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Introduction

It is with pleasure that I present to you the annual Australian Dental Research Foundation's (ADRF) Special Research Supplement of the *Australian Dental Journal* for 2016.

Evidence-based dentistry is the mantra of the dental profession and research provides this evidence.

The Research Supplement showcases the research being undertaken by undergraduate, postgraduate, early career, senior researchers and private practitioners in Australia and the ADRF is one of the major organisations carrying the responsibility to fund dental research in Australia.

I would like to thank the *Australian Dental Journal* for again publishing this special Research Supplement providing insight into the excellent work being undertaken.

The number of applications for research grants is increasing yearly and ADRF is struggling to meet the requests for grants. The Finance Committee, headed by Dr F Shane Fryer, keeps a prudent eye on the expenses and our investments but financial returns are currently low.

To actively assist with fundraising, the Marketing Fundraising Committee (MFC) has been established.

The MFC conducted a very successful raffle at the *ADX16 Sydney* dental exhibition in March of this year, raising awareness of the work of ADRF and $3,300 for the Foundation.

The Australian Dental Industry Association (ADIA) donated the raffle prize and also $67,000 raised from the fee charged to attend the lecture programme held in conjunction with the *ADX16 Sydney* dental exhibition. We look forward to further fundraising activities from the MFC to build our investment capital.

The Research Advisory Committee (RAC) undertakes the enormous task of reviewing the grant applications, grading them according to rigorous criteria and then awarding grants to the highest scoring applications. The RAC spends countless hours poring over the 100+ applications received and the work of the eight volunteer academics is priceless.

The RAC has been expertly chaired by Professor Camile Farah for the past eight years and Professor Farah has done an excellent job in leading the RAC. Thank you, Camile. We welcomed Professor Michael McCullough to the role of RAC Chair in January 2016 and as he was an active member of the RAC for the past six years the transition has gone smoothly.

ADRF has been a volunteer based research institution for over 40 years and we rely on support from the profession and industry for financial support.

I would like to thank the current donors and supporters for their generosity in supporting the work of the ADRF.

Currently, we can only fund approximately 25% of the grant applications and I ask you to please consider becoming a donor or supporter of ADRF.

Pamela Clark
Chair
Australian Dental Research Foundation

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Effect of experimental jaw muscle pain on jaw muscle activity in higher catastrophizers

R Akhter,* P Svensson,† M Nicholas,‡ C Peck,§ G Murray§

It is hypothesized that pain inhibits recruitment of motor programmes and the associated motor cortical activation, and that the psychological response to pain modulates this inhibitory effect. In particular, high catastrophizing leads to enhancement of the inhibitory influence on motor programme recruitment and motor cortex activation so that larger regions of the motor cortex are inhibited. In the orofacial motor system, research showed that experimental or clinical orofacial pain may be associated with an increased variability of jaw movements which suggests changes to motor unit recruitment patterns and these changes may be of relevance in the transition from acute to chronic pain states. This raises the possibility that the orofacial motor system may be an appropriate model system to study the possible effects of catastrophizing on changes in motor coordination occurring in pain. The aims were to (a) determine pain effects on jaw muscle electromyographic (EMG) activity and (b) examine whether pain catastrophizing correlates with any such effects.

Twenty-five asymptomatic adults (35±7 years) were categorized as high (n=11) or low (n=14) catastrophizers (Pain Catastrophizing Scale (PCS)). Muscle electromyographic (EMG) activity using bipolar surface electrodes from right (R) and left (L) masseter (M), anterior temporalis (AT), digastric (D) and sternocleidomastoid (SCM) muscles was recorded during standardized empty chewing (i.e. no food bolus and standardized by visual feedback chewing cycle frequency and amplitude). Jaw muscle activity was calculated in each subject as the difference between induced moderate pain (5% saline tonic infusion into right masseter muscle) and non-pain (isotonic saline infusion) conditions of root mean square EMG activity at every 0.5 mm jaw displacement of the chewing cycle. Mean muscle activity for each group was compared with paired samples t-test.

In all subjects, the LAT EMG activity ($P<0.01$) was significantly lower during pain in comparison with non-pain conditions. During opening, muscle activities of the LD ($P<0.05$) during opening and the RM ($P<0.05$) and LAT ($P<0.01$) during closing phases of chewing were significantly less in high catastrophizers. PCS scores were significantly negatively correlated with muscle activities of LD ($P=0.02$) and RSCM ($P=0.04$) during opening, and LAT ($P=0.01$) during closing phases.

Pain catastrophizing may play an important role in influencing pain's effect on jaw muscle activity. These findings may have clinical implications because not only pain but also related catastrophic thoughts may need to be addressed in the management plan.

This abstract is based on research that was funded partially by the Australian Dental Research Foundation and an outside source: Australian National Health and Medical Research Council.

The findings of the research were presented at the 93rd General Session and Exhibition of the International Association for Dental Research (IADR) Boston, Massachusetts, USA, March 2015.

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The effects of Resolvin E1 on inflammation associated human osteoclast and osteoblast activity *in vitro*

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Periodontal disease (PD) is a set of inflammatory conditions of the periodontium which can affect up to 90% of the adult population in its benign form (gingivitis). However, PD can present as an aggressive disease, causing destruction of the hard and soft tissues that support the teeth, leading to tooth mobility or loss. A major feature of PD is the altered bone metabolism within the inflamed periodontal lesion, with enhanced osteoclastic resorption overwhelming the bone forming capacity of resident osteoblasts.

Interestingly, molecules involved in the resolution phase of an inflammatory reaction, such as Resolvins, are suggested to effect bone cells and their metabolic processes. Resolvin E1 (RvE1) is reported to reduce both hard and soft tissue destruction in periodontal models of disease with reduced numbers of osteoclasts forming within the gingiva. Further investigations are required to identify its effectiveness within human PD, specifically its direct activity on human bone cells. Therefore, this study aimed to assess the effects of RvE1 on inflammatory cytokine induced human osteoclasts and osteoblasts *in vitro*.

Human osteoclasts derived from peripheral blood monocytes and human osteoblasts obtained from trabecular coxal samples were used for the *in vitro* assessment of RvE1 on cells exposed to the inflammatory cytokine, tumour necrosis factor-α (TNF-α). Markers of bone cell formation and bone turnover (TRAP staining, resorption pit analysis, alkaline phosphatase (ALP) activity, and mineralization quantification with alizarin red stain), along with gene expression analyses, were used to identify the effects of RvE1 on bone cell formation and activity.

RvE1 dose dependently reduced human osteoclast formation and dentine resorption both in the presence and absence of TNF-α ($p < 0.05$). Osteoclast specific gene expression analyses for NFATc1, TRAF6, CatK and CTR were inconsistent between donors and RvE1 treatment. Interestingly, RvE1 had minimal anabolic effects, as human osteoblastic mineralization and ALP activity was unchanged, despite an increase in the expression of the major transcription factor, RUNX2, after one week of treatment (10 ng/ml; $p = 0.0051$).

These findings suggest that the capacity of RvE1 to resolve tissue destruction during an inflammatory response, as seen in PD, may be involved with the regulatory control over the catabolic processes of osteoclastic resorption, and not through promoting new bone formation.

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Assessment and validation of a diagnostic scale, oral care protocol, the prevention and treatment of oral mucositis in a paediatric population receiving cancer therapy

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Childhood cancer affects 1 in 500 children and is the second most common cause of death of Australian children. Oral mucositis is a frequent and severe complication of chemotherapy in children with cancer, with a reported prevalence of up to 80%. Oral mucositis can result in pain, infection, depression, prolonged admission, treatment delays, increase in patient morbidity and increased overall costs.

To record the prevalence and severity of oral mucositis among paediatric inpatients and explore the relationship of risks factors and the development of oral mucositis.

During an 18-month period, 117 children aged 3 months to 17 years were receiving chemotherapy treatment for haematological malignancies or solid tumours at the Women's and Children's Hospital. A total of 643 clinical inpatient assessments on 73 children who were admitted and had received chemotherapy in the last 14 days were completed. Statistical analysis was completed with SAS computer software.

There were 43 episodes of oral mucositis identified in 31 patients (26.5% of total population and 42.5% of inpatients assessed). WHO assessment identified 32.6% were grade 1; 34.9% were grade 2; 14.0% were grade 3; and 18.6% were grade 4. Analysis revealed statistically significant association between patient diagnosis and oral mucositis $P<0.0001$, chemotherapy cycles and oral mucositis $p<0.0001$, day 8 and 9 of the chemotherapy cycle and oral mucositis $p=<0.05$ and neutropenia and oral mucositis $p<0.0001$. Children had increased length of admission with increasing severity of oral mucositis $p=0.05$.

The prevalence of oral mucositis was 42.5% among inpatients on a standardized oral health protocol over the study period. Patient diagnosis, chemotherapy protocol and days since chemotherapy were shown to influence the risk of developing oral mucositis. Further investigation of risk factors for the development of oral mucositis is planned.

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The findings of this research were presented at the Australasian Academy of Paediatric Dentistry Meeting, Adelaide, South Australia, November 2015 and the Australian and New Zealand Society of Paediatric Dentistry Biennial Congress, November 2015.

Genetic prediction of orthodontic root resorption

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This study attempted to evaluate the expression of candidate cytokines and genes involved in orthodontic root resorption in response to heavy orthodontic force application of 225 g.

This project was been approved by the Human Research Ethics Committee (Protocol No: X11-0028 & HREC/11/RPAH/37). This was a split-mouth study involving nine patients requiring mandibular first premolar extractions for orthodontic reasons. The teeth in the test group received 225 g of controlled buccal tipping force, using buccally-activated springs placed between the first molars and first premolars, for an experimental period of two weeks. The contra-lateral premolars served as control with brackets bonded to it, but with no orthodontic force.

Gingival crevicular fluid (GCF) was collected from test and control teeth at the end of two weeks, the amount of protein in GCF was quantified and analyzed with Human Multiplex Bead Panel immunoassay (Millipore) to determine bone remodelling cytokine expression in GCF. Additionally, at the end of two weeks, the control and experimental teeth, which received heavy orthodontic force of 225 g were extracted and immediately stored in RNA*Later* RNA Stabilization solution (Ambion, Australia) for sample processing later. Total RNA was isolated from the compression side (buccal) and tension side (lingual) periodontal ligamanet (PDL) from the extracted first premolars using RNeasy Micro Kit (Bioline, Sydney, Australia). The quantity and integrity of RNA was checked using Nanodrop1000 instrument (Thermoscientific, Australia) prior to doing the gene-expression screening.

Out of 13 cytokines measured using the immunoassay, only three cytokines (TNF-alpha, OPG & IL-1beta) showed consistent expression in all patients. Expression of TNF-alpha ($p = 0.15$), OPG ($p = 0.2$) and IL-1Beta ($p = 0.1$) was slightly increased in the experimental side when compared to the control side, however there was no statistical significance. The RNA samples collected directly from the PDL tissues did not meet the quality requirements and were either of insufficient quantity or degraded quality.

The cytokines TNF-alpha, OPG and Interleukin-1Beta showed an increased trend in the experimental group. However, the data was statistically not significant and warrants a bigger sample size. The methodology for RNA isolation directly from PDL tissues proved to be unsuccessful as the sample quality was unfavourable for downstream gene expression analysis. Hence the protocol will have to be re-visited by isolating RNA from primary culture of PDL cells after force application, in order to obtain enough quantity and quality of RNA samples.

This study was funded by the Australian Dental Research Foundation.

The findings of this research were presented at the Australian Society of Orthodontists Clinical Meeting, Hobart, Tasmania, March 2014.

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Vibration enhanced orthodontics: an *in vitro* characterization

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In order to reduce orthodontic treatment duration, a device called Acceledent, which generates 30 Hz, 20 g vibration has been introduced to use in conjunction with orthodontic treatment. The study aims to establish a baseline *in vitro* vibration model to study bone remodelling and assess cell proliferation, apoptosis and proteomic profile relevant to orthodontic bone remodelling immediately following vibration stimulus with different frequencies and durations.

Human foetal osteoblast (CRL-11372, ATCC, USA) were cultured up to third passage in triplicates and were subjected to vibration frequencies of either 10 Hz, 20 Hz, 30 Hz, 40 Hz or no vibration (control) for a duration of either 10 min or 20 min. Live cell imaging (IncuCyte ZOOM) was performed for a period of one full cell cycle for up to 42 hours. Bone-remodelling proteins were analyzed using multiplex ELISA assay. A focused gene expression study was done. Caspase assay to ascertain the apoptotic activity in the vibration-induced cells were carried out and green fluorescent apoptotic signals were quantified. The effects were compared using ordinary least squares regression analysis, ANOVA and student t-test.

In the 10 min vibration treatment, cell proliferation was higher in the 10 Hz and 20 Hz (*p = 0.030; *p = 0.027). The rate of proliferation was lower in the 30 Hz (*p = 0.01) and 40 Hz (NS) and in the 20 min vibration group, the rate of cell proliferation was significantly lower in the 20 Hz (**p = 0.000), 30 Hz (**p = 0.000) and 40 Hz (**p = 0.000). In the 10 min vibration group, an increased rate of apoptosis per two hours was noted in 10 Hz group (*p = 0.01). A similar pattern of apoptosis is observed in the 20 min duration. Differential gene expression of COL1A1, Cathepsin K, Beta-catenin were noted in after 10 and 20 min vibration treatment. Protein expression studies showed that in the 10 min group, up-regulation of proteins such as DKK1 (*p = 0.04) and TNF-alpha (*p = 0.04) and down-regulation of IL6 (*p = 0.037), IL-1B (*p = 0.02), SOST (*p = 0.05) was observed. In the 20 min 30 Hz group, up-regulation of IL6 (*p = 0.01), SOST (*p = 0.001) and down-regulation of OC (*p = 0.000) was observed.

After both 10 min and 20 min vibration treatment, cells proliferate faster in the lesser frequency range (10 Hz and 20 Hz) but grow relatively slower in the higher frequency range (30 Hz and 40 Hz). Early gene signals show bone homeostasis in both 10 min and 20 min vibration treatment. Protein expression data suggests a bone-resorption activity may be higher, immediately after 20 min vibration. An opposite trend is noted in the 10 min group. Apoptosis is hampered in 20–40 Hz in both the 10 min and 20 min group which negatively regulates bone formation. This study has effectively provided baseline information for immediate osteoblast response to vibration stimulus using a reproducible vibration set-up.

This study was funded by the Australian Dental Research Foundation Grant (# 57-2013).

The findings of this research were presented at the Faculty Research Day, Westmead, New South Wales, 17 September 2015.

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Dental enamel defects, caries experience and oral health-related quality of life: a cohort study

P Arrow*

Limited information is available on the longitudinal outcomes of first permanent molars affected with enamel defects and the oral health-related quality of life of children affected with enamel defects of the first permanent molars. The impact of enamel defects of the first permanent molars on caries experience and child oral health-related quality of life was evaluated in a cohort study.

Children who participated in a study of enamel defects of the first permanent molars eight years earlier were invited for a follow-up assessment. Consenting children completed the Child Perception Questionnaire (CPQ_{11-14}) and the faces Modified Child Dental Anxiety Scale (MCDAS), and were examined by two calibrated examiners. ANOVA,

Kruskal-Wallis, negative binomial and logistic regression were used for data analyses.

A completed questionnaire was returned by 111 children and 91 were clinically examined. The mean DMFT of children with enamel defects were; sound = 0.9 (SD 1.4), diffuse defects = 0.8 (SD 1.7), demarcated defects = 1.5 (SD 1.4), pit defects = 1.3 (SD 2.3), Kruskal-Wallis, p = 0.05. Logistic regression of first permanent molar caries found higher odds of caries experience with baseline primary tooth caries experience (OR=1.5, p = 0.01), and the number of teeth affected with enamel defects (OR = 1.9, p = 0.05), and lower odds with the presence of diffuse enamel defects (OR = 0.1, p = 0.04).

The presence of diffuse enamel defects was associated with lower odds of caries experience, and increased primary tooth caries experience was associated with increased risk of permanent tooth caries experience.

The researchers acknowledge the support of the Australian Dental Research Foundation.

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The findings of this research were presented at the International Association for Dental Research (IADR), Seoul, Republic of Korea, June 2016.

The interaction between EphB2/EphrinB1 influences DPSC proliferation and mineralization

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The potential use of stem cells therapy for the treatment of dental caries and oral disease have become of paramount interest. Dental pulp stem cells (DPSC) are the precursors of odontoblast cells that form the mineralized dentine matrix. It is proposed that DPSC are an ideal candidate for stem cell therapy to treat dental caries. We have demonstrated that the Eph receptor tyrosine kinase molecule, EphB2, is able to stimulate and increase the proliferation and mineral formation of DPSC isolated from human molars and specifically ephrinB1 is down-regulated following caries injury potentially to release DPSC from their niche. From these observations, it was our objective to further investigate the functional importance of the interaction between EphB2 and ephrinB1 within human DPSC proliferation and mineralization.

Proliferation and mineralization assays were conducted *in vitro* using DPSC that were modified to down-regulate the expression of EphB2 or over-express or down-regulate the expression of ephrinB1. Cells were cultured in the presence of the receptor EphB2 using a functional EphB2-Fc molecule or human-Fc control at biologically relevant concentrations for the mineralization assay.

DPSC treated with 12 pmol of EphB2 or ephrinB1 siRNA resulted in a 78% and 96% knockdown, respectively. While cells transduced with ephrinB1 full length construct resulted in a minimum of a 100-fold increase in ephrinB1 expression. Proliferation studies demonstrated that knockdown of EphB2 or over-expression of ephrinB1 significantly reduced proliferation capacity as demonstrated by BrdU labelling (Student t-test $p<0.03$) and population doubling experiments (Student t-test $p<0.05$), respectively. Furthermore, following osteogenic induction, ephrinB1 siRNA treated DPSC in the presence of EphB2-Fc significantly decreased calcium production compared to the scramble control treated DPSC (Student t-test, $p<0.05$).

These observations imply that the interaction between EphB2 and ephrinB1 expressed by DPSC is important for DPSC proliferation and mineral formation. These observations strengthen our hypothesis that following caries injury the reason ephrinB1 is down-regulated is to allow the proliferation of DPSC, migration to the injury site and potential mineral formation to assist in the repair of the caries. While under steady-state conditions DPSC are retained within their niche by EphB2/ephrinB1 inhibiting DPSC proliferation, migration and differentiation.

This study was funded by the Australian Dental Research Foundation research grant (44-2014).

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The application of novel nanoengineered implants for craniosynostosis therapy

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Craniosynostosis is characterized by pathologic premature fusion of skull sutures early in development, necessitating multiple surgical interventions. Bone morphogenetic protein (BMP) antagonists such as glypicans (GPC1 and GPC3) can delay growth and have an enormous potential to minimize the need for recurrent re-operations, but they need an effective and viable delivery platform. This project focuses on applying a novel therapeutic-delivery system for sustained release of glypicans to delay coronal suture fusion in Crouzon model of resynostosis by using a Titania nanotube (TNT)-based protein-delivery system. The aims of the study were: (i) to test protein release pharmacokinetics *in vitro*; (ii) to test the functionality of the released glypicans *ex vivo* (in human suture cells); and (iii) to analyze the biocompatibility and bone growth around the TNT implants *in vivo* in a murine model.

TNTs were prepared by two-step electrochemical anodization of 3 mm titanium discs in an organic electrolyte. The glypicans (GPC1, GPC3, GPC1 + GPC3) were loaded under vacuum before the release pharmacokinetics were measured using a fluorimeter. Then, a dual-luciferase (DLR) reporter assay was performed on transfected human suture cells to check glypican functionality. In addition, murine experiments were carried out by placing the implants loaded with or without glypicans over the 3 mm critical sized defect punched into the skull around the coronal sutures. Histological and micro-CT analyses were used to assess the tissue compatibility and bone formation.

Scanning electron microscopy showed hexagonally aligned TNTs with lengths of ~35 μm and pore diameters of 120 nm. There was a rapid elution of the GPCs (burst release), followed by a slower elution phase (sustained release) for up to 20 days. The DLR assay data showed repressed BMP2 bioactivity by almost 40–50%. Histological images and micro-CT scans showed no signs of inflammation or adverse reactions in skull bones or scalp. The critical-sized defect in the skulls with glypican loaded implants showed decreased bone volume after 90 days compared with the control groups.

Novel nanoengineered titanium implants are suitable for controlled delivery of biologically active therapeutic proteins to delay resynostosis. This approach holds promise to lead to minimally-invasive future therapy for craniosynostosis.

This project was supported by Australian Dental Research Foundation (18/2013), the Australian Craniomaxillofacial Foundation, and ARC –FT110100711. This work was performed in part at the OptoFab node of the Australian National Fabrication Facility utilizing Commonwealth and SA State government funding. A/Professor Barry Powell also provided technical support to aspects of the *ex vivo* and *in vitro* studies.

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The findings of this research were presented at the International Association for Dental Research ANZ Division 55th Annual Scientific meeting, Dunedin, New Zealand, August 2015 and the 6th International Nanomedicine Conference, Sydney, Australia, July 2015.

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Associations between school aspects and child oral and general health

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Schooling forms a large part of a child's life experience and characteristics of schools have been associated with various health outcomes. The association between aspects of schools and child oral and general health outcomes were assessed for a sample of children from NSW, SA and ACT.

Parents of a random sample of 3044 children aged 9–14 years responded to self-complete surveys. Parents rated child's oral and general health on 5-point Likert-type scales, dichotomized to good/fair/poor vs excellent/very good. Parent perceptions of their child's school were also collected. Administrative data were collected from the MySchool website for participating schools. Multilevel, multivariate logistic regression analyses were conducted on parent ratings of good/fair/poor child oral health and general health, using child sociodemographic information, MySchool school information and parent perception of schools at the individual (collected) level and at the school level (amalgamated).

Reference models showed a significant school-level variance in child general (Median Odds Ratio MOR 1.41) and oral health (MOR 1.18). After controlling for all other variables, a good/fair/poor rating of child general health was associated negatively with parent perceptions of the school social environment (Odds Ratio OR 0.59 good, 0.41 good-medium vs poor). For child oral health, a good/fair/poor rating was associated negatively with parent perceptions of the school at the individual level; social environment (OR 0.70 good, 0.59 good-medium vs poor), health promoting environment (OR 0.70 good vs poor), quality of buildings/grounds (OR 0.70 good vs poor) and quality of teachers (OR 0.70 medium vs poor). A school-level variable, relations, was also negatively associated with good/fair/poor child oral health (OR 0.59 good vs poor).

There was significant variation across schools in parent-rated child oral and general health. School aspects were associated with parental perceptions of child general and oral health, controlling for individual level factors.

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The findings of this research were presented at the International Association for Dental Research ANZ Division 55th Annual Scientific Meeting, Dunedin, New Zealand, August 2015.

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Lysine acetylation is a common post-translational modification of key metabolic pathway enzymes of the anaerobe *Porphyromonas gingivalis*

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Lysine acetylation is a post-translational modification of proteins where the addition of an acetyl group to a lysine removes this amino acid's positive charge and can induce changes in a protein's secondary structure and reactivity. It has been well studied in eukaryotes, where it plays a vital role in diverse mammalian cellular physiology. Dysregulation of this modification and its regulatory enzymes has been linked to ageing and several major diseases such as cancer, cardiovascular diseases and neurodegenerative disorders. More recently, lysine acetylation been found to occur in prokaryotes, where it appears to modify enzymes required for central metabolism. The aim of this work was to determine whether lysine acetylation occurred in the oral anaerobe *Porphyromonas gingivalis* and if so, to identify the targets of this post-translational modification.

A proteomics based approach combining immune-affinity enrichment with high sensitivity orbitrap mass spectrometry was used to identify peptides containing acetylated lysines from *P. gingivalis* W50.

One hundred and thirty lysine acetylated peptides were identified from 92 *P. gingivalis* proteins. The majority of these peptides (71) were attributed to 45 proteins with predicted metabolic activity; these proteins could be mapped to several *P. gingivalis* metabolic pathways where enzymes catalyzing sequential reactions within the same pathway were often found acetylated. In particular, the catabolic pathways of complex anaerobic fermentation of amino acids to produce energy had 12 enzymes lysine acetylated.

The results suggest that lysine acetylation may be an important mechanism in metabolic regulation in *P. gingivalis*, which is vital for *P. gingivalis* survival and adaptation of its metabolism throughout infection.

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The findings of this research were presented at the BacPath13: Molecular Analysis of Bacterial Pathogens Conference, San Remo, Victoria, Australia, September 2015.

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Proteomic analysis of membrane proteins of *Enterococcus faecalis* V583 and an oral clinical isolate grown in continuous culture at pH 8 and pH 11

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Enterococcus faecalis survives in a number of biological niches which are often nutrient limited and in which the pH can vary greatly. Endodontic treatment of a tooth with a severely inflamed or necrotic pulp usually involves the chemo-mechanical debridement of the canal and placement of an inter-appointment medicament such as calcium hydroxide (pH 12). *E. faecalis* is commonly recovered from endodontic infections that have persisted following treatment with Ca $(OH)_2$. Expression of the cell membrane proteins under alkaline conditions at a biologically relevant growth rate may increase our understanding of how *E. faecalis* can adapt and persist.

Aims:

1. Determine the phenotypic changes of *E. faecalis* when grown at pH 11.
2. To compare plasma membrane protein expression of *E. faecalis*, at pH 11 and pH 8 at an imposed low growth rate.

E. faecalis ATCC V583 was grown in a chemostat at pH 8 and pH 11 and the maximum growth rates (μmax) were determined. An imposed growth rate of one-tenth the organism's μmax was used for growth at pH 8 or pH 11. Membrane proteins were fractionated by ultracentrifugation, homogenisation in carbonate buffer, and membrane shaving. Heavy- or light-isotope-coding protein labels (ICPL) were added to pH 8 or pH 11 samples respectively and combined. Membrane protein expression was quantified using liquid chromatography, electrospray ionization mass spectrometry and MaxQuant analysis. Proteins that deviated by more than one standard deviation (SD) from the mean were considered to be up- or down-regulated.

Mean generation time at pH 8 and pH 11 was 1.2 and 7.7 hours respectively. The extreme alkaline conditions produced co-aggregation of the cells into flocs with the appearance of an extracellular matrix. These observations are consistent with a shift towards biofilm formation.

Six proteins had a $\log_2$ H/L ratio (pH 11/pH 8) greater than one SD of the mean including: polysaccharide biosynthesis family protein EF0669, glycosyl hydrolase, family 20 EF0114, glycerol uptake facilitator protein EF1927. Five proteins had a $\log_2$ ratio one SD less of the mean: PTS system IIC component EF1838, PTS system IID component EF0456, C4-dicarboxylate transporter EF0108, PTS system mannose-specific IID component EF0022.

When cultured at an imposed slow growth rate, extreme alkaline conditions resulted in a reduced mean generation time and altered expression of several membrane proteins. Collectively these membrane proteins appear to be involved in the transition to biofilm formation seen at pH 11.

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In vitro fracture strength and patterns in root filled teeth restored with different base materials

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To compare the fracture strengths and patterns of root filled teeth restored with intermediate bases of glass ionomer cement (GIC), zinc polycarboxylate cement (ZPC), dual-cure resin core (DCRC) or Biodentine® under direct resin composite (RC) restorations.

Standardized mesio-occlusal-distal and endodontic access cavities were prepared in 100 extracted human maxillary and mandibular premolars, and root canal treatment was performed. The teeth were stratified and randomly allocated to five groups (n = 20): 1. GIC; 2. ZPC; 3. DCRC; 4. Biodentine; and 5.

Prepared and unrestored (control). The teeth were subjected to an oblique, ramped load until facture. The fracture loads, level and location were recorded. Mode of failure was determined using the scanning electron microscope (SEM). Statistical analyses included one-way analysis of variance, Chi-square test and Fisher's exact test.

The mean fracture strengths of all restored groups were significantly higher than the unrestored group ($P < 0.001$), but were not significantly different amongst the groups. There were significant overall effects on mean fracture strength for tooth type ($P = 0.002$) and bucco-lingual width of the crown ($P = 0.001$).

The four restorative materials can be considered as appropriate intermediate bases under direct RC restorations in root filled premolars. The laminate restorative technique promoted fracture strengths that can withstand normal and maximum masticatory function. The choice of base material can influence the failure mode, which may have implications for the clinical presentation of structural failures of root filled teeth.

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Fish oil as an adjunct therapy for periodontitis: A pilot study to determine whether there are acute and chronic benefits

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Periodontitis is a chronic inflammatory condition characterized by destruction of the supporting apparatus of teeth, including bone and the periodontal ligament. Long chain Omega-3 polyunsaturated fatty acids (LCn-3PUFA) in fish oil are beneficial for several inflammatory conditions with mixed findings in periodontitis.

We evaluated the clinical efficacy of fish oil supplementation as an adjunct to standard therapy for advanced chronic periodontitis.

In a 13 month double blind, randomized, controlled parallel study participants with advanced chronic periodontitis (10M/23F, mean age 52 ± 10 years) consumed either fish oil (1884 mg LCn-3PUFA/day) or placebo (2000 mg soy oil/day). Patients were assessed at baseline, after 4, 7, 10 and 13 months for clinical outcomes, viz. probing pocket depth (PPD) and clinical attachment (CAL). In addition, inflammatory cytokines (TNF-alpha, IL-1beta and C-reactive protein (CRP)) in gingival crevicular fluid (GCF) and CRP in plasma were measured. Fasting blood samples were assessed for LCn-3PUFA erythrocyte content to determine compliance at 0, 4 and 13 months.

Following non-surgical treatment and 4 months of oil supplementation both groups had improvements in clinical outcomes, with significant reductions in PPD and CAL gain. Groups did not differ in the percentage of sites that had ≥2 mm gain of CAL ($P = 0.229$) or reduction in PPD ($P = 0.264$) after 4 months. There were no significant changes in cytokines in GCF or plasma in either group. Compliance to supplementation was good as indicated by erythrocyte LCn-3PUFA, increasing by 50% in the fish oil supplemented group and no change in the placebo.

Whilst periodontal treatment was effective in both groups, no additional benefit was observed with fish oil supplementation for 4 months. Additional analyses are planned for data from later time points.

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High-resolution profilometric assessment of early enamel erosion

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Most existing techniques in dental erosion research are capable of detecting profilometric changes in enamel erosion after 7 to 10 minutes of erosive demineralization. The aim of this *in vitro* study was to assess the effect of short, repetitive erosive challenges on the erosion depth and surface roughness of enamel under conditions simulating an acidic diet.

Polished enamel surfaces of human third molar teeth (n = 7) were deposited with chrome coating by evaporation. The specimens were subjected to repetitive erosive challenges at pH 3.0 at three stages, including baseline (t = 0 sec), stage 1 (t = 10 sec) and stage 2 (t = 20 sec). Changes in the erosion depth, average roughness (R*a*), core roughness (R*k*), reduced peak height (R*pk*) and reduced valley depth (R*vk*) values were assessed longitudinally on three-dimensional reconstructions using laser confocal microscopy at a magnification of ×100.

Repeated measures multi-factorial MANOVA confirmed the significant effects of stage on erosion depth and surface roughness (R*a*, R*k* and R*vk*) ($p < 0.05$), with significant increases occurring in erosion depths from baseline to stage 1 by 26.1 ± 6.9 nm (mean ± SE), and baseline to stage 2 by 48.6 ± 12.5 nm ($p < 0.05$). Increasing trends were also noted in other roughness parameters over time. After stage 2, multiple regression analyses demonstrated significant correlations between erosion depth and R*a* ($r = 0.62$, $p < 0.05$), and between other roughness parameters (R*a* and R*pk*, $r = 0.77$, $p<0.01$; R*a* and R*vk*, $r = 0.91$, $p<0.01$; and R*pk* and R*vk*, $r = 0.87$, $p<0.01$).

This study demonstrates nanoscale changes in erosion depth and surface roughness under conditions simulating early stages of enamel erosion. These findings have the potential to provide a foundation for the development of new clinical diagnostic tools and preventive strategies.

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Towards selective removal of *Porphyromonas gingivalis* from dental plaque

SA Dingsdag,*† N Hunter*†

To selectively control *Porphyromonas gingivalis,* a porphyrin auxotroph sensitive to nitroimidazole antibiotics, deuteroporphyrin-amide linked (Yap *et al*., OBC, 2009) and –lysine linked nitroimidazole adducts were synthesised (Dingsdag *et al*., OBC, 2015). The aim of this study was to study deuteroporphyrin-nitroimidazole adducts in complex bacterial populations.

The response of bacteria to deuteroporphyrin adducts was studied in liquid broth media using single species growth curves and confirmed in a bacterial growth curve model using illumina paired end 'deep sequencing' of the 16S ribosomal DNA.

P. gingivalis ATCC 33277 was killed by amide linked deuteroporphyrin-nitroimidazole adduct (termed 'P5', at 3 μM) at levels similar to metronidazole (1 μM). None of the other examined anaerobic bacteria were killed by P5 in enriched brain heart infusion (enriched BHI) media. However, it was concluded that enriched BHI growth media contained sources of porphyrin, which may have come from foetal bovine serum component and that porphyrin sources may interfere with uptake and processing of P5.

The effect of P5 was instead investigated in BHI media which did not contain detectable porphyrin. As expected, none the facultative bacteria [Actinobacteria, (n = 3), Proteobacteria (n = 2), *Lactobacillales* (n = 2)] were killed by P5 or metronidazole at 20 μM. Porphyromonads (n = 2) and *Prevotella* (n = 2) were

killed by metronidazole (mean = 1 μM and 1.5 μM respectively) and P5 (mean = 1 μM and 3 μM respectively). Surprisingly, when P5 was titrated below inhibitory concentrations, P5 supported the growth of *Prevotella* (all strains). A mixed-species bacterial growth curve model confirmed these results. This seemingly perplexing finding was explained upon a re-examination of the literature, which clarified that porphyrin auxotrophy is an extensive feature of not only Porphyromonads but also *Prevotella*.

In this study, some anaerobic bacteria were affected by adducts, indicating the nitroimidazole was not delivered to *P. gingivalis* alone. These findings indicate *Prevotella* and have previously unrecognized flexibility for the types of porphyrin that are recognized and metabolized. Lastly, these results confirmed porphyrin auxotrophy is an extensive feature of anaerobic Bacteroidetes.

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Learning by observing: effects of multi-tasking and stress on performance in endodontics

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Routine use of evidence for supporting learning of fine motor skills in dentistry is limited. Recent evidence from studies concerning fine motor skill learning indicates that learning by implicit approaches (e.g. observation with physical guidance) can result in positive and sustained outcomes, even under stressful conditions, in comparison to commonly used/explicit approaches (e.g. video with verbal instructions). This study aimed to evaluate the effect of learning by observation on the acquisition of fine motor skills associated with root-canal hand instrumentation. We hypothesized that learning by observation with guidance would result in minimal decrement in performance under pressured or stressful conditions.

Novice dental students learned endodontic hand-skills by preparing standardized canals of different diameters and curvatures. Learning methods involved silent-video and hand-guidance (n = 23), video with verbal instructions (n = 23), or silent-video (n = 13). Students who had completed the usual endodontic-simulation unit provided comparative data (n = 16). During testing, all participants prepared the distal canal on a plastic tooth, then completed the same task under multi-tasking or stressful conditions.

Performance was assessed by preparation accuracy, completion times, errors, and reported instructions. Differences were assessed using ANOVA or non-parametric tests as indicated (significance = $p < 0.05$).

Performance in the three experimental groups was similar during learning. When tested, all observation groups reported significantly increased stress levels. However, accuracy did not differ significantly within or between observation and comparative groups. Instructed-observation group reported significantly more instructions than guided-observation and observation-only groups ($p < 0.05$).

Our findings showed that performance following learning by observation with guidance (i.e. high error rate during learning and low accuracy during testing), were inconsistent with learning implicitly. These outcomes highlight difficulties with designing implicit learning approaches. Alternative approaches to learning dental motor skills need investigating as they may provide better outcomes, especially in stressful environments.

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Implant surface debridement using lasers

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Conventional methods of cleaning implants have used a variety of instruments to remove bacterial deposits from the surface. However, this approach is not particularly effective and can damage the surface of the implant. Middle infrared lasers have been suggested for use in cleaning implants, this study evaluated the effects of the Er:YAG laser on the surface of titanium.

A free running pulsed Er:YAG laser system was used (2940 nm wavelength) with a window handpiece, with plain optical fibres or with conical tip optical fibres. Surface characteristics of machined and micro-roughened surfaces were assessed. Discs of implant material were subjected to laser irradiation under varying pulse energies with samples at a fixed distance from the laser tip, either dry (without water) or wet (with water). Samples were subjected to light microscopy then scanned using a 3D non-contact laser profilometer, to assess surface roughness, volume of peaks and the maximum diameter of the ablated area.

While low energy doses did not alter the surfaces of titanium, high energy levels were found to cause modification to the surface. The characteristics and extent of surface modification varied according to the implant surface type, the presence or absence of irrigation and the type of fibre optic tip used. Conical tip designs which distributed laser energy caused no surface effects at any power setting on either machined or micro-roughened implant surfaces regardless of the irrigation conditions. For the Er:YAG laser system used, which had a pulse duration of 350 μs, surface cleaning actions from the generation of plasma by the laser pulse occurred at energy densities of 32 J/cm^2 (peak power 500 W with 0.8 mm spot size and 350 mJ/pulse) while surface melting occurred from 120 J/cm^2 upwards (peak power 1714 W with 0.8 mm spot size and 600 mJ/pulse). The parameters which cause surface melting are well above those which would be used for debridement (energy density 12 J/cm^2, peak power 170 W, 60 mJ per pulse).

We conclude that use of high energy densities (120 J/cm^2 upwards) on roughened implant surfaces should be avoided as this may cause surface melting and other modifications of the surface characteristics which may be undesirable. Conversely, the use of the Er:YAG laser delivered with plain or conical fibre tips on machined or roughened surfaces appears safe since it causes little or no surface changes whilst still generating desired actions from plasma formation and cavitation which assist debridement.

This work was supported by the Australian Dental Research Foundation.

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The aetiology of chronic pain

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There is emerging evidence that central anatomical changes within the brain may underlie the development and maintenance of chronic pain in the spinal system. It is unknown if similar anatomical changes occur in the orofacial system in individuals with different forms of chronic orofacial pain. Furthermore, the orofacial system provides a unique opportunity to explore central changes at the level of the primary afferent synapse which cannot be explored in the spinal system. The aim of this project was to explore anatomical changes in the brainstem and in higher brain centres in two chronic orofacial pain conditions.

Using voxel based morphometry of T1-weighted anatomical images and diffusion weighted images we compared regional grey matter volumes and mean diffusivity of the brainstem and higher brain centres in individuals with trigeminal neuropathy and painful temporomandibular disorder with pain-free, age and gender matched controls.

We found that trigeminal neuropathy was associated with significant grey matter reductions in the region of the somatosensory thalamus, the nucleus accumbens and the primary somatosensory cortex. In contrast, painful temporomandibular disorder was not

associated with any change in higher brain regions. However, both orofacial pain conditions were associated with grey matter volume reductions as well as reductions in water diffusivity in the region of the spinal trigeminal nucleus.

These results show that chronic orofacial pain and in particular that of neuropathic origin, is associated with changes in regional brain anatomy in the ascending pain pathway. We have since found that the changes within the spinal trigeminal nucleus are also associated with alterations in resting brain oscillations that likely result from activation of astrocytes and their associated release of gliotransmitters following nerve injury. We suggest that if the activation of astrocytes following nerve injury can be modulated, this might restore the altered oscillatory activity pattern to control levels and prevent the development of pain.

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The effect of bisphosphonates on osseointegration of implants with different surface topography: an experimental study in rats

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To evaluate the influence of surface topography on osseointegration of titanium implants in rat maxillae following systemic bisphosphonate use.

Twenty Sprague-Dawley rats were divided into two groups – test (systemic bisphosphonate use) and control (no bisphosphonate administered). Bisphosphonate administration began three weeks prior to implant placement with thrice-weekly doses of zoledronic acid (66 μg/kg). Forty endosseous implants (two per animal placed bilaterally) with a moderately rough surface (20 implants) or a turned surface (20 implants) were placed in the extraction sockets of maxillary molars. Animals were sacrificed at postoperative times of 14 and 28 days and the implant and surrounding material harvested for histological and histomorphometric analysis. Statistical analysis consisted of unpaired t-test with a level of significance set at $P \leq 0.05$.

The quantitative bone-to-implant ratio (BIC) analysis (mean ± standard error of measurement) of moderately rough surfaced and turned surfaced implants at 14/28 days were: test group -18.94 ± 2.92, 11.42 ± 0.37 / 28.23 ± 2.76, 13.66 ± 2.43; control group – a 46.36 ± 2.27, 33.29 ± 3.97 / 72.99 ± 2.95, 47.62 ± 8.14. Histomorphometric analysis indicated higher BIC values on moderately rough compared to turned surfaced implants. Higher BIC values in control group compared to test group was demonstrated to be statistically significant in both implant surfaces and at all time points. Histological observation within control and test groups demonstrated initial bone formation around moderately rough surfaced implants not only at the parent bone, as was the case with the turned surfaced implants, but also along the implant surface itself. Test group specimens illustrated less bone remodelling activity at 14 and 28 days after implants placement, compared with control specimens.

Osseointegration occurred around all implants, irrespective of surface treatment or zoledronate administration. Systemic zoledronate administration negatively influences the rate of osseointegration. Osseointegration is enhanced adjacent to moderately rough surface implants compared to turned surface implants in both the presence and absence of systemic zoledronate administration.

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Inflammatory cytokines in saliva in oral cGvHD after allogeneic HSCT: incidence, impact on quality of life and associated salivary cytokine profile

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Chronic Graft-versus-Host Disease (cGvHD) remains one of the most significant long-term complications after allogeneic haemopoietic stem cell transplantation (HSCT) with the oral cavity one of the most frequently affected sites. Often recalcitrant to treatment, oral cGvHD may cause a significant and detrimental impact on oral health, function and quality of life (QoL). This study aimed to assess the incidence plus transplant features which predispose to oral cGvHD plus the chronic inflammatory cytokine profile within subjects' saliva to determine if this differs from other common oral mucosal inflammatory conditions.

Adults, planned for allogeneic HSCT were enrolled in this multicentre study from June to December 2013. Comprehensive clinician- and patient-based clinical measures, plus a saline mouth rinse sample, were collected pre-transplant and repeated at days 100, 180 and 270. Patients who developed oral cGvHD had additional assessment at onset and, when possible, resolution of oral cGvHD. A cytometric bead array kit was used, cytokines consisted of those commonly associated with chronic inflammation namely IL-2, IL-4, IL-6, IL-10, INF-γ, TNF and IL-17A. Cytokines were measured in mouth rinse samples of participants plus a small cohort of subjects with other common oral inflammatory conditions such as oral lichen planus, Sjögrens syndrome and healthy controls.

Forty-eight patients were enrolled; six subjects developed oral cGvHD. No significant differences were seen pre-HSCT however, when oral cGvHD developed, a significant increase in oral sensitivity, pain and dryness ($p = 0.004$, $p = 0.005$, $p = 0.004$) was reported. After clinical resolution perceived sensitivity and dryness ($p = 0.188$, $p = 0.177$ respectively) remained elevated. A significantly raised (poor) QoL score was seen ($p = 0.008$) at the time of cGvHD onset which persisted at disease resolution ($p = 0.055$). No significant difference in salivary cytokine profile was demonstrated in those who developed oral cGvHD during the study period. When compared to other common inflammatory conditions, subjects with oral lichen planus exhibited raised levels of IFN, IL-10, IL-4 and IL-2 (all $p < 0.001$) compared to all other mucosal conditions and healthy controls.

Oral cGvHD is associated with significant morbidity, patients describing significant oral dryness, pain, sensitivity and poor QoL with many symptoms persisting beyond disease resolution. Of the chronic inflammatory cytokines tested, no individual cytokine was significantly elevated in the saliva of patients who developed oral cGvHD, this finding requiring further testing in a larger study with longer clinical follow up.

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Rapid prototyped bilayered porous scaffold combined with osteogenic growth factor for vertical bone augmentation

PT Sudheesh Kumar,* C Vaquette,† S Ivanovski*

Orofacial bone loss, particularly that of the maxillary and mandibular jaws, is a common condition which is the result of tooth loss (edentulousness), dental pathology, etc. Following tooth loss, the resorption of alveolar bone is a physiological process, and therefore jaw bone loss is an inevitable outcome of

edentulousness. The jaw bone defect can create major problems for subsequent placement of dental implants. So, it is a very important issue to be addressed. In this study, we investigated the vertical bone regeneration potential of a 3D printed porous scaffold which contained growth factor loaded hydrogel.

The bilayered porous scaffold was made of a 3D printed porous polycaprolactone (PCL) scaffold and a melt electrospun PCL fibre mesh. Bone morphogenetic protein 2 (BMP2) and human osteoblast cells loaded hyaluronic acid hydrogel injected on to the fibre mesh which was then inserted in to the PCL porous scaffold to develop the final construct. *In vitro* evaluations including BMP2 release, cell viability, proliferation and quantitative RT-PCR for gene expression analysis were conducted. The bilayered scaffold loaded with BMP2 extraskeletally implanted on the calvarium of a rabbit animal model. The implanted scaffold was harvested after eight weeks to assess the bone formation.

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The bilayered scaffold showed controlled and sustained release of BMP2 up to four weeks. Cell viability and proliferation study showed the cells were viable and proliferating on the scaffold without BMP2 whereas cells found viable but not proliferating on the scaffolds loaded with BMP2. The differentiation of cells on the BMP2 loaded scaffold was confirmed by qRT-PCR which showed the significant upregulation of osteocalcin, osteopontin and collagen 1A compared to the control. The scaffold showed vertical bone regeneration after eight weeks implantation in rabbits. The presence of BMP2 assisted the bone formation extraskeletally on the calvarium even though newly formed bone quantity was small.

This proof of concept study demonstrated the use of BMP2 loaded bilayered scaffold can overcome the limitations of current treatment modalities utilized for vertical bone regeneration. The presence of BMP2 was necessary for inducing bone formation even though the quantity of newly formed bone was relatively less which might be due to poor vascularization on the extraskeletal site.

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The effect of diabetes on osseointegration of different implant surfaces in rats

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To investigate the effect of metabolic condition on osseointegration along the different Ti implant surfaces (SLA vs modSLA) in rats with type 2 diabetes (Goto-Kakizaki).

Six Sprague-Dawley and six Goto-Kakizaki rats were included, serving as healthy control (H) and diabetic test (D) groups. Each group was then further divided into two sub-groups according to either 14 and 28 days of healing period. Each group contained six animals each. Twenty four implant discs with SLA surface (12 implants) or modSLA surface (12 implants) were placed on critical-sized calvarial defects in the rats. Animals were sacrificed at postoperative times of 14 and 28 days for histological and histomorphometric analysis. Statistical analysis consisted of two-way ANOVA (post-hoc analysis) with a level of significance set at $P \leq 0.05$.

The quantitative bone-to-implant contact ratio (BIC) analysis of SLA and modSLA implant surfaces at 14 and 28 days in the healthy and diabetic groups was calculated. Histomorphometric analysis indicated significantly higher BIC values (%) on the modSLA surface compared to the SLA surface in both healthy and diabetic groups at 14 and 28 days ($p < 0.05$). However, less BIC values (%) were observed in the diabetic group compared to the healthy group for both implant surface types at 14 and 28 days.

Osseointegration occurred around all implants, irrespective of surface treatment or diabetic condition. Systemic metabolic condition adversely influenced the rate of osseointegration, but osseointegration was improved adjacent to the modSLA surface compared to the SLA surface implants in both healthy and diabetic condition.

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The findings of this research were presented at the International Association for Dental Research, General Meeting, ANZ Branch, Brisbane 2015.

The effect of altering the lateral occlusion scheme on peri-implant strain: a laboratory study

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Although the overall implant treatment outcome is favourable, implant-related mechanical and biological complications are still frequently encountered. One of the contributing factors that influence the longevity of implant prosthesis is dental occlusion. The purpose of this study is to investigate the effects of four different lateral occlusion schemes and different excursive positions on peri-implant strains of a maxillary canine implant.

A resin model of a maxillary dental arch was fabricated. A single implant (5.0 mm diameter, 13 mm long Branemark Mk III external hex implant, Nobel Biocare) was inserted in the region of the maxillary right canine. Four metal implant crowns with different occlusion schemes were fabricated from casting cobalt-chromium alloy. The included schemes were canine-guided (CG) occlusion, group function (GF) occlusion, long centric (LC) occlusion and implant-protected (IP) occlusion. In order to simulate physiological loading conditions, each crown was loaded in three sites that correspond to maximal intercuspation (MI), 1 mm excursion and 2 mm excursion. A load of 140 N was applied on each site. The peri-implant strain was recorded by a rosette strain gauge that was attached on the resin model buccal to the implant. For each loading condition, the maximum shear strain value was calculated from principle strain values.

The different schemes and excursive positions impacted on the peri-implant strains. At MI and 1 mm positions, the GF had the least strains followed by IP, CG and LC respectively. At 2 mm, the least strains were associated with GF followed by CG, LC and IP respectively. However, regardless of the occlusion scheme, as the excursion increases, a linear increase of peri-implant strains was detected.

The peri-implant strain is susceptible to occlusal factors. The eccentric location is much more influential on peri-implant strains than the occlusion scheme. Therefore, adopting an occlusion scheme that will reduce the occurrence of occlusal contacts laterally may be beneficial in minimizing peri-implant strains.

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Functional characterization of genes specific for dental mesenchymal stem cells with high proliferation and differentiation capacity

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Previously, we have characterized clonal populations of mesenchymal stem cells (MSC) derived from bone marrow, periodontal ligament and dental pulp to identify potential biomarkers associated with long lived, multi-potential stem cell subsets. Global gene expression profiles of high growth/multi-potential

clones versus low growth potential clones (across the three stromal tissues) identified 24 genes, which were differentially up-regulated in all tissues. Notably, transcription factors, E2F2, PTTG1 and transcriptional co-factor, LDB2, each with critical roles in cell growth and survival, were highly expressed in highly potent stem cell populations. These findings provided basis for further investigation into precise roles of above mentioned factors in MSC proliferation and differentiation, properties fundamental to their clinical utilisation.

The aim of the study was to investigate the regulatory role of E2F2, PTTG1 and LDB2 in processes governing growth and
development of MSC derived from bone and dental tissues.

- MSC, previously derived from six donors, from three stromal tissues (bone marrow, periodontal ligament and dental pulp), were used in the study.
- Expression of E2F2, PTTG1 and LDB2 was transiently down-regulated in MSC populations using silencer select siRNAs (according to Invitrogen protocol).
- Proliferation and differentiation studies were conducted on test and control MSC populations, as previously described (1).
- All experiments were conducted in triplicate with the inclusion of appropriate biological and experimental controls. Statistical analysis and statistical significance was determined using GraphPad Prism v6 6.0.0.289.

Upon assessment of functional capacity *in vitro*, proliferation and differentiation potentials of MSC populations appear to have been altered by modification of expression of the abovementioned genes.

- E2F2 knockdown resulted in reduced MSC survival and proliferation and as such limited further analysis of MSC differentiation capacity.
- Knockdown of PTTG1 and LDB2 expression resulted in enhanced osteogenic and adipogenic differentiation in MSC population in comparison to experimental controls.

This study confirmed that E2F2, PTTG1 and LDB2, hold pivotal roles in cell survival and/or proliferation and differentiation of MSCs. Further, stable knockdown analysis is required to assess altered-MSC properties in long term growth and development (*in vitro* and *in vivo*) models and identify associated underlying mechanisms.

The promising results obtained in this study require further investigation prior to publication.

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Variation in dental morphology associated with altered expression of bone regulators

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Glypican-1 (GPC1) and glypican-3 (GPC3) inhibit bone morphogenetic protein (BMP) signalling and are important regulators of cranial suture closure. Alterations in GPC1 and GPC3 levels have been linked to craniosynostosis as well as a range of other skeletal dysmorphologies, including altered mandibular morphology. However, their effects on tooth size are unclear. The aim of the present study was to compare dental dimensions between GPC1-knockout ($GPC1^-$), GPC3 knockout ($GPC3^-$) and double GPC1/GPC3 knockout ($GPC1^-/GPC3^-$) mice.

Micro-CT images of 35 mice with different genotypes (wildtype = 10, $GPC1^-$ = 10, $GPC3^-$ = 5, $GPC1^-/GPC3^-$ = 10) were obtained using a micro-CT scanner (SkyScan 1076, Kontich, Belgium) at a resolution of 8.65 μm at 74 kV source voltage, 135 μA current, 0.8 rotation step, 0.5 A filter and 1767 ms exposure time. The images were reconstructed using the SkyScan NRecon software package and the following tooth dimensions were recorded: crown height (CH), crown width (CW), mesial root length (MRL), distal root length (DRL) and enamel-dentine thickness (EDT) in first molar teeth.

Factorial MANOVA confirmed significant effects of genotype on crown height, crown width, mesial root length, distal root length and enamel-dentine thickness ($p < 0.05$ for each comparison). The following trend was observed in tooth dimensions between the genotypes: $GPC3^-$ > wildtype > $GPC1^-/GPC3^-$ > $GPC1^-$. The $GPC3^-$ mice displayed significantly greater MRL than wildtype mice, greater CH and EDT than $GPC1^-/GPC3^-$ mice, and greater CH, CW and EDT than $GPC1^-$ mice ($p < 0.05$ for each comparison). The wildtype mice displayed significantly greater CH and

EDT than either GPC1$^-$/GPC3$^-$ mice or GPC1$^-$ mice ($p < 0.05$ for each comparison). The GPC1$^-$/GPC3$^-$ mice displayed significantly larger crown height than GPC1$^-$ mice ($p < 0.05$).

The results of this study indicate that GPC1 and GPC3 have important roles in determining tooth size, including crown height, width and root length. GPC3 mutation tends to increase tooth size, while GPC1-mutation opposes this effect. These results complement previous parallel observations of the effects of GPC1 and GPC3 mutations and mandibular morphology in mice, implying the possible roles of glypican-mutations in causing malocclusion in syndromic craniosynostosis.

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The effects of tooth disinfection and cementum removal on bovine incisor root permeability to hydroxide ions *ex vivo*

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To evaluate the effect of thermal and chemical tooth disinfection protocols, and the effect of cementum removal, on the permeability of bovine roots to hydroxide ions from a calcium hydroxide root canal medicament.

Freshly extracted bovine incisors (n = 180) were randomly disinfected by steam autoclaving or by storage for seven or 28 days in one of three chemical disinfectants; 10% formalin, 1% chloramine-T or 4% NaOCl. To test the effect of cementum removal, the formalin groups were duplicated but with a root-surface cavity in these teeth. After disinfection procedures, teeth were endodontically instrumented and medicated with $Ca(OH)_2$ or 0.9% saline as a control, resulting in 18 groups (n = 10 per group). Teeth were stored in 10 mL 0.9% unbuffered saline, the pH of which was measured over 28 days using a combination pH electrode and pH meter. Data were analyzed using linear mixed models (GenStat 16, $\alpha = 0.05$).

Teeth stored in 4% NaOCl ($P < 0.001$) or 10% formalin with a root-surface cavity ($P = 0.02$) showed significant pH increases over time when medicated with calcium hydroxide paste, however this was not observed in the 1% chloramine-T or 10% formalin groups with intact cementum. Duration of formalin storage had a very modest but statistically significant influence on pH over time ($P = 0.02$). In a pilot study, disinfection with 4% NaOCl or autoclaving caused cracks and damaged the structural integrity of the extracted teeth. During the 28-day observation period, turbid growth developed in the saline storage solution for 90% of autoclaved teeth and 20% of teeth disinfected in 1% chloramine-T for seven days.

Intact cementum can hinder hydroxide ion diffusion through bovine roots. Excluding the teeth in the groups exhibiting turbid growth, disinfecting extracted bovine teeth in 10% formalin for up to 28 days was the most appropriate protocol to use prior to hydroxide ion diffusion experiments, while in a pilot study autoclaving or disinfection in 4% NaOCl were found to be detrimental. Future research should investigate the disinfection efficacy of autoclaving extracted teeth or storing them in 1% chloramine-T.

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Effect of azithromycin on a red complex biofilm

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Mechanical debridement of bacterial biofilm is the first line treatment for periodontal diseases. Antibiotic supplementation is warranted in certain cases to enhance treatment outcomes. The aim of this study was to evaluate the effect of azithromycin on biofilms comprised of *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia* in comparison to the amoxicillin and metronidazole combination.

Planktonic cultures of *P. gingivalis* W50, *T. denticola* ATCC35405 and *T. forsythia* ATCC43037 were grown under anaerobic conditions at 37°C. Growth was monitored by measuring absorbance at a wavelength of 650 nm (AU_{650}). Bacterial cultures were aliquoted into 96-well flat-bottom plates in different combinations with addition of azithromycin or amoxicillin + metronidazole at various concentrations. For the biofilm assay, the plates were incubated at 37°C anaerobically for 48 h after which the biofilms were stained with crystal violet, and measured for absorbance at AU_{620}. Susceptibility of planktonic polymicrobial culture of *P. gingivalis* + *T. denticola* + *T. forsythia* and mono- and polymicrobial biofilms to antibiotics were evaluated. For each bacterial combination and antibiotics concentration, biofilm formation in the presence of azithromycin was compared to that of amoxicillin + metronidazole using Student's paired *t*-test. Significance level was set at 5%.

In this model, there was microbial synergism for biofilm formation between *P. gingivalis* + *T. denticola*, *P. gingivalis* + *T. forsythia* and *T. denticola* + *T. forsythia*. Combination of all three bacteria markedly enhanced biofilm formation. The minimal inhibitory concentration (MIC) of the planktonic polymicrobial culture was 1.52 mg/L for azithromycin and 0.17 mg/L for the amoxicillin + metronidazole combination. Azithromycin demonstrated a minimal biofilm inhibitory concentration (MBIC) of 10.6 mg/L, while the amoxicillin + metronidazole combination was more effective in inhibiting biofilm formation with an MBIC of 1.63 mg/L for the polymicrobial biofilm. The effect of azithromycin and amoxicillin + metronidazole on monomicrobial biofilm formation varied, with amoxicillin + metronidazole being more efficacious than azithromycin. The amoxicillin + metronidazole combination effect was most pronounced in cultures containing *P. gingivalis*.

Synergistic biofilm formation was demonstrated by combination of all three red complex bacteria. Azithromycin was ineffective in preventing biofilm formation within a clinically achievable concentration, whereas the combination of amoxicillin and metronidazole was more effective for this purpose.

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Fit of CAD/CAM frameworks to conical connection implants as determined by micro-CT

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Multi-unit restorations on conical connection implants have become increasingly common, yet information on the quality of framework fit, and an evidence-based test for the same, is lacking. The aim of this study was to evaluate and compare the fit of multi-unit frameworks to conical connection implants with and without intermediate abutments.

Five implant level frameworks (ILF) and five abutment level frameworks (ALF) were fabricated from a model with two conical connection implants in parallel configuration. The frameworks were fastened to the model and evaluated with micro-CT following tightening a single screw to 10 Ncm (passive fit), and again with both screws torqued to 15 Ncm (ALF) or

25 Ncm (ILF) (final fit). Cross-sectional slices were generated and micro-gaps recorded. The collated data was statistically analyzed using t-tests for independent samples.

Passive fit: At the unfastened end, the observed difference between the mean detectable micro-gap (MDMG) for the ILF (29.7 μm ± 1.2 μm) and ALF (29.0 μm ± 2.3 μm) was small (P=0.5). At the fastened end, a difference in the MDMG of ALF (13.6 μm ± 2.3 μm) and ILF (10.1 μm ± 1.6 μm) was statistically significant (P=0.004). Final fit: The MDMG of the ILF (7.7 μm ± 0.7 μm) was smaller than the ALF (10.4 μm ± 0.5 μm) (P<0.001).

The ILF displayed smaller mean detectable micro-gaps than ALF, yet both met currently accepted definitions of framework fit. The micro-gaps in ILF exceeded those present in single implant-abutment studies. Further research is required to appreciate the biologic and mechanical consequences of these findings, if any. Micro-CT appears to be a suitable method for evaluating the internal fit of implant framework connections, but would benefit from improvements in image resolution.

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MicroCT image analysis of Apert human teeth

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Apert syndrome is caused by a mutation in the fibroblast growth factor receptor (FGFR2) gene, with early closure of cranial bone sutures (referred to as craniosynostosis) as well as anomalies of the viscera, skeleton and the nervous system. The aim of this study was to undertake the qualitative and quantitative assessments of Apert tooth morphology in humans by using micro-computed tomography (microCT).

In stage 1, five Apert teeth were scanned at a resolution of around 20 μm using the Xradia MicroXCT-400 imaging system (FEI, Visualization Sciences Group, Burlington, USA) to develop the protocols for three-dimensional (3D) image analysis. From the 3D volumetric data, surface (*.stl) files were generated for the external enamel surface, the dentino-enamel junction (DEJ) and the pulp chamber and then the 3D models were exported into Adobe 3D Reviewer (Adobe Systems Inc., California, USA). In stage 2, 25 Apert primary teeth (including all tooth types except mandibular incisors) and 40 primary control teeth (n = 5 for each tooth type) from male subjects were scanned using Skyscan 1076 microCT scanner (Skyscan, Kontich, Belgium) at a resolution of 20 μm.

Apert teeth displayed twisted crown morphology and prominent cusps and marginal ridges, with the DEJ and the pulp chamber following the external tooth morphology. A large proportion of the Apert teeth either fell below the 2.5^{th} percentile or above the 97.5^{th} percentile for both the mesiodistal and buccolingual widths, except the maxillary primary molars. The strongest trends were noted in the buccolingual width of mandibular primary molars (n = 6), of which 1 (16.7%) fell below the 2.5^{th} percentile and 5 (55.6%) fell above the 97.5^{th} percentile.

Alteration in tooth morphology in Apert syndrome, including increased tooth size and twisted crown shape, has implications in clinical orthodontics and phenomics research.

The support of the Australian Dental Research Foundation and the Australian Craniomaxillofacial Foundation for this study is gratefully acknowledged. Aspects of the micro-CT work related to Xradia MicroXCT-400 imaging system was performed at the South Australian node of the Australian National Fabrication Facility under the National Collaborative Research Infrastructure Strategy to provide nano and microfabrication facilities for Australia's researchers. The authors also acknowledge the support provided by Ruth Williams from Adelaide Microscopy for microCT imaging (using Skyscan 1076 microCT scanner) and subsequent data analysis.

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The role of foetal hyperglycaemia in the formation of cleft lip and maxillary hypoplasia

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Phenytoin is the most common drug-induced cause of cleft lip and maxillary hypoplasia. Many females need to take this drug to control epilepsy and must remain on the drug during pregnancy despite the increased risk of foetal malformations such as facial clefting. The cause of the malformations is unknown but may include hypoxia following embryonic bradycardia and more recently, hyperglycaemia. Using an animal model developed by the authors, our first aim was to examine the role of hyperglycaemia and induction of malformations. The second aim was to further explore the effect of phenytoin on embryonic heart rate.

To confirm the induction of hyperglycaemia, glucose levels were monitored in pregnant rats following a teratogenic dose of phenytoin. Another group of pregnant rats were co-administered insulin and phenytoin and the effect on incidence of cleft lip was determined. To examine the effect on embryonic heart rate, pregnant rats were dosed with phenytoin, their embryos removed, cultured and examined under a dissecting microscope. A preliminary proof-of-method study was also performed in a second group of rats dosed with a known cardioactive drug (dofetilide). The embryos of these rats were examined using high frequency ultrasound.

Study 1 showed that phenytoin induced maternal hyperglycaemia (three times pre-dosing levels). Blood levels peaked two hours after dosing and remained above control concentrations for up to 12 hours. The incidence of facial clefting and/or severe maxillary hypoplasia was 27%. When rats were co-administered insulin, maternal blood glucose levels were no different than control levels. There were no cases of facial clefting in the offspring and the incidence of severe maxillary hypoplasia was half that of non-insulin treated dams (10%). The second study showed that phenytoin was not associated with a change in embryonic heart rate. We were also able to successfully measure the heart rate of early rat embryos using high frequency ultrasound.

Phenytoin is associated with increased blood glucose for a prolonged period of time. Co-treatment with insulin reduced maternal blood glucose levels and was associated with decreased incidence and severity of facial clefting and maxillary hypoplasia. The mechanism of action is not known but *in vitro* studies suggest that it does not appear to involve embryonic bradycardia. This will be confirmed *in vivo* at a later date using high frequency ultrasound.

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Effect of histone deacetylase inhibitors in a combined periodontitis and collagen antibody induced arthritis mouse model

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Periodontitis (PD) and rheumatoid arthritis (RA) are chronic inflammatory diseases characterized by inflammation and associated bone destruction. A bidirectional relationship exists between the diseases, where each enhances the other's disease severity. Despite advances in understanding bone metabolism, there are few effective therapies that control bone erosion. Histone deacetylases (HDAC) are involved in the epigenetic regulation of gene expression and different HDACs are upregulated in human PD gingiva and RA synovia. HDAC inhibitors (HDACi) have been shown to suppress osteoclastic bone resorption and inflammatory cytokine expression both *in vitro* and *in vivo*, in PD and arthritis mouse models. The effect of HDACi in a combined PD/arthritis mouse model is yet to be investigated. This study assessed the effects of two novel HDACi (broad acting HDACi 1179.4b and specific acting HDACi NW-21) on

inflammation and bone loss in a combination PD/arthritis mouse model.

PD was first induced by oral inoculations of the bacterial pathogens *Porphyromonas gingivalis* and *Fusobacterium nucleatum* followed by injections of a monoclonal antibody against Type II collagen and LPS to induce arthritis. HDACi suspended in olive oil or olive oil alone were administered daily via oral gavage. Changes in inflammation was assessed visually by daily paw inflammation scoring and systemically through serum levels of C-reactive protein. Changes in alveolar bone and radiocarpal joints were assessed using high-resolution live animal micro-CT scans and a CTX-1 ELISA which measured systemic bone resorption.

HDACi 1179.4b treatment during PD induction reduced subsequent paw inflammation ($p>0.05$) but interestingly increased radiocarpal joint bone loss ($p>0.05$). NW-21 treatment during the arthritis stage unexpectedly increased paw inflammation and radiocarpal joint bone loss ($p>0.05$), but had no effect on alveolar bone loss.

These findings suggest the HDACi may work differently in the presence of both diseases, likely due to the involvement of different HDACs in the combined PD/arthritis mouse model. Further studies will be required to assess HDAC expression in the combined PD/arthritis model, allowing for effective targeting with specific HDACi.

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Gingipain inhibition using gingipain propeptides

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The proteases RgpA, RgpB, (Arg-gingipains) and Kgp (Lys-gingipain) are major virulence factors of *Porphyromonas gingivalis*, an important bacterial pathogen of periodontal disease. Gingipains are secreted from *P. gingivalis* as inactive prodomain-bearing precursors, which render the proteases inactive until they are cleaved. Previously we demonstrated that recombinant RgpB propeptide (rRgpB-PP) and recombinant Kgp propeptide (rKgp-PP) produced in *Escherichia coli* and purified were able to inhibit RgpB and Kgp respectively. Furthermore, the propeptides inhibited whole cell gingipain activity and inhibited *P. gingivalis* growth when added exogenously. Thus gingipain propeptides are promising for use to treat or prevent periodontitis. Although mature RgpA and RgpB proteases are 97% identical the propeptides are only 76% identical. Thus Arg-gingipain inhibition by a recombinant RgpA propeptide (rRgpA-PP) may differ to rRgpB-PP inhibition.

The aim of this study was to produce and purify rRgpA-PP and examine the inhibitory effect on whole cell Arg-gingipain activity with comparison to rRgpB-PP.

The rRgpA-PP was produced with a His-tag at the N-terminus in *E. coli* and purified using nickel affinity and size exclusion chromatography. Protease assays were monitored at 405 nm and conducted in 200 µl in a microtitre plate incubated at 37°C. Assays contained 50mM Tris-HCl, 150 mM NaCl, 5 mM $CaCl_2$, 1 mM Nα-benzoyl-L-arginine 4-nitroanilide hydrochloride (BApNA) chromogenic substrate, 20 mM cysteine, 300 mM glycylglycine and 1.9 x 10^7 cells/ml, at pH 8. *P. gingivalis* strains used were W50 and ATCC 3277. Inhibition dose-response curves were fitted to a 4-parameter logistic non-linear regression model equation in Kaleidagraph (Hearne Scientific Software Pty. Ltd).

The apparent Km values for BApNA were 78.92 ± 7.1 µM and 55 ± 4.34 µM for the strains W50 and ATCC 33277 respectively. The concentration of rRgpA-PP required to obtain 50% Arg-gingipain inhibition (IC_{50}) for the W50 and ATCC 33277 strains were 132.4–142.2 nM and 164.6–174.3 nM respectively at 95% confidence levels. In contrast, the IC_{50} of rRgpB-PP for W50 and ATCC 33277 strains were 524.9–587.1 nM and 445.4–943.8 nM respectively at 95% confidence levels.

The differential Km and IC_{50} values indicate that the ratios and/or substrate affinities of RgpA and RgpB of strains W50 and ATCC 33277 differ. Using this assay both rRgpA-PP and rRgpB-PP are effective

at whole cell Arg-gingipain inhibition. Notably rRgpA-PP is four times more effective than rRgpB-PP and will be a useful addition to a propeptide-based therapeutic.

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Towards enamel biomimetics: remineralization effectiveness of self-assembling peptide scaffold resembling enamel matrix proteins (Stage I and II)

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A central goal of modern dentistry is to find new methods for remineralizing caries lesions using non-invasive methods. In the current study, our aim was to investigate the mineral parameters and structural properties of natural carious enamel lesions as well as naturally arrested enamel caries in order to understand the possible obstacles and the natural model for caries remineralization process. Our second goal was to explore the efficacy of a self-assembling peptide scaffold for the remineralization of natural and artificial enamel lesions.

Extracted teeth with natural fissural and proximal white spot lesions and teeth with naturally arrested brown spot enamel lesions were collected from the surgery department at Sydney Dental Hospital. Imaging of the specimens was undertaken using a high-resolution desktop micro-computed tomography system. A calibration equation was used to transform the grey level values of images into true mineral density values. The value of lesion parameters including the mineral density and the thickness of the surface layer of the enamel lesion were extracted from mineral density profiles.

At the next stage the remineralization efficacy of a self-assembling peptide scaffold was examined by applying the scaffold on the lesion surface and immersing the lesion in artificial saliva. Mineral density of lesions before and after remineralization treatment was quantified using micro-CT and an image processing programme and compared with control groups.

The mineral maps of natural proximal and fissural enamel lesions revealed the characteristic features of enamel lesions with a high density surface layer on the lesion surface. The study of naturally arrested brown spot lesions showed significant level of remineralization in internal and surface areas of the lesion indicating the possibility of remineralization in advanced stage enamel lesions.

The study of the remineralization efficacy of the peptide scaffold showed some degrees of mineral gain in artificial lesions treated with the peptide scaffold and the control remineralization solution. However, there was no significant difference in the remineralization level of the specimens treated with the peptide scaffold and the control remineralization solution. In natural lesions, there was no considerable remineralization in any of the treatment groups.

In conclusion, the results of this study showed the possibility of subsurface remineralization in natural enamel lesions. However, the remineralization of natural enamel lesions with externally sourced minerals and peptides did not show considerable mineral gain in enamel lesions possibly due to the presence of a high density surface layer in these lesions.

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Zoledronic acid effects wound healing and angiogenesis resulting in BRONJ: An *in vitro* and *in vivo* study

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Recent literature suggests that suppressed angiogenesis contributes significantly to the pathogenesis of bisphosphonate (BP)-related osteonecrosis of the jaw (BRONJ) by effecting local vasculature. This *in vitro* and *in vivo* study examined the mechanisms by which the anti-angiogenic properties of BP's could lead to the occurrence of BRONJ lesions.

The *in vitro* effects of BP's (clodronate 2 μM, alendronate 1 μM or zoledronate ZA 0.5 μM) on endothelial differentiation by placenta-derived mesenchymal stem cells (pMSCs) were assessed using Von Willebrand factor staining and an angiogenesis assay. *In vivo*, a BRONJ model using Sprague-Dawley rats was established by weekly intraperitoneal injection of zoledronic acid (ZA) for three weeks, followed by extraction of the maxillary first two molars. The rats were subsequently sacrificed after two or four weeks of healing and gross examination and micro-CT were used to confirm BRONJ. The amount of osteonecrosis, inflammatory infiltrate, total vascularity and microvessels were quantified in the BRONJ animals and compared to that of control animals which did not receive ZA.

BP's were shown to significantly affect endothelial differentiation of pMSCs, demonstrated using Von Willebrand factor staining and angiogenesis assay. The animal study confirmed the presence of a BRONJ lesion in the extraction defect sites. Histologically, the two-week healing ZA rats showed significantly higher osteonecrosis (25 048 ± 3489 vs 4076 ± 905.7 μm^2, $p < 0.0001$) that remained significantly higher (80 669 ± 13 420 vs 2168 ± 277.2 μm^2, $p < 0.0001$) after four weeks of healing. In addition, significantly higher inflammatory areas in the ZA group, at both two weeks (22 945 ± 2481 vs 2794 ± 252.3 μm^2, $p < 0.0001$) and four weeks (59 999 ± 7110 vs 6820 ± 787.9 μm^2, $p < 0.0001$) were evident. In contrast, total vascularity was significantly reduced in the ZA treated rats (0.1749 ± 0.01324 vs 0.2792 ± 0.02 μm, $p = 0.0011$) at two weeks which decreased further at four weeks (0.1050 ± 0.01vs 0.1889 ± 0.01 μm, $p = 0.0010$). Furthermore, the proportion of microvessels was significantly supressed in two week ZA-rats (30.06 ± 4.23% vs 76.09 ± 4.14%, $p < 0.0001$) that reduced further at four weeks (33.84 ± 4.78% vs 68.01 ± 2.42%, $p < 0.0001$) suggesting that ZA has a direct effect on the microvessels in healing bone.

The *in vitro* study confirmed that nitrogen-containing BP's inhibit the differentiation of multipotent stem cells into cells of an endothelial lineage and affect the downstream functional capability of these cells, thereby supressing the replenishment of vascular cells essential for initial bone healing. Further, the *in vivo* rat model established the critical role of ZA-induced local anti-angiogenesis as a significant contributing factor in the etiopathogenesis of BRONJ.

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A retrospective analysis of *Candida* prevalence in potentially malignant oral lichen planus biopsies

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Oral lichen planus (OLP), a chronic inflammatory mucocutaneous disorder affecting 1–2% of the general adult population, may have possible malignancy potential. Antifungal agents are clinically helpful for symptoms of OLP, indicating a role for *Candida* species. This pilot study assessed specialized staining techniques to investigate the accuracy of re-assessing archival tissue for the presence of *Candida*, in addition to retrospectively

analyzing the relevance of *Candida* presence to the malignant progression of patients with OLP.

To re-assess *Candida* presence, 23 biopsy specimens previously diagnosed as yeast positive were age and gender matched with 23 OLP specimens diagnosed as yeast negative. The archived blocks were retrieved, sectioned at a thickness of three microns, mounted onto slides and stained by Periodic Acid-Schiff (PAS). These slides were de-identified, randomized and observed via light microscopy for the presence of hyphae.

To assess the relevance of the presence of oral yeast in archival biopsy material to the malignant progression of patients with OLP, PAS staining was performed on specimens from 18 patients with a single biopsy diagnosed with OLP, and four patients with multiple biopsies and whose diagnoses subsequently worsened to either increased levels of dysplasia or frank squamous cell carcinoma (SCC).

Twelve biopsy samples that were initially yeast positive remained so, while two of the OLP specimens initially yeast negative stained positive in the archival tissue. Thus, this resulted in a sensitivity of 52% and specificity of 91% for observing yeast via PAS staining of archival tissue. Of the 18 patients with a single biopsy of OLP, three had the presence of yeast noted on PAS stained archival sections, two of which had very large amounts of hyphae present. All four of the patients whose lesions progressed had oral yeast noted on at least one of their biopsy specimens; with two patients this was on their first biopsy, while the other two patients had yeast noted on subsequent biopsies.

The low sensitivity of PAS staining for presence of oral yeast may, due to loss of hyphae in the tissue after taking multiple sections subsequent to the initial diagnosis. This, along with the specificity result, indicates that PAS remains an easy and reliable staining method for assessing the presence of yeast in archival tissue. Finally, these preliminary results indicate that the presence of oral yeast may be important for patients whose oral mucosal disease progresses in severity or develops malignancy.

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Detection of human herpes viruses and HIV in patients from Southeast India

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DNA Genotek OMNIgene™·DISCOVER (OM-505) kits stabilize microbial DNA and RNA in saliva at room temperature for 14 months and three months respectively. Human herpesviruses (HHVs) are common in India, except for Kaposi's sarcoma and other HHV-8 diseases. We determined the seroprevalence and prevalence of six HHVs in Southeast India as well as compared HIV salivary viral loads (SVL) to plasma viral loads (PVL).

HHVs were detected from 188 ART-naïve HIV-positive patients from Southeast India who visited YRG CARE from 2012-2014. Seroprevalence for past (IgG) and current (IgM) HSV-1, HSV-2, VZV, EBV, and HCMV infections were determined via commercial kits. ELISA for HHV-8 ORF65 and ORF K8.1 were also performed. Prevalence was determined on blood (plasma and PBMC) and saliva (OM-505) via in-house rtPCR assays.

HIV SVL and PVL were determined on 64 HIV-positive ART-naïve patients. Saliva was collected in OM-505 and incubated at 50°C/1 hour. Samples (800 μL) were well-vortexed and centrifuged at 2,000 xg for five minutes before 70 μL isopropanol was added to the supernatant. From OM-505 and plasma, RNA was extracted automatically on Abbott m2000sp with viral loads determined on Abbott m2000rt.

Seroprevalence for past and current HSV-1, HSV-2, VZV, EBV, and HCMV infections were 92.55% and 0.00%, 68.09% and 9.57%, 81.91% and 3.72%, 100% and 5.85%, and 98.94% and 13.30% respectively. HHV-8 seroprevalence was 10.64%. HHV-8 rtPCR was negative in plasma, but was positive in seven saliva samples.

For HIV, calibration curves (2-fold dilutions) were linear (R^2=0.9951) from 57,273-621 HIV copies/mL. In clinical isolates, PVL averaged 330,141 HIV copies/mL (range: 62-7,604,620) whilst SVL averaged 29,115 HIV copies/mL (range: 153-220,104). SVL was not detected in 24 samples and not determined in nine due to viscosity/cellular debris. In 26/31 patients SVL was lower than PVL, higher in 2/31 and equivalent in 3/31. Extracting RNA from supernatants increased the SVL and prevented clogging during automated extraction.

Regarding HHV-8, this is the first detection of the virus in India. Despite the absence of HHV-8 disease the virus is in India at a seroprevalence of 10.64% in the HIV-positive population. Whilst the virus is not detected in plasma it can be detected in saliva, which is understandable as the oropharynx is the reservoir for infection and the virus is transmitted via saliva.

Regarding HIV, oral shedding is low/non-existent. If present, HIV can be detected accurately to 621 HIV-copies/mL. SVL does not correlate with PVL and cannot be used to determine HIV carriage. Nonetheless, when detected in saliva, HIV is intact free virion. This has clear implications for transmission.

This research was possible through funding obtained through the Australian Dental Research Foundation and Griffith University.

The findings of this research were presented at IADR/AADR/CADR 93rd General Session, Boston, Massachusetts, USA. March 2015; the 7th World Workshop on Oral Health and Disease in AIDS. Hyderabad, India, November 2014. Awarded the best poster at the WW7 Conference. 2nd International Science Symposium on HIV and Infectious Diseases (HIV SCIENCE 2014) Chennai, India. 30 January – 1 February 2014.

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[Correction added on 27 December 2016, after first online publication: the affiliations of DJ Speicher and NW Johnson have been updated and the conference "2nd International Science Symposium on HIV and Infectious Diseases (HIV SCIENCE 2014) Chennai, India. 30 January – 1 February 2014" has been added.]

Effects of Tooth Mousse Plus on remineralization and fluoride uptake in artificially demineralized enamel: an *in situ* study using electron probe microanalysis

C Tran,* L Chai,* H Ngo,*† LJ Walsh*

This small scale human *in situ* clinical study aimed to establish the value of a new model for assessing remineralization using white spot lesions (WSL) mount into orthodontic brackets, which can be placed in different sites within the mouth.

Backscatter electron imaging (BSE) and electron probe microanalysis (EMPA) were used to compare the clinical effects of two weeks of twice daily application onto 100 μm deep enamel white spot lesions mounted into orthodontic brackets located on the buccal surfaces of the left maxillary second premolar and first molar teeth. The subjects used in a random order Tooth Mousse (TM), Tooth Mousse Plus (TMP), TMP-Enhanced, or a vehicle paste of TMP containing 900 ppm fluoride, with washout periods between products. Subjects were blinded to the products being used, which had identical flavours.

All products induced changes in the white spots lesions compared to the baseline. Alterations in the BSE grey scale levels from the enamel surface through the lesion correlated with calcium and phosphorus levels assessed using EPMA. The ranking of products in the order of increasing subsurface remineralization was the fluoride vehicle < TM < TMP = TMP mineral enhanced. Minor differences between the two forms of TMP were found by EMPA in terms of calcium and phosphorus levels in the outermost enamel.

The study is a useful proof of concept for the use of BSE as a means for quantifying the effect of various agents on remineralization. The clinical factors in this model include high access to parotid saliva, regular brushing of enamel surfaces and twice daily topical use of products by highly compliant healthy subjects.

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Novel debridement methods of dental implant surfaces contaminated by a dental biofilm: a proof of concept study

C Tran,* C Yip,* M Wei,* N Meredith,* LJ Walsh*

The purpose of this study was to assess cleaning of implant surfaces and associated surface damage. Biofilms were grown on 10 mm grade 4 titanium discs with abraded surfaces over 96 hours in BHI broth using human saliva as the inoculum. An Acteon ultrasonic scaler with carbon composite, titanium or stainless steel tips was used for 10 to 60 seconds with low water spray at the recommended power setting of 2/10. Samples were

fixed and examined using SEM at 15 kV at magnifications up to 10,000x. Images were scored blind by two calibrated examiners for debris and damage.

A series of 4.1 x 10 mm grade 4 threaded titanium implants (ITC 410, Southern Implants) were coated in indelible ink to replicate biofilm and mounted in a bone replica material with saucer-shaped defects. Implants were debrided for two mins with hand scalers (plastic, titanium, carbon fibre, stainless steel), an ultrasonic scaler (plastic or stainless steel tips), an air abrasion unit (calcium carbonate powder) (NSK) or a saline swab. Fixture surfaces were photographed using a custom jig so that 12 images of each implant could be digitally stitched to give a single rectangular image of the entire implant surface in perfect focus. Residual ink area was calculated as a percentage of the total surface.

When an ultrasonic scaler was used on the flat discs, the best biofilm removal occurred for carbon composite tip, followed by titanium and then stainless steel tips. The extent of damage was in the reverse order, with the most extensive damage to the surface caused by stainless steel tips. Longer treatment times increased the extent of damage with all methods. No cleaning method removed all traces of biofilm or ink from the discs or implant surfaces respectively. Using the ink model, the best cleaning result was achieved with air abrasion device, followed by the ultrasonic scaler with a metal tip and the plastic tip (4.46%, 15.02% and 49.54% residual area, respectively). There was no significant difference in cleaning between the hand scalers (78.48–98.17%) and the saline swab control (92.72%).

Thus, once biofilm has developed on abraded Ti surfaces, it is extremely difficult to remove using currently available devices. Abrasive powders are superior to ultrasonic scalers and hand instruments, with better cleaning and less damage.

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Phenotypic effects of exchange between fibroblasts and malignant cells

H Zoellner,* KCL Kwong,* M Lu,* E Kelly*

We earlier reported the exchange of membrane and cytoplasm between fibroblasts (F) and malignant cells (MC), and corresponding changes in MC cell shape at the population level (J Pathol, 2012 228:495), as well as altered cytokine synthesis (Cytokine, 2013 62:48). Based on observations by others we assumed this exchange was via tunneling nanotubes (TNT).

We aimed to confirm a role for TNT, and also to determine, at the level of individual cells, if MC morphological change corresponded with uptake of F contents at the single cell level. Further, we wished to examine the effect of uptake of F material on MC proliferation and migration, processes relevant to cancer.

MC and F were cultured alone or together, and studied using a combination of confocal laser scanning microscopy (CLSM), time-lapse microscopy, fluorescence activated cell sorting analysis (FACS), and FACS sorting. An image analysis system was developed for relating the uptake of F fluorescent label by MC, to the cell surface area profile and circularity of individual cells. In addition, F as well as MC with high as opposed to low levels of F labelling, were studied in bioassays for cell proliferation, colony formation and migration.

Contrary to expectation, TNT were not involved. We found instead, that F organelles were injected into MC via F filopodia. Studying co-cultured cells following FACS separation, we found that MC with high F labelling had: greater migration, slower proliferation, and reduced colony formation; compared with MC with low levels of F label. In addition, changes in MC circularity and cell surface area profile corresponded with uptake F label.

Data suggest that F transfer cytoplasm to MC via filopodia. Data also indicate that uptake of F material results in substantial phenotypic change in MC, such that MC acquire a morphological and functional phenotype intermediate between that of MC and F cultured alone. Reduced proliferation and colony formation of MC with high levels of F labelling, suggests a role in chemotherapy evasion, while increased migration implies a separate effect increasing invasion and metastasis. Further work is required to properly elucidate the clinical implications of this study.

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Summaries of observations were presented to the collected Faculty of Dentistry in October 2015.

ADRF Research Grant Reports published as full papers in the *Australian Dental Journal* in 2016

Aust Dent J 2016;61:16–20
A content analysis of oral health messages in Australian mass media
K Jones, J Merrick, C Beasley

Aust Dent J 2016;61:174–182
Job satisfaction among 'migrant dentists' in Australia: implications for dentist migration and workforce policy
M Balasubramanian, AJ Spencer, SD Short, K Watkins, S Chrisopoulos, DS Brennan

Aust Dent J 2016;61:183–189
Prevalence of infraocclusion of primary molars determined using a new 2D image analysis methodology
R Odeh, S Mihailidis, G Townsend, R Lähdesmäki, T Hughes, A Brook

Aust Dent J 2016;61:288–297
Immunolocalization and distribution of proteoglycans in carious dentine
K Stankoska, L Sarram, S Smith, AK Bedran-Russo, CB Little, MV Swain, LE Bertassoni

ADRF Dental Student Research Grant Abstracts

The associations between self-perceived orofacial pain symptoms with social and physical variables in regional New South Wales

G Hilton;* R Akhter,† N Hassan*(Supervisors)

The aims of this study were to examine: (i) the prevalence of orofacial pain in community members attending dental clinics in regional New South Wales, (ii) to examine associations between self-reported orofacial pain symptoms and physical and psychosocial factors.

A total of 119 participants (age; male = 32, female = 87) were recruited for this study from student dental clinics. Participants completed an interview and questionnaires to obtain information on socio-demographics, orofacial pain symptoms and physical and psychological factors. The psychological measures included the Depression, Anxiety and Stress Scale (DASS), Pain Catastrophizing Scale (PCS), Satisfaction with Life Scale (SWLS) and Rosenberg Self-Esteem Scale (RSE).

The prevalence of reported orofacial pain was 35.3%. Individuals who reported oral habits and symptoms such as a jaw click ($p < 0.05$), jaw pain on waking ($p < 0.01$) and bruxism ($p < 0.05$) were significantly more likely to report orofacial pain. Psychological factors assessed in this study showed no significant differences between those with and without orofacial pain. The presence of two or more orofacial pain symptoms was significantly ($p < 0.05$) associated with the duration, character, severity and effect on daily activities.

This study showed a high prevalence of self-perceived orofacial pain symptoms in community members attending dental clinics in regional New South Wales. Individuals who reported oral habits and symptoms such as a jaw click, jaw pain on waking and bruxism were significantly more likely to report orofacial pain. These findings can be used to improve the management of orofacial pain in regional New South Wales.

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This study was presented at the 94th The International Association of Dental Research (IADR), Seoul, Korea June 2016.

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Removable prosthodontics: patient-reported treatment outcomes in the University of Sydney student clinics

CM Kluner;* V Hanna,* S King,* A Ellakwa,* I Klineberg* (Supervisors)

The aims of this pilot study were to assess the profile of patients selected for the year three Removable Dental Prostheses (RDP) clinics at the University of Sydney Faculty of Dentistry, to evaluate patient satisfaction and denture use one-year post construction, and identify potential predictors of patient satisfaction. The study included patients from four consecutive years of the programme (2012-2015).

Data for each patient was gathered prior, during, and at one-year post denture construction. Information across three domains was collected: patient profile (age, sex, medical history, smoking history), oral health status (Sulcus Bleeding Index (SBI), Approximal Plaque Index (API), and remaining teeth), and denture information (previous denture history, denture base material, Kennedy Classification, denture use and level of satisfaction one-year post denture issue).

A total of 92 patients were included in the study (44 male and 48 female). The mean age was 69 years with 89% of these patients having had previous dentures. Smoking history revealed 25% as current smokers and 23% as previous smokers. At initial presentation, a mean SBI of 27% and API of 72% were found and the average number of remaining teeth was nine. The dentures constructed in the programme included 70 complete dental prostheses (CDPs) of which 10 were mandibular and 60 were maxillary. There were 93 removable dental prostheses (RDPs) of which 68 were mandibular and 25 maxillary. There were a total of 58 cobalt-chrome RDPs and 35 acrylic RDPs of which the majority (49) were Kennedy Class I. One-year follow-up data revealed that 87% of patients were satisfied with and wearing their dentures.

This investigation has provided a patient profile and information about the type, classification, and success of the dentures made through the RDP programme. Students were exposed to a wide range of patient cases. The majority of patients followed-up were satisfied with and wearing their dentures one year post issue. However, a small sample size and incomplete data hindered calculation of statistical correlations. Continued patient follow-up will allow for increased sample sizes across the different categories and enable meaningful analysis of the data to help identify factors that may help improve the clinical outcomes of the programme.

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The findings of this research were presented at the 54th Annual Scientific Meeting of the International Association for Dental Research (IADR) ANZ Division, Brisbane, Queensland, Australia, September – October 2014.

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Assessment of anxiety and depression among university students and its impact on temporomandibular disorders

A Murray;* R Akhter,† NMM Hassan*(Supervisors)

The aim of this study was to assess the presence of anxiety and depression among university students and its subsequent impact on temporomandibular disorders (TMD).

One hundred and forty-five students (58 male and 87 female) participated in the study. The study surveyed Bachelor of Dental Science students from Years 1–4 at Charles Sturt University, Orange Campus. Each participant completed the Depression, Anxiety and Stress Scale (DASS), the Tampa Scale for Kinesiophobia (TSK), and Pain Catastrophizing Scale (PCS) questionnaires. The Research Diagnostic Screening Criteria for Temporomandibular Disorders (RDC/TMD) was used to determine the presence or absence of TMD symptoms. SPSS 22.0 was used for statistical analysis and a $P<0.05$ was considered as statistically significant.

Among 200 students, 145 students returned completed survey questionnaires and 40.7% reported the presence of jaw click, 37.2% reported the presence of joint pain and 17.9% reported difficulty in opening mouth. Bivariate analysis showed a significant relation between female students who self-reported jaw click and those who did not (53.5% and 69.5%, $P < 0.05$

respectively). There was a significant association between self-reported all TMD symptoms such as jaw click ($P < 0.05$), jaw pain ($P < 0.01$) and difficulty on opening ($P < 0.05$) and DASS total scores. There were no significant relations with self-reported TMD symptoms and other psychological variables.

This study showed that headache, depression, anxiety and stress are significant risk factors for TMD. This finding could also be used to justify the provision of better education for students in general relaxation and stress management techniques as well as information about exercises for relief of TMD and additional treatment when required.

This research project was made possible with the funding from the Australian Dental Research Foundation Undergraduate Dental Student Grant. The Charles Sturt University School of Dentistry and Health Sciences (Orange Campus) academic staff for help with organising times for survey completion and encouragement. The students from the Bachelor of Dental Science Years 1–4 for their participation in the surveys. The authors would also like to thank Professor Boyen Huang (Head of School) for his support in this project.

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Knowledge, awareness, and opinions regarding bisphosphonates and other anti-resorptive agents: a survey of dentists and dental students

P Ponna;* J Mitchell,* M Schifter,*† T Prvan‡ (Supervisors)

To assess the current knowledge and opinions of Australian dentists and dental students regarding patients taking bisphosphonates and other anti-resorptive medications (ARMs), as well as analyze methods used to obtain updated information.

A cross-sectional survey was used to obtain information from dental practitioners and students in regards to patients taking ARMs.

Although a number of areas in our survey were commonly and correctly answered by the majority of respondents, many areas were answered incorrectly highlighting considerable gaps in knowledge which is important for dentists and dental students to be aware of when treating patients taking ARMs. Furthermore, methods for prevention and treatment of BRONJ varied considerably among respondents.

Dentists and dental students require further information regarding patient populations most at risk of bisphosphonate-related osteonecrosis of the jaw (BRONJ), and which medications are capable of causing BRONJ. In addition, there is a need for further research to produce a widely recognized, evidence-based guideline for all dental professionals to follow as a standard of care for BRONJ prevention and treatment. Almost all respondents indicated they would like to receive updates on information and guidelines related to BRONJ.

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Effectiveness of implant surface debridement using particle beams at differing air pressures

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

Implant surface decontamination is challenging and air powder abrasive systems are a novel treatment approach. This *in vitro* study investigated the effectiveness of different powder formulations and air pressures in cleaning implant surfaces and the extent of surface damage.

A validated ink model of implant biofilm was used. Sterile 4.1 × 10 mm Grade 4 titanium implants were coated in a blue indelible ink to form a uniform, visually detectable biofilm-like layer over the implant threads and mounted into a bone replica material with bony defects to approximate peri-implantitis. Air powder abrasive treatments were undertaken using glycine, sodium bicarbonate or calcium carbonate powder at air pressures of 25, 35, 45 and 55 psi. Digital macro photographs of the threads were stitched to give composite images of the

threads, so the amount of ink remaining could be quantified as the residual area and expressed as a percentage. Implant surfaces were also examined with scanning electron microscopy to grade the surface changes.

No treatment cleaned all the surface of the threads. The powders were ranked in order of decreasing effectiveness and decreasing surface change into the same sequence of calcium carbonate followed by sodium bicarbonate followed by glycine. Higher air pressure improved cleaning and increased surface change, with a plateau effect evident. All powders caused some level of surface alteration, with rounding of surface projections most evident.

With air powder abrasive systems, there is a trade-off between cleaning and surface damage. Sodium bicarbonate and calcium carbonate powders were the most effective for surface cleaning when used at air pressures as low as 25 psi and these seem promising for further investigation.

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Colin Cormie Grant

Investigation of the effectiveness of D-amino acids to disrupt *Enterococcus faecalis* biofilms for root canal treatment

V Butnejski,* P Zilm,* G Rossi-Fedele*

Enterococcus faecalis is the most frequent species present in failed obturated root canals and plays a significant role in persistent periapical infections following root canal treatment due to its ability to form biofilms. The presence of certain D-amino acids (DAA's) has been shown to disrupt and prevent the formation of a biofilm in *Bacillus subtilis*.[1,2] The mis-incorporation of DAA's into proteins responsible for anchoring biofilms to a surface may also provide a means of disrupting biofilms of *E. faecalis* and may lead to success rate improvements in current root canal therapy.

The main investigation aims were as follows:

- To determine if DAA's had the ability to disrupt biofilm formation in initial and late biofilm growth stages for ten *E. faecalis* strains.
- To test their efficacy in disrupting *E. faecalis* biofilms grown in sub minimum inhibitory concentrations (MIC) levels of endodontic irrigants/medicaments.
- If DAA's were effective at disrupting *E. faecalis* biofilms, explore their incorporation into current endodontic irrigants/medicaments.

Thirty-seven *E. faecalis* strains were screened for their ability to produce biofilms using a crystal violet staining protocol and the ten 'strongest' biofilm producers were used to test the ability of DAA's (D-Leucine, D-Methionine, D-Tyrosine and D-Tryptophan at concentrations of 0.25 mM, 2.5 mM and 25 mM) to influence biofilm growth over a period of 24, 72 and 144 hours.

DAA's were also compared to their conjugate L-amino acids for their ability to disrupt and reduce *E. faecalis* biofilms. DAA's at a final concentration of 25 mM were tested for their effectiveness to reduce biofilm growth in sub MIC 0.031% NaOCl and 0.25% (w/v) for Odontocide™, and in the presence of 0.25% (w/v) Odontopaste™. An unpaired t-test was used to determine statistical significance ($p<0.05$) between experimental and control groups.

The presence of the DAA's was determined to significantly disrupt and reduce biofilm formation for all strains tested *in vitro*. As the concentration of the DAA's was increased, greater reductions of *E. faecalis* biofilms were demonstrated.

LAA's showed no significant ability to disrupt and reduce *E. faecalis* biofilms. DAA's were also shown to be effective in disrupting and reducing *E. faecalis* biofilms in the presence of Odontopaste™ and sub MIC levels of sodium hypochlorite and Odontocide™.

The use of D-amino acids may have application in root canal treatment. The inclusion of DAA's into current endodontic procedures may increase the success of retreatment cases and reduce the occurrence of secondary endodontic infection.

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Reginald and Pamela Hession Award

The influence of titanium surface characteristics on diabetic bone healing: a proteomic analysis

S Hamlet,* S Ivanovski*

Adsorption of serum proteins onto implant surfaces is one of the first steps in the blood-coagulation cascade following placement. Studies have shown that the physicochemical and topographical properties of implants can modulate protein adsorption and the subsequent adhesion, migration, and differentiation of osteogenic cells suggesting that the composition and functional state of the adsorbed protein layer plays a critical role in later cellular responses leading to implant osseointegration. As impaired healing of bone in subjects with systemic conditions such as diabetes mellitus significantly increases the risk of implant failure, the aim of this study therefore was to use a diabetic animal model to determine *in vivo,* whether titanium surface topography (presence of nanostructures) and/or chemistry (hydrophilicity vs. hydrophobicity), could promote the adsorption of serum proteins onto titanium that would subsequently enhance downstream osteogenic cell adhesion.

Proteomic analysis of 1) material adsorbed onto sand-blasted acid etched (SLA) and hydrophilic-modified SLA (modSLA) titanium discs covering a critical sized calvarial defect and 2) the serum exudate beneath these discs, was assessed in healthy Sprague-Dawley and Type 2 diabetic Goto-Kakizaki rats using either liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS) or SWATH (sequential window acquisition of all theoretical spectra) MS respectively. The protein and peptide data obtained were then searched against the Swissprot Rat database to determine their biological function(s).

Qualitatively, more fibrinogen and the osteoblast integrin ligand, fibronectin, was adsorbed onto the modSLA than SLA surface in both healthy and diabetic animals. This effect was more pronounced in the diabetic animals. In the serum exudate collected from the healing defect beneath the modSLA surface of diabetic animals, fibronectin levels were significantly down-regulated compared to those in the SLA exudate. Anti-inflammatory proteins such as SPA3N, SPA3K, ANXA1 and S10A9 were also significantly up-regulated within the modSLA exudate.

The increased adsorption of fibronectin and fibrinogen, which play important roles in cell adhesion and proliferation, onto modSLA titanium surfaces *in vivo*, coupled with increased levels of anti-inflammatory proteins within the healing milieu, suggests topographically modified hydrophilic titanium surfaces may subsequently improve the rate and degree of osseointegration in diabetic subjects.

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Implicit acquisition of dental drill-manipulation skills using an errorless learning paradigm

T Winning,* N Malhotra,†‡ RSW Masters‡§

Competence in dentistry is defined by successful execution of both technical and non-technical skills (e.g. multi-tasking). Research in motor learning has shown that acquiring technical skills implicitly reduces attention demands compared to explicit learning, which releases resources for concurrent performance of non-technical skills (e.g. Lam *et al*., J Sports Sciences 2010; 28:1543-54; Master & Poolton, 2012, Skill acquisition in sport: Research, theory and practice, pp. 207-228). This study examined the viability of

such an approach in dentistry, using an implicit learning paradigm (i.e. errorless learning).

Novices were randomly assigned to either an errorless (n = 11) or an errorful (n = 8) learning condition to acquire dental drill-manipulation skills. During training, they used a dental drill, in which the bur was replaced by pencil lead, to shade different cavity preparation shapes (n = 8, 22 repetitions each). The errorless learners progressed from the easiest shape (small circle) to the most difficult shape (cross), whereas the errorful learners progressed from most difficult to easiest. In a test phase, they completed simulated cavity preparations in a baseline condition, with additional instructions and when multi-tasking. Performance during learning was computed as the percentage of the shape that was successfully shaded. Performance during the test phase was computed as percentage error in length and depth of the cavity preparations.

Repeated measures ANOVA revealed that, overall, errorless learners were more accurate than errorful learners when shading the shapes during training, F (1,17) = 29.61, $p < .001$. A significant learning condition x shape interaction, F (3,51) = 2.94, $p = .042$, revealed that errorless learners were more accurate than errorful learners when shading difficult shapes but not easy shapes. ANCOVA (with baseline performance as a covariate) showed that in the test phase when participants were provided instructions to process, cavity preparation depth seemed to be drilled more accurately by errorless learners than errorful learners F (1,16) = 4.21, $p = .057$, but length was not, F (1,16) = 0.40, $p = .540$. When multi-tasking, the errorless learners were more accurate at cutting the designated length of the cavity preparation, F (1,16) = 4.59, $p = .048$, but not depth, F (1,16) = 0.01, $p = .920$.

The findings suggest that drill use in dentistry can be acquired implicitly via errorless learning and that it is less attentionally demanding than errorful learning, as showcased by superior ability of errorless learners to process additional relevant instructions and to multi-task.

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