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Introduction

In this my fourth and final year as Chairman of the Australian Dental Research Foundation (ADRF), I am extremely pleased and proud to be able to provide this introduction to the *Australian Dental Journal Special Research Supplement*. This supplement continues to provide readers of the *Australian Dental Journal* with an opportunity to get an overview of the excellence that is currently being undertaken in dental research in Australia. Despite harsh economic times and the many difficulties within universities and research institutions, I am continually amazed at the dedication and commitment of the dental and oral health researchers who produce such high quality research information for the profession. While this supplement only provides abstracts and a short summary of the research achieved, much of what is presented here forms the basis for more extensive publications which provide the evidence base for our clinical practice. The ADRF is now more than ever focusing on supporting dental research in Australia and the evidence of the ADRF's contribution to dental research can be seen in the range and quality of abstracts presented in this supplement. I would also like to acknowledge the enormous efforts of the ADRF Research Advisory Committee, chaired by Associate Professor Camile Farah, for the countless hours they spend not only reviewing grants, but also ensuring final reports and abstracts are presented in such high quality format for publication in this research supplement.

While we continue to see the cost of high quality research increase, and the ADRF has tried to increase funding support to researchers, it is unfortunate that there has been a reduction rather than an increase in the ADRF's ability to provide funds for the many valid research projects being undertaken. While the ADRF Executive and Finance Committees work continuously on improving income sources, it must be realized that ultimately it is the responsibility of every one of us in the profession to ensure dental research in Australia continues. This requires a financial commitment and as such I ask again that you seriously consider becoming a Supporter of the Foundation, or provide a donation to enhance future dental research, and thereby maintain our reputation for providing high quality research information as is presented in this supplement.

Again this year the supplement includes a broad range of topics in a range of disciplines, and is presented in a clear and concise format. I encourage you to read and embrace the information presented in this research supplement and appreciate the benefits of research in maintaining the development of the profession. I am sure you will find information relevant to your clinical practice and in doing so I encourage every reader to realize the essential requirement for research to underpin clinical practice and as such support the ADRF by contributing financial support or donation, and thereby keep innovation alive and assist with keeping dental research active in Australia.

Ian Meyers
Chairman
Australian Dental Research Foundation

CONTENTS

ADRF Special Research Supplement
Volume 59 No 4 December 2014

Contents continued

ADRF Dental Student Research Grant Abstracts

Contents continued

ADIA Award

Colin Cormie Scholarships

Trebitsch Research Grant

ADRF Research Grant Abstracts

Bond strength of composite resin to alumina-reinforced feldspathic ceramic

J Abbott*

The aim of this project was to assess the microtensile bond strength of different composite resin repair methods to an alumina-reinforced feldspathic ceramic.

Discs (2 mm thickness) were prepared from ingots of alumina-reinforced fluorapatite ceramic (Zirpress). These were all polished to a standardized mirror finish. The ceramic surfaces were imaged using a Leica MZ16FA stereo microscope before treatment and after the microtensile bond strength testing. The polished discs were divided into 3 groups: Group 1 – untreated surface (control); Group 2 – etching with Ultradent Porcelain Etch 9% buffered hydrofluoric acid for 4 minutes; Group 3 – airborne particle abrasion with 110 μ aluminium oxide (Erskine Dental) using a fixed pressure chairside air abrasion device (Dento-Prep Aluminium Oxide Microblaster, Ronvig) for 15 seconds. A silane bonding agent (Kerr Silane Primer) was then applied for 5 minutes, followed by an adhesive resin (Adper Scotchbond SE, 3M EPSE) according to the manufacturer's instructions. Each ceramic surface then had 2 separate composite resin deposits (Z100, 3M ESPE) approximately 2 mm thick and light cured for 40 seconds, applied. Specimens were tested according to the specifications outlined in the ISO Standard TR 11405. Data were statistically analysed using one-way analysis of variance followed by Tukey's multiple comparison tests (GraphPad Prism 6). Significance levels were set at $p < 0.05$.

The mean microtensile bond strength was calculated for each group: control (14.2 ± 1.9 MPa), etched with 9% hydrofluoric acid (7.1 ± 0.63 MPa) and air abraded with aluminium oxide particles (6.9 ± 0.48 MPa). Although both treated groups showed unexpectedly significantly lower bond strength values than the control ($p < 0.001$), the difference between the two treatments was not significant ($p > 0.05$). These groups, at the point of fracture, demonstrated a significant amount of composite resin still attached to porcelain from the stereo image analysis, while in the control group it had separated cleanly off the porcelain surface. This would suggest that the microtensile bond strength in the surface treated groups is greater than that measured.

The result obtained with the control is at odds with the anticipated higher microtensile bond strength of the treated ceramic surfaces. Examination of the porcelain surface demonstrated retention of composite to treated surfaces when compared to the untreated surface. Hence, the true strength of this bond is still to be determined and likely to be much higher than that recorded for the treated surfaces. Quantifying this bond strength would require a review of the specimen preparation and testing procedure.

*School of Dentistry, The University of Adelaide, South Australia.
Email: victor.marino@adelaide.edu.au

Cytokine profiling of gingival crevicular fluid following orthodontic force application

R Ahuja, J Chiu, O Dalci, AM Darendeliler*

The study aimed to perform a cytokine profiling of gingival crevicular fluid (GCF) collected from patients after heavy orthodontic force application. The cytokine expression was matched with the extent of orthodontically induced root resorption (OIIRR) which was obtained by evaluating the extracted tooth for resorption craters.

A split mouth study design was used. Eight patients requiring maxillary first premolar extractions received 225 grams of controlled buccal tipping force for 28 days on one side. The contralateral side was used as a control. GCF was collected from both test and control teeth using Periopaper® at six time points. Subsequent analysis of GCF using multiplex bead immunoassay was performed to determine the levels of specific cytokines, which are previously implicated in OIRR.

Statistically significant temporal increase was observed in the heavy force group for cytokines such as TNF-α at 3 hours and 28 days ($p = 0.01$).

IL-1β, IL-4, IFN-γ peaked during 7 days, IL-6, IL-7, TNF-α and GM-CSF peaked at 28 days, while IL-8 showed no fluctuations. Comparing cytokine profile for patients with high and low OIIRR (>0.35 mm^3 and <0.15 mm^3 respectively), the levels of anti-resorptive cytokines IL-4 ($p = 0.07$) and GM-CSF ($p = 0.03$) were higher in low root resorption cases.

The tendency of higher levels of anti-resorptive cytokines IL-4 and GM-CSF in low root resorption cases suggests a potential anti-resorptive role that these cytokines may play in reducing the level of OIIRR. However, for this observation to be clinically significant a larger sample size is warranted.

*Sydney Dental Hospital, Surry Hills, New South Wales and Department of Orthodontics, The University of Sydney, New South Wales.
Email: ali.darendeliler@sydney.edu.au

Collaborative learning: understanding dental students' perceptions and experiences

A Almajed,* V Skinner,* R Peterson,† T Winning*

Collaborative learning (CL) has several advantages; however, it is demanding for students. Few studies have examined students' understandings of learning collaboratively. To design effective CL activities, we need to understand students' perceptions about CL. This qualitative study aimed to generate evidence of dental students' understandings of CL based on participants' constructions of their CL experiences.

Focus groups (5 first-year (n = 14) and 4 fourth-year (n = 14)) were conducted with volunteer dental students at the University of Adelaide (Ethics Approval: HS-2013-001). Open-ended questions were developed following completion of a comprehensive systematic review that identified gaps in our knowledge about students learning collaboratively. The focus group questions addressed what happened during group learning and the importance of group learning to students. Focus groups were audio-recorded and transcribed professionally. Initial coding was based on students' words, e.g. 'same motivation', 'similar personalities', followed by grouping of codes into similar concepts, e.g. 'the right batch of students'. Finally, the codes were examined for larger patterns or themes, e.g. facilitating factors.

Five main themes were generated, namely, 'value of differences', 'facilitating factors', 'inhibiting factors', 'learning processes and outcomes', and 'value of group learning'. Students perceived that having different people with different perspectives were critical factors influencing positive outcomes. Students recognized that differences in views and conflicting knowledge were beneficial for their learning. Students noted that having 'the right batch of students' was important for their group's learning in terms of having similar levels of and attitudes toward group work, and goals and motivations.

Students' learning during group activities was strongly mediated by students teaching and explaining to each other. These interactions helped to clarify their doubts, improve their understanding and reinforce their knowledge. Students reported using key theoretical elements of CL, such as linking, comparing, and building information from each other. However, careful questioning and probing were needed for students to articulate these elements. In general, there was a strong similarity in students' responses across the main themes in both years.

Developing students' awareness regarding learning processes in groups can be used to support students' learning experiences in CL. Having the right mix of students, and enabling group discussion, especially teaching and explaining to each other, were perceived by students to support their learning and satisfaction in group learning contexts.

*School of Dentistry, The University of Adelaide, South Australia.
†Faculty of Health Sciences, The University of Adelaide, South Australia.
Email: abdulaziz.almajed@adelaide.edu.au

The individuality of the human dentition: implications for forensic odontology

A Ashar, T Hughes, J Kaidonis, H James, G Townsend*

Teeth are derived from the interplay of genetic, epigenetic and environmental factors, giving rise to significant natural variation which may provide a mechanism for developing models for forensic identification. The aims of this study were to: (1) describe dental crown size and dental arch size and shape in selected populations; and (2) utilize principal components analysis and discriminant function analysis to assign individuals to their respective populations.

Stone dental models of adult dentitions were available from six populations including Australian Aboriginals (103), Europeans (134), and four major ethnic groups in Malaysia; Malays (110), Indians (113), Chinese (101) and Orang Asli (51).

Standardized 2D digital images were obtained from models. Data collected from these images by a trained and calibrated examiner included dental crown size, dental arch size and dental arch shape. Ten per cent of the sample was randomly re-sampled to quantify inter- and intra-examiner variation. There was no evidence of systematic error and random error was small relative to population variation.

Data were measured on an interval or ratio scale. Descriptive statistics included measures of central tendency and dispersion. Inferential statistics included unpaired t-tests, Levene's test and ANOVA. Principal components analysis (PCA) was used to examine patterns of diversity within the groups, and discriminant function analysis was used to predict group membership. PCA was used to empirically estimate random-match probabilities between unrelated members within a group. This point estimate was then used as a basis for pairwise comparisons between individuals within groups.

Australian Aboriginals displayed the largest dental crown size and dental arch size. Malays and Chinese had similar crown sizes. Europeans and Indians had similar arch shapes. Discriminant function analysis separated Europeans and Australian Aboriginals. The four Malaysian groups clustered together away from Europeans and Australian Aboriginals, making assignment of population membership for these groups challenging. Patterns of separation depended on the number of dental crown variables used.

PCA was capable of discriminating between individuals, even between identical twins reared together. It was able to correctly identify known matches in a small, independent test sample. The model remains to be tested in a large, independent sample to provide estimates of sensitivity and specificity.

This research provides objective support for the use of dental morphology in identification. Results suggest that probabilistic models may be developed from standardized dental data with sufficient power to discriminate between individuals within a group.

*School of Dentistry, Faculty of Health Sciences, The University of Adelaide, South Australia.
Email: toby.hughes@adelaide.edu.au

The international dental graduate (IDG) study: findings from the 2013 national survey in Australia

M Balasubramanian,* AJ Spencer,* SD Short,† K Watkins,‡ DS Brennan*

The aim of this study was to understand the demographic, migration/residence, practice profiles and life-story experience of international dental graduates (IDGs) in Australia.

IDGs registered with the ADA (N = 1872) and those pursuing graduate programmes in dental schools (N = 105) were surveyed. Data were mainly collected through mailed self-completed questionnaires,

between January and May 2013. Characteristics of major IDG entry groupings were compared using chi-square statistics and the level of significance set at $p < 0.05$. Factor analysis was used to examine a battery of 82 life-story experience items.

A total of 1022 IDGs completed the questionnaire; adjusted response rate of 54.5%. Females comprised 41.8% of the respondents. Nearly half of the participants (48.8%) were under 45 years of age, 78.9% were married, and 42.1% had migrated to Australia less than 10 years ago. The average age was 46.2 years; and they worked an average of 35.3 hours a week. Only 9% worked in the most disadvantaged SEIFA quintiles. Comparisons across the direct entry (n = 491), ADC successful (n = 411) and alternative pathway (n = 120) candidates were significant for gender, age group, marital status, years since arrival to Australia, SEIFA disadvantaged quintiles and hours worked. Factor analysis (principal components; varimax rotation; factor loading of ≥0.4) and further reliability analysis provided three factors on home country experience (system, social, job); and two on experiences in Australia (positives, difficulties). ADC successful candidates (OR = 6.45; 2.31–18.02) and alternative pathway (OR = 9.03; 2.25–36.22) were more likely to face system level push factors than directly recognizable candidates. ADC successful candidates are also likely to face more difficulties in Australia (OR = 4.76; 2.82–8.03). In the positive experience scales, the ADC successful candidates had the lowest odds ratio (OR = 0.60; 0.38–0.94). The odds ratio for positive experiences was associated with years since arrival to Australia, with participants having stayed more than 10 to 29 years having an odds ratio of 1.91 (1.14–3.21), and those above 30+ years with an odds ratio of 3.31 (1.40–7.81).

The study has provided valuable evidence on characteristics and experiences of IDGs in Australia.

*Australian Research Centre for Population Oral Health, School of Dentistry, The University of Adelaide, South Australia.
†Faculty of Health Sciences, The University of Sydney, New South Wales.
‡Australian Dental Council, Melbourne, Victoria.
Email: david.brennan@adelaide.edu.au

Nanomechanical and nanostructural properties of human dentine: an improved understanding of the role of proteoglycans

LE Bertassoni, K Stankoska, L Sarran, P Costa Filho, MV Swain*

Dentine is a mineralized nano-structured biological composite that forms the bulk of the tooth. Its matrix is primarily composed of collagen (30%), mineral (50%) and water (20%), thus sharing great compositional similarities to bone and other calcified human tissues. Despite extensive efforts, the structural mechanisms that endow mineralized tissues with outstanding durability and unique mechanical behaviour remain largely a mystery. Our results point towards a novel interpretation whereby nano-scale noncollagenous structures comprising less than 5% of the matrix represent key regulators of the mechanical behaviour of dentine. These nano-scale structures, namely proteoglycans, have long been recognized as primarily responsible for the structural organization of collagen in the extracellular matrix of all vertebrates. Surprisingly, little information about the role of these molecules on the biomechanical response of dentine has been made available thus far, a knowledge gap that our project sought to address.

In this project, a set of structural and mechanical characterization tools were utilized to determine the participation of proteoglycans on dentine. Our studies utilized atomic force microscopy (AFM) and scanning electron microscopy (SEM) to analyse the micro- and nano-morphology of the dentine matrix before and after enzymatic treatments targeting proteoglycan family members and their carbohydrate structural components, namely glycosaminoglycans. These studies were completed with nanoindentation analyses of elastic modulus, hardness, energy dissipation and creep properties.

In brief, this project demonstrated that: (1) hydration and drying may affect nanoindentation properties of dentine; (2) dentine exhibits inherent mechanical anisotropy at different length scales, namely within the collagen fibrils and between the intertubular and peritubular regions; the peritubular mineral, in turn, appears to interact with proteoglycans which form an organic matrix for its deposition during tissue devel-

opment; (3) and finally, and more importantly, our results suggested that proteoglycans and their glycos-aminoglycan side chains may be an important contributor to the nanostructural organization and to the outstanding longevity of dentine and teeth.

With this information, we hope to contribute to the development of optimized strategies towards biomimetic regeneration of dentine and other mineralized tissues of similar composition.

Publications:

1. *Bertassoni LE, Cardoso JC, Manoharan V, et al. Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels. Biofabrication 2014;6:024105.*

2. *Annabi N, Tamayol A, Uqillas JA, et al. 25th anniversary article: Rational design and applications of hydrogels in regenerative medicine. Adv Mater 2014;26:85–123.*

*Biomaterials Research Unit, Faculty of Dentistry, The University of Sydney.
Email: luiz.bertassoni@sydney.edu.au

3. *Guazzato M, Clarke I, Aquilla A, Bertassoni LE, Klineberg I. Surface roughness of crown margin preparations: a comparative analysis of finishing techniques. Int J Periodontics Restorative Dent (in press).*

4. *Bertassoni LE, Marshall GW, Swain MV. Mechanical heterogeneity of dentin at different length scales as determined by AFM phase contrast. Micron 2012;43:1364-1371.*

5. *Bertassoni LE, Orgel JPR, Antipova O, Swain MV. The dentin organic matrix – limitations of restorative dentistry hidden on the nanometer scale. Acta Biomaterialia 2012;8:2419-2433.*

6. *Bertassoni LE, Swain MV. Influence of hydration on nanoindentation induced energy expenditure of dentin. J Biomech 2012;45:1679-1683.*

7. *Bertassoni LE, Stankoska K, Swain MV. Insights into the structure and composition of peritubular dentin organic matrix and the lamina limitans. Micron 2012;43:229-236.*

8. *Bertassoni LE, Swain MV. The contribution of proteoglycans to the mechanical behavior of mineralized tissues. J Mech Behav Biomed Mater 2014;38:91-104.*

Typing of *Porphyromonas gingivalis* isolates from periodontal patients

PS Bird,* LL Blackall,† MP Cullinan,‡ GJ Seymour‡

While *Porphyromonas gingivalis* has been implicated as a key pathogen associated with chronic periodontal disease, a number of reports have suggested that differences in pathogenicity may exist between *P. gingivalis* isolates. Previous studies using Multi-Locus Sequence Typing (MLST) have elucidated clonal lineages within populations of other pathogenic microorganisms, such that MLST may also identify different lineages in *P. gingivalis* clinical isolates.

The aim of this study was to characterize a population of *P. gingivalis* isolates using mutilocus sequence typing (MLST).

Ten *P. gingivalis* isolates, comprising three laboratory strains (ATCC 33277[T], W50, FDC 381) and seven clinical isolates from chronic periodontitis patients were cultured from frozen stocks or from the patient plaque samples using standard anaerobic culture techniques. Development of the MLST required selection of housekeeping genes from the

P. gingivalis genome and the design of primers for the amplification and sequencing of each gene. Accordingly, 11 housekeeping genes were identified, primer sets designed and the PCR optimized for each set. DNA was extracted from each isolate and subjected to PCR amplification. From the 11 genes, seven genes were selected to establish the clonal lineage.

The results were analysed using the Sequence Type Analysis and Recombinational Tests (START) programme available at http://outbreak.ceid.ox.ac.uk/software.htm. The allele profile and frequency for each housekeeping loci ranged from 2 to 10 and represented the sequence type (ST) of that isolate. We demonstrated that allelic types were easily distinguishable for each gene, that the ST was able to be associated with virulent and avirulent strains and that in one patient four different STs could be identified.

Even using this small strain and data set, the MLST technique established definitive characteriza-

tion of the *P. gingivalis* population. Therefore, the results of the present study have shown that using MLST, elucidation of clonal lineages within the

taxon and association of STs with possible pathogenicity is possible.

*School of Veterinary Science, The University of Queensland, Gatton, Queensland.
†Faculty of Life and Social Sciences, Swinburne University of Technology, Hawthorn, Victoria.
‡Department of Oral Sciences, University of Otago, Dunedin, New Zealand.
Email: phil.bird@uq.edu.au

Proteomic analysis of cell surface proteins by *Enterococcus faecalis* in response to stress

P Cathro, P Zilm, N Gully*

The success rate of re-treating endodontic cases that have failed is low. It is established that *Enterococcus faecalis* is the most commonly recovered isolate for failed cases and a clearer understanding of its ability to survive conventional irrigation and medication regimes is required. Calcium hydroxide is the recognized medicament of choice in endodontics due to its high alkalinity, yet *E. faecalis* has been shown to survive elevated pH levels. One explanation for this is that the hydroxyl ions may take several hours or days to penetrate the dentinal tubules and reach the bacterial biofilm. The gradual increase in pH coupled with the buffering effect of dentine may allow *E. faecalis* to respond to the increasing pH.

The aims of this study were to investigate the effects of increasing pH on changes in the expression of cell surface proteins.

Enterococcus faecalis (V583) was grown in a chemostat in Todd Hewitt broth at a growth rate relative (μrel) to one-tenth of the maximum growth rate. After steady-state had been achieved at pH 8.0 and 11.0, bacteria were harvested and pooled for proteomic analysis. Cell membrane proteins were extracted using the membrane shaving and 1D-SDS protocols described by Wolff *et al*. (Mol Cell Proteomics 2008;7:1460–1468) and membrane proteins were identified by mass spectroscopy.

With increasing pH, the growth rate slowed from a generation time of approximately 1 hour (pH 8) to nearly 5 hours (pH 11). The phenotypic change between *E. faecalis* grown at pH 8 and pH 11 were obvious as there was evidence of capsule production at pH 11.

Initial analysis of the results indicated that the method for recovering trans-membrane proteins worked well and that the majority of proteins identified were associated with cellular transport. Of the 58 proteins identified, 98% were membrane associated and represent approximately 10% of the predicted membrane proteome. Thirty-six of the 58 recovered proteins in this study were additional to those already published for *E. faecalis* V583.

The use of continuous culture and altered pH conditions has a direct influence on the trans-membrane expression. Cell surface proteins identified which are differentially expressed in response to increasing pH are potential targets for the development of drugs/medicaments which may improve the efficacy of current endodontic treatments.

*School of Dentistry, The University of Adelaide.
Email: peter.zilm@adelaide.edu.au

The effect of surfactant on the dissolution of porcine pulpal tissue by sodium hypochlorite solutions

RM Clarkson,* B Kidd,* GE Evans,† AJ Moule*‡

In a previous study, Hypochlor (Dentalife Pty Ltd, Ringwood, Victoria, Australia), a surfactant-containing NaOCl solution, performed better than Milton (Milton Australia Pty Ltd, Carole Park, Queensland, Australia) in dissolving pig incisor pulp. It was suggested that the surfactant content of the Hypochlor products may have improved the speed of the dissolution process.

The aim of this study was to assess whether Hypochlor solutions without surfactant would take the same mean time to dissolve dental pulp as the market versions with surfactant.

Hypochlor solutions without surfactant were compared with the retail Hypochlor products containing surfactant and with Milton, for the time taken to dissolve pig incisor pulp samples 2.5 mm in diameter in 25 ml of the chosen NaOCl solution. The elapsed time to total dissolution was measured for 20 samples of each of the four Hypochlor solutions. The results were statistically analysed in R (version 2.12.0) using multiple regression with nominal active chlorine content as a covariate.

The Hypochlor 1% solution containing surfactant took 22 minutes 32 seconds, while the solution without surfactant required 23 minutes 8 seconds. The surfactant had a slightly opposite effect in the

Hypochlor 4% forte solutions, which was not statistically significant (p > 0.1). Here the solution minus surfactant took a mean time of 10 minutes 33 seconds and the 4% solution with surfactant required a mean time of 11 minutes 52 seconds.

In an amorphous tissue such as dental pulp, a large number of samples (n = 20) were required to ensure a statistically significant result. Having the manufacturer prepare test solutions identical in every way except for surfactant content was essential to test the effect of surfactant content on dissolving pulp in NaOCl. Other authors have produced contradictory results but appeared to use solutions from different manufacturers.

The addition of surfactant to otherwise identical hypochlorite solutions does not influence the ability of these solutions to dissolve pulpal tissue.

*School of Dentistry, The University of Queensland, Australia.
†School of Mathematics and Physics, The University of Queensland, Australia.
‡Kuwait University, Kuwait.
Email: rogercla@ozemail.com.au

A prospective study – the prevalence of chronic pain following successful endodontic treatment

Y Cui, L Kim, T Truong, P Duckmanton, C Peck*

Persistent chronic orofacial pain following endodontic treatment presents a significant challenge for dental practitioners and a major source of distress for patients. The condition is poorly described in the literature and little is known of its frequency and pathophysiology. This study aimed to determine the prevalence of chronic pain following successful

endodontic treatment and to evaluate possible contributing variables, and identify risk factors and predictors that may affect the onset of the condition.

The sample population in this study comprised of patients who had received endodontic treatment by dental students and two endodontists at two major public dental hospitals (Sydney Dental Hospital,

Sydney, Australia and Westmead Centre for Oral Health, Sydney, Australia) from May to December 2011. Of the 122 patients/teeth treated, 106 were followed-up and fulfilled the inclusion criteria for successful endodontic treatment. Endodontic procedures included in this study were non-surgical root canal treatment and retreatment. A detailed history of the patient/tooth and treatment regimen was obtained prior to and during treatment. Those that fulfilled the inclusion criteria were followed up three and six months postoperatively.

Data analysis indicated that endodontic treatment performed by students and treatment of anterior teeth was representative of the majority of the data. No cases included in this study fulfilled the diagnostic criteria for neuropathic tooth pain. Furthermore, no patients reported the presence of persistent chronic pain after their treatment. Isolated cases of transient discomfort were reported in two patients, although none lasted beyond the follow-up period. One patient reported a change in the characteristics of her headache disorder, but no evidence in the data suggests that this may be a result of the treatment

Results from this study suggest that the prevalence of chronic pain following successful endodontic treatment may be more uncommon than the previous literature describes. However, further research with a larger patient pool and a greater variation in endodontic treatments (including surgical procedures) is needed to elucidate and characterize the nature of post-endodontic chronic pain and its contributing factors.

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

*Faculty of Dentistry, The University of Sydney.
Email: pduckman@mail.usyd.edu.au

In vitro analysis of cellular and genotoxicity in human oral epithelial cells after treatment with alcoholic mouthwashes

S Currie,*† A Dalley,* CS Farah*†

Alcohol is an established risk factor for oral cancer and has been shown to induce genotoxicity through a number of secondary pathways including the formation of reactive oxygen species during the metabolism of ethanol to acetaldehyde. Ethanol concentrations as high as 27% can be found in some commercially available alcohol-containing mouthwashes, prompting concerns about possible health risks associated with their use. This *in vitro* project aimed to investigate the cellular and molecular changes induced in oral cells by regular exposure to mouthwash.

Normal (OKF6-TERT2) and dysplastic (DOK) cultured oral keratinocytes were exposed to diluted alcohol-containing (ACM) or non-alcoholic (NAM) mouthwash from either of two parent brands. Ethanol-evaporated and ethanol-supplemented versions of each group were also formulated for comparison. Cells were assayed for DNA strand breaks (Comet assay), oxidative DNA damage (8-oxo-dG), lipid peroxidation (lipid peroxidation assay) and CYP2E1 activity (p-Nitrophenol) through a variety of methods.

A series of acute exposure experiments with cultured oral keratinocytes revealed: increased DNA strand breakage of ACM groups compared to NAM groups within parent brands, significantly higher strand breaks with original formulations compared to ethanol-evaporated groups for ACM groups but not NAM groups, and increased strand breakages when ethanol was added to a NAM. Overall, the presence of ethanol in mouthwash formulations increased DNA strand breakage. The adaptive response of cells to repeated exposure to mouthwash formulations was also investigated. Microsomal CYP2E1 enzyme activity was negligible in oral keratinocytes and was not induced by chronic exposure to ACM or NAM solutions.

DNA strand breaks were seen more commonly with ACM formulations than with NAM formulations and ethanol-supplemented groups, while negligible CYP2E1 enzyme activity was observed in oral keratinocytes irrespective of chronic exposure to various mouthwash formulations. Together these results suggest that ACM-induced DNA damage occurs without

the contribution of reactive oxygen species derived specifically from the CYP2E1-mediated breakdown of ethanol to acetaldehyde.

*The University of Queensland Centre for Clinical Research.
†The University of Queensland School of Dentistry.
Email: c.farah@uq.edu.au

Effect of azithromycin on cytokine expression by human gingival fibroblasts

C Doyle, PM Bartold, T Fitzsimmons, C Marchant*

Azithromycin is a macrolide antibiotic that is well known for its antibacterial properties, as well as possessing potential anti-inflammatory and immune modulating effects. This antibiotic has therefore been widely used in medicine for treating conditions ranging from inflammatory pulmonary diseases to dermatologic skin conditions. It has also been shown to be an effective antibiotic against most common periodontal pathogens and is used as an adjunct to treat periodontitis, a condition with bacterial aetiology and an inflammatory pathogenesis. Furthermore, periodontal case studies report regeneration of alveolar bone accompanied by significant reductions in inflammation have been achieved with azithromycin. However, the mechanisms by which these are achieved in the periodontium are largely unknown.

This study aimed to determine the potential anti-inflammatory effect of azithromycin on cytokine and chemokine production by healthy human gingival fibroblasts (HGFs) that were stimulated by *Porphyromonas gingivalis* lipopolysaccharide (*P. gingivalis* LPS).

*School of Dentistry, The University of Adelaide, South Australia.
Email: mark.bartold@adelaide.edu.au

HGFs were isolated from healthy gingiva collected from three donors. The effects of azithromycin at concentrations ranging from 0.1 to 10 μg/mL were tested. Cytokine and chemokine protein levels were assessed using the Luminex® multiplex immunoassay. *P. gingivalis* LPS induced cytokine/chemokine (IL-6, IL-8, MCP-1 and GRO) protein production in HGFs was suppressed by azithromycin at all concentrations tested, and in all three donors.

Suppression by azithromycin of IL-6, IL-8, MCP-1 and GRO *P. gingivalis* LPS protein induction in HGF was statistically significant when all donor results were collated ($p < 0.05$). This study demonstrates that azithromycin suppresses *P. gingivalis* LPS induced cytokine/chemokine protein production in HGFs, which may explain some of the clinical benefits observed with the adjunctive use of azithromycin in the treatment of periodontitis.

Effect of implicit learning on the acquisition of fine motor skills in pre-clinical endodontics

M El-Kishawi,* G Townsend,* P Cathro,* R Masters,† T Winning*

The use of theories or research evidence for designing learning of fine motor skills for root canal preparation is limited. Recent evidence from other disciplines indicates

that implicit learners (unlike explicit learners) are better able to maintain performance when multi-tasking. We hypothesized that root canal preparation skills learnt

implicitly will not deteriorate when multi-tasking. Therefore, this study aimed to investigate the effect of learning implicitly (errorless) or explicitly (errorful), on the acquisition of root canal preparation hand skills.

Participants were volunteer students from the University of Adelaide, with no previous endodontic work/learning experiences (ethics approval: UA: H-2012-117).

Participants performed fundamental root canal hand instrumentation tasks, during learning and testing phases. During learning trials, participants prepared standardized canals with different canal diameters and curvatures. The errorless group (implicit) began training on the simplest task (straight, wide canal) and progressively moved toward the most difficult task (curved, narrow canal). The errorful group (explicit) completed these tasks in reverse order. Other students who completed the normal pre-clinical activities provided comparative (control) performance data. In the testing phase, all participants prepared the distal canal on a plastic tooth.

Performance was assessed by completion time and preparation accuracy of learning and testing trials. T-tests and repeated measures ANOVA, with *post hoc* analyses, were used to assess for differences between and within groups.

Data from 55 participants (25 males and 30 females) were available for analysis (comparative: 15, explicit: 20, and implicit: 20). Participants' ages ranged from 19 to 34 years (mean: 21.95 ± 2.86 years).

Errorless and errorful groups showed similar performance during learning. When tested, however, errorful learners showed a significant deterioration in preparation accuracy when multi-tasking ($p < 0.05$). In contrast, completion time and accuracy of preparation for the errorless and control groups remained stable when multi-tasking. Completion time in the control group was significantly shorter when multi-tasking ($p < 0.05$) related to a longer learning phase (20 h vs 2 h). Other differences in performance in implicit and control groups were not statistically significant ($p > 0.05$).

Our findings confirmed that learning under conditions that limit errors resulted in stable performance when multi-tasking. This finding may be explained by a reduced use of working memory for error detection/correction when learning implicitly.

These findings provide preliminary evidence that implicit approaches can provide an effective method for learning root canal hand skills that remain robust under demanding conditions.

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*School of Dentistry, The University of Adelaide, South Australia.
†Formerly, Institute of Human Performance, The University of Hong Kong, Hong Kong; currently, Department of Sport and Leisure, University of Waikato, New Zealand.
Email: mohamed.el-kishawi@adelaide.edu.au

Gene expression profile alteration during oral cancer progression

CS Farah*

Carcinogenesis is a highly complex, multistep process involving accumulation of genetic alterations that lead to the induction of proteins promoting cell growth (encoded by oncogenes) and the loss of proteins restraining cell proliferation (encoded by tumour suppressor genes). In order to gain an appreciation for the precise nature and sequence of events leading to cancer formation, we hypothesized that undertaking molecular profile analysis of oral cancers in relation to their tumour-free margin and adjacent clinically normal mucosa would reveal a set of markers involved in carcinogenesis for future validation in independent patient cohorts.

Tumour samples were obtained from 20 patients with oral cavity squamous cell carcinoma (OSCC), in addition to samples from the tumour-free surgical margin and adjacent clinically normal mucosa. RNA isolation was undertaken with TRIzol Reagent, and assessment of RNA integrity was carried out on an Agilent 2100 Bioanalyzer, with the RNA 6000 Nano kit. 100 ng of RNA was used as input for the GeneChip 3' IVT Express Kit assay using a standard protocol, followed by hybridization of aRNA to the GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, USA). Data from good quality arrays were normalized using the GeneChip robust

multiarray average (GCRMA) normalization method. The normalized, filtered data were tested for genes significantly differentially expressed in pairwise comparisons using the *maanova* software package.

Genes significantly over-expressed between the tumour and the surgical margin included PPBP, ASPRV1, SLC16A10, while those under-expressed included KRT4, TMPRSS11B, and CRNN. Genes over-expressed in the surgical margins compared to clinically normal tissue included GBP2, while those under-expressed included HBA1, HBA2 and SGCG. Genes known to be involved in late stage carcinogenesis and also metastasis including MMP1, MMP3, MMP10 and MMP12 were significantly over-expressed in tumour compared to clinically normal oral mucosa.

Novel genes have been highlighted in this study which may serve as useful candidate biomarkers for assessment of disease free margins, and for determining early changes to oral epithelial dysplasia (OED), in addition to late stage changes in progression from OED to OSCC. Further validation is required in independent patient cohorts to verify the usefulness of such markers.

*The University of Queensland Centre for Clinical Research and The University of Queensland School of Dentistry.
Email: c.farah@uq.edu.au

The contribution of *Porphyromonas gingivalis* peptidylgarginine deiminase to the pathogenesis of rheumatoid arthritis

N Gully,* R Bright,* V Marino,* C Marchant,* M Cantley,† D Haynes,† C Butler,‡ S Dashper,‡ E Reynolds,‡ M Bartold*

The aim of this study was to investigate the suggested role of *Porphyromonas gingivalis* peptidylarginine deiminase (PAD) in the relationship between the aetiology of periodontal disease and experimentally induced arthritis.

A genetically modified PAD-deficient strain of *P. gingivalis* W50 was produced. The effect of this strain, compared to the wild type, in the mouse model for experimental periodontitis and experimental arthritis was assessed. Experimental periodontitis was induced following oral inoculation with the PAD deficient and wild type strains of *P. gingivalis*. Experimental arthritis was monitored by assessment of paw swelling and micro CT analysis of the radio-carpal joints. Experimental periodontitis was monitored by micro CT scans of the mandible and histological assessment of the periodontal tissues around the mandibular molars. Serum levels of anti-citrullinated protein antibodies (ACPA) and *P. gingivalis* were assessed by ELISA.

Periodontitis was significantly reduced in the presence of the PAD-deficient *P. gingivalis* strain. When experimental arthritis was induced using the PAD-deficient strain there was less paw swelling, less erosive bone damage to the joints and reduced serum ACPA levels when compared to the wild type *P. gingivalis* inoculated group.

This adds further evidence to the potential role for *P. gingivalis* PAD in the pathogenesis of periodontitis and exacerbation of arthritis.

*Colgate Australian Clinical Dental Research Centre, School of Dentistry, The University of Adelaide, South Australia.
†Discipline of Anatomy and Pathology, School of Medical Sciences, The University of Adelaide, South Australia.
‡Oral Health Collaborative Research Centre, Melbourne Dental School, The University of Melbourne, Victoria.
Email: neville.gully@adelaide.edu.au

Analysis of the gene expression changes occurring as induced pluripotent stem cells differentiate into mesenchymal progenitor cells

K Hynes,* S Gronthos,† PM Bartold*

Mesenchymal stem cells (MSC) have emerged as a potential stem cell population for use in tissue regenerative therapy for treatment of defects arising from periodontitis. However, issues surrounding their accessibility and proliferation in culture significantly limit their potential as a mainstream treatment approach. In recent years it has emerged that MSC can be generated from the differentiation of induced pluripotent stem cells (iPSC) and this method could provide a novel and unlimited source of MSC for clinical use. Whilst the differentiation of iPSC to MSC is achievable, it is currently an inefficient, time consuming and unreliable process. The aim of this project was to identify key gene expression changes which occur during the differentiation process; by understanding exactly what changes are occurring through the differentiation process, we will be much better placed to manipulate the process in order to enhance the generation of MSC.

Therefore, our objective was to assess the gene expression changes occurring when iPSC differentiate into MSC in order to identify more efficient methods to generate MSC from iPSC.

MSC were generated by differentiating iPSC in MSC media for two weeks. This was followed by serial passaging to select for fast growing MSC which have the capacity to attach and proliferate in mono-layer cultures, whilst eliminating slow-growing differentiating iPS cells. Flow cytometry analysis was performed using key MSC and pluripotent markers to track the differentiation away from a pluripotent iPSC phenotype and towards an MSC phenotype.

We successfully generated MSC 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. We were unable to identify key gene expression changes that occur during this process due to the high level of heterogeneity in cell types generated through this process.

The differentiation process that occurs when iPSC differentiate into MSC is highly complicated and difficult to dissect on a molecular level.

*Colgate Australian Clinical Dental Research Centre, School of Dentistry, The University of Adelaide.
†Mesenchymal Stem Cell Laboratory, School of Medical Sciences, The University of Adelaide.
Email: kim.hynes@adelaide.edu.au

DNA mismatch repair in oral carcinogenesis

M Jessri,*† A Dalley,* CS Farah*†

Mismatch repair (MMR) pathway plays an important role in preserving the integrity of human genome. Changes in expression of MMR proteins have been associated with oral squamous cell carcinoma (OSCC). Here we studied the combined expression of 4 MMR genes as a panel in oral epithelial dysplasia and OSCC.

A total of 274 formalin fixed paraffin embedded samples (113 OSCC, 13 carcinoma *in situ* (CIS), 21 moderate/severe dysplasia (SD), 37 mild dysplasia (MD) and 90 normal) were immunohistochemically stained for hMLH1, hMSH2, hMSH6 and hPMS2.

Expression of hMLh1, hMSH2 and hPMS2 was decreased in SD, CIS and OSCC compared with normal samples (p < 0.001). Although expression of all MMR proteins tested in this study was decreased in moderately/poorly differentiated OSCC (Md = 75.89 n = 27) compared to well differentiated samples (Md = 51.07 n = 86), only hPMS2 reached statistical significance (U = 651 z = –3.53 p < 0.001). Immunoreactivity of MMR proteins was predominantly limited to the cell nuclei, however cytoplasmic expression of hMSH6 was found in 76 OSCC samples (61 well differentiated and 15 moderately to poorly differentiated OSCC). Also contrary to positive controls, 61.5% (8 out of 13) CIS samples showed loss of expression of hMSH6 in the basal layer in presence of positive cells in the adjacent epithelium. The same finding was observed in 8% (10

out of 113) of OSCC samples, in the marginal areas of the tumour.

Here we demonstrate changes in expression of mismatch repair proteins to be significantly correlated and associated with oral epithelial dysplasia and neoplasia, and suggest a role for genetic instability in oral carcinogenesis.

*The University of Queensland Centre for Clinical Research.
†The University of Queensland School of Dentistry.
Email: c.farah@uq.edu.au

The social construction of oral health in Australia

K Jones, J Merrick, C Beasley*

This research is the second step towards developing a body of theory about the contextual factors, possibly driven by media messages, which may contribute to risk behaviours and attitudes towards oral health.

Twelve focus groups were conducted in three Australian states, and a purposive sample of the public was selected. Participants were selected using a snowballing technique seeded using the social media site Facebook; 8–10 people per group were selected. Groups were intended to be split into one male, one female and one mixed group per state. Due to low uptake by males, all groups were mixed gender.

Analysis is currently still underway. Due to the illness of one of the ethnographic researchers last year for several months, the research schedule was held back and finally completed in February 2014. Reflexive analysis will be used to analyse the transcripts from the focus groups. A 'WPR' (what is the problem represented) approach (developed by Bacci in 2009) to facilitate critical interrogation of public policies or media, serves as the reflexive starting point for analysis of focus group data.

Oral health activity, oral health suggestive or talks about oral health and portrayed oral health risks, behaviours or responsibilities are being explored in the data.

No final data are reported here. More complex textual analysis is yet to be completed. It is anticipated that full analysis will be completed and published in several journals by the end of 2014, to be submitted to the *Australian Dental Journal* and *International Journal of Health Promotion* or the *Australian and New Zealand Journal of Public Health*.

Our preliminary analysis shows that there are confusing messages about oral health as reported by the community. Lack of a cohesive, clear message was evident. Additionally, oral health literacy appears relatively low among certain populations despite high self-efficacy within the groups and a lack of understanding of the reasons for tooth decay appear widespread. Toothbrushing behaviours were high and the role of toothpaste in preventing decay unclear. There was high scepticism regarding messages around good oral health received from the media.

*School of Dentistry, The University of Adelaide, South Australia.
Email: kelly.jones@adelaide.edu.au

Comparison of a remineralizing paste and antibacterial gel to prevent early childhood caries in a birth cohort study: results at five years

R Koh,* ML Pukallus,† LJ Walsh,* WK Seow*

This study reported on the five-year results of a birth cohort study investigating the prevention of early childhood caries in a low socio-economic community in Queensland.

Ethical approval was obtained from Queensland Health and The University of Queensland, and signed informed consent was obtained from the mothers. The clinical trial design had been reported previously. The participants were recruited at birth and randomized to receive either home visits or telephone contacts at ages 6, 12, 18, 30 and 42 months by oral health therapists. At 2, 3, 4 and 5 years, all children were examined at the community dental clinic. Within the home visits and telephone contacts groups, at recruitment, the children were randomly allocated into one of the following groups: 10% casein phosphopeptide amorphous calcium phosphate (CPP-ACP 10% topical cream, Tooth Mousse, GC Corporation, Tokyo, Japan), 0.12% chlorhexidine gel (Curasept, Curaden Swiss, Saranno, Switzerland), or no product (toothpaste only). To determine the general caries prevalence in the same community, clinical data were obtained from a group of 5-year-old children from the same dental clinic.

Overall, children in the chlorhexidine, CPP-ACP and toothpaste groups showed a caries prevalence of 43%, 42% and 48% respectively (p > 0.1). The caries prevalence of the chlorhexidine and toothpaste groups was significantly lower when compared to the 60% prevalence found in the community control group (p = 0.009 and p = 0.033). In the home visits group, the mean dmft (decayed, missing, filled teeth) scores of the chlorhexidine, CPP-ACP and toothpaste groups were significantly less when compared to the community controls (1.39 ± 2.32, 1.24 ± 2.22, 1.74 ± 2.80, vs 3.76 ± 4.62) (p < 0.004 comparing test groups with community controls).

All study groups (chlorhexidine, CPP-ACP and toothpaste controls) showed a significantly lower prevalence of caries and reduced dmft scores compared to the community controls. The lack of additional preventive effect of CPP-ACP and chlorhexidine over toothpaste is probably due to the fact that a ceiling effect had been reached with regular toothbrushing.

*Centre for Paediatric Dentistry, The University of Queensland.
†Oral Health Program (Logan–Beaudesert Division), Metro South Health Service District, Queensland Health.
Email: k.seow@uq.edu.au

Genetic variability associated with severe oral complications in patients treated with fluorouracil

R Logan,* J Coller,† J Bowen,‡ C Karapetis§

The incidence of severe toxicity affecting the gastrointestinal tract (GIT) following fluorouracil treatment is common and can result in poor clinical outcomes. Complications affecting the oral cavity, such as oral mucositis, are associated with decreased quality of life and treatment interruptions. There is no diagnostic marker to predict a patient's GIT toxicity risk prior to treatment. Given the key role of inflammatory signalling in the development of GIT toxicity, this pilot study investigated the association between genetic variability in innate immune signalling on the severity of GIT toxicity in patients treated with fluorouracil.

Patients that had completed treatment with 5-fluorouracil or the oral pro-drug capecitabine at Flinders Medical Centre, South Australia, were identified through pharmacy records and invited to participate in the study. Treatment-induced GIT toxicity data were collected from case note review. The incidence and severity of oral mucositis, nausea/vomiting and diarrhoea were graded according to CTCAE v.3. Each individual toxicity was combined to give an

overall GIT toxicity score per patient for the purpose of analysis. Genomic DNA was isolated from saliva of pre-treated patients and analysed for variants in the following genes using a customized Sequenom Mass-Array assay: *IL-1B, IL-6, IL-10, TNF-a, TGF-b, TLR2, TLR4, MD2, MYD88, BDNF, CRP, ICE*. Logistic regression with stepwise addition created a model describing combined genetic variants and co-factors that were associated with GIT toxicity, and receiver operator curves (ROC) determined the performance of the model in predicting severe toxicity.

To date, 40 patients have participated in the study, of which 10 had severe GIT toxicity. The model that included genetic variability in *TLR2, TNFA* and the co-factor cancer type was best related to toxicity, such that the ROC area under the curve from this model was 0.87. Comparison of patient characteristics between the two groups found that toxicity was asso-ciated with a diagnosis of colorectal cancer ($p = 0.051$), but unrelated to age ($p = 0.27$), gender ($p = 0.11$) and treatment ($p = 1.0$).

These initial findings have revealed for the first time that immune genetic variability is highly predictive of patient risk of severe GIT toxicity. A larger study is ongoing to reach our target sample size of 100. Following validation, translation of these findings may provide a novel diagnostic approach to toxicity risk prediction.

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*School of Dentistry, The University of Adelaide, South Australia.
†Discipline of Pharmacology, The University of Adelaide, South Australia.
‡Discipline of Physiology, The University of Adelaide, South Australia.
§Medical Oncology, Flinders Medical Centre, Adelaide, South Australia.
Email: richard.logan@adelaide.edu.au

Management of deep caries in primary molars – a prospective randomized controlled study

S Mistry,*† S Kazoullis,* WK Seow*†

Despite well documented clinical success rates, indirect pulp therapy (IPT) is not carried out commonly due to a limited number of controlled studies. Pulpotomy is the more common procedure for management of deep caries in primary teeth, even in the absence of pulp exposure. The objective of this study is to compare the outcomes of IPT and pulpotomy in the management of deep caries in primary molar teeth.

Ethical approval and parental consent was obtained for the study. Healthy children aged 2–9 years with deep caries lesion in primary molars, without pulpal exposure and with clinical and radiographic signs and symptoms of healthy or reversible pulpitis were included in the study. A total of 203 primary molars were treated, 176 teeth receiving IPT with randomly allocated agents: mineral trioxide aggregate (MTA) (ProRoot, Dentsply, Tulsa, OK, USA) (N = 48); Ledermix (Sigma Pharmaceuticals Pty Ltd, Victoria, Australia) (N = 58); control glass ionomer cement (GIC) (FIX Capsule, GC Corporation, Tokyo, Japan) (N = 70); and 27 teeth receiving pulpotomy due to pulpal exposure on caries removal, randomly allocated to MTA (N = 13) or Ledermix (N = 14). All teeth were immediately restored with stainless steel crown (SSC). All treatment was carried out by one operator (SM). Teeth were followed up over a period extending to 54 months for clinical and radiographic assessment. Failure was defined as presence of clinical signs/symptoms of inflammation/infection or radiographic signs of bony pathosis.

For the IPT group, the overall clinical and radiographic success rates were 100% for all teeth available for follow-up. Two-tailed Fisher's exact test was carried out for statistical analysis up to 30 months. There were statistically significant differences

(p < 0.05) in clinical and radiographic outcomes with IPT performing better than pulpotomy at 6 and 18 months. The pulpotomy group had 3/14 teeth failing at 6 months (p < 0.01) and 3/15 teeth failing at 18 months (p < 0.05). Moreover, Ledermix had more failures than the MTA in pulpotomy group.

IPT restored with SSC is highly successful for primary molar teeth with deep caries and no caries exposure. Further long-term studies with larger samples are needed to bolster the findings of this study.

*School of Dentistry, The University of Queensland.
†Kingston Oral Health Centre, Metro South Health Service District, Queensland Health.
Email: drmistry@outlook.com

Actions of thymol and related compounds on GABA receptors in human dental pulp

DAS Parker, J Ong, V Marino*

The aim of this study was to examine: (a) the effects of thymol (2-isopropyl-5-methyl-phenol) on noradrenaline (NA) release from sympathetic nerves in human dental pulp *in vitro*; and (b) whether any effects are a consequence of activation of GABA receptors. The actions of a structurally related compound (2-tert-butyl-4-methylphenol; 4MP), were also examined.

Pulp removed from extracted teeth was incubated in gassed (95% O_2–5% CO_2) Krebs solution containing ^{3}HNA (0.6 µM) for 30 minutes and superfused with Krebs solution containing ascorbic acid (0.3 mM), desipramine (DMI; 0.3 µM) and rauwolscine (Ra; 0.1 µM). Sympathetic nerves were stimulated after 70 (S_1) and 115 (S_2) minutes (20 Hz, 1 msec, 40 V). Thymol, 4MP and the GABA receptor antagonists Sch 50911 [(+)-(S)-5, 5-dimethylmorpholinyl-2-acetic acid], bicuculline and picrotoxin were added to the superfusion medium at least 10 minutes prior to S_2. Superfusate was collected and assayed, and the fractional overflow of ^{3}H in each collection period calculated. The effects of agents were determined by comparing the overflow of [^{3}H] at S_2 relative to that at S_1 in their presence with that in their absence. Statistical analysis was conducted using one-way ANOVA, followed by Bonferroni's multiple comparison tests (GraphPad Prism 6). Significance levels were set at p < 0.05.

Thymol inhibited ^{3}HNA release (IC_{50} = 80 µM). At a concentration of 100 µM ^{3}HNA release was inhibited by 19.1%, an action not reversed by the GABA$_B$ receptor antagonist Sch 50911 (10 µM) or the specific GABA$_A$ receptor antagonist, bicuculline (10 µM). Picrotoxin (100 µM), which blocks the Cl$^-$ channels associated with GABA$_A$ receptors, prevented the inhibitory action of thymol (p < 0.05). 4MP had little effect at concentrations less than 600 µM, when it inhibited ^{3}HNA release by 38.0%. This inhibition was reversed by picrotoxin (100 µM), but not Sch 50911 and bicuculline (both 10 µM).

The results of this study imply that Cl$^-$ channels associated with GABA$_A$ receptors mediate the release of ^{3}HNA from sympathetic nerves in human dental pulp but that GABA$_B$ heteroceptors do not. These findings are in accordance with those of a previous study in which GABA was used to inhibit ^{3}HNA release in dental pulp.

*School of Dentistry, The University of Adelaide, South Australia.
Email: victor.marino@adelaide.edu.au

Central neural changes associated with repetitive non-functional tooth contact (RNTC) habits

CC Peck,* AM Youssef,† SM Gustin,† JM Reeves,† ET Petersen,‡ GM Murray,* LA Henderson†

Chronic temporomandibular disorders (TMDs) refer to a group of conditions that affect the temporomandibular joint, jaw muscles and associated structures. TMDs are the most frequent causes of chronic orofacial pain and are likely associated with clenching and grinding teeth. TMD pain probably results from changes within higher neural structures which have already been associated with the coding of acute pain stimuli. The aim of this series of experiments is to determine anatomical and functional changes associated within subjects with painful TMD who report repetitive non-functional tooth contact.

Fifteen subjects with painful TMD and 54 pain-free control subjects were recruited for the study. Using a 3 Tesla MRI scanner, a three-dimensional quantitative arterial spin labelled (qASL) series encompassing the entire brain was collected using a gradient echo, quantitative STAR-labelled (Quasar) sequence. In addition, a T1-weighted anatomical image set was collected. Using SPM8, cerebral blood flow (CBF) maps were created, normalized and smoothed. Significant differences between TMD and controls were determined by 2-sample tests (random effects, uncorrected $p < 0.001$; minimum cluster size 10 voxels, age and gender as nuisance variables).

TMD subjects displayed CBF increases in the dorsolateral prefrontal, cerebellar, premotor and anterior cingulate cortices (ACC), supplementary motor area (SMA), globus pallidus and precuneus. In addition, TMD subjects had significant CBF increases in a number of brainstem regions including the caudalis division of the spinal trigeminal nucleus (SpVc) and the region of the right principal sensory trigeminal nucleus (Vp).

TMD is associated with ongoing activity increases in brain regions that are also activated by acute pain stimuli and are thought to code the emotional and cognitive aspects of acute pain. In addition, brainstem analysis revealed that TMD is associated with increased activation of the caudalis division of the SpVc. These findings are in striking contrast to changes we have previously reported to occur in neuropathic orofacial pain and highlight the differences in neural processing between different forms of chronic pain. Furthermore, these data strongly suggest that non-neuropathic pain such as TMD is likely driven by increased tonic activity within the TMJ and associated muscles.

*Jaw Function and Orofacial Pain Research Unit, Faculty of Dentistry, The University of Sydney, Westmead Hospital, Westmead, New South Wales
†Department of Anatomy and Histology, The University of Sydney, New South Wales.
‡Departments of Radiology and Radiotherapy, University Medical Center Utrecht, The Netherlands.
Email: dentistry.dean@sydney.edu.au

Changes in inflammatory mediators in gingival crevicular fluid following periodontal disease treatment in pregnant women

B Penova-Veselinovic, J Keelan, JP Newnham, CE Pennell*

Periodontal disease (PD) in pregnancy is associated with increased risk of adverse pregnancy outcomes including miscarriage and preterm birth. Controversy surrounds the extent of this association; there is some

evidence that periodontal treatment may reduce inflammatory mediators in gingival crevicular fluid (GCF), which may reduce the risk of inflammation associated pregnancy complications.

The aim of this study was to determine if PD treatment (deep cleaning), during mid-pregnancy alters local inflammation in GCF and has beneficial effects on dental clinical parameters.

Subgroup of n = 80 pregnant women with clinically diagnosed PD were recruited from the SMILE study, a randomized controlled trial run in Perth, Western Australia. The treatment group (n = 40) underwent intensive PD treatment (20–28 weeks GA), whilst the control group (n = 40) underwent the same treatment during the postnatal period. GCF was collected at 20 and 28 weeks gestation, and concentrations of the following cytokines determined utilizing Luminex xMAP Technology: IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, MCP-1 and IL-17.

Periodontal treatment significantly reduced the levels of IL-1β (p < 0.0001), IL-10 (p = 0.003), IL-12p70 (p = 0.003), IL-6 (p = 0.013) and IL-17, but paradoxically increased the levels of MCP-1 (p = 0.028), TNF-α (p = 0.016) and IL-8 compared with controls. Post-treatment clinical parameters improved in the treatment group with significant reductions in bleeding on probing from 80.6% to 20.7% (p < 0.0005), moderate to advanced clinical attachment loss (p < 0.0005) and probing depth (p < 0.0005). No changes in pregnancy related outcomes were observed.

PD treatment in pregnancy reduces levels of some inflammatory mediators in the GCF, and has beneficial effects on dental parameters with no apparent adverse effects.

*School of Women's and Infants' Health, The University of Western Australia, Perth.
Email: craig.pennell@uwa.edu.au

Application of micro-computed tomography in identifying dental anomalies associated with apical periodontitis

S Ranjitkar,* W Cheung,*† R Yong,* M Packianathan,† J Deverell,‡ C Hall,§ D Farmer,* G Heithersay*

Apical periodontitis may be associated with the presence of subtle dental anomalies which allow microbial ingress into the root canal system. Currently available radiographic techniques have limited resolution which may lead to diagnostic dilemmas. High-resolution 3D micro CT that is currently applied only to *in vitro* human studies holds promise to improve our understanding of some dental anomalies which have been associated with apical periodontitis. Accordingly the aim of this study was to use micro CT imaging to assess the morphology of extracted non-carious teeth which had developed apical periodontitis without any apparent predisposing factor/s.

Three teeth associated with odontogenic infections were collected: (1) teeth 73 (n = 2) from two healthy patients and (2) a tooth 37 from a patient with dentine dysplasia Type 1c. Additionally, intact teeth 83, 37 and 47 from healthy patients were used as controls. All teeth were subjected to 3D reconstruction at a resolution of around 20 μm using X-radia Micro-XCT-400 imaging system and VSG Avizo Fire software (FEI, Visualization Sciences Group, Burlington, USA).

One infected tooth 73 displayed porosities (channels) communicating the pulp chamber to the oral cavity via an exposed dentino-enamel junction and the second infected tooth 73 displayed a longitudinal crack extending from the external coronal surface into the pulp chamber. The dentine dysplasia tooth 37 displayed numerous cracks in enamel, with aberrant dentine formation that extended into the pulp chamber. Control teeth did not display abnormal structural morphology or extensive cracks.

Microscopic porosities and enamel cracks in the obscure dental anomalies under investigation would have provided pathways for bacterial ingress into the root canal system, leading to subsequent development of apical periodontitis.

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

*School of Dentistry, The University of Adelaide, South Australia.
†Pediatric Dentistry Department, Women's and Children's Hospital, South Australia.
‡Ian Wark Research Institute, The University of South Australia, South Australia.
§Mawson Institute, The University of South Australia, South Australia.
Email: sarbin.ranjitkar@adelaide.edu.au

An evaluation of chair-side caries tests in the prediction of white spot lesions in patients undergoing orthodontic treatment

B Srirom, WJ Sampson, CW Dreyer, J Kaidonis, N Gully*

The aim of this study was to investigate associations between presence, number and severity of white spot lesions (WSLs) and patient characteristics; saliva properties tested with the Saliva-Check Buffer Kit (GC Corp., Belgium); and the ability of the DIAGNOdent Pen (KaVo, Germany) to identify WSLs.

Ninety-one orthodontic patients were de-identified and date of birth, gender, postcode, age at banding, time in bands, failure to attend (FTA) rate, type of bracket used, reported oral hygiene regimen and number of filled molars were recorded. WSLs were noted on their anterior teeth using the International Caries Detection and Assessment System II (ICDAS II) visual index and a laser-based caries detection device (DIAGNOdent Pen). Fifty patients had saliva properties tested which included hydration, consistency, resting pH, stimulated flow, stimulated pH and buffering capacity.

Brushing less than 14 times a week and the number of filled molars were significant for the development and severity of WSLs when the severity was greater than or equal to an ICDAS II score of 2 ($p < 0.05$). When WSLs were ICDAS II ≥ 3 severity, the FTA rate and brushing less than 14 times per week were significant predictors ($p < 0.05$). WSLs increased with brushing less than 14 times per week or an increased FTA rate ($p < 0.05$). DIAGNOdent Pen scores and ICDAS II scores ($p < 0.0001$) were strongly related. With the Saliva-Check Buffer, pH of stimulated saliva was significant for the presence of WSLs ($p < 0.05$). The pH of stimulated saliva and the quantity of saliva produced in five minutes were significant predictors of WSL severity when severity was greater than or equal to an ICDAS II score of 2 ($p < 0.05$). When the ICDAS II score was ≥ 3, the pH of unstimulated saliva reached significance ($p < 0.05$). No relationship was found between the number of WSLs and the Saliva-Check Buffer.

Reported times per week of brushing indicates the presence, severity and number of white spots. The number of filled molars before treatment might indicate the presence and severity of white spot lesion experience. High FTA rates indicate more WSLs with greater severity. The DIAGNOdent Pen correlates extremely well with the ICDAS II system for grading WSLs. The pH of stimulated saliva and unstimulated saliva plus saliva flow rate indicate susceptibility to WSLs and the severity of the lesions. The Saliva-Check Buffer cannot distinguish between patients with many or few WSLs.

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The findings of this research were presented at the Australian Society of Orthodontists Foundation Meeting, Canberra, March 2013.

*School of Dentistry, The University of Adelaide.
Email: wayne.sampson@adelaide.edu.au

The roles of Runx2 and VEGF during healing of the periodontal ligament following hypothermal insult

T Stewart, WJ Sampson, CW Dreyer, K Dharmapatni*

This study investigates the roles of Runx2 and VEGF following hypothermal insult and subsequent ankylosis in the rat dentoalveolar region. Runt related transcription factor 2 (Runx2) is the master regulator of osteogenesis whilst vascular endothelial growth factor (VEGF) controls angiogenesis.

With ethics approval (M-023-2007) the upper right first molar of 15, 18-week-old male Sprague Dawley rats underwent a 10-minute application of dry ice. Five randomly divided groups were killed at 0, 4, 7, 14 and 28 days post insult. The contralateral molar was the internal control. Three additional rats were the external control. Immunohistochemical staining was performed for Runx2 (mouse anti-rat monoclonal antibody; Abnova Corporation, Taiwan) and VEGF (VG1 monoclonal; Novus Biologicals, USA). Sections were scanned and semiquantitative counting was performed.

Ankylosis was noted on the experimental side of all animals at days 7 and 14, and one animal at day 28 post hypothermal insult. No ankylosis was present on any control teeth or experimental teeth at days 0 and 4.

Statistically significant differences in the percentage of Runx2 positive cells between treated and untreated molar teeth were found in the pulp and alveolar bone but not in the PDL. Non-statistically significant trends of changing Runx2 expression with time were noted in the pulp and alveolar bone. Runx2 expression increased at days 4, 7 and decreased at days 14 and 28 in the pulp whilst in the alveolar bone expression increased at day 4, decreased at days 7 and 14, and slightly increased at day 28.

Statistically significant difference was found between VEGF positive pulp cells on the experimental side compared to the internal control, with more VEGF positive cells on the experimental side at day 7 than at days 14 and 28. In the alveolar bone and PDL, although a statistically significant difference was found between the experimental and control sides, there was no significant interaction with time. VEGF positive cells were fewer in the PDL at days 4, 7 and 14 but greater at day 28. In the alveolar bone, more VEGF positive cells were seen at day 4 than at days 7, 14 and 28.

Changes in Runx2 and VEGF expression were found in the experimental group and were consistent with the formation of a bone-like material in the pulp and PDL.

*School of Dentistry, The University of Adelaide.
Email: wayne.sampson@adelaide.edu.au

A longitudinal study of changes in tooth and dental arch dimensions from the primary to permanent dentitions

WH Tan, WJ Sampson, GC Townsend*

The aims of this study were to investigate tooth size and dental arch dimensional changes from the mixed to permanent dentitions in Australian Aboriginals using a three-dimensional imaging system, and to investigate the extent to which tooth size and dental arch dimensions contribute to dental crowding.

Serial dental models of 49 Australian Aboriginals (28 males and 21 females) from the Yuendumu Study at ages 8, 12 and 15 years (or within a 1-year range) were used.

All the dental models were digitized using a 3D scanner (Optix 400S) and analysed with a 3D modelling software (Rhinoceros® 3D 4.0). Study variables included mesiodistal tooth widths, arch widths, arch lengths, arch depths and tooth size arch length discrepancy (TSALD). Subjects at age 15 years were then

subdivided into crowded (TSALD ≤-1 mm) and non-crowded (TSALD >-1 mm) groups for analysis.

The changes in tooth size and dental arch dimensions from the mixed dentition to permanent dentitions are summarized as follows:
(1) There was a mean reduction in mesiodistal widths of individual teeth ranging from 0.07 mm to 0.24 mm.
(2) The intercanine and intermolar widths increased in males and females from the age of 8 to 15 years.
(3) Arch length and arch depth showed a progressive reduction between 8 and 15 years of age.
(4) Sexual dimorphism was observed whereby males had larger tooth size and dental arch dimensions than females.

(5) In crowded subjects, tooth 15 in males and teeth 31, 36 and 42 in females were statistically significantly larger than the non-crowded subjects.
(6) The lower intercanine width in males and upper intercanine width in females were significantly smaller in crowded subjects.

The findings of the present study showed reduction in mesiodistal tooth width and significant changes in the dentition from the age of 8 to 15 years. No clear distinction could be made in the present study as to whether tooth size or dental arch dimensions play a greater role in contributing to dental crowding. Evidence of reduction in the tooth width over time was found despite adoption of a largely Western diet.

*School of Dentistry, The University of Adelaide.
Email: wayne.sampson@adelaide.edu.au

Development of pictograms for use in age estimation for an Australian population

JA Taylor, MRB Blenkin*

The aim of this study was to develop an atlas style chart, similar to those of Schour and Massler, and Ubelaker, relevant to an Australian population, for use in mass disaster situations.

Each diagram within the charts developed by Ubelaker was analysed using the modified Demirjian method as described by Blenkin, and was treated as a 'case' that needed an age estimation. Each tooth of the right mandibular quadrant (41–47) was assessed and rated according to the Demirjian system. These ratings were converted to numerical scores and estimates of age, including range at the 95% confidence interval, were made using Blenkin's standards. For those diagrams representing ages below the utility of the Blenkin method, data from Fanning were used to estimate the age of the cases depicted. For those diagrams representing ages older than the utility of the Blenkin method, data collected on the development of mandibular third molars by the authors and others were used. Each pictogram was then assigned an 'Australian' age, and diagrams which represented stages with inadequate differentiation were removed. A validation study was undertaken using a sample of 204 OPGs (m = 100, f = 104).

This project reinterpreted the Australian data previously collected by Blenkin and other relevant studies and applied it to a schematic similar to that of Ubelaker to develop a reliable, convenient and contemporary reference for use in age estimation.

The age estimation charts developed provide a useful tool to estimate age at time of death for cases occurring within the Australian population. The datasets on which the charts are based are relevant, contemporaneous, and based upon significant sample sizes and gender specific.

The collaboration of Dr R Bassed and Dr J Graham of the Victorian Institute of Forensic Medicine was much appreciated, as were the efforts of Dr S Chiam of the ACT Department of Health. The researchers also wish to acknowledge Dr D Ubelaker of the Smithsonian Institution for giving permission to use

his diagrams as a basis for this work. This research was, in part, made possible by the award of an Australian Dental Research Foundation grant.

*The University of Newcastle, Ourimbah, New South Wales.
Email: jane.taylor@newcastle.edu.au

A cross-sectional study investigating the association between oral bacteria and jaw osteonecrosis using intravenous bisphosphonates for malignancy

C Tsao, I Darby, G Borromeo, N O'Brien-Simpson*

Osteonecrosis of the jaw has been associated with bisphosphonates, drugs that suppress bone turnover. The pathophysiology is poorly understood though periodontal disease occurs in up to 80% of bisphosphonate associated jaw osteonecrosis (ONJ) cases, suggesting it is a risk factor. However, the role of periodontal disease as a risk factor for ONJ has not been investigated comprehensively.

The study aimed to assess periodontitis associated bacteria in patients with a history of bisphosphonates, and by comparing those with and without ONJ, sought to determine if levels of periodontitis associated bacteria are a risk factor for ONJ.

The cross-sectional study involved participants with a history of intravenous (IV) bisphosphonates for malignancy, as identified by screening pharmacy records at seven specialist dental clinics and oncology clinics in Melbourne. Participants underwent a medical questionnaire, a blood test and a clinical oral examination according to the National Survey of Adult Oral Health (NSAOH) protocol. Serum was tested for IgG titres against four bacteria associated with periodontitis (*Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Actinomycetemcomitans aggrgatibacter*).

Of the 63 participants, 22 had ONJ. No significant differences were found between the groups in terms of the number of teeth missing, untreated caries and the DMF (decayed, missing, filled) indices at both the tooth and surface levels. A significant association was found between specific IgG titres against *P. gingivalis* and ONJ on multivariate analysis (OR 2.72, 95% CI 1.19–6.22, p = 0.018), but not for the other species.

In participants with a history of IV bisphosphonates, levels of *P. gingivalis* periodontal were higher in the group with ONJ compared to those without. Further longitudinal research is required to clarify the association.

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

The effect of polyethylene glycol (PEG) based hydrogels loaded with lipoxin A4 (LXA4) on bone healing in the rat mandible

S Varanasi,* AL Symons,* F Rasoul†

Treatment of bone defects remains a critical challenge in the restoration of form and function of lost osseous tissue. LipoxinA4 (LXA4) has shown to promote reso-lution of inflammation and enhance epithelial wound healing. Polyethylene glycol (PEG) based hydrogels are three dimensional networks of synthetic polymers

developed for the application as scaffolds or as carriers for therapeutic agents. The aims of this study were to determine the effect of an injectable Boltorn™ hydrogel alone and with LXA4 on bone healing of the surgical defect.

Twelve-week-old female Lewis rats were anaesthetized and a standardized surgical defect, with a depth and diameter of 1.0 mm, placed on the left buccal aspect of the mandible. In the treatment groups, the hydrogel alone or with LXA4 (1.0 µg/100 gm body weight) was injected into the surgical defect. To determine the effect of hydrogels or LXA4, animals were sacrificed at days 5, 10, 20 and 30 post-treatment. Undecalcified transverse serial sections of the mandible with surgical defects were stained for histomorphology and for the immunoexpression of ALP, OCN and OPN. Comparisons between the groups were made by analysis of variance (ANOVA).

During normal bone healing, at day 10, the depth of the defect was reduced compared to the initial depth and the defect contained islands of unmineralized bone matrix and mineralized bone. At day 20, the defect contained dense collagenous connective tissue and trabecular bone. By day 30, the defect was occluded with mineralized bone and the buccal contour of the bone was restored.

In the undecalcified tissue section from the day 5 and day 10 hydrogel treatment groups, tearing or loss of soft tissue and hydrogels occurred, leaving artefacts within the defect. This may indicate the lack of integration of the cells into the hydrogel. By day 20 and 30, in the treatment groups, there was a delay in the new bone formation and the defect was partially occluded with new bone, resulting in a decrease in the immunoexpression of bone matrix proteins at day 20.

The hydrogel, either with or without LXA4, did not induce inflammation and the hydrogel degraded *in vivo* within 30 days. However, from day 20 onwards, the hydrogel did not support complete obturation of the defect with new bone formation and there was a decrease in the immunoexpression in the ALP, OCN and OPN compared with controls. In this study, the controlled slow release of LXA4 from hydrogel was not available.

*School of Dentistry, The University of Queensland.
†Australian Institute of Bioengineering and Nanotechnology, The University of Queensland.
Email: a.symons@uq.edu.au

In vitro full field bone strain analysis of implants loaded with axial and cantilevered abutments using reflection photoelasticity: a preliminary study in the dog mandible

SP Watson, RB Judge, JEA Palamara*

The aim of this study was to examine surface strain in the mandible using full field reflection photoelasticity when osseointegrated dental implants, in sites with variable buccal bone quality, are loaded using axial or cantilevered abutments.

Dental implants were placed in unilateral healed molar and premolar extraction sites of the mandibles of two greyhounds. Following 16 weeks of healing and successful osseointegration of the implants, the dogs were euthanized and the mandibles removed and defleshed. Buccal bone loss varied between the four sites from no loss to almost total dehiscence. Quantified occlusal loads were directed onto axial and cantilevered abutments.

Using the reflection photoelastic coating method, full field *in vitro* bone strain patterns were demonstrated. Following calibration, semi-quantitative strain data were calculated at sites of higher strain order.

In this preliminary study, loading the implant with complete peri-implant bone contact, either axially or with a cantilevered abutment, resulted in surface strains that were diffuse and of a low order across the mandible surface. Loading of implants with reduced buccal bone resulted in higher order peri-implant bone strain radiating from the marginal bone of the defect, while the extent and degree of strain redistributed to distant sites was reduced.

High occlusal load on dental implants with satisfactory peri-implant bone conditions does not appear to result in localized concentration of peri-implant bone strain. Instead, the peri-implant bone is contiguous with the mandible, operating as a functional integrated system so that strain is dissipated to distant sites. When buccal bone-implant contact is compromised, strain is concentrated more locally in peri-implant bone on either side of the defect. High loads, particularly when magnified by cantilever moments, may induce pathological strain in the peri-implant bone environment. The reflection photoelastic method described in this study is useful for full field bone strain analyses, and can be adapted to provide semi-quantitative data.

*Melbourne Dental School, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Victoria.
Email: simon_watson@mac.com

Changes in jaw muscle pain, activity and jaw movement after stressful experiences

T Whittle, GM Murray*

Jaw muscle pain is the most common symptom in temporomandibular disorders with >6% of the population seeking treatments that are based on perceived relations with maladjusted occlusion, centrally mediated muscle hyperactivity/spasticity, bruxism or psychological disorders. Stress, implicated in many disorders, is considered a possible cause or facilitator of jaw muscle pain. This project investigates the effect of two stressful experiences, a personally-relevant and a cognitive experience, on jaw muscle activity and movement, and their effects on jaw muscle pain perception.

A within-subject double-stressor cross-over design recorded activity bilaterally from the masseter and temporalis, and right digastric muscles in 30 participants: 15 chronic jaw muscle pain patients (CP) and 15 no-pain control participants (PF). The participants were age and gender matched, screened for medical or psychological disorders, and self-reported on their mood and levels of stress, fear and anxiety during the recording session. Personally-relevant stressors were a self-made tape recording of a previous stressful experience and the cognitive stressor was a computer generated math task which matched level of difficulty with math competency. Jaw movement and pain perception were recorded during rest, open, close, free chewing (FC) of gum and controlled chewing (CC) of gum tasks. Jaw muscle electromyographic (EMG) activity was recorded bilaterally from the masseter, anterior temporalis and right digastric muscles. Statistical significance was set at $p < 0.05$.

Jaw movement analysis found significant stressor effects in both the FC and CC tasks for opening and closing amplitude and velocity although no significant group differences were found. EMG analysis showed no activity during rest although significant main effects of stressor were shown in the bilateral elevator muscles in most movements. Only right digastric muscle activity between baseline and cognitive stressor were significantly different between the groups in both FC and CC in opening and closing movements. The CP and PF groups did not differ in age, gender, levels of stress, fear, or anxiety, personality traits, mood or psychological assessment with the exception of somatization and intensity of symptomatic distress where CP patients reporting greater levels than controls.

This study found that acute stressful experiences, whether emotional or cognitive, have an effect on jaw muscle activity and kinematics. Interestingly, and contrary to past research, few differences were found between the chronic jaw pain sufferers and pain free controls. Thus, considering patient stress in dental treatments should not be isolated to pain patients.

*Jaw Function and Orofacial Pain Research Unit, Faculty of Dentistry, The University of Sydney.
Email: terry.whittle@sydney.edu.au

The findings of this research were presented at the IASP 14th World Congress, Milan, Italy, 2012.

Effect of salivary cortisol levels by a stressful experience in chronic jaw muscle pain patients

T Whittle, GM Murray*

Temporomandibular disorders (TMD) are common with >1.2 million of the population seeking treatment. Although studies have suggested an association between stress and chronic muscle pain disorders, the exact nature of the relationship is not known. Consensus points to functional dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis which relates to symptoms such as recurrent pain, fatigue and sleep disturbances. Several studies with fibromyalgia patients suggest a blunting of the stress response or hypocortisolism in chronic muscle pain sufferers. The current study compared the level of salivary cortisol after two stressful experiences, a cognitive stressor and a personally-relevant stressor, in chronic jaw muscle pain patients (CP) and healthy pain-free controls (PF).

Thirty participants (15 CP and 15 PF) participated in a cross-over within-subject study where 2 stressors, a personally-relevant pre-tape of a stressful experience (tape) and a cognitively oriented computer-generated math task that adjusted for math competency (math), were experienced for 5 minutes each. The stressors were separated by jaw movement tasks and a 5-minute rest period. Saliva was sampled at 6 time points throughout the procedure using commercial sterile cotton rolls which were outsourced to a laboratory for cortisol analysis. Changes in cortisol levels from baseline were calculated for each sample to account for individual/diurnal cortisol differences between participants. Participants self-reported levels of anxiety, fear, stress and pain perception at each time point.

No significant differences between the CP and PF groups were found in age, gender, personality traits, mood or psychological assessment. No significant differences in cortisol change scores were found between the math or tape stressors. A significant effect of group was found across all samples with the CP patients showing a decrease in cortisol that was significantly different to PF participants after the second stressor. Changes in cortisol levels were associated with early levels of self-reported anxiety but not with fear, stress or pain perception.

This study has shown a possible dysfunction of the HPA axis in diagnosed TMD patients similar to findings from studies in other chronic muscle pain disorders. Although the type of stressor seemed irrelevant, the experience of concurrent stressful events affected the TMD patient's stress response. Further research with TMD patients to detect plasma levels of cortisol, and other adrenal-related hormones, is needed to explain these novel results.

*Jaw Function and Orofacial Pain Research Unit, Faculty of Dentistry, The University of Sydney.
Email: terry.whittle@sydney.edu.au

Evaluation of a novel approach in the prevention of white spot lesions around orthodontic brackets

J Yap,* S Naser-ud Din,* HC Ngo,* LJ Walsh,* DJ Manton†

The purpose of this study was to evaluate and compare the relative efficacy of a resin fissure sealant, nano-filled self-adhesive protective coating, resin infiltrant, glass ionomer cement (GIC) and GIC containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) in preventing the formation of subsurface lesions of enamel (SLE) adjacent to orthodontic brackets by acting as an enamel surface sealant (ESS).

Eighty-five enamel specimens with molar tubes bonded at their centre were randomly divided into five groups, each treated with a different material at the bracket's periphery. Specimens were stored in an acetate demineralization solution at pH 4.5 for 7 days at

37 °C then imaged using QLF to determine the differences in fluorescence (ΔF) between sound- and acid-exposed enamel. Cross-sections of the lesion areas were then examined using SEM in back-scattered mode to measure lesion depth.

The use of GIC alone or with the incorporation of CPP-ACP significantly reduced ΔF compared with the other materials. Back-scattered SEM images showed no measureable demineralization for samples treated with either GIC material compared with the other materials.

Based on the results of this *in vitro* study, the fluoride-releasing effects and CPP-ACP benefits of the GIC materials show promise as an effective enamel surface sealant in inhibiting enamel demineralization adjacent to orthodontic brackets.

*School of Dentistry, The University of Queensland.
†Melbourne Dental School, The University of Melbourne.
Email: Jason_balwyn@yahoo.com

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ADRF Dental Student Research Grant Abstracts

Sterilization of rotary NiTi instruments when inserted into endodontic sponges

HWA Chan;* K Tan,*† SG Daspher,*† P Parashos* (Supervisors)

The aim of this study was to determine whether endodontic sponges can be sterilized by autoclaving and whether rotary nickel-titanium (NiTi) endodontic instruments inserted into endodontic sponges can be sterilized.

Sixty-four samples of eight different endodontic sponges (n = 512) were placed into Brain Heart Infusion Broth (BHI) solution for 72 hours. An aliquot of this was then spread onto Horse Blood Agar (HBA) and cultured aerobically and anaerobically to test sterility at purchase.

Sterile sponges (n = 512) were contaminated with *Enterococcus faecalis, Porphyromonas gingivalis* and *Geobacillus stearothermophilus* BHI bacterial suspensions; 256 contaminated sponges were autoclaved and the other 256 acted as positive control. Samples were placed into BHI for 72 hours that was then spread onto HBA and cultured aerobically and anaerobically.

Rotary NiTi instruments (n = 512) were contaminated with *E. faecalis, P. gingivalis* and *G. stearothermophilus* BHI bacterial suspensions. Of these, 128 were inserted into sterile endodontic sponges and another 128 were not. The remaining 256 instruments were immersed in the bacterial suspension but without inserting into sponges. The 128 contaminated instrument-sponge complexes and 128 NiTi contaminated instruments were then autoclaved at 134 °C for 3 minutes. The remaining 256 contaminated instruments acted as controls. All 256 rotary instruments were placed into separate sterile containers containing BHI solution and incubated at 37 °C for 72 hours. The instruments were then inserted into HBA and cultured aerobically and anaerobically. The success of sterilization was measured qualitatively as either growth or no-growth.

The experiment was repeated with clinically used rotary NiTi instruments (n = 512) obtained from three endodontic clinics.

ADM Endofoam PVP sponges and Gunz blue round sponges exhibited microbial growth when aerobically cultured. All of the 256 autoclaved endodontic sponges showed no microbial growth. All control sponges (n = 256) showed microbial growth.

None of the autoclaved endodontic instruments with or without insertion into endodontic sponges showed microbial growth when aerobically and anaerobically cultured. All control samples showed microbial growth.

Not all endodontic sponges were sterile on purchase but all could be successfully sterilized by autoclaving. Therefore, sponges should be autoclaved before clinical use to achieve an acceptable sterility assurance level.

Rotary NiTi endodontic instruments can be sterilized with or without insertion into endodontic sponges. Therefore, it would be clinically efficient and cost-effective to sterilize endodontic instruments in endodontic sponges.

*Melbourne Dental School, The University of Melbourne, Victoria.
†Oral Health Cooperative Research Centre, The University of Melbourne, Victoria.
Email: parashos@unimelb.edu.au

Dental anxiety in patients attending a university dental clinic

AB Hua; J Kroon (Supervisor)*

Dental anxiety is a debilitating barrier to oral health care access in Australia. Its impact on patients attending a university dental clinic has yet to be investigated in detail. The purpose of this study was to describe both the prevalence of dental anxiety in a clinical setting and to explore the relationships between dental anxiety and several demographic variables.

A cross-sectional study (n = 368) was conducted on patients older than 18, attending the Griffith University Dental Clinic. It included a questionnaire developed specifically to measure aspects of patient demographics, dental visits and dental anxiety. It incorporated the Index of Dental Anxiety and Fear (IDAF-4C) developed by Armfield (2010). All participants were interviewed individually.

The prevalence of high dental anxiety in the study population was 25.6% (n = 94). The majority of participants were middle-aged and from low socio-economic backgrounds. The greatest prevalence of high dental anxiety occurred for those with no tertiary education, who had very poor self-rated oral health, who had not visited a dental professional for over 2 years and who demonstrated symptomatic visitation patterns. Financial cost was identified as the greatest barrier to dental care. To help reduce dental anxiety, most patients wanted more information regarding the actual dental procedure.

Patients attending a university dental clinic have significantly higher levels of dental anxiety compared to previous studies in other clinical settings. Numerous differences between individuals with high dental anxiety and low dental anxiety in terms of education, self-rated oral health perception, dental visits and barriers to access were identified. Early identification and effective management of such patients is critical to help minimize dental avoidance and consequently poorer oral health.

*School of Dentistry and Oral Health, Gold Coast Campus, Griffith University, Queensland.
Email: j.kroon@griffith.edu.au

Cortical thickness in normal human femora: its relevance to the understanding of atypical femoral fracture as another complication of bisphosphonate therapy

C Jovanovic; JG Clement, CD Thomas (Supervisors)*

Bisphosphonates are drugs that inhibit bone resorption, primarily via effects on osteoclasts. However, long-term use of nitrogen-containing bisphosphonates may have unwanted side effects and two have been identified as potentially serious problems: osteonecrosis of the jaw (ONJ) and atypical femoral fracture (AFF). Changes in the geometry of femora that have been exposed to bisphosphonates are poorly understood. This study aimed to contribute baseline data from normal subjects for comparison with that from cases of AFF.

Three hundred and fourteen femoral computed tomography scanned specimens were analysed by means of mapping the cortical thickness at four distinct regions of interest (ROIs) along the femur, being the lesser trochanter, greater third diaphysis, mid-diaphysis and the lesser third diaphysis. At each ROI, an additional four locations were selected to represent posterior, anterior, lateral and medial aspects. Cortical thickness estimates were achieved using the technique implemented in Stradwin software (v4.6, http://mi.eng.cam.ac.uk/~rwp/stradwin/) and data were analysed statistically using the Statistics Toolbox in MATLAB (v6.1, The MathWorks Inc., Natick, MA, 2000). Comparisons were made between: (1) male and female specimens for all ROIs at all locations; (2) left and right femurs of the same sample for all ROIs at all locations; and (3) medial and lateral locations at all ROIs for both left and right femurs.

It was found that femoral cortical thickness in males was significantly greater than in females for all ROIs at all locations (posterior, anterior, lateral and medial) (where $p < 0.05$). No other consistent significant difference was determined from the statistical comparisons conducted.

The results from this study provide important evidence for the understanding of normal human femoral geometry for comparison with that in cases of AFF. Future investigations will help clinicians understand how bisphosphonate therapy may act on the relative contributions of organ anatomy and the changed

tissue qualities of normal bone that predispose to destructive oral conditions such as ONJ.

*Melbourne Dental School, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Victoria.
Email: christina.j.jovanovic@gmail.com

Enamel softening during early stages of wine erosion

S Kwek,* M Mian;* C Hall,† R Yong,* J Kaidonis,* GC Townsend,*
S Ranjitkar* (Supervisors)

Extensive wine tasting by professional winemakers and wine judges is an occupational hazard, but little is understood about the effects of early stages of wine erosion on the tribological behaviour of bioceramic materials including enamel and dentine. The aim of the present *in vitro* study was to investigate early wear behaviour of enamel from wine erosion over a period of 10 minutes.

Flat, polished enamel specimens (n = 10) were bathed in artificial saliva for two hours before baseline nanoscratches were placed. Each specimen was then subjected to alternate demineralization–remineralization cycles for 10 episodes, with each episode comprising one minute of wine erosion (pH 3.0) followed by one minute of remineralization in artificial saliva. Nanoscratches were placed with a spherical tip (20 μm radius) in a nanoindenter under a load of 100 mN at baseline (stage 1), after a one-erosion episode (stage 2) and after 10-erosion episodes (stage 3). A linear mixed model analysis was used to determine whether the mean scratch depths and surface roughness (Rq) values were significantly different between the three stages described.

The mean ± SE values at the three stages for scratch depth were 0.71 ± 0.06 μm, 0.66 ± 0.03 μm and 1.00 ± 0.10 μm, and for surface roughness were 0.012 ± 0.003 μm, 0.013 ± 0.003 μm, 0.032 ± 0.002 μm. There were significant effects of erosion stages on both these parameters ($p < 0.001$). *Post hoc* paired *t*-tests showed significant differences in both parameters between stages 1 and 3 ($p < 0.001$), and between stages 2 and 3 ($p < 0.01$).

Enamel softening after 10 minutes of wine erosion indicates the vulnerability of this bioceramic material to wear rapidly from mechanical wear during and after wine tasting. This emphasizes the need to implement preventive strategies against mechanical wear from attrition, toothbrushing and dietary abrasion when protective host factors are compromised.

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The findings of this research were presented at the Biomouth 2013 Symposium, University of Otago, Otago, New Zealand, 9 October 2013.

*School of Dentistry, The University of Adelaide, Adelaide.
†Mawson Institute, The University of South Australia, Mawson Lakes.
Email: sarbin.ranjitkar@adelaide.edu.au

The effects of the Y chromosome and intrauterine male hormones on tooth size and shape

F Lam;* AH Brook,*† S Ranjitkar,* R Yong,* T Hughes,* GC Townsend* (Supervisors)

Dental development is a Complex Adaptive System in which genetic, epigenetic and environmental factors (including genes on the Y chromosome and intrauterine male hormones) interact during different stages to influence tooth size and shape. This ongoing study aims to quantify the influences of these two factors on the primary and permanent dentitions.

Using an advanced 2D image analysis system, each tooth in the primary and permanent dentitions of 30 females from dizygotic opposite-sex (OS) twin pairs and 30 females from dizygotic same-sex (SS) twin pairs is being studied. The mesiodistal, buccolingual, crown height and intercuspal linear dimensions are being measured. Furthermore, tooth size and shape are being analysed by calculating perimeters and surface areas, and customized macros define the 25th, 50th, 75th percentiles along linear planes. Paired t-tests have shown no significant differences between left and right sides, so data have been combined for statistical analysis. MANOVA, Principal Components Analysis and Euclidian Distance Measurement Analysis are being utilized to quantify the differences in tooth size and shape.

Analyses have shown that females in the OS cohort have larger tooth size than their SS counterparts. This trend has been observed in all variables analysed (i.e. mesiodistal, perimeter and surface area from both occlusal and vestibular views). The perimeters and surface areas of permanent incisors and first molars have been found to be significantly greater in the OS cohort than the SS cohort ($p < 0.05$). Permanent canines in the OS cohort have also been found to be significantly wider in the mesiodistal dimension from the occlusal view ($p < 0.05$).

Findings for the permanent dentition have shown a trend of increased tooth size in females from OS twins compared to their SS counterparts. Utilization of tooth measurement data can contribute to the biological understanding of the impact of genetic, epigenetic and environmental factors, and have significance in paediatric clinical practice and research.

*School of Dentistry, The University of Adelaide, South Australia.
†Institute of Dentistry, Queen Mary University of London, United Kingdom.
Email: alan.brook@adelaide.edu.au

Description of total population hospital admissions for cleft lip and/or palate in Australia

JYJ Lo;* N Kilpatrick,*† LM Slack-Smith* (Supervisors)

Orofacial clefts are among the most common observed congenital malformations worldwide. Management for these conditions can require multiple hospital admissions over an affected individual's lifespan. The purpose of this study was to describe admission rates to hospitals for orofacial clefts across Australia over a 10-year period using total population data. Average length of stay along with associations between different age groups, gender and year were investigated to determine any influence on admission rates.

Data for 11 618 hospital admissions from public and private hospitals were obtained from the Australian Institute of Health and Welfare National Hospital Morbidity Database for the financial years of 2000 to 2010 inclusive. The outcome variable examined was hospital admission with the principal diagnosis of isolated cleft palate (CP), isolated cleft lip (CL) and concurrent cleft lip and palate (CLP) classified according to ICD-10-AM diagnosis codes Q35–Q37 using estimated resident population figures for each year as the

denominator for rates. Statistically significant trends over time between gender and cleft type were investigated using negative binomial regression measured with Stata v13.1.

Throughout the 10-year period, 4913 admissions for CLP (0.25 per 10 000 people per year), 4454 admissions for CP (0.22 per 10 000 people per year) and 2251 admissions for CL (0.11 per 10 000 people per year) were recorded throughout Australia for those aged 0 ≤79. Statistically significant trends over time for groups (cleft type and gender) using negative binomial regression were admissions of female CP (p < 0.0270), female CL (p < 0.0081) and male CLP (p < 0.0022). Overall, males were more likely to be admitted to hospital than females and the average length of stay was highest for those with CLP. The majority of admissions occurred prior to adolescence in CP and CLP, although CLP showed higher admission rates until late teens. Admissions continued across the lifespan for all groups, with a steady decline in rate after ages 20–24.

This study will provide useful total population level data on admissions for orofacial clefts in Australia.

*School of Dentistry, The University of Western Australia, Perth.
†Murdoch Childrens Research Institute, Melbourne.
Email: linda.slack-smith@uwa.edu.au

Understanding the factors influencing preschool child oral health

D Lucas; A Arora (Supervisor)*

This study aimed to explore the knowledge and views of mothers living in South-West Sydney regarding risk and preventive factors that can have an impact on childrens' oral health. The aim was to find any common information that was unknown amongst subjects to get a better understanding of the information mothers are not receiving regarding their child's oral health.

Mother-infant dyads (n = 21) were selected from a population based cohort study that begun in 2010 focusing on preschool children living in disadvantaged areas of Sydney's South-West. The caretakers were invited to take part in a qualitative study, where a semi-structured, in-depth interview technique was utilized to explore their views on factors influencing early childhood oral health. Interviews were audio-recorded, transcribed verbatim, and subsequently analysed using thematic coding.

Four themes were identified: (1) importance of the first set of teeth; (2) importance of the first dental visit; (3) getting to know if the child had dental problems; and (4) the importance of diet and spoon sharing. Responses that represent the majority of those interviewed have been discussed in order to identify particular factors related to oral health in preschool children which are poorly understood by mothers in Sydney.

Mothers in South-West Sydney require more information regarding how to maintain their child's oral health, as well as the roles of the dentist, fluoride and oral hygiene. Information from this report may be useful for future health promotion programmes providing pregnant women or new mothers with the information they need to reduce the risk of early childhood caries and optimize their child's oral health.

The findings of this research were presented at the Sydney University Faculty of Dentistry Research Day.

*Faculty of Dentistry, The University of Sydney, New South Wales.
Email: amit.arora@sydney.edu.au

Three-dimensional imaging of microwear texture in anterior human teeth

C Mann;* S Ranjitkar,* AH Brook,*† JA Kaidonis,* D Lekkas,* GC Townsend* (Supervisors)

Erosive tooth wear is an issue of growing concern, as its tendency for slow progression means that it is often only detected clinically after extensive tooth wear has occurred. Therefore, greater emphasis should be placed on early diagnosis and prevention before restorative management is considered. However, current methods of assessing tooth wear lack sensitivity to detect small amounts of wear. By calculating microwear characteristics of worn surfaces from high-resolution 3D reconstructions, the aim of the present study was to characterize wear patterns associated with attrition and erosion.

Thirty-six extracted human anterior teeth with pre-existing attrition, erosion and combined wear were selected (n = 12 per group). Attrition facets were limited to enamel, except in one tooth, whereas erosion and combined wear involved both enamel and dentine. The worn surfaces were scanned using confocal microscopy to generate three-dimensional models (at an optical resolution of 0.15 μm) that were subjected to complex mathematical modelling to calculate microwear complexity (i.e. measurement of peak to valley distances) and anisotropy (i.e. measurement of the variation in scratch direction).

Kruskal–Wallis one-way ANOVA showed an overall effect of wear type on both microwear complexity and anisotropy for enamel. Enamel complexity in both the erosion group (median, IQR; 4.1, 1.8–5.9) and the combined wear group (4.9, 2.0–6.2) was significantly greater than that in the attrition group (0.7, 0.4–2.9) ($p < 0.05$). There was a trend for complexity in the erosion group to be lower in enamel (4.1, 1.8–5.9) than dentine (5.7, 4.0–12.1) ($p = 0.06$). Mann–Whitney U test showed that anisotropy was greater in enamel than dentine in both the erosion group (0.0030, 0.0023–0.0037 vs 0.0014, 0.0010–0.0018) ($p < 0.001$) and the combined wear group (0.0025, 0.0018–0.0026 vs 0.0015, 0.0012–0.0018) ($p < 0.05$).

This is the first report on quantitative assessments of attrition, erosion and combined wear in the form of microwear complexity and anisotropy values in anterior human teeth. Further refinement of the confocal microscopy imaging technique has the potential to provide a new sensitive diagnostic clinical tool for longitudinal assessment of tooth wear.

*School of Dentistry, The University of Adelaide, Adelaide, South Australia, Australia.
†Queen Mary University of London, London, United Kingdom.
Email: chelsea.mann@gmail.com

A review of the literature describing the training of medical practitioners in oral health – implications for Australia

ND Naren; N Li, R Cho, S Naoum, D Walker (Supervisors)*

Oral diseases are some of the most prevalent diseases in Australia, and have been shown to affect the general health of a patient, as well as nutrition, speech and socialization. It is well documented that Australians residing in rural and remote Australia, as well as Indigenous Australians, have less access to dental care, as both a result of socio-economic factors and availability of dental practitioners. In these communities, there is a burden on medical practitioners in treating patients who present with oral disease. Training of medical practitioners in knowledge and treatment of oral diseases has been poorly documented. This review aims to address this gap in knowledge.

A literature search for articles from 1990 and beyond on the training of medical practitioners in oral diseases was conducted using Medline via OvidSP. Analysis of titles, abstracts and full texts were conducted, and the articles relevant to this literature review were selected.

Of the 817 articles which resulted from the literature search, 41 were deemed relevant to the training and level of knowledge of oral diseases of medical practitioners after analysis of titles and abstracts. Full text analysis was conducted for the 41 relevant articles. The articles were broadly categorized into four categories, namely: current level of knowledge and training in oral health; training in screening for oral health; training in oral health promotion; and training in clinical oral health care. The analysis showed that whilst in rural, remote and Indigenous communities patients often present to medical practitioners with oral diseases, there is very limited training in oral health conditions for these practitioners.

This review of the current literature describing the oral health knowledge and training of medical practitioners identifies very limited training in oral health, during both their undergraduate and post-registration careers. This is of particular concern for medical practitioners serving rural, remote and Indigenous communities with very limited access to dental personnel. There is a need for recognition of the problem by stakeholders, and for the development of formal, structured training programmes to equip medical practitioners in the management of basic dental conditions that may present to them. This review is a first step in that direction.

*Faculty of Dentistry, The University of Sydney.
Email: nikinaren@gmail.com

Effect of ultrasonication on physical properties of mineral trioxide aggregate

A Phoon; C Sathorn, P Parashos (Supervisors)*

The aim of this study was to evaluate the effect on physical properties of mineral trioxide aggregate (MTA) when using direct hand compaction during placement and when using hand compaction with indirect ultrasonic activation with different application times.

One hundred acrylic canals were obturated in 3 increments with MTA in sample sizes of 10. One group was obturated by hand with an endodontic plugger and the remainder obturated with indirect ultrasonic application, with times ranging from 2 seconds to 18 seconds per increment. Microhardness values, dye penetration depths and radiographs of the samples were evaluated.

As ultrasonic application time per increment increased, microhardness values fell significantly while dye penetration values increased. Microhardness of MTA ultrasonicated for 2 seconds was significantly higher than hand compaction. Most radiographic voids were visible in the hand-compacted group, which also had higher dye penetration depths than the 2-second ultrasonicated samples. Ultrasonication of MTA for 10–18 seconds resulted in significantly more voids than 2–8 seconds of ultrasonication.

The use of ultrasonics with MTA improved the compaction and flow of MTA, but excessive ultrasonication adversely affected MTA properties. A time of 2 seconds of ultrasonication per increment presented the best compromise between microhardness values, dye penetration depths and lack of radiographic voids.

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

Immunolocalization and distribution of proteoglycans in healthy and carious dentine

K Stankoska,* L Sarram;* S Smith,† MV Swain,‡ CB Little,† LE Bertassoni‡ (Supervisors)

Dentine is a mineralized tissue underlying the tooth enamel that is significantly affected by caries. Proteoglycans, along with collagen and certain non-collagenous proteins represent an important building block of the dentine organic matrix, where these structures have been recognized to regulate dentinogenesis and play important structural and mechanical roles. While the presence of proteoglycans in dentine has been extensively studied, their specific distribution and involvement in the progression of caries has not been established in the literature. Therefore, the aim of this study was to investigate and compare the immunolocalization of three small collagen-binding proteoglycans, biglycan, fibromodulin and lumican, as well as collagens type I and VI in adult permanent healthy and carious dentine.

Extracted teeth were fixed in 10% formalin, decalcified in 5% formic acid and paraffin embedded. Mid coronal longitudinal sections of the entire tooth were immunostained with antibodies recognizing the specific proteoglycan core proteins and collagens.

Results demonstrated that in healthy dentine, proteoglycans appeared to be more abundant near the tubule walls and directly under the cusps. Conversely, in carious dentine, specific locations appeared to be more prone to proteoglycan degradation than others. These degradation patterns were well correlated with the progression of caries into the tissue, which also appeared to trigger interesting morphological and compositional changes, such as the deformation of dentine tubules near highly infected areas and the lower concentration of proteoglycans in tertiary dentine.

In summary, this study presents new insights into the involvement of proteoglycans in the progression of caries, which provide an improved understanding of the disease and how tooth structures may respond to it.

The findings of this research were presented at the Sydney University Faculty of Dentistry Research Day, 19 September 2013.

*Faculty of Dentistry, The University of Sydney.
†Raymond Purves Research Laboratory, The University of Sydney.
‡Biomaterials Research Unit, Faculty of Dentistry, The University of Sydney.
Email: luiz.bertassoni@sydney.edu.au

Using 3D *ex vivo* fluorescence model to evaluate the efficacy of 980 nm laser and colloidal silver compounds upon bacterial biofilms grown on root treated teeth

DK Thalagala; R George (Supervisor)*

Inadequate bacterial biofilm eradication from the canal space during chemomechanical preparation is a major contributor to poor root canal treatment outcome. This study evaluated the efficacy of two different concentrations of colloidal silver and 980 nm diode laser therapy to eliminate *Enterococcus faecalis* across the depth and surface of the root canal, using 3D *ex vivo* fluorescence model.

Eighty-seven extracted human single rooted teeth were chemomechanically prepared according to standard root canal protocol, prior to establishing an *E. faecalis* biofilm on the external root surface of each sample over six days. This external root surface model provided the treatment protocols with a challenge to diffuse through the internal root canal anatomy and establish bacterial kill on the external root surface. It was hypothesized that this model would allow for better elevation of bacterial kill across the thickness and surface of dentine. This study used seven different intracanal treatment protocols to evaluate efficacy of bacterial kill: Group 1 (5.2 ppm electrolyte silver colloid); Group 2 (10 ppm silver citrate colloid); Group

3 (980 nm laser irradiation for 15 seconds – laser control); Group 4 (Group 1+ laser for 15 seconds); Group 5 (Group 2+ laser for 15 seconds); Group 6 (negative control – no treatment); Group 7 (calcium hydroxide). To evaluate the viability of *E. faecalis* on the external root surface over different time periods (24 hours, 48 hours and 72 hours), all samples were stained with *Bac*light^{TM} (Invitrogen Corporation, Carlsbad, CA, USA) containing SYTO9 and propidium iodide and assessed using confocal fluorescent microscopy.

Groups 1–5 showed significant bacterial kill (p < 0.001) when compared to Groups 6 and 7 over 72 hours. Group 4 showed significantly (p < 0.01) greater bacterial kill at the 72 hour time point, when compared to Groups 3, 6 and 7.

*School of Dentistry and Oral Health, Griffith University.
Email: drroygeorge@gmail.com

The results of this study indicate that colloidal silver compounds and 980 nm laser therapy may provide an alternative to current endodontic disinfection protocols. The novel 3D fluorescence model allows for real time evaluation of bacterial kill over different time periods.

The findings of this research were presented at the International Association of Dental Research, Brisbane, 28 September 2014.

Analysis of the nutritional content and health claims of infant and toddler ready-to-eat foods and its implications to early childhood caries

N Trisna; TA Pinchin, A Arora (Supervisors)*

The purpose of this study was to analyse the nutritional content of infant and toddler foods readily available in leading Australian supermarkets and its potential impact on preschool child oral health.

'Nutrition facts' labels of infant and toddler food (n = 317) were analysed to assess the nutritional quality based on the US Center for Science in the Public Interest's 'Foods of Poor Nutritional Quality' criteria. Bivariate analysis was conducted to determine an association between nutritional quality and health claims of baby foods.

Twenty-two per cent of the infant foods analysed were of a poor nutritional quality. Of the poorly nutritious foods, 63% were not nutritious due to their saturated fat and sugar content. Most snack foods (77%) for children under the age of six years were poor in nutritional quality. Interestingly, 48% of foods with poor nutritional content had some sort of a health claim on the packaging whilst 14% of foods that were healthy had claims (χ^2 = 31.43, p < 0.001).

This evidence increases the need for government bodies to regulate the health claims on infants and children food. More evidence is needed to investigate the relationship between the increasing availability of ready-to-eat infant and toddler foods, and its influence on child oral health.

*Faculty of Dentistry, The University of Sydney.
Email: amit.arora@sydney.edu.au

The efficacy of alkali solutions for decontaminating titanium surfaces investigated with SEM: a pilot study

M Wei; C Tran, A Kazoullis, C Yip, LJ Walsh (Supervisors)*

Biofilm on dental implants plays an important role in the aetiology of peri-implantitis. Alkali solutions may exert proteolytic actions which could disrupt biofilms on implant surfaces. This study evaluated the effectiveness of several alkali solutions in removing biofilm from etched titanium discs.

Seven alkali solutions were used, allowing comparison of conventional and nanoparticle calcium hydroxide in water; calcium hydroxide in polyethylene glycol (PEG) 200 or 400; calcium hydroxide in glycerol; sodium hypochlorite; and calcium hypochlorite in water. Distilled water served as the negative control. Biofilm was grown on titanium discs which had been etched with 0.2% hydrofluoric acid for 2 minutes and then placed in an *in situ* model. Treated surfaces were examined using scanning electron microscopy and the area of biofilm remaining calculated as a percentage from backscatter images.

All alkali-treated solutions showed less biofilm remaining than samples immersed in distilled water ($p < 0.001$). In descending order, the percentage area of biofilm remaining was 31.0% for water; 22.7% for 7.5% calcium hypochlorite in water; 21.2% for 7.5% sodium hypochlorite; 16.9% for 1.6% calcium hydroxide in glycerol; 15.9% for 1.6% calcium hydroxide in PEG 400; 11.3% for 0.4% calcium hydroxide in water; 10.1% for 1.6% calcium hydroxide in PEG 200; and 4.7% for 0.4% nano calcium hydroxide in water.

While all alkali solutions tested were more effective in removing biofilm from etched titanium discs than water, the preparation using nanoparticle calcium hydroxide had the highest efficacy in this model system.

This project was funded by an undergraduate research grant from the Australian Dental Research Foundation.

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

Assessment of alkaline agents on cleaning biofilm contaminated titanium surfaces using protein quantification: a pilot study

C Yip; C Tran, A Kazoullis, M Wei, LJ Walsh (Supervisors)*

Due to the rising popularity of dental implants to treat missing teeth, peri-implant diseases such as peri-implantitis have become increasingly common disorders observed in clinical practice. The aetiology of this group of diseases lies in the accumulation of a pathogenic biofilm around the implant surface. The aim of this present study was to assess the effectiveness of various alkaline agents at removing biofilm from contaminated titanium surfaces, according to protein concentration.

Ninety-six hour mixed biofilms were cultivated on 0.2% hydrofluoric acid-etched titanium discs using human saliva as the inoculum. The biofilms were treated with one of seven alkaline treatment solutions using immersion: (1) 0.4% calcium hydroxide ($Ca(OH)_2$); (2) 0.4% $Ca(OH)_2$ nanoparticles in water; (3) 1.6% $Ca(OH)_2$ in polyethylene glycol (PEG) 200; (4) 1.6% $Ca(OH)_2$ in PEG 400; (5) 1.6% $Ca(OH)_2$ in glycerol; (6) 7.5% sodium hypochlorite (NaOCl); or (7) 7.5% calcium hypochlorite ($Ca(OCl)_2$). Distilled water was used as the negative control, whilst NaOCl was regarded as the positive control. After treatment, the discs were transferred into a phosphate buffered saline (PBS) solution and then sonicated for 15 minutes to disperse any biofilm remaining on the disc. A Bradford protein assay was used to quantify the concentration of protein remaining in the buffer.

NaOCl and $Ca(OCl)_2$ removed significantly more biofilm than distilled water. The best $Ca(OH)_2$ vehicle was PEG 200. The viscous vehicles of PEG 400 and glycerol did not exert significant effects on biofilm reduction in this model compared to distilled water.

Alkaline chemical agents, such as hypochlorites and certain preparations of calcium hydroxide, can help

remove biofilm from titanium surfaces. They have potential for augmenting mechanical methods of surface cleaning, provided biocompatibility issues can be addressed.

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

The effect of topical capsaicin induced intraoral mucosal pain on human jaw function

NN Zhao; GM Murray, A Forte, CC Peck (Supervisors)*

The aim of this study was to determine whether mucosal pain, evoked through a novel topical capsaicin model applied to the gingival mucosa, has an effect on jaw movement and jaw muscle activity.

Twenty-six asymptomatic subjects were recruited and allocated to either a pain study group (6 male, 7 female, mean age $\pm$ SD 29.5 $\pm$ 4.9 yrs) or a control group (6 male, 7 female, mean age $\pm$ SD 29.6 $\pm$ 3.4 yrs). Jaw movement (velocity, amplitude and chewing rate) and average (root mean square values) electromyographic activity from five jaw muscles (bilateral anterior temporalis muscles, bilateral masseter muscles and right digastric muscle) were recorded from all the subjects during the following tasks: free jaw open and close, resistant jaw open, and free and standardized chewing. Recordings were obtained at baseline and test sessions while wearing a custom upper mouth guard coated with either 1% capsaicin (for pain group) or inert placebo cream (for control). The changes in jaw movement and muscle activity from baseline to test between pain and control group were compared statistically with linear mixed model analysis.

In comparison with control, experimental mucosal pain did not have a statistically significant effect on amplitude and velocity of jaw movement during open/close movements and during chewing ($p > 0.05$, independent t-test) but did result in a significant increase in chewing rate during free and standardized chewing ($p < 0.05$). All muscles' activities did not show a significant mucosal pain-induced effect ($p > 0.05$).

Capsaicin induced mucosal pain did not result in a significant reduction in amplitude or velocity of jaw movement. This is not consistent with the Pain Adaptation Model which proposes, in pain, decreases in jaw movement amplitude and velocity to help protect the jaw system. Our subjects were able to achieve the goal-direct tasks despite the presence of pain. Indeed, with mucosal pain, subjects chewed more quickly than without pain. This may be a result of motivation where the subjects wish to complete the task as quickly as possible. These findings have implications on management strategies and suggest a focus on improving jaw function in mucosal pain subjects may not be necessary.

Part of the results have been published in: Zhao NN, Whittle T, Murray GM, Peck CC. The effects of capsaicin-induced intraoral mucosal pain on jaw movements in humans. J Orofac Pain 2012;26:277-287.

The findings of this research were presented at the 9th IASP (International Association for the Pain of Study) Research Symposium, Shanghai, China, 15 October 2011.

*Faculty of Dentistry, The University of Sydney.
Email: greg.murray@sydney.edu.au

ADIA Award

Effects of decomposition on DNA within dentine and cementum, and the consequences for human identification

D Higgins,* J Kaidonis,† G Townsend,† T Hughes,* JJ Austin*

Teeth are frequently used as a source of DNA for identification of human skeletal remains but these endeavours are not always successful or predictable. This study aimed to increase knowledge of the effects of post-mortem DNA degradation in the hard tissues of teeth to inform optimal sampling and DNA extraction protocols to increase successful human identification.

Healthy third molar teeth were collected from donors aged 15 to 65 years and then buried for periods from 0 to 16 months. DNA was extracted from cementum, coronal dentine and root dentine using a commercial silica column kit, and DNA was subsequently quantified using real time PCR. To assess the value of nuclear DNA retrieved, profiling by analysis of short tandem repeats (STRs) was performed. Ante-mortem (chronological age and gender of the donor) and post-mortem effects (post-mortem interval and burial temperature) were evaluated to assess their influence on the yield of both nuclear and mitochondrial DNA. A sub-sample of teeth was also decalcified, paraffin imbedded, sliced and stained with H & E to visualize loss of cellular material and structural changes in the tissues.

Cementum was found to be the best tissue for recovery of nuclear DNA at all stages of decomposition, whereas root dentine was the best source of mitochondrial DNA. It was found that small samples of cementum from decomposed teeth could be used to successfully generate nuclear STR profiles, enabling identification of the donor. Histological examination revealed structural breakdown of the dentine with complete loss of the predentine layer by 16 months but little structural change in the cementum. Increasing post-mortem interval and soil temperature were found to have significant negative effects on the yield of both mitochondrial and nuclear DNA. In respect to nuclear DNA, increasing chronological age of the donor had a significant positive effect on yield. It is hypothesized that this is due to a decrease in permeability of teeth with age, slowing down the penetration and action of soil microorganisms.

The roots of human molar teeth were shown to be valuable for genetic analysis and, with careful sub-sampling, can lead to successful identification of remains.

*Australian Centre for Ancient DNA, School of Earth and Environmental Sciences and Environment Institute, The University of Adelaide, South Australia.
†School of Dentistry, The University of Adelaide, South Australia.
Email: denice.higgins@adelaide.edu.au

Colin Cormie Scholarships

Prevalence of HHV-8 in an HIV-negative population on the Gold Coast, Australia

V Kashchuk;* DJ Speicher,†‡ NW Johnson†‡ (Supervisors)

First identified in 1994, Human herpesvirus 8 (HHV-8) is the aetiological agent of Kaposi sarcoma (KS), Castleman's disease (MCD) and primary effusion lymphoma (PEL). Incidence of KS has been described following both organ transplantation and blood transfusion. The Australian Red Cross Blood service (ARCBS) does not currently test for HHV-8 due to the low incidence of disease. Therefore, we launched the first study aimed at determining the prevalence of HHV-8 in healthy blood donors in South-East Queensland.

Study population consisted of plasma collected through the ARCBS from 480 HIV-negative blood donors stratified by decade of age (males:females 40:40 per decade) between 16 to 75 years old. Samples were spiked with 10^6 copies of EHV (exogenous control) and then extracted via High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Australia). Viral loads for HHV-8 and EHV were determined with in-house MIQE compliant quantitative PCR (qPCR) assays on the Roche LightCycler® 480. All samples and calibration curves were run in triplicate.

All plasma samples were HHV-8 negative. As qPCR dynamics, i.e. linearity (0.9984 and 0.9939) and efficiency (1.874 and 1.675) were acceptable for both HHV-8 ORF73 and ORF26 qPCR assays respectively, the results appear true. EHV qPCR revealed consistent viral load recovery suggesting DNA extractions worked.

A negative result for all plasma suggests that all donors do not have active HHV-8 infection. Whilst this may indicate a low risk of viral transmission, further serological testing is required to determine how many patients have a latent HHV-8 infection. Although it is difficult to deduce the risk for Australia as a whole, it is very likely similar results would be obtained nationwide.

*School of Dentistry and Oral Health, Griffith Health Institute, Griffith University, Gold Coast, Queensland.
†Molecular Basis of Disease Research Program, Griffith Health Institute, Griffith University, Queensland, Australia.
‡Population and Social Health Research Program, Griffith Health Institute, Griffith University, Queensland, Australia.
Email: victoria.kashchuk@hotmail.com

Effect of experience on dental clinicians' proficiency in determining the presence and extent of proximal enamel iatrogenic damage, and the possibility of using a low viscosity resin to protect damaged surfaces

T Milic;* M Valentine,† S Shetty,* LJ Walsh* (Supervisors)

Even with the utmost care, many adjacent tooth surfaces sustain iatrogenic damage during Class II cavity and crown preparations. This laboratory study investigated how operators' experience influences their ability to detect the presence and extent of damage, and explored the degree to which small surface defects could be repaired using low-viscosity nano-filled adhesive resin.

A total of 17 second year dental students and 10 experienced dentists scored surface damage in 40 premolar and second molar teeth mounted in 5 phantom heads, where the first molars had been previously prepared with Class II cavity preparations by different operators. Detection and correct classification rates were then analysed. Ten extracted natural teeth with varying types of iatrogenic damage were treated with a layer of GC G-Coat Plus and examined using SEM.

There was no difference in the proficiency of experienced dentists and second year dental students in detecting surface damage; however, experienced dentists were significantly better at classifying the type of damage present. Applying low-viscosity resin was found to uniformly coat and occlude shallow to moderate defects.

The study indicates that both experienced dentists and dental students can detect iatrogenic damage in approximately 80% of instances. From experience gained over time, dentists can reliably classify the type of such damage. The high detectability of damage may enable practitioners to proactively mitigate the risks associated with surface damage. Coating of shallow iatrogenic defects with an adhesive resin could be a viable clinical option, and is worthy of further examination.

*School of Dentistry, The University of Queensland, Brisbane.
†School of Mathematical and Physical Sciences, The University of Newcastle, Callaghan.
Email: tihana.milic@uqconnect.edu.au

Evaluation and prevention of enamel surface damage during dental restorative procedures

T Milic;* R George,† LJ Walsh* (Supervisors)

This study examined the effect of operator experience, dominance, tooth position (maxillary vs mandibular), and access (MO vs DO), on frequency and extent of iatrogenic damage to approximal tooth surfaces during Class II cavity preparations, and the effectiveness of novel and existing protective devices in preventing and minimizing damage. In addition, the clinical relevance of the results obtained on typodont teeth was examined.

Ten students and 10 experienced dentists each prepared 24 Class II cavity preparations in typodont teeth without protection, 10 utilizing stainless steel matrix bands and 10 utilizing protective interproximal wedges with a stainless steel plate. The frequency and extent of damage was analysed with respect to operator experience, use of a guard, dominance, tooth position and access. The second part of the study utilized 20 natural teeth and 20 typodont teeth to establish the relationship in depth of damage caused by a high-speed diamond bur on plastic typodont versus natural teeth under precisely controlled forces applied on the bur. To simulate an accidental slip of a bur during preparation of proximal surfaces, teeth were in brief contact with the bur (approximately 200 milliseconds).

Iatrogenic damage caused by experienced dentists was reduced from 74% for cavities prepared without a guard to 50% and 46% when bands and wedges were used as guards, respectively. The corresponding reduction of damage for students was from 94% without a guard, and 80% and 44%. The use of guards confined damage to <60 μm in depth for experienced dentists in 81% and 94% of cases for bands and wedges respectively, while for students, this occurred in 49% and 76% of cases for bands and wedges respectively. The second part of the study showed no statistically significant difference in the depth of damage caused by the same cutting force on the two types of teeth with a very short contact time, confirming the clinical relevance of the results of the first part of the study.

This investigation demonstrated that operator experience and the use of protective devices reduces iatrogenic damage to proximal surfaces during preparation with high-speed rotary instruments.

*School of Dentistry, The University of Queensland, Brisbane.
†School of Dentistry and Oral Health, Griffith University, Gold Coast.
Email: tihana.milic@uqconnect.edu.au

The practical application of protective stainless steel bands and wedges for prevention of iatrogenic damage during Class II cavity preparations

T Milic; LJ Walsh (Supervisor)*

This investigation examined the feasibility of using interproximal protective devices for preventing iatrogenic damage to adjacent teeth during Class II cavity preparations. It assessed potential adverse effects or risks associated with the use of such devices; e.g. whether protective devices impair direct vision of the cavity, resulting in unnecessary increase in preparation size, and whether their use prolongs preparation time.

Ten dentists and 10 senior dental students participated in the study. Each prepared Class II cavities on 10 first molar typodont teeth as a baseline, followed by 10 Class II preparations using metal matrix bands as guards on the adjacent teeth, and 10 preparations utilizing interproximal protective wedges with a built-in proximal guard strip (FenderWedge®). The time taken was recorded, and the typodont teeth were assessed for the widths and depths of the cavity preparations. The effect of operator experience as a variable was also investigated.

No significant difference was found in cavity preparation width or depth for operator experience (p = 0.261, p = 0.952) and use of either guard (p = 0.519, p = 0.238). Students took significantly more

time to prepare cavities of a similar quality to those prepared by dentists, both with and without the use of guards. The cavity preparation time was reduced with the use of guards for students, but remained unaffected by use of guards for dentists.

The study demonstrated that use of guards did not result in a significant increase in cavity width, depth, or preparation time for either students or experienced dentists. The majority of operators did not perceive the guards as a hindrance and less experienced operators often felt more confident when a guard was in place. The potential benefits of preventing iatrogenic damage during Class II cavity preparations outweigh any perceived disadvantages associated with the use of guards for this purpose.

This study was fully funded by the Australian Dental Research Foundation through both a research grant and the 2013 Colin Cormie Grant, and their support is gratefully acknowledged.

*School of Dentistry, The University of Queensland, Brisbane.
Email: tihana.milic@uqconnect.edu.au

Trebitsch Research Grant

An investigation into the antibiotic prescribing practices of Western Australian dental practitioners

JC Tan;* TV Riley,† LM Slack-Smith* (Supervisors)

The aim of this study was to determine the antibiotic prescribing habits of members of the Western Australian branch of the Australian Dental Association.

Members were invited to complete an online survey. Of the 1289 invitations sent, 119 surveys were completed, giving an overall response rate of 9.2%. The data were analysed using Stata Version 13.

The mean duration of practice was 22 years, with dental specialists constituting 15.1% of respondents. The majority of practitioners prescribed on a weekly (30.8%) or fortnightly basis (31.8%) and averaged between 2 to 3 prescriptions per week. Prescribed dosages were generally consistent with guideline recommendations. Amoxycillin was the most highly favoured antibiotic (71.4%), followed by phenoxymethylpenicillin (8.9%). Clindamycin was generally preferred for patients with an allergy to the first choice (50%). Most prescribed for gross or diffuse facial swelling (87.9%), facial cellulitis (88.8%) and acute necrotizing ulcerative gingivitis (64.5%). However, many unnecessarily prescribed for pericoronitis (42.1%), localized swelling (22.4%) and various endodontic conditions.

The majority of practitioners (73.5%) had never utilized microbiological diagnostic services to guide therapy, with turnaround time being the main deterrent. Eighty per cent of these practitioners reported their workplace not possessing the necessary sampling equipment and 50% were not aware of such services available to their workplace. Despite extensive awareness of the national dental therapeutic guidelines (95%), 15.8% either rarely or never referred to it. However, 68.3% of practitioners felt that they fully abided by the guidelines. 38.2% felt that their undergraduate dental course did not arm them with sufficient prescribing knowledge. While nearly all respondents were concerned about inappropriate prescribing, 88.1% felt that the dental profession contributed minimally towards antibiotic resistance.

This survey demonstrated moderate adherence to professional guidelines for prescribing antibiotics. Practitioners generally demonstrated adherence to guidelines in terms of antibiotic dosage, prophylaxis and choice when hypersensitivity was encountered. However, there was a heavy reliance on moderate- and broad-spectrum antibiotics, with many being prescribed for conditions for which they are contraindicated. Despite the perceived impracticality of laboratory diagnostics, the lack of sampling equipment limits the ability to definitively treat deep infections. The main limitation of this study was non-response bias as respondents were unlikely to be representative of all survey invitees. With important implications for practice, this study sought to encourage practitioners to take greater care when prescribing and, in doing so, combat bacterial resistance.

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*School of Dentistry, The University of Western Australia.
†Microbiology and Immunology, The University of Western Australia and Department of Microbiology PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre, Nedlands, Western Australia.
Email: joeltan@lei.org.au