

Volume 60
Number 4
December 2015

Australian Dental Journal

*The official journal of the
Australian Dental Association*

SPECIAL
RESEARCH
SUPPLEMENT

Australian Dental Research Foundation



AUSTRALIAN DENTAL
ASSOCIATION INC.

ISSN 00450421

WILEY

Introduction

As Chair of the Australian Dental Research Foundation (ADRF), I am pleased to present to you the annual ADRF Special Research Supplement of the *Australian Dental Journal* for 2015. The Research Supplement features the hard work of numerous researchers, academics and students that was funded either entirely or in part by the ADRF. Several organizations, including the Australian Dental Industry Association Limited (ADIA), International College of Dentists – Australasian Section, Oral Medicine Academy of Australasia and GC Australasia Dental Pty Ltd have established named grants adding to the ADRF grant pool. We are also privileged to have generous support from many individuals who are named in our ADRF Annual Report and to whom we are extremely thankful. You will find abstracts from two of the GC Australasia Dental Pty Ltd Award winners enclosed within these pages.

With funding from such generous donations, the ADRF has in recent years granted in excess of \$250,000 per annum. In selecting the successful applicants, the Research Advisory Committee, chaired by Professor Camile S Farah, and aided by seven highly experienced and qualified academics, spent countless hours evaluating many hundreds of grant applications. Very rigorous selection criteria are applied to each application and each application receives two independent external reviews in addition to the Research Advisory Committee's review. Thousands of volunteer hours have been spent to find the highest scoring applications every year.

In 2014, there were also eight highly deserving students of the Dental Student Grants, and the ADRF thanks the supervisors who assisted the students with their research studies, offering support, encouragement and oversight.

As you study the reports presented, please remember that research is vital to advances in the dental profession and research needs your financial support. Medical research funding is very scarce and it's a competitive field.

Whilst most of the grant applications are worthy of funding, unfortunately the ADRF is only able to currently provide funding for approximately one-third of all applications.

Most of the funds come from interest on our invested capital and, as you are aware, interest rates are at historically low levels.

You can become a supporter of ADRF or make a donation to continue the research work that provides the evidence underpinning your daily dental practice.

Let's accept the obligation of supporting the dental research community to the benefit of all Australians.

In closing, I would like to sincerely thank the Editor and Editorial Advisory Board of the *Australian Dental Journal* for recognizing the importance of this Supplement and continuing to support its publication. The abstracts not only feature within the ADRF Special Research Supplement but many full manuscripts are published in the *Australian Dental Journal* and other leading Australian and international journals.

Pam Clark

Chair

Australian Dental Research Foundation

CONTENTS

ADRF Special Research Supplement

Volume 60 No 4 December 2015

ADRF Research Grant Abstracts

- S4 A histomorphometric assessment of collagen stabilized anorganic bovine bone mineral in maxillary sinus augmentation: a controlled clinical trial
J Alayan, C Vaquette, DW Huttmacher, CS Farah, S Ivanovski
- S4 An *in vitro* pilot study of a wireless telemetry system for the measurement of bone strain
F Angelis, RB Judge, J Palamara
- S5 Management of dentine hypersensitivity: clinical assessment and treatment modalities
A Bradshaw, I Meyers
- S6 Fish oil as an adjunct therapy for periodontitis: a pilot study
B Chee, B Park, A Coates, P Howe, PM Bartold, T Fitzsimmons
- S6 The effect of surface treatment on the strength of zirconia after artificial ageing: an *in vitro* study
S Chundururu, M Guazzato, IJ Klineberg
- S7 Advanced tissue engineering scaffold design for periodontal regeneration: an ectopic *in vivo* study
PF Costa, C Vaquette, Q Zhang, RL Reis, S Ivanovski, DW Huttmacher
- S8 Towards selective removal of *Porphyromonas gingivalis* from dental plaque
SA Dingsdag, N Hunter
- S8 A clinical evaluation of Class II posterior composite resins placed using different interproximal matrix systems
E Fisher, I Meyers
- S9 A TonB-dependent outer membrane protein HusB, the specific receptor of haemophore-like protein HusA in *Porphyromonas gingivalis*
J Gao
- S10 Exploration of dentists' characteristics associated with caring for disadvantaged patients: Stage 2 of a mixed-methods approach
S Gardner, K Roberts-Thomson, T Winning, R Peterson
- S11 Quantitative strain analysis of a titanium-zirconium alloy and titanium narrow diameter implants: an *in vitro* comparison
DS Goonawardhana, RB Judge, JEA Palamara, J Abduo
- S11 Particle size influences the properties of mineral trioxide aggregate
WN Ha, DP Bentz, B Kahler, LJ Walsh
- S12 Assessment of the immunomodulatory properties of mesenchymal stem cells derived from induced pluripotent stem cells and their utility for inhibiting bone loss in periodontal disease
K Hynes, J Ng, V Marino, R Bright, S Gronthos, PM Bartold
- S13 Bacterial profile of dentine caries and the impact of pH on bacterial population diversity
N Kianoush, CJ Adler, K-AT Nguyen, GV Browne, M Simonian, N Hunter
- S14 A simple way to make bulk amounts of highly regular apatite crystals
T Lawson, P Nesterenko, B Paull
- S14 A new approach to dental morphology analysis
S Mihailidis, S Ranjitkar, T Hughes, C Hall, M Bockmann, G Townsend
- S15 Effects of dentine permeability with direct adhesive restorations
K Nguyen, R Wong, J Palamara, MF Burrow
- S16 Interface strength and aesthetics of ceramic coated titanium
R Oberoi-Gutteridge, R Judge, JEA Palamara, J Abduo
- S16 Chinese dental beliefs, socioeconomic status and dental visits among Chinese migrants
HP Tan, D Brennan
- S17 A laboratory and clinical evaluation of the surface wear and marginal integrity of a ring-opening polymer composite restorative material compared with a conventional composite resin in posterior teeth
Z Vorster, I Meyers

Contents continued

- S17 The effect of simulated dentine tubule fluid on remineralization of demineralized dentine: using AFM-based nanoindentation to investigate the mineralized dentine
MA Vu Thanh, JM McIntyre, L Richards, R Zoehrer
- S18 A five-year retrospective assay of implant treatments and complications in private practice: the restorative complications of single and short span implant supported fixed prostheses
HJ Wang, RB Judge, D Bailey
- S19 A five-year retrospective assay of implant treatments and complications in private practice: the restorative treatment profiles of single and short span implant supported fixed prostheses
HJ Wang, RB Judge, D Bailey
- S20 Chemotherapy causes tight junction defects in the oral cavity of patients, which coincide with elevated proinflammatory cytokines and MMPs
HR Wardill, RM Logan, JM Bowen, RJ Gibson
-

- S21 ADRF Research Grant Reports published as full papers in the *Australian Dental Journal* in 2015
-

ADRF Dental Student Research Grant Abstracts

- S21 Comparison of facial characteristics between adolescent orthodontic patients prior to treatment and their parent
D Deall; A Docherty, M Skilbeck, MA Darendeliler (Supervisors)
- S22 Content analysis of nutritional information in paediatric oral health education leaflets
JN Doan; A Arora (Supervisor)
- S22 Touch-imprint cytology of impacted third molars to detect the presence of inflammation
CS Franco; KR Iyengar, YH Gim, A Ariyawardana (Supervisors)
- S23 Environmental stress affects development: evidence from the dental morphogenesis in a Romano-British population
KSB Koh, VKL Toh; AH Brook (Supervisor)
- S24 Mandibular morphology in mutant glypican 1- and glypican 3- knockout mice: a micro-CT investigation
M Mian; S Ranjitkar, GC Townsend, T Hughes, PJ Anderson (Supervisors)
- S24 A scanning electron microscope pilot evaluation of novel phosphoric acid preparations and techniques for enamel etching
P Tran; C Tran, LJ Walsh (Supervisors)
- S25 Root canal treatment success factors in regional Australia from 2009 to 2014: a retrospective study
JJ Zachar; A Sadr, NM Hassan, R Akhter (Supervisors)
- S26 A retrospective analysis of the short-term survival of zirconia and porcelain fused-to-metal crowns fitted in a university dental clinic
C Zhang; MR Baig, AT Flatau (Supervisors)
-

Colin Cormie Scholarships

- S26 Knowledge, behaviours and attitudes of mothers regarding vertical transmission of mutans streptococci: a qualitative case study
A Bansal; J Mills, R Boase (Supervisors)
- S27 Immunohistochemical investigation of toll-like receptor expression in a rat model of chemotherapy-induced mucositis
H McInnes, A Stringer, B Mayo, R Logan
- S28 Toll-like receptors and oral microflora in mucositis: report of a cell culture experiment in a rat model
H McInnes, A Stringer, B Vanhoecke, B Mayo, C Lucchesi, R Logan
-

GC Australasia Dental Pty Ltd, Minimum Intervention Awards

- S29 Patients' and dental student-clinicians' demographics: are these associated with patients' ratings of communication?
T Winning, A Kinnell, M Stanners, D Schönwetter

Contents continued

- S30 Synthesis of stabilized hydroxyapatite nanoparticles for enamel remineralization
M Shahmoradi, F Sonvico, M Ghadiri, M Swain, R Rohanzadeh
-

Trebitsch Research Grant

- S30 The development of a chairside paper-based assay for the assessment of salivary acetaldehyde levels after 'therapeutic' use of an alcohol-containing mouthwash
A Fam; AN Ramdzan, MI Almeida, SD Kolev, M McCullough (Supervisors)

ADRF Research Grant Abstracts

A histomorphometric assessment of collagen stabilized anorganic bovine bone mineral in maxillary sinus augmentation: a controlled clinical trial

J Alayan,* C Vaquette,† DW Hutmacher,† CS Farah,‡ S Ivanovski*

The aim of this study was to histomorphometrically compare the use of collagen stabilized anorganic bovine bone (ABBM-C) (test) to anorganic bovine bone + autogenous bone (ABBM+AB) (control) in maxillary sinus augmentation.

Forty (n = 40 sinuses) patients underwent sinus augmentation and received either control (20 sinuses) or test bone graft (20 sinuses). Bone samples were harvested from the augmented sinuses five months post-grafting. The samples were processed for histomorphometry, which assessed within the region of interest (ROI-1), the area fraction of new bone (% NB), graft particle osseointegration (% OI), residual graft (% RG), and soft tissue components (% STM). The same analysis was also carried out in a second region of interest (ROI-2) located in a zone 1 mm proximal to the previous maxillary sinus floor.

In both ROI-1 and ROI-2, the mean % NB, % OI, % RG and % STM in the control group were similar to mean values in the test group ($p > 0.05$). However, the % OI was greater in the control group when compared to the test group in ROI-2 but did not reach a level that is statistically significant. No statistically significant differences were seen when ROI-1 and ROI-2 are compared within each group.

ABBM-C exhibited very similar histomorphometric parameters to the composite graft of ABBM+AB. ABBM-C is a suitable bone substitute for the purposes of maxillary sinus augmentation. Its clinical utility may be in cases of sinus membrane perforation and insufficient autogenous bone in the local area

This abstract is based on research that was funded entirely by an Australian Dental Research Foundation grant (RM#: 2010001012).

*School of Dentistry and Oral Health, Griffith University, Queensland.

†Institute for Health and Biomedical Innovation, Queensland University of Technology, Queensland.

‡School of Dentistry, The University of Western Australia, Western Australia.

Email: s.ivanovski@griffith.edu.au

Published: Alayan J, Vaquette C, Farah C, Ivanovski S. A histomorphometric assessment of collagen-stabilized anorganic bovine bone mineral in maxillary sinus augmentation – a prospective clinical trial. Clin Oral Implants Res 2015 Sep 16. doi:10.1111/clr.12694.

An *in vitro* pilot study of a wireless telemetry system for the measurement of bone strain

F Angelis, RB Judge, J Palamara*

The aim of this study was to develop and validate via comparison to conventional wired strain gauge data an electronics system capable of measuring and wirelessly transmitting strain in the mandible of a greyhound skull *in vitro*.

Two printed circuit board (PCB) components were designed, an implantable device and an external induction based wireless power source, a programme was written in Visual Basic to capture the data. Due to errors introduced during construction of the implant PCB, the power supply and radio frequency (RF) transmitter were non-operational. To compen-

sate, a Nordic board was soldered to the implant PCB to provide power and wireless transmission. A 1000 Ohm rosette gauge was calibrated, bonded to a greyhound mandible and the three pairs of terminals soldered directly to the implant PCB via coloured Teflon coated wires (red, grey, blue). The skull was loaded and data collected via wireless transmission, the strain gauge disconnected from the implant PCB, resoldered to a National Instruments SC-2345 Carrier/SCC-SG04 module data capture board and the experiment repeated via conventional wired means.

Multiple linear regression modelling produced separate linear equations for each of the strain gauge channels which related the wireless and the wired data with high correlation (R^2 values red = 0.97, grey = 0.88, blue = 0.74). These equations allow the calculation of actual strain values from the wirelessly collected data. This validated the prototype and demonstrated its ability to produce comparable strain

*Melbourne Dental School, The University of Melbourne.
Email: roybj@unimelb.edu.au

data to conventional wired systems which provides a partial solution to the problem of long term *in vivo* wireless strain measurement.

The wirelessly transmitting prototype PCB described in this study successfully transmitted comparable strain data to conventional wired technology. This system, if rendered biocompatible, could be implanted into a living greyhound and facilitate long-term *in vivo* strain measurement and analysis.

This abstract is based on research that was funded entirely or partially by an outside source: the Australian Dental Research Foundation Inc.

Management of dentine hypersensitivity: clinical assessment and treatment modalities

A Bradshaw, I Meyers*

Dentine hypersensitivity has been defined as a short, sharp pain arising from exposed dentine in response to stimuli such as thermal, evaporative, tactile, osmotic or chemical, which cannot be ascribed to any other dental defect or pathology. Due to the large array of factors that contribute to the pathogenesis of dentine hypersensitivity, diagnosis and management can be challenging for the clinician. A number of in-office and home care products exist that may assist in the management and prevention of hypersensitivity; however, the selection and recommendation of products varies between practitioners. This cross-sectional questionnaire study aimed to investigate the diagnostic principles and management strategies undertaken by dentists and oral hygienists in Queensland.

A total of 192 oral hygienists and 808 dentists were sampled at random. These individuals were sent an information sheet and questionnaire and invited to participate in the study. The questionnaire presented participants with 14 questions focused around practice specifics, education, diagnosis/identification and management of dentine hypersensitivity. Participants were asked to rank in preference various strategies for diagnosis and management, and responses were recorded using a preferential scoring system.

The response rate was 20.3% for hygienists and 21.8% for dentists. A greater proportion of the respondents worked in private practice (87%). During

the diagnosis/identification phase, both dentists and oral hygienists utilized a number of clinical observations and tests to confirm the diagnosis of dentine hypersensitivity. The most favoured diagnostic techniques were a clinical history of symptoms, observing the presence of exposed dentine, and patient sensitivity on stimulation of dentine with an air blast. The median of the estimated prevalence of hypersensitivity given by dentists and hygienists was 10% and 30% respectively. The most favoured recommendations for short-term management were substituting the patient's regular toothpaste for one containing a desensitizing agent, application of in-office fluoride varnish, the use of products containing casein phosphopeptide and amorphous calcium phosphate (CPP-ACP) and diet modification to minimize exposure to exogenous acid sources.

From this study it can be concluded that similar diagnostic and management strategies are utilized by both dentists and dental hygienists in the clinical diagnosis and management of dentine hypersensitivity. The responses of the study indicated that dental hygienists conducted a more thorough screening for both dentine hypersensitivity and its risk factors.

The support of the Australian Dental Research Foundation for assistance with this project is greatly appreciated.

The findings of this research were presented at The University of Queensland School of Dentistry Research Day 2009.

*School of Dentistry, The University of Queensland.
Email: ian.meyers@uq.edu.au

Fish oil as an adjunct therapy for periodontitis: a pilot study

B Chee,* B Park,* A Coates,† P Howe,‡ PM Bartold,§ T Fitzsimmons§

The aim of this study was to evaluate the clinical efficacy of fish oil supplementation rich in long chain Omega-3 polyunsaturated fatty acids (LCn3PUFA) as an adjunct to non-surgical periodontal therapy in the treatment of advanced chronic periodontitis.

Thirty-four subjects (10 male, 24 female; mean age 50.1 years) with advanced chronic periodontitis were recruited for this parallel group double-blind placebo-controlled randomized trial. All participants received non-surgical periodontal therapy and were randomly allocated to receive either adjunctive dietary fish oil supplements (6 x 500 mg capsules equivalent of 2 g LCn3PUFA per day) or placebo (6 x 500 mg capsules of vegetable oil). Clinical parameters were recorded at baseline, 4, 7, 10 and 13 months. At each recall visit gingival crevicular fluid (GCF) was obtained for determination of C-reactive protein, TNF- α and IL-1 β levels using magnetic luminex performance assay. Additionally, erythrocytes were isolated from fasting blood samples to allow assessment of fatty acid biomarkers including EPA, DHA, Omega-3 Index (EPA + DHA) and total LCn3PUFAs. Data for the 4-month follow-up are presented in this initial report.

There was a statistically significant increase in erythrocyte membrane levels of total long chain omega-3

fatty acid and Omega-3 Index from baseline to 4 months in the fish oil group only. Both treatment groups demonstrated improved clinical outcomes evidenced by significant reduction of full-mouth bleeding scores, probing pocket depth reduction and clinical attachment gain. At the 4-month follow-up, no significant difference was recorded between groups for the percentage of sites that had ≥ 2 mm gain of clinical attachment or reduction in probing pocket depth. Mean number of sites with residual pocket depth ≥ 5 mm at follow-up were not significantly different for the test group or placebo group. The concentration of IL-1 β in GCF was increased in both the placebo and fish oil groups at 4 months compared to baseline levels. However, these changes were not statistically significant, either within the group or between groups. CRP in GCF decreased in the placebo group but remained unchanged in the fish oil group.

Within the limitations of this pilot study, the findings of this study do not support an additional benefit of adjunctive LCn3PUFA supplementation for the treatment of advanced chronic periodontitis. There is a need for further research to establish the clinical efficacy of LCn3PUFA as a host modulatory therapy for the treatment of periodontitis, particularly larger multi-centre randomized controlled trials.

The support of the Australian Dental Research Foundation is gratefully acknowledged.

The findings of this study were presented at The University of Adelaide School of Dentistry Research Day 2014.

*School of Dentistry, The University of Adelaide, South Australia.

†Alliance for Research in Exercise, Nutrition and Activity, The University of South Australia.

‡Clinical Nutrition Research Centre, The University of Newcastle, New South Wales.

§Colgate Australian Clinical Dental Research Centre, School of Dentistry, The University of Adelaide, South Australia.

Email: mark.bartold@adelaide.edu.au

The effect of surface treatment on the strength of zirconia after artificial ageing: an *in vitro* study

S Chunduru, M Guazzato, IJ Klineberg*

Y-TZP is now increasingly used as both layered and full-contour restorations which may be subject to hydrothermal degradation in the presence of oral fluids. This is in conjunction with the need for clinical adjustment of Y-TZP which may add additional mechanical stresses to the material. The aims of this study were to assess how different surface adjustments and polishing can affect the long-term flexural strength of Y-TZP.

A total of 510 beam shaped Y-TZP specimens were subjected to 10 different surface preparation protocols and three durations of ageing (0, 3 and 6 hours) in an autoclave (134 °C at 0.2 MPa). One sample from each group was tested with XRD to assess the surface monoclinic content. Uniaxial flexural strength was tested by a three-point bend test with an Instron 5655 universal testing machine. Statistical analysis was conducted with two-way ANOVA with Bonferroni

pairwise comparisons followed up with linear regression analysis.

Grinding using the green band diamond bur ($p < 0.001$) and red band diamond bur ($p = 0.057$) decreased the flexural strength of Y-TZP (659 and 786 MPa respectively) and was subsequently reversed if followed by a polishing kit. With the exception of these groups (without ageing), all remaining groups had mean flexural strengths greater than 950 MPa with most groups having no statistically significant change in flexural strength after artificial ageing. The zirconia polishing kit had the least amount of $t \rightarrow m$ phase transformation even with artificial ageing and the control group started with no detectable mono-

clinic phase and showed the greatest $t \rightarrow m$ phase transformation after artificial ageing.

Surface adjustment with diamond burs is best followed up with polishing burs. Artificial ageing did not appear to cause a decrease in flexural strength. Surface monoclinic content of Y-TZP did not appear to relate to changes in flexural strength.

This abstract was based on research that was partially funded by Australian Dental Research Foundation (ADRF) and Australian Prosthodontic Society (APS). The authors would also like to acknowledge Vita Zahnfabrik for provision of Y-TZP specimens, W&H for provision of laboratory handpieces and EVE GmbH for provision of polishing kits.

The findings of this research were presented at the AANZP Academy Scientific Meeting, Melbourne, 2014.

*School of Dentistry, The University of Sydney, New South Wales.
Email: ivenk@mail.usyd.edu.au

Advanced tissue engineering scaffold design for periodontal regeneration: an ectopic *in vivo* study

PF Costa,*† C Vaquette,† Q Zhang,†‡ RL Reis,* S Ivanovski,§ DW Hutmacher*

This study investigated the performance of a second generation biphasic scaffold combined with cell sheet technology for the regeneration of the periodontium complex composed of cementum, periodontal ligament and alveolar bone.

The biphasic scaffold was manufactured utilizing a rapid prototyping technique called fused deposition modelling and melt electrospinning. This resulted in the production of a bi-compartmentalized scaffold into which the FDM scaffold permits bone ingrowth (due to its macroscopic pore size and excellent interconnectivity) and the fibrous melt electrospun scaffold enables the support of periodontal ligament fibroblast cell sheets. To enhance the osteoconductivity of the bone compartment, a layer of inorganic material, that is calcium phosphate (CaP), was deposited onto the polymer filament. Ovine periodontal ligament fibroblast cell

sheets were formed *in vitro* and harvested using the biphasic scaffold previously cultured with osteoblasts for 6 weeks. This aimed at assessing the performance of the cell sheet towards ligament attachment and the impact of the calcium phosphate towards mineralization and osteoconduction both *in vitro* and *in vivo*. The *in vivo* model consisted of placing the tri-layered cell sheet and the scaffold onto an ovine dentine block subcutaneously implanted in athymic rats.

The *in vitro* study revealed that cell sheet harvesting was facilitated by the presence of the melt electrospun membrane. The bone compartment supported higher levels of alkaline phosphatase activity and *in vitro* mineralization for the CaP-coated specimens. The samples were subsequently implanted and then retrieved after 8 weeks post-surgery. Micro-computed tomography confirmed the *in vitro* findings and demonstrated a higher amount of new bone in the CaP coated samples. Histological staining showed that the large pore size of the periodontal compartment permitted vascularization of the cell sheets and periodontal attachment was achieved at the dentine interface.

This work demonstrates that the combination of cell sheet technology, together with an osteoconductive biphasic scaffold could be utilized to address the limitations of current periodontal regeneration techniques.

*B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal; 2ICVS/3B's – PT Government Associate Laboratory, Braga, Portugal.

†Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia.

‡College of Chemical Engineering, Sichuan University, Chengdu, China.

§Griffith Health Institute, School of Dentistry and Oral Health, Griffith Health Institute, Griffith University, Southport, Australia.
Email: cedryck.vaquette@qut.edu.au

This abstract is based on work funded by the National Health and Medical Research Council, the Australian Research Council and the Australian Dental Research Foundation. This study was additionally supported by Pedro Costa's PhD grant from the

Portuguese Foundation for Science and Technology (SFRH/BD/62452/2009).

The findings of this research were presented at the European Society for Biomaterials, Madrid, Spain 2013.

Towards selective removal of *Porphyromonas gingivalis* from dental plaque

SA Dingsdag,*† N Hunter*†

The periodontal pathogen *Porphyromonas gingivalis* requires porphyrin supplementation for growth. *P. gingivalis* is also sensitive to nitroimidazoles such as metronidazole, which are prodrugs inactive until taken up and reduced by ferredoxin linked metabolic processes. In order to exploit porphyrin auxotrophy, 'Trojan horse' amide-linked (see Yap and Hunter, 2009) and lysine-linked deuterporphyrin-nitroimidazole adducts (see Dingsdag and Hunter, 2015) were previously synthesized, designed to deliver nitroimidazoles into *P. gingivalis*. The aims of this study were to determine the effect of porphyrin-metronidazole adducts on representative anaerobic bacteria and secondly to determine the effect of porphyrin-metronidazole adducts in mixed bacterial growth curves.

Five anaerobic bacteria were studied by growth curve analyses in enriched brain heart infusion broth (enriched BHI) in an anaerobic chamber [Don Whitley Scientific, CO₂ (5%), H₂ (10%) and N₂ (85%)] at 37 °C. Enriched BHI media contained brain heart infusion (37 g L⁻¹, Oxoid, pH 7.7), yeast extract (5g L⁻¹, Oxoid) and L-cysteine (0.5 mg L⁻¹, Fluka), which was autoclaved before the addition of filter sterilised menadione (2 mg L⁻¹, Sigma), fetal bovine serum (FBS, 5% v/v) and N-acetylmuramic acid (NAM, 10 mg L⁻¹, Sigma).

In enriched brain heart infusion growth media, none of the anaerobic bacteria (*Prevotella melaninogenica* ATCC 25845, *Tannerella forsythia* ATCC 43037, *Bacteroides fragilis* ATCC 25285 and *Fusobacterium nucleatum* ATCC 25586) were killed by amide or lysine adducts (at 20 µM), whilst metronidazole (average of 5 µM) killed these anaerobes. The growth of *T. forsythia* and *P. melaninogenica* was delayed in the presence of 20 µM adducts, indicating these bacteria were taking up adducts. Secondly, growth of *P. gingivalis* in DMSO carrier control was similar to DPIX treatment, indicating there were sources of porphyrin in enriched BHI media, perhaps from media components such as fetal bovine serum. In *P. gingivalis* co-cultured with other bacteria, where it was known that the nitro (-NO₂) group on deuteroporphyrin-nitroimidazole adducts was activated, limited 'by-stander damage' was detected.

In this study, some anaerobic bacteria were affected by adducts, indicating the nitroimidazole was not delivered to *P. gingivalis* alone. Nevertheless all human-derived anaerobes tested were killed by metronidazole, yet none were killed by adducts, indicating some success in specific elimination of *P. gingivalis*.

We thank the Australian Dental Research Foundation for funding the research.

Published: Dingsdag SA, Yap BC, Hunter N, Crossley MJ. Amino acid-linked porphyrin-nitroimidazole antibiotics targeting Porphyromonas gingivalis. Org Biomol Chem 2015;13:98-109.

*Institute of Dental Research, Westmead Millennium Institute and Centre for Oral Health, Westmead, New South Wales.
†Faculty of Dentistry, The University of Sydney, New South Wales.
Email: sdin2365@uni.sydney.edu.au

A clinical evaluation of Class II posterior composite resins placed using different interproximal matrix systems

E Fisher, I Meyers*

The purpose of this clinical evaluation was to compare the Sectional Retainer Matrix System (3M ESPE) and the V-Ring System (Triodent) with regard to their abil-

ity to predictably create anatomically correct proximal contours and tight contact areas during the restoration of posterior interproximal (Class II) cavities.

A total of 38 Class II restorations were placed, by two trained operators, in the permanent teeth of patients recruited from The University of Queensland School of Dentistry. Clinical evaluations of the restorations were performed at the initial appointment and at 1, 3 and 6 months after placement. The restorations were assessed for retention, interproximal anatomic form, interproximal contact, marginal ridge, occurrence of food trapping and the adjacent gingival condition. Ease of placement and patient comfort with regard to the matrices were also examined upon restoration. Clinical photographs and PVS impressions were taken at each appointment to visually document the effectiveness of the matrix systems in the placement of the Class II restorations. Epoxy resin models were then fabricated from the impressions and photographed under a light microscope to further supplement the clinical findings.

At the end of the six-month period no statistically significant differences were observed between the dif-

ferent matrices according to the assessment criteria of restoration retention, patient comfort, ease of placement, interproximal anatomic form, interproximal contact or marginal ridges. Significantly more food trapping was experienced by restorations placed with the V-Ring System at the six-month review. The gingival condition was significantly worse at the six-month review than at the baseline review in restorations placed with the Sectional Retainer Matrix System.

Based on the clinical evaluation of this sample of Class II restorations, it is apparent that the Sectional Retainer Matrix System (3M ESPE) and the V-Ring System (Triodent) perform similarly with regard to their ability to create tight contact areas and anatomically correct proximal contours for the composite resin restoration of posterior interproximal cavities.

The support of the Australian Dental Research Foundation for assistance with this project is greatly appreciated.

The findings of this research were presented at The University of Queensland School of Dentistry Research Day 2009.

*School of Dentistry, The University of Queensland.
Email: ian.meyers@uq.edu.au

A TonB-dependent outer membrane protein HusB, the specific receptor of haemophore-like protein HusA in *Porphyromonas gingivalis*

J Gao*

Porphyromonas gingivalis, a black-pigmented, Gram-negative anaerobic bacterium, has been implicated as the keystone pathogen in the development and progression of chronic periodontitis. Due to the absence of various enzymes required for synthesis of the protoporphyrin ring *de novo*, *P. gingivalis* depends on exogenous haem to survive. This haem auxotroph bacterium secretes haemophore-like protein HusA (haem uptake system component A), a high affinity extracellular haem scavenger, which is critical for *P. gingivalis* to grow in haem limited environment. Like other haemophores, the haem uptake mediated by HusA requires a specific outer membrane receptor. Perusal of the annotated genomic sequences available for *P. gingivalis* revealed the presence of a putative TonB-dependent outer membrane protein termed HusB.

This study aimed to characterize the role of HusB in the novel Hus haem uptake system and verify the structural model of HusB as an outer membrane receptor using site-directed mutagenesis.

In this study, the HusB was evidenced to form a protein complex with HusA haemophore in *P. gingi-*

valis detected by *in vivo* cross-linking and pull-down analysis. Under haem limited conditions, the *husB* deleted *P. gingivalis* mutant failed to grow. Similarly to *husA*, the expression of *husB* gene was detected to be up-regulated in response to haem limitation and also co-transcribed with the *husA* haemophore gene. To further characterize the HusB as an outer membrane receptor, a 3D structure of HusB was modelled using Phyre2 software which presented as a typical integral membrane protein with 20 transmembrane domains and an N-terminal plug domain. This topology model was further validated using octapeptide inserted into the predicted membrane spanning regions which disrupted localization of HusB inserts. The subcellular location of HusB was determined by the fractionation. Western blot using anti-HusB antibody showed the presence of HusB only in extracts from the outer membrane fraction.

Results from this study suggested the HusB was a specific outer membrane receptor of HusA haemophore which played an essential role in haem uptake. The greater understanding of the Hus haem uptake system

in *P. gingivalis* would provide novel targets to design a specific antimicrobial agent which leads to opportunities to control the infection process by this organism with subsequent health and economic benefits.

*Institute of Dental Research, Faculty of Dentistry, The University of Sydney, New South Wales.
Email: jinlong.gao@sydney.edu.au

This abstract is based on research that was funded entirely by the Australian Dental Research Foundation.

The findings of this research were presented at the Tetrapyrroles, Chemistry and Biology of Gordon Research Conference, Newport, USA, July, 2014.

Exploration of dentists' characteristics associated with caring for disadvantaged patients: Stage 2 of a mixed-methods approach

S Gardner,* K Roberts-Thomson,* T Winning,* R Peterson†

Disparities in access to dental services have been reported for particular groups of Australians, resulting in a greater burden of oral disease compared with the general population. The reasons why dentists do not undertake and/or sustain working with disadvantaged patients (DPs) are well known, but not so is what drives those who do. This project aims to better understand the characteristics of dentists whose practice orientation is toward disadvantaged groups. This will supplement existing knowledge of dentists' career decision making, allow a more targeted recruitment of dental applicants, and inform admissions committees and dental educators in how best to prepare students with the skills, attributes and experiences necessary to serve all Australians.

The aim of this research project was the second stage (Stage 2) of a sequential mixed-method study design to explore the characteristics, values, beliefs, and motivations of dentists who work with DPs and compare the findings with those who treat mainly general patients.

A self-report questionnaire was mailed to a random sample of registered dentists in Australia. Univariate, bivariate and multivariate analyses were performed. The categorical outcome variable was 'dentists treating $\geq 50\%$ or $< 50\%$ DPs'.

A total of 1523 questionnaires were returned, yielding an adjusted response rate of 62.6%. The adjusted odds of dentists treating DPs was around twice that for those treating $< 50\%$ DPs for being motivated by 'status' (OR 2.4, 95% CI: 1.32, 4.35), 'to help' (OR 1.8, 95% CI: 1.03, 3.16), 'a challenging career' (OR 2.1, 95% CI: 1.01, 4.40) and 'second choice to medicine' (OR 2.1, 95% CI: 1.11, 3.95). They were 11 times more likely to work in government clinics (OR 11.6, 95% CI: 5.2, 26.0) and had three times the odds of working in the defence force or tertiary institutions (OR 3.0, 95% CI: 1.0, 9.2) than in private solo practices. Treating DPs was associated with neutral attitudes towards oral health therapists (OR 2.31; 95% CI: 1.09–4.91), being religious (OR 2.23; 95% CI: 1.12–4.42) and working in remote locations (OR 8.60, 95% CI: 2.21–33.48).

Stage 2 showed that career choice motivation, religious affiliation, type and location of practice were associated with treating DPs. However, while empathy and resilience were key themes from the qualitative study, socioeconomic factors, demographics and dental school experience were not found to be associated after adjusting for other variables.

The authors gratefully acknowledge the support of the Australian Dental Research Foundation for partly funding this project.

The findings of this research were presented at the International Association for Dental Research (IADR), Africa/Middle East Divisional Regional Meeting, Cape Town, South Africa, June 2014.

*School of Dentistry, The University of Adelaide, South Australia.

†Faculty of Health Sciences, The University of Adelaide, South Australia.

Email: sue.gardner@adelaide.edu.au

Quantitative strain analysis of a titanium-zirconium alloy and titanium narrow diameter implants: an *in vitro* comparison

DS Goonawardhana, RB Judge, JEA Palamara, J Abduo*

A new material for narrow diameter implants – titanium-zirconium alloy (TiZr) – has been advocated due to its superior mechanical properties compared to commercially pure titanium (Ti) grade IV. Validation is required regarding the perceived mechanical behaviour under loading of implants made from this material compared to its current alternatives. The study will determine whether the stiffer material (TiZr alloy) in a smaller diameter (3.3 mm) will produce similar peri-implant strains as a regular diameter implant (4.1 mm) made from commercially pure titanium grade IV (less stiff material).

A total of 18 implants – 6 each of the TiZr 3.3 x 10.0 mm implants (Roxolid®), Ti 3.3 x 10.0 mm implants (Ti Narrow) and Ti 4.1 x 10.0 mm implants (Ti Regular) were mounted and embedded in epoxy resin. A strain gauge was bonded at the crestal level adjacent to the implant. Identical titanium direct-to-implant crowns of a maxillary lateral incisor were torqued onto the implants and loaded at 30 degrees (to the implant) in a gradual loading pattern up to 200 N. Data from the strain gauge were recorded for each specimen and statistically analysed and compared.

The mean peri-implant strains obtained for the TiZr implant was 723.7 $\mu\epsilon$ higher than the Ti Regular implant, but only 156.3 $\mu\epsilon$ higher than the Ti Narrow implant (both these mean differences were statistically significant). Higher peri-implant strains generally correlate to poorer stress distribution, which is related to, amongst many parameters, implant mechanical properties, geometry as well as the angle the implant is loaded. TiZr implant showed marginally poorer crestal peri-implant stress distribution under oblique load compared to the Ti Narrow diameter implant. The Ti Regular implant showed much lower mean strains than both the narrow diameter implants, proving that there was better stress dissipation of this implant under load.

The reported increased stiffness of the TiZr implant did not translate to lower peri-implant strains of this implant when tested under loading *in vitro*. Rather the diameter of the implant (bulk properties), than mechanical property of the material seems to affect the amount of peri-implant strain observed at the crestal level.

This project was supported by the Australian Dental Research Foundation, the Melbourne Dental School (The University of Melbourne, Victoria), and the Australian Prosthodontic Society Inc.

*Melbourne Dental School, The University of Melbourne, Victoria.
Email: roybj@unimelb.edu.au

The findings of this research were presented at the ADAVB Convention, Melbourne 2014.

Particle size influences the properties of mineral trioxide aggregate

WN Ha,* DP Bentz,† B Kahler,* LJ Walsh*

The aims of this study were to verify that the reduction of particle size reduces the setting time of mineral trioxide aggregate (MTA) and assess the subsequent influences to its strength. The traditional method to assess setting time is to perform indentation tests using arbitrary weights. In conjunction with indentation tests, rheological measurements are an alternative way to determine the setting time of MTA. After the defined 'setting time', MTA continues to harden over a period of a month, which can be assessed using compressive strength and flexural strength testing.

Two different experimental MTAs (M1, M2) were created using two PCs with different particle sizes mixed with Bismuth oxide (P1, P2). Their particle sizes were assessed using laser diffraction. The setting times of the experimental MTAs and respective PCs were tested using indentation testing as per C266-03 of ASTM. Furthermore, throughout the setting process, the change in elastic and viscous modulus over time using a strain controlled rheometer was also analysed.

Compressive strength was assessed as per ISO 9917.1 and flexural strength assessed as per 4049. The samples were tested at 1 day, 1 week and 3 weeks.

From the four experimental cements, their d90s are summarized as P1 29.1; M1: 16.5 and M2: 37.1.

Using indentation testing, initial setting times were ranked M1 < P1 < P2 < M2 while final setting times were ranked P1 < M1 < P2 < M2, with all differences between groups being statistically significant ($p < 0.05$). Using rheological assessment, the time required to reach 95% of the elastic modulus plateau group sequence was ranked P1 < M1 < P2 < M2. These rankings corresponded with the elastic modulus plateau of P1 > M1 > P2 > M2.

Finer cements (M1, P1) had greater compressive strength than the larger cements (M2, P2) at 1 day; however, there was no difference at 1 week or 3 weeks. There was no difference in flexural strength amongst any of the cements at any of the time periods.

Smaller particle sizes result in faster indentation setting times and a quicker time to reach 95% of the elastic modulus plateau. However, there is no

difference in the final elastic modulus and, at 3 weeks, the compressive or flexural strength. The inclusion of bismuth oxide to PC, to create MTA, results in slower setting times, and lesser initial compressive strengths.

This work was supported by the Australian Dental Research Foundation and the Australian Society of Endodontology.

Published:

1. Ha WN, Kahler B, Walsh LJ. Particle size changes in unsealed mineral trioxide aggregate powder. *J Endod* 2014;40:423–426.
2. Ha WN, Kahler B, Walsh LJ. Clinical manipulation of mineral trioxide aggregate. *J Can Dent Assoc* 2015;81:f4.
3. Ha WN, Bentz DP, Kahler B, Walsh LJ. D90: the strongest contributor to setting time in MTA and Portland cement. *J Endod* 2015;41:1146–1150.

Aspects of this research were presented at The University of Queensland's Research Day (2011–2014), and the Scientific Meetings of the International Association for Dental Research (Bangkok 2013, Brisbane 2014 and Dunedin 2015).

*School of Dentistry, The University of Queensland, Brisbane, Australia.
 †National Institute of Standards and Technology, Gaithersburg, Maryland, USA.
 Email: w.ha@uq.edu.au

Assessment of the immunomodulatory properties of mesenchymal stem cells derived from induced pluripotent stem cells and their utility for inhibiting bone loss in periodontal disease

K Hynes,* J Ng,* V Marino,* R Bright,* S Gronthos,† PM Bartold*

Mesenchymal stem cells (MSC) have emerged in recent years as a potential cell population for use in novel treatment approaches to chronic inflammatory diseases such as periodontal disease. However, issues surrounding their accessibility and proliferation in culture significantly limit their ability to be used as a mainstream treatment approach. Therefore, it is important that alternative easily accessible and safe populations of stem cells are identified for use in the treatment of chronic inflammatory diseases. Controlled induction of induced pluripotent stem cells (iPSC) into mesenchymal progenitor cells (MPC) is emerging as an attractive source for obtaining large populations of stem cells for regenerative medicine.

The objective of this study was to establish if MPC derived from iPSC have the ability to control the chronic inflammatory response that leads to tissue destruction in periodontal disease.

MPC were generated by differentiating iPSC cells in MSC media for two weeks. This was followed by

serial passaging to select for fast growing MPC which have the capacity to attach and proliferate in monolayer cultures, whilst eliminating slow-growing differentiating iPSC cells. The resulting MPC were injected into mice in which periodontal disease had been induced through oral inoculation with *Porphyromonas ginigivalis*. Micro CT analysis was used to determine the extent of bone loss that occurred in mice which received MPC compared to those that did not receive cells.

We successfully generated MPC which satisfied the International Society of Cellular Therapy's minimal criteria for defining multipotent MSC, as they had plastic adherent properties, expressed key MSC associated markers and had the capacity to undergo tri-lineage differentiation. Injections of these MPCs into mice with chronic periodontitis led to an inhibition of the chronic inflammatory response and ultimately resulted in a statistically significant decrease in the amount of bone loss.

Together our results demonstrate that MPC can be derived from iPSC and these cells have the capacity to control the chronic inflammatory response that leads to tissue destruction in periodontal disease. Therefore, MPC are a promising novel source of readily accessi-

ble stem cells which could be used in the treatment of periodontal disease.

The authors would like to acknowledge the support of the Australian Dental Research Foundation (grant #37/2013) and the National Health and Medical Council (Australia; grant # 1023747) for funding this research.

The findings of this research were presented at The University of Adelaide School of Dentistry Research Day 2014.

*School of Dentistry, Colgate Australian Clinical Dental Research Centre, The University of Adelaide, South Australia.

†Mesenchymal Stem Cell Laboratory, School of Medical Sciences, The University of Adelaide, South Australian Health and Medical Research Institute, South Australia.
Email: kim.hynes@adelaide.edu.au

Bacterial profile of dentine caries and the impact of pH on bacterial population diversity

N Kianoush,* † ‡ CJ Adler,* † K-AT Nguyen,* † GV Browne,* M Simonian,* N Hunter* †

Dental caries is caused by the release of organic acids from fermentative bacteria, which results in the dissolution of hydroxyapatite matrices of enamel and dentine. While low environmental pH is proposed to cause a shift in the consortium of oral bacteria, favouring the development of caries, the impact of this variable has been overlooked in microbial population studies.

This study aimed to detail the zonal composition of the microbiota associated with carious dentine lesions with reference to pH.

We used 454 sequencing of the 16S rRNA gene (V3–V4 region) to compare microbial communities in layers ranging in pH from 4.5 to 7.8 from 25 teeth with advanced dentine caries.

Pyrosequencing of the amplicons yielded 449 762 sequences. Nine phyla, 97 genera and 409 species were identified from the quality-filtered, de-noised and chimera-free sequences. Among the microbiota associated with dentinal caries, the most abundant taxa included *Lactobacillus sp.*, *Prevotella sp.*, *Atopobium sp.*, *Olsenella sp.* and *Actinomyces sp.* We found a disparity between microbial communities localized at acidic versus neutral pH strata. Acidic conditions were associated with low diversity microbial populations, with *Lactobacillus* species including *L. fermentum*, *L. rhamnosus* and *L. crispatus* to be distinctive species at low pH ranges (4.5–5.5) in carious lesions.

In comparison, the distinctive species of a more diverse flora associated with neutral pH regions of carious lesions included *Alloprevotella tanerrae*, *Leptothrix sp.*, *Sphingomonas sp.* and *Streptococcus anginosus*. While certain bacteria were affected by the pH gradient, we also found that ~60% of the taxa associated with caries were present across the investigated pH range, representing a substantial core.

Our proposed ‘substantial core model’ identifies pH-distinctive taxa and demonstrates bacterial diversity changes from acidic to neutral pH gradients. The presence of numerous species that are not discriminatory of pH and are omnipresent in this ecology represents the core of the bacterial consortium in dentine caries with respect to pH.

We demonstrated that some bacterial species implicated in caries progression show selective clustering with respect to pH gradient, providing a basis for specific therapeutic strategies. The present in-depth report of bacterial community constitution affirms the ecological plaque hypothesis at the *in vivo* level.

The authors acknowledge funding from NIH/NIDCR (Grant #R01DE015272-07), the Australian National Health and Medical Research Council (NHMRC) (Grant #512524.3) and the Australian Dental Research Foundation (ADRF) (Grant #92-2012).

Published: Kianoush N, Adler CJ, Nguyen K-AT, Browne GV, Simonian M, Hunter N. Bacterial profile of dentine caries and the impact of pH on bacterial population diversity. PLoS One 2014;9:e92940. doi:10.1371/journal.pone.0092940.

*Institute of Dental Research, Westmead Centre for Oral Health and Westmead Millennium Institute, Westmead, Sydney, New South Wales.

†Department of Oral Biology, Faculty of Dentistry, The University of Sydney, New South Wales.

‡Department of Periodontics, Faculty of Dentistry, The University of Sydney, New South Wales.
Email: nima.kianoush@sydney.edu.au

A simple way to make bulk amounts of highly regular apatite crystals

T Lawson, P Nesterenko, B Paull*

The aim of this project was to synthesize in bulk amounts apatite crystals of regular size and shape.

A simple and rapid hydrothermal method that makes bulk amounts of highly regular spheroidal apatite crystals is described here. Crystals were synthesized from commonly found chemicals that were dissolved in water and then incubated over five hours under mild conditions. The regularity of the crystals is not dependent on a lengthy filtering or separation steps.

Crystals with a highly regular size and shape were produced at a concentration approximately 100 times greater than that described elsewhere. Hydroxyapatite and fluoroapatite crystals about 1 µm in length by half

micron in width were made. Their particular shape can be controlled so that rods or dumbbells can be produced and, depending upon the reaction conditions, so they can contain a nanometer anisotropic microstructure.

A simple method is described, so that large amounts of highly regular apatite crystals can be produced. Because of the yield and scale of this method, dense and tough ceramics could be produced in bulk for use in the body. This method might be of interest to researchers in orthopaedics, dentistry and material science.

The authors wish to acknowledge Ioan Jones, Oral Health Services Tasmania, the Australian Dental Research Foundation (ADRF), the University of Tasmania and the Australian Government.

*The University of Tasmania.
Email: tomxlawson@gmail.com

A new approach to dental morphology analysis

S Mihailidis,* S Ranjitkar,* T Hughes,* C Hall,† M Bockmann,* G Townsend*

The application of micro-CT scanning in this study has enabled the characterization of dental phenotypes in greater detail compared with traditional methods. Where previous studies have generally assumed that the morphological appearance of the outer enamel surface (OES) reflects the morphology of the dentino-enamel junction (DEJ), our study tests the hypothesis that the morphology of the DEJ provides a more faithful reflection of an individual's underlying genotype than the OES. The main aim of this study is to describe the appearance of selected dental traits, such as Carabelli trait, at the DEJ and also at the OES in pairs of twins to clarify the roles of genetic, epigenetic and environmental influences on observed variation.

A sample of 6 intact upper primary second molar teeth from 3 monozygotic twin pairs, excluding teeth with any evidence of wear on the OES, were selected. CT scans showing three-dimensional reconstructions of the DEJ and the OES were produced using the Xradia Micro XCT-400 (360° of rotation, automated) computed tomographic scanner.

Following extensive efforts toward developing the expertise and processes needed for the acquisition of good quality three-dimensional CT reconstructions, images of primary teeth from twin pairs have been obtained. As hypothesized, our findings show that the

morphology of the Carabelli trait, originates at the DEJ. This finding was consistent in the other scans collected.

We have developed an effective process for acquiring good quality CT scans of primary molar teeth. Preliminary findings from a small sample show the outline of Carabelli cusp is evident at the DEJ and similar to that at the OES. Next, we intend to increase the sample size and number of scans, and to conduct 3D geometric morphometric assessments, so that genetic modelling can be carried out to determine the relative contributions of genetic, epigenetic and environmental contributions to phenotypic variation.

We gratefully acknowledge the support of the Australian Dental Research Foundation and the participation of the twins and their families in our study. The micro-CT work was performed by Dr Jeremy Deverell at the South Australian node of the Australian National Fabrication Facility under the National Collaborative Research Infrastructure Strategy to provide nano and microfabrication facilities for Australia's researchers.

Published:

1. Hughes TE, Townsend GC, Pinkerton SK, et al. *The teeth and faces of twins: providing insights*

into dentofacial development and oral health for practising oral health professionals. *Aust Dent J* 2014;59:(1 Suppl):101–116.

2. Hughes T, Bockmann M, Mihailidis S, et al. Genetic, epigenetic, and environmental influences on dentofacial structures and oral health: ongoing studies of Australian twins and their families. *Twin Res Hum Genet* 2013;16:43–51.

*School of Dentistry, The University of Adelaide, South Australia.
 †Ian Wark Research Institute, The University of South Australia, South Australia.
 Email: suzanna.mihailidis@adelaide.edu.au

3. Mihailidis S, Ashar A, Hughes T, Bockmann M, Brook A, Townsend G. Dental phenomics: high-tech scans reveal similarities and differences in monozygotic twins. *Dental Tribune* 2013; April, A4–A5.
4. Townsend G, Bockmann M, Hughes T, Brook A. Genetic, environmental and epigenetic influences in variation in human tooth number, size and shape. *Odontology* 2012;100:1–9.

The findings of this research were presented at the 28th Annual Australasian Society for Human Biology Conference, 10–12 December 2014, Adelaide.

Effects of dentine permeability with direct adhesive restorations

K Nguyen,* R Wong,* J Palamara,* MF Burrow†

Contraction stress at the bonding interfaces may occur as a result of polymerization shrinkage of resin composite. This may affect the restorative seal. Observing the fluid flow within dentine can be used to assess the seal of a restoration.

The aim of this study was to investigate effects on the restoration-dentinal seal using different placement methods with various direct adhesive systems and restorative materials.

Mesio-occluso-distal cavities were prepared on extracted third molars ($n = 42$). Prepared teeth were divided into 6 groups and restored with either: sonic activated bulk-fill resin composite, SonicFill™; conventional nano-hybrid resin composite, Herculite® Ultra; Herculite® Ultra with a resin-modified glass-ionomer cement (RMGIC) base, Riva light-cure HV; Herculite® Ultra with a glass-ionomer cement (GIC) base, Riva self-cure HV; conventional nano-hybrid resin composite, Filtek™ Supreme XTE with selective enamel etch-and-rinse adhesive; and Filtek™ Supreme XTE with no separate etch-and-rinse step. Either a two-step self-etch bonding system, Optibond XTR and a one-step bonding system, Scotchbond Universal Adhesive was used. Using an automated flow-recording device, fluid flow was assessed before,

during, and at periods after restoration, at 24 hours, 1 week, 1 month and 4 months. Fluid flow rates of the different restorative procedures, and post-restoration time intervals were analysed using ANOVA. The changes in flow rates between the different restorations were compared using Tukey's test ($p < 0.05$).

There were no statistically significant differences in the mean percentage change in fluid flow rates between the restorative procedures after placement of the bonding agent, with or without a base. Immediately post-restoration, all test groups had increased fluid flow rates compared with baseline values.

None of the different adhesive restorative materials were able to provide a complete seal to prevent fluid movement. Placement of RMGIC/GIC base produces a restorative seal that is comparable to that of teeth restored with only resin composite.

This study is supported by the Australian Dental Research Foundation, a National Health and Medical Research Council grant (1039604) and the Australian Prosthodontic Society Inc.

The findings of this research were presented as an oral presentation at the IADR General Meeting, Seattle, USA, March 2013. Abstract reference: Nguyen K, Wong R, Palamara J, Burrow MF. Sealing ability of direct adhesive restorations in posterior teeth. J Dent Res 2013;92(A) (Abstract 276).

*Melbourne Dental School, The University of Melbourne, Victoria.
 †Faculty of Dentistry, University of Hong Kong, Hong Kong.
 Email: rhkwong@unimelb.edu.au

Interface strength and aesthetics of ceramic coated titanium

R Oberoi-Gutteridge, R Judge, JEA Palamara, J Abduo*

This study compared the bond characteristics between a titanium alloy and veneering ceramics, with an intermediate coating of zirconia to a commercially available titanium-ceramic system and a benchmark 'gold-based' ceramo-metal system. This included studying the flexural bond strength and microstructure of the coatings and fractured surfaces.

Forty-four specimens of machined titanium – 6 aluminum – 4 vanadium alloy were divided into two groups according to the intermediate bond layer used. The titanium-zirconia-veneering ceramic group (Gp TZC) was plasma sprayed with yttrium-stabilized tetragonal zirconia (YTZ); the titanium-bonder-veneering ceramic group (Gp TBC) used GC Tibonder as an intermediate layer. GC ceramics were used for both groups (n = 22). The control group had cast gold alloy with conventional veneering ceramics (Gp AuC) (n = 10). Bond strength was measured using three-

point flexural configuration, according to ISO 9693 requirements. The fractured specimens were evaluated by scanning electron microscopy (SEM) and energy dispersive x-ray spectrometry (EDS). Bond strength results were statistically analysed with one-way ANOVA test and Fisher test ($\alpha = 0.05$).

The descending order of bond strength was Gp AuC (59.8MPa) > Gp TZC (40.3MPa) > Gp TBC (29.9MPa). SEM and EDS analysis indicated mainly cohesive fractures in Gp AuC and Gp TZC, but mainly adhesive fracture in Gp TBC. All three groups had bond strengths higher than minimal ISO requirements.

Ti-alloy with YTZ surface coating demonstrated improved bond strength when compared to a commercially available system. It was, however, lower than the bond strength of gold-based ceramic system. Further research could lead to improvement and integration of this technique into dentistry.

We thank the Australian Dental Research Foundation for support provided for this research project.

*Melbourne Dental School, The University of Melbourne, Victoria.
Email: roybj@unimelb.edu.au

Chinese dental beliefs, socioeconomic status and dental visits among Chinese migrants

HP Tan, D Brennan*

The aim of this study was to investigate the influences of Chinese dental beliefs and the socioeconomic status (SES) on dental visits among Chinese migrants in Australia.

The addresses of the participants were randomly selected from the White Pages of residents in metropolitan South Australia, Victoria, Queensland and New South Wales by selecting people with names that appear to be Chinese. Mailed self-complete questionnaires written in both English and Chinese were used to collect data. Demographic information, SES, Chinese dental beliefs, time since the last dental visit and reason for the dental visit were asked in the questionnaire. Multivariable logistic regression was used to investigate the influences of Chinese dental beliefs and the SES on dental visits among Chinese migrants. Outcome variables were time since the last dental visit and reason for the dental visit.

Over 900 participants completed the questionnaires. Results of multivariable logistic regression after con-

trolling age group, gender and years living in Australia, showed that Chinese migrants with weak or moderate Chinese dental beliefs and those in the highest household income tertile were more likely to see the dentist within the last 12 months compared with those with strong Chinese dental beliefs and those in the middle household income tertile ($p < 0.05$). The reason for last visit was not significantly affected by Chinese dental beliefs but household income had an effect – those in the lowest and middle household income tertiles were more likely to make a problem-based visit compared with those in the highest household income tertile ($p < 0.05$).

The time since the last dental visit was significantly influenced by Chinese dental belief and household income but reason for the dental visit was mainly influenced by household income among Chinese migrants.

The support of the Australian Dental Research Foundation is gratefully acknowledged.

The findings of this research were presented at the International Association for Dental Research meeting, Brisbane, 2014.

*Australian Research Centre for Population Oral Health, School of Dentistry, The University of Adelaide, South Australia.
Email: haiping.tan@adelaide.edu.au

A laboratory and clinical evaluation of the surface wear and marginal integrity of a ring-opening polymer composite restorative material compared with a conventional composite resin in posterior teeth

Z Vorster, I Meyers*

Polymerization shrinkage remains as one of the most common causative factors leading to the need for replacement of posterior composite resin restorations. Changes to the molecular weight of the monomer, the degree of monomer conversion, and the percentage filler loading have been the main focus areas in endeavours towards minimizing polymerization shrinkage. Recent changes in the monomer component, replacing the common Bis-GMA resin with a Silorane resin, have opened new avenues for preventing the loss of marginal seal and reducing polymerization stress. The purpose of this clinical evaluation is to evaluate posterior restorations restored using the new 3M ESPE silorane restorative resin, Filtek Silorane, and its dedicated priming and bonding solution with similar restorations restored with Filtek Z250 restorative resin, over a six-month period.

Thirty-seven Class II restorations were placed in patients recruited from The University of Queensland, School of Dentistry. Clinical evaluation of the restorations was completed at baseline, 1 month, 3 month, and 6 month reviews with regard to restoration retention, anatomical form, margin integrity, margin discolouration, colour match, staining and secondary

caries. Results were analysed for significance using chi-square and Fisher's exact tests.

At the end of the six-month period, from the 26 restorations that were available for review, no statistical significance was found between the materials for any of the categories tested. There was, however, some evidence of restoration degradation occurring within both materials as a function of time. Margin integrity was found to be significantly reduced in the Z250 restorations over the six-month period, whilst there was a statistically significant increase in the margin discolouration of both Z250 and Silorane restorations.

After analyses of the results, the null hypothesis can be accepted in that 3M ESPE Z250 and Silorane perform comparably in Class II posterior restorations with regards to surface wear, margin integrity and margin discolouration. Deterioration of the restorations seen over time indicates the need for a longer observation period to assess the clinical significance of these changes.

The support of the Australian Dental Research Foundation for assistance with this project is greatly appreciated.

The findings of this research were presented at The University of Queensland School of Dentistry Research Day 2009.

*School of Dentistry, The University of Queensland.
Email: ian.meyers@uq.edu.au

The effect of simulated dentine tubule fluid on remineralization of demineralized dentine: using AFM-based nanoindentation to investigate the mineralized dentine

MA Vu Thanh,* JM McIntyre,* L Richards,* R Zoehrer†

The aim of this study was to determine whether increased strontium/calcium level increased nanohardness in remineralized dentine.

Class one cavities of similar dimension were cut into the occlusal surface of 12 extracted, intact third molar teeth. These teeth were painted with nail varnish, leaving the cavity floors exposed for the generation of artificial caries for 7 days, 14 days and 21

days of demineralization. One-half of the demineralized cavity floor was protected with nail varnish (control side) and Fuji IX restorations placed. The remaining demineralized dentine contacted to Fuji IX became the test sides. A reservoir was connected to the individual tooth to supply simulated dentine tubular fluid (SDTF) under recognized hydrostatic pressure to each pulp chamber for six weeks. During this per-

iod, ion-exchange proceeded between Fuji IX and demineralized dentine in the presence of SDTF. After six weeks, teeth were detached from the system, sectioned and embedded in epoxy resin for polishing. Samples underwent nanohardness analysis and were then carbon coated for electron probe analysis to determine profiles of Ca, P, F and Sr.

Relative weight percent of calcium and phosphorus on the test sides were greater than those on the control sides in most cases (significant at 5% level). Nanohardness was greater at lesion front of most samples. High nanohardness values coincided with high levels of strontium at lesion front. Calcium

changes did not seem to affect the nanohardness values.

The increase in calcium and other elements (strontium, fluorine, phosphorus) showed a positive trend of remineralization of the demineralized dentine, leading to an increase in nanohardness at lesion front. Comparing this data with those from previous *in vitro* studies, it was concluded that SDTF in the presence of Fuji IX restorations enhanced the remineralization process and hence increased nanohardness at the lesion front of remineralized dentine.

The support of the Australian Dental Research Foundation is gratefully acknowledged.

*School of Dentistry, The University of Adelaide, South Australia.
†School of Chemical and Physical Sciences, Flinders University, South Australia.
Email: myanh.vuthanh@adelaide.edu.au

The findings of this research were presented at the International Association for Dental Research meeting, Boston, USA, March 2015.

A five-year retrospective assay of implant treatments and complications in private practice: the restorative complications of single and short span implant supported fixed prostheses

HJ Wang,* RB Judge,* D Bailey†

This report aims to describe the restorative outcome of 5491 implant supported single crowns, fixed partial dentures (FPD) and splinted restorations which were prescribed or had implant placement during the study period. Timing of the complications and the relationship between the complications and different factors (practitioner, patient and restoration) were examined.

Dental clinicians qualified on or before December 2004 registered in Victoria, and placing and/or restoring implants in private practice were invited to participate in the study. Data extraction was conducted by two trained and calibrated research assistants. Prostheses average time observed/in function was calculated by using the difference between the definitive restoration date and the patient record examination date or the date of implant/restoration lost. Both descriptive statistic and generalized linear mixed modelling were used to describe the restorative complications.

Over the study period a total of 499 mechanical complications were recorded. Single implant crowns had the largest sample size (4760) and recorded the rate of complication at 2.56 per 100 prostheses per

year. In single implant crowns abutment screw loosening occurred at the rate of 0.07 per 100 per year while unspecified screw loosening occurred at the rate of 0.53. Lateral screw loosening was more common in lateral screw retained implant crowns (1.06) than decementation was in cement retained implant crowns (0.57). Aesthetics (0.25), veneer chipping or fracture (0.41) and food packing/contact point issues (0.53) also represent significant part of the restorative complications. Clustering within the first year was common. The ratio of screw loosening between the group who prescribed between 1 to 100 during the study period and those who prescribed more than 501 implant restorations was 1:0.15 ($p = 0.005$). Patient with operator reported attrition had double the rate of veneer fracture ($p = 0.005$). Contact point issues occurred approximately 3 times more commonly in the posterior segment ($p = 0.001$).

During the period January 2005 to December 2009 the complications of screw loosening, lateral screw loosening, decementation, aesthetic complication, veneer chipping or fracture and food packing/contact point issues were recorded at different rates for differ-

ent types of prosthesis in the private practices included in this study. Clusters of several complications within the first year were observed. For single implant crowns, screw loosening complications were less frequent in the more experienced group. Operator reported attrition was related to higher rate of veneering material frac-

*Prosthodontics Department, Melbourne Dental School, The University of Melbourne, Victoria.

†Oral Health CRC, Melbourne Dental School, The University of Melbourne, Victoria.

Email: roybj@unimelb.edu.au

A five-year retrospective assay of implant treatments and complications in private practice: the restorative treatment profiles of single and short span implant supported fixed prostheses

HJ Wang,* RB Judge,* D Bailey†

The aim of this study was to describe and analyse the restoration profiles of 5491 implant supported single crowns, fixed partial dentures (FPD) and splinted restorations which were prescribed during the period January 2005 to December 2009.

Dental clinicians qualified on or before December 2004 registered in Victoria, and placing and/or restoring implants in private practice were invited to participate in the study. Data extraction was conducted by two trained and calibrated research assistants from dental records. Cross-tabulation was carried out in SPSS™ to provide descriptive statistics on clinician profiles, patient demographics and restoration information.

A total of 34 practitioners participated in the study; 25 general dentists and 9 specialists. Clinicians that graduated between the years 1970–1989 (16–40 years' experience) prescribed most of the prostheses (82.0%). Female patients were present in higher numbers across most age and restoration groups. 5491 of implant supported prostheses fitted the criteria of 'simple basic restoration' to be included in this report. The majority of the prostheses (86.7%) were single

tooth restorations. Metal ceramic was the material of choice for the majority of the restorations (3382/5491). The use of implant single crowns in the anterior mandible was rare. Over 65% of the implant supported restorations in this study were direct to fixture screw retained. The specialist clinicians within this study largely used the screw retained method to retain single implant crowns (82.0%). As a ratio general dentists provided 1066% more cement retained single implant crowns.

This abstract is based on research that was partially funded by the eViDent Foundation and the Australian Dental Research Foundation (ADRF).

Published: Wang HJ, Judge RB, Bailey D. A five-year retrospective assay of implant treatments and complications in private practice: the restorative complications of single and short span implant supported fixed prostheses. Int J Prosthodont (in press).

tooth restorations. Metal ceramic was the material of choice for the majority of the restorations (3382/5491). The use of implant single crowns in the anterior mandible was rare. Over 65% of the implant supported restorations in this study were direct to fixture screw retained. The specialist clinicians within this study largely used the screw retained method to retain single implant crowns (82.0%). As a ratio general dentists provided 1066% more cement retained single implant crowns.

This report showed during the period January 2005 to December 2009, private practice clinicians in Victoria, Australia favoured PFM material over all ceramics for implant restorations. Most implant restorations were single crowns. More female patients received implant restorations in the posterior region of the mouth. The clinicians in this study, particularly the specialists, generally favoured a retrievable design in choosing the method of retention for the prostheses.

This abstract is based on research that was partially funded by the eViDent Foundation and the Australian Dental Research Foundation (ADRF).

Published: Wang HJ, Judge RB, Bailey D. A five-year retrospective assay of implant treatments and complications in private practice: the restorative treatment profiles of single and short span implant supported fixed prostheses. Int J Prosthodont (in press).

*Prosthodontics Department, Melbourne Dental School, The University of Melbourne, Victoria.

†Oral Health CRC, Melbourne Dental School, The University of Melbourne, Victoria.

Email: roybj@unimelb.edu.au

Chemotherapy causes tight junction defects in the oral cavity of patients, which coincide with elevated proinflammatory cytokines and MMPs

HR Wardill,* RM Logan,† JM Bowen,‡ RJ Gibson*

Modification of tight junction (TJ) proteins following chemotherapy is well-documented. In the setting of mucositis, the interaction between proinflammatory cytokines, matrix metalloproteinases (MMPs) and TJs is compelling given the strong inflammatory component of mucositis. We have previously shown chemotherapy is able to modulate tight junction expression in the gut; however, the effect of chemotherapy on oral epithelial TJs remains unclear.

Our study determined the molecular integrity of oral epithelial TJs in patients receiving chemotherapy and correlated these with changes in proinflammatory cytokines and MMP profiles.

Archival patient samples were used. Patients (n = 23) undergoing chemotherapy were recruited from the Royal Adelaide Hospital between 2000 to 2003. Patients had two buccal biopsies; one prior to chemotherapy and one up to 11 days after chemotherapy. Buccal biopsies were also taken from five healthy volunteers. Standard H&E analysis was performed and epithelial thickness quantified. Immunohistochemical analysis was conducted for three TJ proteins (occludin, claudin-1, ZO-1), IL-1 β , IL-6, TNF, MMP-2 and MMP-9.

Significant epithelial atrophy was seen in cancer patients both prior to ($p = 0.0002$) and following chemotherapy ($p < 0.0001$). Epithelial thickness prior to chemotherapy correlated with the number of previous chemotherapy cycles patients underwent ($r^2 = 0.66$). Increased expression of proinflammatory cytokines and MMP-2 was seen in patients following chemotherapy corresponding with decreased expression of ZO-1, particularly in the basal epithelium. Redistribution of ZO-1 staining from the membrane

to the cytoplasm was also seen. Altered distribution of claudin-1 was also seen in the basal epithelium following chemotherapy.

Histopathology: our study identified a strong correlation between epithelial thickness and the number of previous chemotherapy cycles each patient had undergone. This suggests epithelial atrophy observed in patients prior to chemotherapy is likely to be due to residual damage from previous chemotherapy treatment.

Immunohistochemical: although no significant changes in staining intensity were seen, redistribution of the TJ proteins ZO-1 and claudin-1 was clearly evident. This is a well-documented aspect of TJ modulation, and is known to alter both TJ integrity and function. Importantly, the TJ defects seen in patients coincided with elevations in proinflammatory cytokines and MMP-2 indicating possible interaction. These results are consistent with changes in the gut following chemotherapy highlighting common mechanisms for both oral and gastrointestinal manifestations of mucositis.

Chemotherapy causes significant epithelial atrophy in the oral epithelium and is coupled with disruption of TJs, elevated proinflammatory cytokines and altered MMP profiles. The interaction between cytokines, MMPs and TJs warrants further investigation.

This study was funded by the Australian Dental Research Foundation.

Published online: Wardill HR, Logan RM, Bowen JM, Van Sebille Y, Gibson RJ. Tight junction defects are seen in the buccal mucosa of patients receiving standard dose chemotherapy for cancer. Support Care Cancer 2015; doi: 10.1007/s00520-015-2964-6 [Epub ahead of print].

The findings of this research were presented at the Multinational Association in Supportive Care in Cancer 2015 Symposium.

*Discipline of Anatomy and Pathology, School of Medicine, The University of Adelaide, South Australia.

†School of Dentistry, The University of Adelaide, South Australia.

‡Discipline of Physiology, School of Medicine, The University of Adelaide, South Australia.

Email: richard.logan@adelaide.edu.au

ADRF Research Grant Reports published as full papers in the *Australian Dental Journal* in 2015

Aust Dent J 2015;60:12–17

Nanoscratch testing for the assessment of enamel demineralization under conditions simulating wine erosion
SXR Kwek, M Mian, C Hall, Z Xie, R Yong, J Kaidonis, GC Townsend, S Ranjitkar

Aust Dent J 2015;60:294–300

The shortened dental arch concept: awareness and opinion of dentists in Victoria, Australia
MA Abuzar, AJ Humplik, N Shahim

Aust Dent J 2015;60:301–308

Evaluation and prevention of enamel surface damage during dental restorative procedures
T Milic, R George, LJ Walsh

Aust Dent J 2015;60:426–433

Odontogenic facial swelling of unknown origin
S Ranjitkar, W Cheung, R Yong, J Deverell, M Packianathan, C Hall

ADRF Dental Student Research Grant Abstracts

Comparison of facial characteristics between adolescent orthodontic patients prior to treatment and their parent

D Deall;* A Docherty,* M Skilbeck,* MA Darendeliler† (Supervisors)

There is a growing body of evidence that suggests facial soft tissue characteristics are heritable. This study aims to identify if there is a correlation between parent and offspring facial features, the degree and variation of such correlation and if child age influences it. This information may be used in the treatment planning of adolescent orthodontic patients by using their parents' soft tissue characteristics as a predictor of growth.

Following ethics approval, 178 cases were accessed from the Sydney Dental Hospital Orthodontic Department's photographic records. Each case contained lateral and frontal photographs of the patient and one parent. Following exclusion there were 101 cases and comparisons were made between children and their

parent based on 12 facial soft tissue anthropometric variables. Child soft tissue facial characteristics were correlated with their parent's using Pearson's correlation and linear regression.

All variables except mandibular prominence, middle facial height:lower facial height and nasofrontal angle were significantly (p -value <0.05) positively correlated between children and parents. Mothers had a more significant positive correlation with their offspring than fathers; however, the data collected for fathers demonstrates limitations due to low case numbers.

A considerable number of soft tissue characteristics of the face correlated strongly between child and parent, suggesting heritability. Mothers tended to correlate stronger with their offspring than fathers. Child age had no influence over correlations between children and parents. This research will hopefully provide the basis for future studies into the correlation of soft tissue characteristics with treatment outcomes and failures.

*Final year dental student and investigator, Faculty of Dentistry, The University of Sydney, New South Wales.

†Professor and Head of Discipline of Orthodontics, The University of Sydney, New South Wales.
Email: ali.darendeliler@sydney.edu.au

This abstract is based on research that was funded entirely by the Australian Dental Research Foundation. Support with statistical analysis was provided by

Peter Petocz, Associate Professor of Statistics, Department of Statistics, Macquarie University, Sydney, Australia.

Content analysis of nutritional information in paediatric oral health education leaflets

JN Doan; A Arora (Supervisor)*

The aim of this study was to determine if the nutritional content in Australian paediatric oral health education leaflets complied with the Australian Dietary Guidelines and Infant Feeding Guidelines.

Thirty leaflets aimed at parents were sourced in 2013 and a content analysis was performed. Recommendations on food and drink type, consumption frequency and general diet and nutrition advice were considered and cross-referenced with the Australian Dietary Guidelines to identify areas of consistency and discrepancy.

Thirteen leaflets (43%) recommended reducing the consumption of sugary and/or acidic food, while 17 leaflets (57%) recommended reducing the consumption of sugary and/or acidic drinks. The majority of the leaflets advised water ($n = 26$) and milk ($n = 16$)

to drink. Although 23 leaflets encouraged a healthy diet, five did not specify what a healthy diet was. Twenty-five leaflets aimed at parents of infants (0–2 years) included advice on bottle feeding. Four leaflets provided general information about oral health and nutrition but did not follow up with any recommendations. Confusing messages were found across the range of leaflets, with ambiguous recommendations that were open to individual interpretation.

While there were some consistent recommendations across all leaflets, there were also vague messages that may confuse parents. Regulatory authorities, such as health departments, should ensure that nutritional messages are consistent across dental leaflets and with relevant national guidelines.

The study was supported by National and Health Medical Research Council funds (1033213 and 1069861) and an Australian Dental Research Foundation Dental Student Research Grant.

*Faculty of Dentistry, The University of Sydney, Westmead, New South Wales.
Email: a.arora@westernsydney.edu.au

Touch-imprint cytology of impacted third molars to detect the presence of inflammation

CS Franco;* KR Iyengar,† YH Gim,* A Ariyawardana* (Supervisors)

The aim of this study was twofold. Firstly, to identify the presence of inflammatory cells from the roots of extracted impacted third molars to compare the findings with non-impacted teeth and to correlate clinical and radiographic findings through the touch-imprint cytology (TIC) method. Secondly, to ascertain the usefulness of TIC as an alternative method to recognize inflammatory cells on root surfaces of extracted teeth.

A prospective clinical, radiographic and cytopathological study was undertaken using roots of 45 permanent posterior teeth extracted for various indications in 34 patients. Two groups, those undergoing extractions of a third molar and those undergoing extrac-

tion of any other posterior tooth were assessed. Imprint smears were examined by a pathologist blinded to clinical and radiographic findings.

The TIC method detected inflammation in 56% of teeth with clinically and radiographically confirmed pathologies. The presence of neutrophils, lymphocytes and histiocytes were statistically significant in teeth with periapical lesions compared to those without ($p < 0.05$). There was a statistically significant association between the presence of neutrophils and abnormal periodontal ligament space ($p < 0.05$). By cytologic assessment alone, the presence of bacteria in inflammation was marginally significant ($p = 0.052$) in comparison to those samples without inflammation.

The results of this study suggest a correlation between the presence of inflammatory cells and abnormal clinical and radiographic findings. Therefore, it can be concluded that TIC is a simple alternative to histopathology in identifying inflammation associated

*College of Medicine and Dentistry, James Cook University, Cairns, Queensland.

†Anatomical Pathology, Queensland Health.

Email: rkrish66@gmail.com

Environmental stress affects development: evidence from the dental morphogenesis in a Romano-British population

KSB Koh,* VKL Toh;* AH Brook*† (Supervisor)

The human dentition shows general characteristics of a complex adaptive system; interactions at a molecular level of genetic, epigenetic and environmental factors lead to the emergence of tooth germs. Dental development also records environmental influences from 6 weeks *in utero* to 20 years of age, and is therefore a valuable paradigm of general development. The main aims of this project are to evaluate the data from studies of the dentition of a group of Romano-Britons, comparing them to modern Britons, to investigate the effects of the 3 identified major environmental factors on dental and systemic development, and to explore the possible mechanisms of their effect.

The data evaluated was from the British Museum of Natural History, and consisted of 486 skulls with complete dentitions from Poundbury, Dorset, UK, living between 200–400 AD. Visual examination and direct measurements had been made for tooth number, size, shape and enamel defects. Using radiographs, the number of teeth formed and the root size and shape had been confirmed. An extensive search of the literature on factors in environmental stress, their interactions and mechanisms of action was now undertaken.

The findings were that the patterning of the dentition, the sexual dimorphism and the types of developmental defects were similar to modern Britons. However, the Romano-Britons had a threefold increase in hypodontia and microdontia, smaller tooth size and

with periapical pathology. However, small sample size is the main limitation of this study, and therefore it is recommended that an expanded study be undertaken with a larger sample.

This study was funded by the Australian Dental Research Foundation Inc (Grant no. 8-2013-2014). The authors acknowledge and thank Sullivan Nicolaides Pathology of Cairns, Queensland, for their invaluable assistance in this study.

changes in shape, smaller root size and changes in root shape, and a threefold increase in enamel hypoplasia. The literature search revealed valuable new information on the effects, and interactions of the major environmental insults.

The effects of environmental stress, arising from excess lead ingestion, poor nutrition, and recurrent infections, occurred over a long period of time and affected tissues with different genetic backgrounds. The Romano-British dentition showed evidence of insults occurring at all stages of tooth development. The environmental insults showed synergistic effects, and also probably affected the immune and endocrine systems. Together, these changes gave rise to the variation in tooth number, crown size and shape, root morphology and deposition of enamel matrix.

This study demonstrates the value of investigating the dentition as a complex adaptive system and as a model for general development.

We thank Theya Molleson of the British Museum of Natural History who enabled access to the material and gave guidance in the original studies. A grant from the Australian Dental Research Foundation (ADRF) was awarded to the first author.

Preliminary findings of this research were presented at the Australasian Society for Human Biology (ASHB) conference 2014, Glenelg, Adelaide, Australia. Further results were presented at the Wessex Institute of Technology Complex Systems 2015 conference, Southampton, United Kingdom, and at the IADR ANZ Conference 2015, Dunedin, New Zealand. A full referenced paper is published in the International Journal of Design & Nature and Ecodynamics 2015.

*School of Dentistry, The University of Adelaide, South Australia, Australia.

†Institute of Dentistry, Queen Mary University of London, United Kingdom.

Email: alan.brook@adelaide.edu.au

Mandibular morphology in mutant glypican 1- and glypican 3- knockout mice: a micro-CT investigation

M Mian,* S Ranjitkar,* GC Townsend,* T Hughes,* PJ Anderson*† (Supervisors)

Glypicans are a group of six proteoglycans known to act as coregulators of bone formation. Two of these molecules, glypicans 1 and 3, have recently been implicated in preventing craniosynostosis via inhibition of the bone morphogenic proteins. By using a knockout-mouse model to investigate these novel molecules, the objectives of this study were: (1) to assess the validity and reliability of micro-CT against traditional caliper based measurements in murine craniofacial cephalometrics; (2) to determine the effects of glypicans 1 and 3 on mandibular bony morphology; and (3) to determine the effect of genetic background (strain purity) on the mandibular phenotype of the GPC3-knockout mouse.

The mandibular morphology of wildtype, GPC1-knockout, GPC1/GPC3-knockout and GPC3-knockout groups at 72% and 43% strain purity (N = 40) was compared through analysis of five landmark based 3D linear dimensions: anterior and posterior lengths and ascending, descending and posterior heights. Measurements were recorded by 3D microcomputed tomography (micro-CT) and verified with hand calipers.

Micro-CT and caliper analysis showed similar levels of technical error (0.3% of total variance), and the difference between the two techniques were insignificant ($p > 0.05$). GPC3-knockout mandibles were sig-

nificantly larger compared with wildtype for all dimensions, with mandibular height being most affected ($p < 0.05$). Analysis of GPC1-knockout and GPC1/GPC3-knockout mandibles indicated that GPC1 was attenuating the effect of GPC3. Genetic background had significant effects of the expression of the GPC3-knockout phenotype; with 72% C57Bl strains showing increased descending height compared with 43% C57Bl mice.

Micro-CT is an excellent method of analysis in murine cephalometrics, with a high degree of validity and reliability. GPC3-knockout mandibles showed alterations in size and growth patterns consistent with the dentofacial characteristics of analogous human mutations. Our investigations of glypican 1 and glypican 3 provide novel insights into the effects and interactions of these molecules on a macroscopic scale and have the potential to assist in the development of molecular-based strategies for future treatment of craniosynostosis.

This project was supported by the Australian Dental Research Foundation Inc. in the form of an undergraduate research scholarship to the first author.

The findings of this research were presented at the 10th Asia Pacific Craniofacial Conference; the 36th Australian Dental Congress – SCADA Competition (1st Prize); the 2015 University of Adelaide, School of Dentistry Research Day; and the 2015 Beacon Conference of Undergraduate Research, University of Adelaide.

*School of Dentistry, The University of Adelaide, South Australia.

†Australian Craniofacial Unit, Women and Children's Hospital, Adelaide, South Australia.

Email: haemro2@hotmail.com

A scanning electron microscope pilot evaluation of novel phosphoric acid preparations and techniques for enamel etching

P Tran; C Tran, LJ Walsh (Supervisors)*

This *in vitro* study using scanning electron microscopy (SEM) investigated whether alterations in etchant solutions or in agitation techniques influence the surface changes created in human enamel.

A total of 40 permanent human molars were selected and prepared with windows of acid resistant varnish to create control and experimental regions. Specimens were allocated randomly into 8 experimental groups, which varied by both the etching solution used and/or

the use of agitation. Etchant solutions were 37% phosphoric (orthophosphoric) acid only, 37% phosphoric acid enriched with 3% (0.48 M) acetic acid, and 37% phosphoric acid enriched with 10% (0.48 M) citric acid. The agitation techniques included no agitation, microbrush agitation or ultrasonic agitation. Specimen surfaces were examined using SEM.

Etching of enamel surfaces resulted in observable surface changes that were predominantly character-

ized by one of three Silverstone etch patterns. SEM evaluation of acetic acid modified phosphoric acid and citric acid modified phosphoric acid revealed changes in enamel surface morphology similar to those of phosphoric acid only but were variable as to the outcome of Silverstone etch pattern. The effect of agitation with a microbrush or ultrasonic tip did not give a consistent change in the Silverstone etch morphology.

Based on the SEM profiles, both plain phosphoric acid, and the same solution enriched with acetic acid

or citric acid will give a morphologically roughened and acid etched surface. The benefits of the change in etchant composition or agitation technique were not consistent in terms of the surface morphology created, so it is necessary to now apply profilometry techniques to evaluate the surface changes at a greater level of detail and to quantify them. Trials of bonding to these surfaces would be needed to establish whether any subtle changes in surface morphology as a result of alternative etchant composition or agitation technique affect adhesion of resin materials.

This work was supported by a dental student research scholarship from the Australian Dental Research Foundation Inc.

*School of Dentistry, The University of Queensland.
Email: l.walsh@uq.edu.au

Root canal treatment success factors in regional Australia from 2009 to 2014: a retrospective study

JJ Zachar; A Sadr, NM Hassan, R Akhter (Supervisors)*

This project retrospectively examines dental health from periapical radiographs (with regard to periapical status, quality of root canal fillings and coronal restorations) in an adult population in regional Australia, and determines whether the quality of the coronal restoration is more significant than the quality of root canal treatment in eliminating apical periodontitis.

Participants were recruited from the Charles Sturt University Dental Oral Health Clinic in Orange and Bathurst that had received root canal treatment by an undergraduate dental student in the period 2009–2014. They were contacted by mail and phone for a follow-up review appointment. The recall appointment included a clinical examination of the root canal treated tooth, as well as having a follow-up periapical radiograph taken to determine the postoperative success of the treatment. The radiographic data was deidentified by a non-assessor in a double-blinded fashion and then calibrated by two assessors. The periapical status, the length and homogeneity of the root canal fillings and coronal restoration was reviewed on all root canal treated teeth ($n = 88$) and were statistically analysed using the chi-squared test.

The correlation between the coronal seal and the quality of the root canal filling variables demonstrated no significant difference ($p > 0.005$) in eliminating apical periodontitis. The 88 root canal treated teeth (25 anterior and 63 posterior) only showed signs of either, an absence of periapical radiolucency or a decrease in size ($AP = 1$) and a size of no more than 3 mm (size of $AP = 1$). Radiographically, 70.7% of the teeth had an adequate length (>2 mm from radiographic apex), 48.3% had an adequate density and 68.5% demonstrated an adequate coronal restoration.

There was no significant relationship in whether the coronal restoration or the quality of the root canal filling had a greater impact in eliminating apical periodontitis (AP). A longer retrospective time period and a larger number of root canal treated teeth for analysis is recommended for future studies within this field in order to determine a more definitive result. Overall, the findings of this study do not indicate that either factor should be undervalued in relation to the other as all root canal treated teeth showed signs of success within the nature of the short retrospective timeline.

This research project was funded entirely by the Australian Dental Research Foundation Dental Student Grant and supported by the clinical facilities provided by the Charles Sturt University School of Dentistry and Health Sciences, Dental and Oral Health Clinic.

*School of Dentistry and Health Sciences, Charles Sturt University, Orange, New South Wales.
Email: asadr@csu.edu.au

A retrospective analysis of the short-term survival of zirconia and porcelain fused-to-metal crowns fitted in a university dental clinic

C Zhang; MR Baig, AT Flatau (Supervisors)*

The study aimed to assess the 6–24 month clinical outcomes of zirconia-based crowns and conventional PFM (porcelain fused-to-metal) crowns in a university dental clinic.

The sample study included patients treated with porcelain-veneered zirconia single crowns, monolithic zirconia crowns and PFM crowns. These crowns were issued in the Charles Sturt University dental clinic by clinical undergraduate students during the period January 2012 to July 2014. Thirty-one patients were interviewed regarding patient satisfaction and 50 crowns were clinically examined at the 6–24 month recall appointment.

Three out of five abutment teeth with zirconia monolithic crowns exhibited BOP (bleeding on prob-

ing). Eleven teeth with zirconia layered crowns out of 20 crowns in this category exhibited BOP and one crown showed early sign of dental caries. In comparison, 8 out of 20 abutment teeth with PFM crowns exhibited BOP and none had any sign of dental caries. Two 3-unit metal ceramic FDPs had more problematic clinical outcomes compared with single crowns. The most superior clinical outcomes were achieved with full metal coverage crowns. No significant adverse outcomes were found with these crowns.

With respect to short-term survival, zirconia-based crowns were favourably comparable with metal ceramic crowns.

This abstract is based on research that was funded by the Australian Dental Research Foundation Inc. and supported by Professor David Wilson (former Head of School), Dr Heather Cameron (Clinical Director) and Charles Sturt University clinic staff from both Orange and Bathurst clinics.

*School of Dentistry and Health Sciences, Charles Sturt University, Orange, New South Wales.
Email: mbaig@csu.edu.au

Colin Cormie Scholarships

Knowledge, behaviours and attitudes of mothers regarding vertical transmission of *mutans streptococci*: a qualitative case study

A Bansal,* J Mills,† R Boase* (Supervisors)

Mutans streptococci (MS) are the key bacterial species responsible for the development of early childhood caries. Studies have shown that these bacteria can be transmitted from mother to child via a pathway known as vertical transmission. However, a paucity of research has been found on mothers' knowledge, behaviours and attitudes of this issue. The aim of this research project was to determine the knowledge, behaviours and attitudes of mothers regarding vertical transmission of MS from mother to child, and to determine external influences on mothers' awareness of this issue. To assess this, a qualitative case study methodology of an exploratory nature was chosen. The case was defined as mothers' awareness regarding vertical transmission of MS from mother to child.

Several embedded units of analysis were identified as being relevant to the chosen case. These included

25 mothers, 2 oral health therapists, 1 government report and 2 health promotional documents. All data were manually coded and analysed.

It was found that mothers' awareness of vertical transmission of MS was minimal when compared to other risk factors for dental caries. Behaviours promoting vertical transmission are widely practised – sharing of utensils and food, cleaning the child's dummy in the mother's mouth and kissing on the mouth. Also, most mothers reported that had they known earlier about vertical transmission, they would have changed their behaviours and attitudes. The oral health therapists also reported similar patterns. Despite the lack of information regarding this issue, there were minimal documents from government and dental associations which promoted awareness of vertical transmission of MS from mother to child.

Further research is recommended to investigate the relationship between the accessibility of promotional documents and education by oral health professionals regarding vertical transmission of MS, and the awareness and action taken by mothers. This should be conducted using a quantitative approach which is statistically representative of a larger population. Results will aid in the formulation of appropriate health promotion and educational strategies to target mothers. By doing so, it is hoped that the likelihood

of early MS colonization in child will be reduced, thus delaying the onset of dental caries.

We would like to thank the Australian Dental Research Foundation for awarding the author the 2014 Colin Cormie Research Grant. Also, Dr Robert Game and James Cook University (JCU) Dental. In addition, the 2012 fourth year JCU Dentistry student research group: Aaron Tamayo, Mischa Dummel, Cornelius Richter and Mayumi Inaba.

The findings of this research were presented at the North Queensland Festival of Life Sciences 2014 Division of Tropical Health and Medicine – ‘so you think you can research’ student research presentation competition, Townsville, 9 October 2014.

*College of Medicine and Dentistry, James Cook University, Queensland.

†College of Healthcare Sciences, James Cook University, Queensland.

Email: jane.mills@jcu.edu.au

Immunohistochemical investigation of toll-like receptor expression in a rat model of chemotherapy-induced mucositis

H McInnes,* A Stringer,† B Mayo,† R Logan*

Alimentary mucositis (AM) is a frequent and potentially debilitating side-effect of chemotherapy and radiotherapy, with serious clinical and economic consequences. Therapies to prevent and manage AM remain limited, as does understanding of its pathogenesis. This project aims to elucidate the involvement of toll-like receptors (TLRs) at various time points in the development and progression of mucositis. TLRs are activators of the innate immune system, and are involved in the initiation and ulceration phases of AM. Given its small sample size, this study is intended as a pilot project for further research in this area.

This study tested archival samples of buccal mucosa from tumour-bearing Dark Agouti rats treated with the chemotherapeutic agent irinotecan (n = 27). A control group received the chemotherapy drug vehicle, without the active ingredient (n = 2). The rats were euthanized in groups at time points of 6, 24, 48, 72, 96 and 120 hours after drug administration. Samples of buccal mucosa were collected and standard immunohistochemical techniques were used to identify TLRs 2, 4, 5 and 9 in the tissues. Slides were visually assessed by a single examiner in a blinded fashion, using NanoZoomer Digital Pathology software. A

staining intensity score from zero (no stain) to four (intense stain) was assigned.

The control group had mean staining intensity grades of 1.5 (TLRs 2 and 4), 2.5 (TLR 5) and 1 (TLR 9). TLR 2 and 9 expression peaked at 24 hours (grade 2.5 and 2.67 respectively). TLR 5 expression was varied throughout the time groups, with the control group at greatest intensity. TLR 4 expression was highest at the 6-hour time point (grade 2.33).

TLRs have been implicated in the pathogenesis of AM by a number of previous studies. Better understanding their role may enable development of targeted interventions for prevention and management of mucositis. The overall value of the results produced in this study is diminished by its significant limitations. No conclusions can be drawn from the present data. However, as a pilot project, this study was valuable in demonstrating weaknesses in project design and execution that should be avoided in future studies of this type.

Helen McInnes received grants from the Australian Dental Research Foundation and the Royal College of Pathologists of Australasia. Andrea Stringer was supported by a National Health and Medical Research Council Early Career Fellowship. Bronwen Mayo is supported by a scholarship from the University of South Australia.

The findings of this research were presented at the Australian Society for Medical Research South Australia meeting, Adelaide, 2014.

*School of Dentistry, The University of Adelaide, South Australia.

†School of Pharmacy and Medical Sciences, The University of South Australia.

Email: hmcinnes89@gmail.com

Toll-like receptors and oral microflora in mucositis: report of a cell culture experiment in a rat model

H McInnes,* A Stringer,† B Vanhoecke,‡ B Mayo,† C Lucchesi,§ R Logan*

Mucositis is a common side-effect of cytotoxic cancer therapy, affecting both oral and gastrointestinal mucosa. Toll-like receptors (TLRs) are immune system activators implicated in the pathogenesis of mucositis. The study objectives are to investigate expression of TLRs and the influence of oral microflora in chemotherapy-induced mucositis.

Non-transformed rat intestinal epithelial cells (IEC-6) were seeded into culture plates. Transwell® permeable membrane inserts were suspended over each well. Buccal swab microbial samples (healthy patient) in brain heart infusion broth were added to the inserts, and SN-38 (irinotecan active metabolite) was added to the wells, in the following configurations for each plate:

- (1) 3 wells with both microbial sample and SN-38;
- (2) 3 wells each with either the microbial sample or SN-38;
- (3) 3 double negative controls.

After incubation, samples were prepared and SYBR-Green based RT-PCR was performed. Pfaffl's model for relative quantification was used. Results were subjected to one- or two-way ANOVA and Tukey's multiple comparison tests.

TLR2: At 24 hours, SN-38 was associated with significantly increased expression of TLR2 (>4 fold increase, $p < 0.05$, 95% CI).

TLR4: At 24 hours, the microbes-only group showed a 1.5 fold increase in TLR4 expression when compared to the negative control ($p < 0.05$, 95% CI). However, at 48 hours, results were reversed, with microbe presence associated with significantly lower expression of TLR4 ($p < 0.05$, 95% CI).

TLR5: At 48 hours, the microbes-only group showed a 3.6 fold increase in TLR5 expression compared to the negative control ($p < 0.0001$, 95% CI). However, in the presence of chemotherapy, the addition of microbes did not significantly alter TLR5 expression.

This study suggests that TLR2 expression is significantly increased by SN-38 in this model. While TLR4 expression was increased by the presence of microbes in the 24-hour group, an inverse relationship was noted at the later time point. TLR5 expression was increased by the presence of microbes in the 48-hour group, but this difference was negated by the addition of SN-38. Further research is required to better delineate the relationship between TLRs and resident flora in chemotherapy-induced mucositis.

Helen McInnes received grants from the Australian Dental Research Foundation and the Royal College of Pathologists of Australasia. Andrea Stringer was supported by a National Health and Medical Research Council Early Career Fellowship. Bronwen Mayo is supported by a scholarship from The University of South Australia. Vanhoecke's research leading to these results has received funding from the Seventh Framework Program (FP7/2011) under grant agreement no. 299169 (Mucositis Platform).

The findings of this research were presented at the International Association for Dental Research ANZ meeting, Brisbane, 2014.

*School of Dentistry, The University of Adelaide, South Australia, Australia.

†School of Pharmacy and Medical Sciences, The University of South Australia, Adelaide, Australia.

‡Laboratory of Microbial Ecology and Technology, University of Ghent, Belgium.

§Faculty of Sciences, The University of Adelaide, South Australia, Australia.

Email: hmcinnes89@gmail.com

GC Australasia Dental Pty Ltd, Minimum Intervention Awards

Patients' and dental student-clinicians' demographics: are these associated with patients' ratings of communication?

T Winning,* A Kinnell,* M Stanners,* D Schönwetter†

Demographic factors (age, gender, ethnicity) have been reported to influence the dynamics of clinical communication in medicine. However, there has been only limited investigation of these factors in oral healthcare settings. Understanding which factors influence communication dynamics is important to inform our oral healthcare curricula. This study aimed to investigate patterns of variation in the quality of communication by dental student-clinicians as assessed by their patients.

After a minimum of two appointments, patients (n = 922; 55.3% female; 77% Australian:European and European; 64% >50y) who received care from student-clinicians (n = 183; 59% female; 74% Asian and Australian:Asian) at the Adelaide School of Dentistry completed the dental-specific Patient Communication Assessment Instrument (PCAI; University of Manitoba) (ethics approval: UA: H-071-2009). This instrument consists of four subscales: caring and respect, sharing information, tending to patient comfort, and student/team interaction. Patient care experience for student-clinicians varied, ranging from 15 weeks (3rd y: 2009, 2012, 2013) to 60 weeks (5th y: 2009, 2011). Ratings of student-clinicians' communication were included provided at least three patients had rated their student-clinician. Percent excellent ratings for the four PCAI subscales were calculated. Multi-level modelling was used to identify associations between patient ratings of their student-clinician's communication, and patients' gender, age, education, and ethnicity, and student-clinicians' gender and ethnicity ($p < 0.05$).

Patients rated female student-clinicians' communication for the subscale 'caring and respect', significantly

higher than male student-clinicians. Students who had participated in the revised communication curriculum received significantly higher patient ratings across three out of the four subscales compared with students from the previous curriculum (subscales: caring and respect, tending to patient comfort and student/team interaction). For two subscales (sharing information and student/team interaction), older patients rated student-clinicians' communication significantly lower. Discordant patient:student ethnicities were associated with lower patient ratings across several subscales.

Student gender, participation in the revised communication curriculum, patient age and discordant patient:student ethnicities were associated with variations in patient ratings of dental student communication. These findings are consistent with other healthcare professions in terms of factors associated with variations in communication experiences. Differences in patient and student 'explanatory models of sickness' may contribute to these preliminary results of variations in communication ratings related to age and ethnicity. Analyses of larger cohorts and other oral healthcare educational settings are needed to confirm these findings.

We wish to thank the patients and students; the Australian Dental Research Foundation; the Centre for Orofacial Research and Learning (The University of Adelaide); the Network for Oral Research Training and Health; and the Canadian Institutes of Health Research International Opportunity Program. This project was supported by the GC Australasia Dental Pty Ltd, Minimum Intervention Award 2012.

The findings of this research were presented at the European Association of Communication in Healthcare, September 2014.

*The University of Adelaide, South Australia.

†The University of Manitoba, Canada.
Email: tracey.winning@adelaide.edu.au

Synthesis of stabilized hydroxyapatite nanoparticles for enamel remineralization

M Shahmoradi,* F Sonvico,† M Ghadiri,‡ M Swain,* R Rohanizadeh‡

An important goal of contemporary dental research is to explore novel approaches for treating and remineralizing caries lesions without surgical drilling and filling. The aim of this study was to develop and evaluate a method for synthesizing a stable hydroxyapatite nanosuspension and to test and evaluate the remineralization efficacy of these nanoparticles for remineralization of artificial enamel lesions.

Hydroxyapatite (HA) particles were synthesized using wet chemistry by mixing Ca and PO₄ precursors at pH 10 and 80 °C. The mixture was aged for 2 hours and then filtered and dried using 100% ethanol. The prepared HA was characterized using X-ray diffraction (XRD) and scanning electron microscopy (SEM). A top-down approach was employed to prepare stable hydroxyapatite nanosuspension using high-pressure Emulsiflex-C5 valve homogenizer in the presence of different stabilizers. Particle size and size distribution of HA nanoparticles were determined using dynamic laser scattering (DLS) technique. The morphology and chemical elements of the nanoparticles were also determined using SEM, energy dispersive spectroscopy and atomic absorption. Using a demineralization acidic solution, artificial lesions were prepared on the extracted healthy teeth and then the teeth were remineralized in HA nanosuspension. Min-

eral density of lesions before and after remineralization treatment as well as the quantification of the remineralization treatment were carried out using a microcomputed tomography system and an image procession programme.

XRD confirmed the apatite structure of the prepared particles. The high-pressure homogenizer resulted in the formation of HA nanoparticles with 20 to 40 nm in size. This has been confirmed by SEM. Lesions treated with HA nanosuspension showed mineral gain in some areas of teeth. In most of the cases, the remineralization took place in areas with the lowest level of mineral content and highest amount of demineralization. However, in none of the remineralized teeth, complete or near complete remineralization leading to mineral recovery up to the level of sound enamel was observed.

In conclusion, we showed a novel and effective method for making a stable hydroxyapatite nanosuspension with particle size of 20 to 40 nm. We also showed the application of our proposed segmentation and quantification method for analysing dental MicroCT images. Finally, we showed levels of remineralization in artificial enamel lesions following treatment using the prepared hydroxyapatite nanosuspension. However, it seems that further optimization and development of new methods are needed for more effective remineralization of enamel lesions.

This abstract is based on research that was funded by a GC Australasia Dental Pty Ltd Minimum Intervention Award and the Australian Dental Research Foundation (ADRF).

*Faculty of Dentistry, The University of Sydney, New South Wales.

†School of Pharmacy, The University of Technology, Sydney, New South Wales.

‡Faculty of Pharmacy, The University of Sydney, New South Wales.
Email: ramin.rohanizadeh@sydney.edu.au

Trebitsch Research Grant

The development of a chairside paper-based assay for the assessment of salivary acetaldehyde levels after 'therapeutic' use of an alcohol-containing mouthwash

A Fam;* AN Ramdzan,† MI Almeida,† SD Kolev,† M McCullough* (Supervisors)

Literature from the past few decades has demonstrated the mutagenic effects of acetaldehyde (the first metabolite of ethanol) in high concentrations. This led to the hypothesis that widespread ingestion of alco-

holic beverages and use of alcohol-containing mouthrinses in modern society may be contributing to the increase of oral cancer. Current methodology employed is time-consuming and technique-sensitive,

and involves preserving saliva collections with perchloric acid and measuring salivary acetaldehyde levels by headspace gas chromatography. This project aimed to develop a paper-based device (PBD) for instantaneous, chairside oral cancer risk assessment in the clinical setting without the need of specialized apparatuses and reagents.

The PBD consisting of 15 pairs of hydrophilic circular zones, separated by a Teflon interleaving sheet, were printed onto Whatman Grade 4 filter paper using a wax printer. The hydrophilic zones of each pair were impregnated with 3-methyl-2-benzothiazoline hydrazone (MBTH) and Fe(III) solutions, respectively. Samples were introduced in the MBTH zones and after a predetermined period of time the interleaving sheet was removed to allow the reaction between Fe(III), MBTH and its adduct with the aldehydes to take place producing a blue coloured compound in the MBTH reagent zone. The intensity of the colour developed was measured by scanning the MBTH zones and processing the corresponding images using Image J software. The difference between the colour

intensity after and prior to using the alcohol-containing mouthwash corresponded to the concentration of salivary acetaldehyde. Concurrently, an algorithm based on salivary acetaldehyde concentration, determined by the proposed PBD, was constructed by taking into account known oral cancer risk factors that include smoking status, drinking status, usage of alcohol-containing mouthwash, and presence of mucosal lesions.

The main parameters of the PBD were optimized with respect to sensitivity. Under optimal conditions the PBD is characterized by a linear concentration range of up to 5 mg L⁻¹ and a limit of detection of 0.64 mg L⁻¹. Successful recovery tests using spiked with acetaldehyde saliva samples were conducted.

There is a great need for an oral cancer risk assessment that incorporates habits, oral examination, and patient ability to metabolize alcohol, to assess patient risk of cancer development. This paper-based assay system in conjunction with the algorithm holds promise for clinical use as a risk assessment and patient education tool.

This research was funded by an Australian Dental Research Foundation Trebitsch Research Grant and support from the School of Chemistry, The University of Melbourne.

*Oral Medicine, Melbourne Dental School, The University of Melbourne, Victoria.

†School of Chemistry, The University of Melbourne, Victoria.
Email: m.mccullough@unimelb.edu.au