, HC Kim, YJ Kim, J Choi. "Isolation of mesenchymal stem cells from cryopreserved human umbilical cord blood". *Int J Hematol,* vol. 81, pp. 126-130, 2005.
3. O Téclès, P Laurent, S Zygouritsas, AS Berger, J camps, J Dejou, I About. "Activation of human dental pulp progenitor/stem cells in response to odontoblasts injury". *Arch Oral Biol*, vol. 50, pp. 103-108, 20005
4. S Gronthos, J Brahim, W Li, LW Fisher, N Cherman, A Boyde, P DenBesten, P Gehron Robey, and S Shi. "Stem Cell Properties of Human Dental Pulp Stem Cells". *J Dent Res*, vol. 81, pp. 531-535, 2002.
5. S Gronthos, M Mankani, J Berahim, P Gehron Robey, and S. Shi. "Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo", *Proc Natl Acad Sci*, vol. 97, pp. 13625-13630, 2000.

7th SCIENTIFIC MEETING OF IADR MALAYSIAN SECTION
AND
9th ANNUAL GENERAL MEETING

Research in Your Daily Practice

23 FEBRUARY 2008



OP1

Dental Materials Effect on SHED Following Pulp Capping

A. N. LUTFI, S.N.F MOHD NOOR, S. JAAFAR, T.P. KANNAN
School of Dental Sciences. Universiti Sains Malaysia Health Campus. Kelantan.

Objective: To assess Stem cells from Human Extracted Deciduous teeth (SHED) proliferative activity following indirect pulp capping (IPC) procedure. **Method**: Healthy patients aged between 9-11 years old with carious primary molars without pulp exposure were selected. Fourteen teeth were divided into two groups: *Group 1* seven teeth were restored with Glass Ionomer Cement (GIC) and for *Group 2* seven teeth were lined by Dycal® and restored with GIC. The teeth were reviewed up to 180 days before being extracted. Laboratory procedures were carried out according to standardized protocol for dental pulp stem cell isolation. The cell cultures were observed daily under microscope. Immunocytochemistry confirmation was done using mesenchymal stem cell markers CD105 and CD166. The proliferative activity of the SHED from the two groups were determined using alamarBlue™ assay. **Results**: Stem cells were found positive for CD105 and CD166. Proliferative activity of SHED was higher in *Group 2* (alamarBlue reduction 72%) as compared to *Group 1* (alamarBlue reduction 66%). **Conclusion**: Using calcium hydroxide cements liner such as Dycal® is beneficial in IPC procedure for deep cavities since the stem cell activity is maintained. This approach can be used in tissue engineering to aid healing and stimulate pulp-dentin regeneration.

This study was supported by the Universiti Sains Malaysia Grant (304/PPSG/6131527).

OP2

Occlusal Features of 13-17 years old Iraqi Adolescents

N.M. ABDULLAH
Faculty of Dentistry, Universiti Teknologi Mara

Objective: This study was conducted to assess the occlusal features among Iraqi adolescents. **Methods**: The study included 600 adolescents in three age groups, 13 years, 15 years, and 17 years. The assessment was by an intra-oral examination to assess the occlusal features. The instruments used in the intra-oral examination were: Vernier with depth gauge, Dental Vernier, Metal feeler gauges. The following occlusal features were registered over jet, over bite, open bite, molar occlusion,, crowding, spacing and medial diastema. Results: The results showed that the over jet mean value was 3.17 mm, over bite mean value was 3.82mm, while the open bite was detected in 4.5% of the sample. Class I molar occlusion was in 74% of the sample. Spacing was mainly in the upper posterior segment (33% of sample); while crowding was mainly in the lower anterior segment (37% of sample). Maxillary medial diastema was detected in 14% of the sample. **Conclusions:** This assessment indicates a reduction in over jet, over bite, and open bite with age. Iraqi adolescents showed lower over jet mean value, and higher over bite mean value compared with studies in other populations. Class I molar relation in this sample was higher when compared with studies on European population.

Presenter	Topic	Type of presentation	Time	Presentation number
1. DR. ALAA NASSES LUTFI	Dental Materials Effect on SHED Following Pulp Capping	Oral	9.00–9.15 am	OP1
2. DR. FOUAD HUSSIAN M.H BAYATY	Effects of Smoking on Alveolar Bone Loss of Dental Undergraduates Students in University of Malaya	Oral	9.00–9.15 am	OP8
3. DR. NAGHAM MOHAMMED ABDULLAH	Occlusal features of 13-17 old Iraqi adolescents	Oral	9.15–9.30 am	OP2
4. DR. MA'EN HUSSNI ZREAQAT	Facial Dimensions of Repaired Unilateral Cleft Lip and Palate Malay Children in Kelantan	Oral	9.30–9.45 am	OP3
5. DR. LAITH MAHMOUD ABDUL HADI	A Scoring Scale For Prognosis In Fully Edentate Geriatric Patient. A Pilot Study	Oral	9.45–10.0 am	OP4
6. DR. ASILAH YUSOFF	Inter-population comparisons of craniofacial morphology based on 3D-CT	Oral	9.15–9.30 am	OP9
7. DR. ZIYAD KAMAL MAHMOOD MOHAMMAD	Microleakage in Class II Composite Restorations Bonded With Self-Etch Adhesive	Oral	11.45–12.00 pm	OP6
8. DR. RASHEED ABDUL SALAM ABDULLAH	Molar Classification among 14 Year Old Yemeni Adolescent	Oral	9.30–9.45 am	OP10
9. DR. OMAR F. TAWFIQ	Relevance of some Facial Dimensions to Interior Teeth Proportions	Oral	9.45–10.0 am	OP11
10. ASSOCIATE PROF. DR. ZAKIAH MOHD ISA	Relationship of Complete Denture maxillary Incisors to the Incisive Papilla	Oral	11.30–11.45 am	OP5
11. DR. ZAINUL AHMAD RAJION	Application of Digital Morphometric Software in Facial Analysis	Oral	11.30–11.45 am	OP12
12. DR. MARHAZLINDA JAMALUDIN	Quality of Life Among Repaired Cleft Lip and Palate Patients	Oral	11.45–12.00 pm	OP13
13. DR. MOHD ARAD JELON	Anthropometric Measurements of Craniofacial Region amongst Healthy Malay	Oral	12.00–12.15 pm	OP7
14. DR. NURLIDIA MOHD GHAZALI	Occurrence of Mandibular Fractures in Johor Bahru, Johor	Oral	12.15–12.30 pm	OP14

Note: OP1-OP4 and OP5-OP7 : venue: Dewan Kuliah Alamanda
OP8-OP11 and OP12-OP14 : venue : Dewan Kuliah Fakulti Perubatan

12th INTERNATIONAL CONFERENCE OF ASIA PACIFIC ASSOCIATION OF SURGICAL TISSUE BANKS

"What's New in Tissue Banking"

2 - 6 June 2008

Renaissance Kuala Lumpur Hotel
Kuala Lumpur Malaysia

Organised by

MALAYSIAN NUCLEAR AGENCY (NUCLEAR MALAYSIA)

MOSTI MOH MOHE

Under the auspices of

With the support of

S6.4

SHED PROLIFERATIVE ACTIVITY RESPONSE TO DENTISTRY MATERIALS

Alaa Nasser Lutfi, Siti Noor Fazliah Mohd Noor,
Saidi Jaafar and T.P. Kannan
*School of Dental Sciences, Universiti Sains Malaysia Health Campus
Kubang Kerian, Kelantan, Malaysia*

The objective of this study is to assess the proliferative activity of Stem cells from Human Extracted Deciduous teeth (SHED) following indirect pulp capping (IPC) procedure. Healthy patients aged between 9-11 years old with carious primary molars without pulp exposure were selected. The teeth were divided into two groups: Group 1 the teeth were restored with Glass Ionomer Cement (GIC) and for Group 2 the teeth were lined by Dycal® and restored with GIC. The teeth were reviewed up to 180 days before being extracted. Laboratory procedures were carried out according to standardized protocol for dental pulp stem cell isolation. The cell cultures were observed daily under microscope. Immunocytochemistry confirmation was made using fluorescein conjugated cell markers CD105 and CD166. The proliferative activity of the SHED from the two groups were determined using alamarBlue™ assay. Stem cells were found positive for CD105 and CD166. Proliferative activity of SHED was higher in Group 2 (93%) as compared to Group 1 (84%). Using calcium hydroxide cements liner such as Dycal® is beneficial in IPC procedure for deep cavities since the stem activity is maintained. This approach can be used in tissue engineering to aid healing and stimulate pulp-dentin regeneration.

5th WORLD CONGRESS ON TISSUE BANKING

In conjunction with

12th INTERNATIONAL CONFERENCE OF ASIA PACIFIC ASSOCIATION OF SURGICAL TISSUE BANKS

2 - 6 June 2008 - Kuala Lumpur, MALAYSIA

Organised by:

GOVERNMENT OF MALAYSIA

MALAYSIAN NUCLEAR AGENCY

UKM

Under the auspices of:

APASTB

FATB

EATB

ALaBaT

With the support of:

MOSTI

MOH

IAEA

UNESCO

10 Mac 2008

Dr. Alaa Nasser Abed Al Rahman Lutfi
School of Dental Sciences,
Universiti Sains Malaysia,
Health Campus,
16150 Kubang Kerian,
Kelantan,

Dear Dr. Alaa Nasser Abed Al Rahman Lutfi,

ACCEPTANCE OF ABSTRACT FOR ORAL PRESENTATION
"5th WORLD CONGRESS ON TISSUE BANKING (WCTB 08) in conjunction with THE 12th INTERNATIONAL CONFERENCE OF ASIA PACIFIC ASSOCIATION OF SURGICAL TISSUE BANKS (APASTB)", 02 – 06 JUNE 2008, KUALA LUMPUR, MALAYSIA

We are pleased to inform you that your abstract entiled:

SHED PROLIFERATIVE ACTIVITY RESPONSE TO DENTISTRY MATERIALS

has been accepted for Oral Presentation.

Kindly ensure that you have registered with the Conference Secretariat and the registration fee is paid in full. **Please inform us by 1st April 2008 if you are unable to make it to the meeting for one reason or another.**

Thank you very much for submitting your paper and your participation in the Congress.
We look forward to seeing you in June 2008.

Yours sincerely,

Secretariat of WCTB 08

Congress Secretariat
"5th World Congress on Tissue Banking"
c/o Dr. Norimah Yusof
Malaysian Nuclear Agency,
Bangi, 43000 Kajang
Selangor, Malaysia
Tel: 603-89282911
Fax: 603-89282956
Email: wctb09@gmail.com
Website: www.wctb09.com

P2849

UNIVERSITI SAINS MALAYSIA
PUSAT PENGAJIAN SAINS PERGIGIAN
SCHOOL OF DENTAL SCIENCES

RECEIVED
- 5 SEP 2007
TNC (P & I)
USM

4 September 2007

Kepada:

Yg. Berbhg. Dato' Profesor Muhammad Idris Salleh
Timbalan Naib Canselor (Penyelidikan dan Inovasi)
Universiti Sains Malaysia
11800 Universiti Sains Malaysia, Minden
Pulau Pinang

Yg. Berbhg. Dato',

Permohonan Kelulusan Pembelian Alatan Di bawah Vot 35000 bagi 'Elipar Freelight 11 230v GB'
Tajuk Projek: Evaluasi dan respon sel stem pergigian dalam tisu pulpa selepas rawatan pulpa menggunakan CaOH dan GIC atau prosedur restoratif menggunakan GIC bagi gigi molar primari
No akaun: 304/PPSG/6131527

Dengan segala hormatnya saya merujuk kepada perkara di atas. Surat saya bertarikh 9 Ogos 2007 adalah dibatalkan.

Sukacita dimaklumkan bahawa kami ingin memohon kelulusan Yg. Berusaha Dato' Prof untuk membeli peralatan seperti yang dinyatakan di bawah kerana alat ini amat penting bagi kegunaan penyelidikan. Alat ini dianggarkan bernilai RM 5000.00 sahaja.

Pertimbangan daripada Yg. Berusaha Dato' Prof amat diperlukan dan saya dahului dengan ucapan terima kasih.

Semoga mendapat perhatian dan keizinan pihak Yg. Berusaha Dato' Prof.

Sekian, terima kasih.

Yang benar,

(Dr Siti Noor Fazliah Mohd Noor)
Ketua Projek

s.k. Encik Zulkifli Mohamed
Timbalan Bendahari
Jabatan Bendahari
Kampus Kesihatan USM

06/09/07

DATO' PROF. MUHAMMAD IDRIS SALEH
Timbalan Naib Canselor
(Penyelidikan & Inovasi)

KAMPUS KESIHATAN ▲ HEALTH CAMPUS
Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan.
Tel: 609 766 3000 / 766 3500 ext. 3700: Fax: 609 764 2026: Website: www.usm.my