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Introduction

This is my first year as Chairman of the Australian Dental Research Foundation (ADRF). I have served on the Board and Executive of the Foundation since 2011, as Chairman of the Research Advisory Committee (RAC) from 2011 to 2016, and as a Member of the RAC from 2007 to 2011. As Chairman, I am grateful for the opportunity to showcase the work of our researchers in the *Australian Dental Journal* (ADJ) ADRF Special Research Supplement.

This Supplement provides a snapshot of the completed research projects undertaken by students, researchers and academics from across the country. It provides the busy clinician reader of the ADJ with distilled insight into the efforts of the dental research community, while simultaneously disseminating the findings from these projects to industry and the wider scientific community. Not only is the Supplement an important witness to the work of our researchers and the funding provided by the Foundation to support them; it also acknowledges the individuals, industry and organisations who have supported the Foundation through named research awards and grants.

This Supplement covers a wide range of material from motor impairment and oral health-related quality of life issues in children with Cerebral Palsy, through to the exomic profile of oral precancer and the genotoxic effects of alcohol-containing mouthwashes on dysplastic oral keratinocytes. The projects cover the broad areas of basic, clinical and translational research.

Bereza and colleagues demonstrate that mice with a genetic mutation in *EFNB1* demonstrate increased skull size, eye socket distance and nasal bone width, promoting this as a model for patients with Craniofontonasal Dysplasia. Hamlet and Ivanovski use a diabetic animal model to demonstrate that proteins associated with wound healing and the regulation of inflammation are preferentially adsorbed onto titanium with a micro-rough hydrophilic surface suggesting implants with this surface may better integrate with bone in diabetic subjects. Finally, using modern genetic methods exploring the risk for dental caries in children, Johnson and colleagues found that new bacteria associated with severe decay depended more on a child's environment, especially diet, than on maternal influences, and that the mix of bacteria in the mouth could be changed by topical applications of disinfectant and fluoride resulting in a health-associated flora which could last as long as a year.

The Board of the Foundation is indebted to the Australian Dental Journal and its Editor, Professor Mark Bartold for supporting this initiative. I am also appreciative of the work of the Chairman of the RAC, Professor Saso Ivanovski and the ADRF Services Officer, Jane Levey for assisting with its compilation.

On behalf of the Board, I appreciate the opportunity to present this ADRF Special Research Supplement and its abstracts to you.

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ADRF Research Grant Abstracts

Effect of probiotic treatment in an experimental model of periodontitis

S Gatej, M Bartold, N Gully, P Zilm, C Christophersen

Previous studies have shown that probiotics may play a role in the management of periodontitis. The aim of this study was to investigate the impact of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* inoculation firstly on intestinal inflammation and secondly on the structure and diversity of the intestinal microbiome in an *in vivo* mouse model. The role of the probiotic *Lactobacillus rhamnosus* GG in altering these changes was also investigated.

Thirty-six mice were allocated into six groups. Experimental bone loss was induced in mice by oral inoculation with *P. gingivalis* and *F. nucleatum* over a period of 44 days. The probiotic LGG was administered via oral inoculation or oral gavage prior to and during disease induction. The probiotic treated groups were compared with animals with experimental bone loss and with controls. Intestinal tissue changes were assessed using histological analysis. Interleukin 6 (IL-6) expression in gut tissue was assessed via immunohistochemistry. The phylogenetic structure and diversity of the intestinal microbiota were analysed by sequencing the 16S rRNA genes of the caecal content. Statistical differences between groups were identified using a permutational multivariate ANOVA pseudo F test for beta diversity, a pairwise Kruskal–Wallis test for alpha diversity, analysis of the composition of microbes for detection of specific taxa associated with different treatments, and a pairwise Kruskal–Wallis test followed by Dunn’s multiple comparisons test to determine histology and IL-6 expression. Statistical significance was accepted at $P < 0.05$.

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Inoculation with *P. gingivalis* and *F. nucleatum* induced inflammation throughout the gastrointestinal tract (duodenum $P = 0.0143$, jejunum $P = 0.0009$, ileum $P = 0.0017$, colon $P = 0.0442$), increased expression of IL-6 in the ileum ($P = 0.052$), and significantly altered the gut microbiome ($P < 0.05$) in experimental mice compared with controls.

Mice treated with LGG had significantly reduced tissue inflammation in the duodenum ($P = 0.0437$) and significantly lowered levels of IL-6 in the ileum ($P = 0.048$) when compared with disease. LGG therapy prevented gut microbiome changes associated with *P. gingivalis* and *F. nucleatum* inoculation irrespective of the probiotic mode of administration. *P. gingivalis* or *F. nucleatum* DNA were not detected in caecum or faecal samples.

Porphyromonas gingivalis and *F. nucleatum* inoculation induced changes in intestinal inflammation and in the phylogenetic structure and diversity of the intestinal microbiome. Oral gavage with LGG prior to *P. gingivalis* and *F. nucleatum* inoculation exerted a protective effect against intestinal inflammation. Pre-treatment with LGG prevented gut microbiome changes associated with *P. gingivalis* and *F. nucleatum* inoculation irrespective of the probiotic mode of administration.

This research was funded by a grant from the Australian Dental Research Foundation.

The findings of this research were submitted for publication in the Journal of the International Academy of Periodontology and were presented at IADR 2018 and Adelaide Dental School Research Day 2018.

Transcriptomic alterations in head and neck cell lines

S Fox,* S Currie,† A Dalley,† CS Farah*†

Transcriptomic sequencing was performed on immortalised cell lines derived from normal and mildly dysplastic oral cavity tissue with and without exposure to alcoholic mouthwash representing a model for oral carcinogenesis to investigate individualized and common transcriptomic expression alterations in two oral cell lines.

Two brands of alcohol-containing mouthwash and their alcohol-free counterparts were used to treat two oral cell lines derived from normal (OKF6-TERT) and dysplastic (DOK) tissues. Genotoxicity was determined by Comet assay. RNA-sequencing was performed using the Ion Torrent platform. Bioinformatics analysis was conducted using R/Bioconductor

packages with differential expression using DEseq2. Pathway enrichment analysis was performed via EnrichR with WikiPathways and Kyoto Encyclopedia of Genes and Genomes databases.

Comet assay revealed that both cell lines displayed dose-dependent DNA damage in response to acute exposure to ethanol and alcohol-containing mouthwashes as well as alcohol-free mouthwashes reconstituted with ethanol. The transcriptomic effects of alcohol-containing mouthwash exposure were more complex with significant differential gene expression

ranging from >2000 genes in DOK cells to <100 genes in OKF6-TERT cells. Pathway enrichment analysis in DOK cells revealed common features between the two brands of alcohol-containing mouthwashes used, including DNA damage response and cancer-associated pathways. In OKF6-TERT cells the most significantly enriched pathways involved inflammatory signalling.

Alcohol-containing mouthwashes are genotoxic *in vitro* to normal and dysplastic oral keratinocytes and induce widespread changes in gene expression. Dysplastic cells are more susceptible to the transcriptomic effects of mouthwash.

This research was funded by a grant from the Australian Dental Research Foundation.

The findings of this research were published in the Journal of Oral Pathology & Medicine.

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Impact of early childhood caries on the oral health-related quality of life of preschool children: A population based study

DH Ha, N Hariyani, AJ Spencer, LG Do

Early childhood caries (ECC) remains the most prevalent chronic disease in children, with over a third of Australian preschool children affected. However, the impact of ECC on children and society has not been comprehensively quantified. Identifying the magnitude of the impact of ECC on oral health-related quality of life (OHRQoL) will be useful for informing effective oral health promotion programs. This study investigated the impact of ECC on the OHRQoL of young children.

Data was collected from a population-based prospective birth cohort study of newborn children in Adelaide. The study sample was recruited during 2013–2014. Questionnaire data were collected at birth and at different ages. When the children reached 24 months of age, both the children and their mothers were invited to oral epidemiological examinations to assess evidence of dental caries. The explanatory variables of this analysis were the prevalence of ECC and the number of primary tooth surfaces detected with caries. The outcome was parent-reported OHRQoL when children reached 5 years of age. Household

income at baseline, parental education, and country of birth were covariates. Analysis was conducted progressively from bivariate to multivariable log-binomial regression with robust standard error estimation to evaluate an association between ECC at 24 months and OHRQoL reported at age five.

A total of 1040 children were examined. Those whose oral health was perceived as fair/poor at age five had higher mean caries experience at age two than those whose oral health was excellent/very good/good ($\beta = 0.53$). In the multivariable regression model, the presence of ECC at age two was a significant predictor for having negative oral symptoms at age five (prevalence ratio = 1.78, 95% confidence interval: 1.12–2.81).

The study provides evidence of a relationship between ECC and child OHRQoL at the start of school. Preventive programs need to reach and benefit children early in life, providing them the opportunity to stay healthy and to maximise their life potential.

This research was funded by grants from the Australian Dental Research Foundation and the National Health and Medical Research Council.

The findings of this research were published in Community Dentistry and Oral Epidemiology.

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Influence of oral cavity tissue preservation on birefringence contrast using polarization-sensitive optical coherence tomography

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A detailed comparison with corresponding histology is needed to further advance our understanding of polarization-sensitive optical coherence tomography (PS-OCT) contrast. The utility of fixed samples from an oral cancer tissue bank could accelerate and broaden the scope of such investigations to a wide range of pathologies. However, it has been reported that tissue fixation may diminish the visibility of some features when measured with OCT and no reports have measured the effect with PS-OCT. The objective of this study was to investigate the effects of tissue fixation on birefringence contrast assessed with PS-OCT measurements.

We measured 12 freshly excised oral cavity tissue samples from different locations covering a range of pathologies. Excised samples were imaged with polarization-sensitive OCT. Following the PS-OCT measurement, the samples were immersed in fixative solution (formalin) and stored. The PS-OCT measurements were repeated at 24 and 48 h after excision. Mueller–Jones formalism and differential Mueller matrix algorithm were constructed to extract the local birefringence. Birefringence contrast was investigated both visually and statistically.

In general, despite some changes due to the tissue remodelling induced by the fixation procedure, the birefringence contrast is preserved. Regardless of the differences in birefringence contrast ($C_{\Delta n}$) between the samples, the contrast change during investigated fixation process time is not significant. Moreover, $C_{\Delta n}$, even if reduced slightly, offers improved contrast compared to OCT intensity (C_{int}). On average the $C_{\Delta n}$ is three times higher than the C_{int} . Statistically significant differences were found only between fresh and +48 h measurements for three samples (P values of 0.049, 0.037 and 0.048). However, in those cases, the differences related to improvement of the birefringence contrast, which is a positive effect considering the future utility of samples from the oral cancer tissue bank.

Our results show that fixation of oral cavity tissue samples does not diminish the contrast provided by the polarization sensitive measurement. Promisingly, these results offer an opportunity for a deeper understanding of polarization contrast for oral tissue and research of numerous fixed samples might lead to processing methods to further enhance contrast and differentiation between normal and abnormal tissue.

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Injectable bone extracellular matrix protein extract-polyethylene glycol diacrylate hybrid hydrogel scaffold for growth factor delivery and vascularization in bone tissue engineering

F Obregon, A Spahr

Ridge preservation techniques aim to keep the bone volume after teeth extraction or at least reduce the bone resorption of surrounding tissues. Previous reports have shown that at 3 months post-extraction, dimensional changes of up to 63% horizontally and up to 22% vertically occur. Therefore, ridge

preservation may help to reduce the need for subsequent bone augmentation in order to allow implant placement and/or complex rehabilitation.

For this purpose, several bone substitutes have been used, including autografts, allografts, xenografts, and alloplasts, as well as growth factors and platelet-rich

fibrin. However, bone substitutes usually lack bioactivity and their scaffolding properties are limited. To solve this problem, hydrogels have been explored as suitable candidates because of their capability to achieve bioactivity and their modifiable physicochemical properties.

In this study the bone regenerative capacity and stimulation of vascularisation of the previously optimized injectable pddeECM/PEGDA hydrogel blend when combined with fibroblast growth factor 2 (FGF2) in the post-extraction sockets of rats was investigated.

A previously fabricated interpenetrating polymeric network hydrogel comprised of demineralized porcine and digested bone extracellular matrix (0.1% w/v pddeECM) blended with 20% w/v PEGDA (Mn 700) and the same blend in combination with FGF2 was injected into the post-extraction sockets (first mandibular molars) of 30 Sprague–Dawley rats. Untreated defects and Bio-Oss Collagen were used as negative and positive controls, respectively. After eight and 12 weeks, the rats were euthanized, the mandibles containing the defects removed, fixed in 4% paraformaldehyde for 8 h, demineralized in 0.5 M ethylene-diamine-tetraacetic acid for 6 weeks, and

embedded in paraffin blocks for further sectioning (6 µm). Histology (H&E) and immunohistochemistry (bone morphogenetic protein 2 (BMP-2) and Laminin) were then performed. (N = 5, statistically analysed ANOVA, Bonferroni, * $P < 0.05$).

The histological analyses of the post-extraction sockets treated with 0.1% w/v pddeECM/20% w/v PEGDA and 0.1% w/v pddeECM/20% w/v PEGDA with FGF2 showed marked bone formation at eight and 12 weeks post-injection. Histomorphometric analysis showed no statistical differences between the two gel groups and the positive control. Significant shrinkage was observed in untreated sockets at 12 weeks compared to grafted sockets. Immunohistochemistry revealed increased vascularization in the hydrogel groups compared to the positive and negative controls.

The injectable pddeECM/PEGDA hydrogel blend is an inexpensive bone substitute and a suitable candidate for ridge preservation as it regenerates a substantial proportion of new bone and can be used as a carrier for growth and/or differentiation factors enabling a sustained release.

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The findings of this research were submitted to the Journal of Biomedical Materials.

The salivary microbiome associated with dental caries in Australian children

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The study was conducted to determine risk factors for dental caries in two cohorts of Queensland children: ~6 year olds and their mothers (n = 174 dyads), and all consenting school-going children from a remote Indigenous community (n ~ 400).

Epidemiological screening was performed using International Caries Detection and Assessment II (ICDAS-II) criteria. The Indigenous children were rendered dentally fit, received a single annual preventative intervention of fissure sealant, topical povidone-iodine and fluoride varnish, and were followed for 2 years.

In the mother–child dyads, past caries experience ($\beta = 0.332$, $P = 0.018$), and salivary counts of mutans streptococci ($\beta = 0.215$, $P = 0.032$) were positively associated with untreated decay at the time of examination. With a trend toward significance, children

whose mothers had reported taking iron supplements during pregnancy experienced lower levels of past caries ($\beta = -0.137$, $P = 0.068$) and current untreated dental caries ($\beta = -0.046$, $P = 0.051$). Preliminary work on the microbiome reveals an increase in bacterial diversity with higher caries levels: there are many shared taxa between mother–child pairs, but the microbial composition of mothers and children are dissimilar, suggesting that environment has a greater influence on the saliva microbiome than genetics.

In the Indigenous children, the relative abundance of *Lactobacillus salivarius*, *Lactobacillus reuteri*, *Lactobacillus gasseri*, *Prevotella multisaccharivorax*, *Parascardovia denticolens*, and *Mitsuokella HMT 131* species were significantly increased in children with severe caries. Lower taxonomic diversity and abundance was observed a year after application of

disinfectant and F varnish (Shannon index, Bray–Curtis; $P < 0.05$)

Caries-associated microbiota are more complex than health-associated flora; are not dominated by mutans streptococci; and can be returned to a sustained, more health-associated flora by this topical preventative intervention.

This research was funded by grants from the Australian Dental Research Foundation and the National Health and Medical Research Council.

The findings of this research project were included in a PhD thesis (S Fernando, Griffith University, August 2017, accessible at <<https://research-repository.griffith.edu.au/handle/10072/371955>>) and have

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Evaluation of the bond strength between various pre-treated zirconia and composite cement through three bond strength tests

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The study was conducted to: (i) assess and compare the mean bond strength between composite cement and zirconia, treated with two pre-treatment techniques: alumina air abrasion versus DCMHotbond coating, by three bond strength tests; and (ii) to correlate the mean bond strength results of three test methodologies.

Zirconia blocks were fabricated with different dimensions to satisfy the parameters of three bond strength test methodologies: micro-tensile, shear, and strain energy release rate tests. Two pre-treatment techniques were prepared on the zirconia surface: (i) GC- 50 µm alumina air-abrasion followed by G-Multi Primer and G-Cem Linkforce Cement, and (ii) HOT-DCMHotbond ceramic coating followed by hydrofluoric acid etching, G-Multi Primer, and G-Cem LinkForce Cement. Bilayered specimens of each bond strength test were stored in distilled water for 24 h and then divided into three aging conditions: (i) immediate test, and (ii) thermocycling for 5000

been presented at the ANZ Division of IADR, Adelaide 2017, IADR London 2018, and IADR Washington 2020. Findings are published in:

Indicators of Risk for Dental Caries in Children: A Holistic Approach. Fernando S, Tadakamadla SK, Bakr M, Scuffham PA, Johnson NW. *J Dent Res Clin Trans Res.* 2019 Oct;4(4):333-341. doi: 10.1177/2380084419834236. Epub 2019 Apr 30. PMID: 31039050

Children's untreated decay is positively associated with past caries experience and with current salivary loads of mutans Streptococci; negatively with self-reported maternal iron supplements during pregnancy: a multifactorial analysis.

Fernando S, Kumar S, Bakr M, Speicher D, Lea R, Scuffham PA, Johnson NW. J Public Health Dent. 2019 Mar;79(2):109-115. doi: 10.1111/jphd.12301. Epub 2018 Dec 14. PMID: 30551255

and (iii) 10 000 cycles. The specimens were then tested in tensile, shear, and four-point bending according to the parameters of each test methodology. Failure mode analysis was also performed with light and scanning electron microscopes.

GC groups recorded higher mean bond strength than HOT groups in micro-tensile and strain energy release rate testing for the majority of aging conditions. In the case of the shear bond test, there was no significant difference in the mean bond strength between GC and HOT groups at any aging conditions. In addition, thermocycling did not affect the mean bond strength in either group. A moderate correlation ($r^2 = 0.67$) between micro-tensile bond strength and strain energy release rate was observed and found to be significant ($P = 0.047$).

Micro-tensile and strain energy release rate testing showed a similar trend. The bond strength of GC groups was higher than HOT groups on the bonding between zirconia and composite resin cement.

This research was funded by grants from the Australian Dental Research Foundation and GC Corp.

The findings of this research were published in the Journal of Adhesive Dentistry.

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Evaluation of thermal conduction in all-ceramic crowns

KN Tran, RM Love, R George

Pulp-sensibility testing of teeth restored with a full-coverage restoration may be influenced by the conductivity of the restorative material. A cold test is the most commonly conducted test because of its reliability and simplicity. However, there is limited data available in the literature on the thermal conduction behaviour of all-ceramic crowns. This *in vitro* pilot study investigated the thermal conduction profile of a cold stimulus through all-ceramic crowns to build on the current understanding of cold testing through a full-coverage restoration.

Two different types of all-ceramic milled crown were investigated: a lithium disilicate (IPS e.max) and a zirconium oxide (zirconia). Cold stimulus was generated by using a dimethyl ether refrigerant spray (ADM Frostbite). The cold test was carried out using three different types of cotton carrier: pellet, tip, or roll. The carriers were saturated with Frostbite spray and applied directly on the occlusal surface of the crown for 60 s. The thermal conduction profiles were measured and data was collected in real time using a multimeter. Mean and standard deviations from a

minimum of three test runs were analysed for each testing condition.

Both e.max and zirconia crowns had similar thermal conduction profiles. The maximum change in temperature occurred in the first 30 s, with the most rapid change in temperature occurring in the first 10 s. The volume of the carrier had a strong correlation with the net stored energy. Cotton roll stored the most energy, followed by cotton tip and cotton pellet. However, cotton tip has the most effective rate of heat transfer.

This pilot study confirmed the viability of a cold test as a diagnostic tool for assessing the current pulpal status of teeth restored with full-coverage all-ceramic crowns. The clinical guidelines to achieve the most effective cold test are to select the carrier with adequate volume, fully saturate the carrier, and rapidly transfer the carrier to the conducting surface. Testing carriers that can transfer more energy may help to illicit a response in special cases involving thicker restorations. Future research using infra-red and high-speed cameras could help better assess in real-time the thermal conduction pattern of teeth subjected to thermal sensibility testing.

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This research was funded by a grant from the Australian Dental Research Foundation.

Soft microlithography and melt electrospinning for the development of a systematic fibre guiding biphasic scaffold for periodontal regeneration

C Vaquette, S Ivanovki

The objective of this research was to manufacture a fibre-guiding biphasic scaffold combined with cell sheet technology for the purpose of promoting bone formation, enhanced functional periodontal attachment, and regeneration. In this biphasic scaffold, the periodontal compartment featured aligned channels with widths ranging from 20 to 100 μm , thus capable of inducing excellent cell alignment and more closely mimicking the hierarchical and highly organised architecture of the native periodontal complex.

The micro channels were prepared by standard photolithography using a

polydimethylsiloxane (PDMS) mould that was subsequently utilised to create silk fibroin micropatterned membranes with channel widths of 20, 45, 70, or 100 microns. The bone compartment was manufactured

using polycaprolactone, a biodegradable polymer using melt electrospinning writing with a 0/90 pattern. The orientation of the fibres was obtained utilising an x,y stage translating at 2 m/min. Human periodontal cells (20 000 in 100 μL of media) were seeded on the membrane and the degree of cellular alignment along with collagen production was qualitatively assessed via confocal microscopy. The fibre-guiding efficacy was assessed subcutaneously in a rodent model, involving the placement of the biphasic scaffold with a trilayer human periodontal cell sheet onto a dentine chip. In order to recapitulate the regenerative events of periodontal healing, 50 μg of bone morphogenetic protein 2 encapsulated in a hyaluronic acid/gelatine hydrogel were injected into the bone compartment. The regenerative performance was

assessed at four weeks post-implantation by computed tomography and histology.

All micropatterned channels were capable of aligning the cells, although the 20 microns were particularly efficient for this purpose. Interestingly, the collagen deposited by the cells was also aligned in the channels and there was qualitatively more collagen in the larger grooves (70 and 100 μm). The *in vivo* study demonstrated the maintenance of a periodontal gap between the newly formed bone and the dentin block, indicating that the compartmentalisation was preserved and no ankylosis was observed. The histology revealed that the

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Nano-engineered membrane as an efficient growth factor delivery system for periodontal tissue regeneration

C Xu, QS Ye

Guided tissue regeneration (GTR) is an efficient technique to repair lost periodontal tissues and restore functions where a membrane plays an important role. Compared to existing bare membrane, bioactive membrane with growth factors that mimic the physiological microenvironment of periodontal tissues show great potential. However, the loss of bioactivities and the fast drug release rate in conventional membranes limit their performance. In this project, nano-engineered membranes with a growth factor delivery system were developed with better encapsulation capacity, bioactivity, and controlled release for efficient guided tissue regeneration.

A novel membrane with core-shell nanofibers containing growth factor (recombinant bone morphology protein-2, rhBMP-2) loaded nanoparticles using coaxial electrospinning method were fabricated. The growth factor release profile and activity after released were tested. The effects of the nano-engineered membrane on the differentiation of bone marrow mesenchymal stem cells (BMSCs) were evaluated. Cell Counting Kit 8, alkaline phosphatase (ALP) staining and activity, and Alizarin Red staining were used to test cell proliferation and osteogenic differentiation. Quantitative real-time polymerase chain reaction analysis was performed to determine the expression of runt-related transcription factor 2 (Runx2) and other osteogenesis-related genes. Membranes with added

larger channels enabled newly formed extracellular alignment perpendicularly to the dentin, while the smaller channels prevented cellular infiltration. Cementogenesis was not observed in any of the samples.

The micropatterned membranes, although efficient at inducing fibre-guiding capacity to the biphasic scaffold, do not seem to be an appropriate approach for periodontal regeneration because of the lack of proper integration with the newly formed bone.

This research was funded by a grant from the Australian Dental Research Foundation.

The findings of this research were presented at the Biomaterials World Congress in Montreal, Canada in 2016.

growth factors without nanoparticles and bare membranes without growth factors were used as controls.

Core-shell nanofibers with embedded growth factor loaded nanoparticles were successfully fabricated and the structures were confirmed by transmission electron microscope and scanning electron microscopy. The membranes were nontoxic to BMSC growth and cells were observed to grow well on the surface. Sustained release behaviour of growth factor was observed and the membrane showed better regeneration abilities toward BMSCs. BMSCs cultured with nano-engineering membranes exhibited the highest ALP activity compared to control groups. Osteo-related genes (*Runx2*, collagenase (*ColAL*), osteocalcin (*OCN*), osteopontin (*OPN*), and integrin binding sialoprotein (*IBSP*)) were significantly up-regulated after stimulation for seven and 14 days. Alizarin Red results showed that BMSCs on nano-engineered membranes formed a significantly higher calcium component after 21 days of osteogenic induction.

Novel membranes with the ability to encapsulate growth factors, preserve bioactivity and control their release behaviour were successfully fabricated using nanoparticles and the electrospinning method. We also showed that BMP-2 loaded membranes have better bone regeneration ability.

This research was funded by a grant from the Australian Dental Research Foundation.

The findings of this research were submitted to Advanced Healthcare Materials, and were presented at IADR ANZ 2018 and the UQ CCR Workshop 2018.

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Predicting the presence of oral squamous cell carcinoma using commonly dysregulated microRNA in oral swirls

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Oral swirls are a non-invasive, rapidly collected source of salivary microRNA (miRNA) potentially useful in the early detection of disease states, particularly oral squamous cell carcinoma (OSCC). The aim of this study was to predict the presence of OSCC using a panel of OSCC-related dysregulated miRNA found in oral swirls, identified jointly in data from formalin-fixed paraffin-embedded (FFPE) and fresh frozen specimens.

Next-generation sequencing (NGS) was used to determine miRNA fold changes in FFPE OSCC specimens relative to histologically normal epithelium. These data were placed with NGS of fresh frozen tissue data from The Cancer Genome Atlas to select a panel of commonly dysregulated miRNA. This panel

was then analysed by real-time-quantitative polymerase chain reaction in RNA extracted from oral swirls collected from 30 patients with OSCC and 30 controls.

Up-regulation of miR-31 and miR-21 and down-regulation of miR-99a, let-7c, miR-125b, and miR-100 were found between OSCC and controls in both FFPE and fresh frozen samples. These miRNAs were studied in a training set of 15 OSCC versus 15 control oral swirls to develop a dysregulation score (area under the curve (AUC) 0.95; 95% confidence interval (CI): 0.88–1.03) and classification tree. A test cohort of 15 OSCC versus 15 control oral swirls yielded a dysregulation AUC of 0.86 (95% CI: 0.79–1.00) with the classification tree identifying 100% (15/15) of OSCC and 67% (10/15) of controls.

This study debuts the use of OSCC-associated miRNA, commonly dysregulated in both FFPE and frozen specimens, in oral swirls to indicate the presence of OSCC with high accuracy.

This research was funded by grants from the Australian Dental Research Foundation and *the Australia and New Zealand Head and Neck Cancer Society Research Foundation*.

The findings of this research were published in Cancer Prevention Research and presented at IAOO 2017.

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Non-invasive screening of a microRNA-based dysregulation signature in oral cancer and oral potentially malignant disorders

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We have previously shown that oral swirls are a robust source of microRNA protected by extracellular vesicles, potentially useful to detect oral squamous cell carcinoma (OSCC)-associated molecular aberration. This study was conducted to develop a dysregulation score and risk classification algorithm based upon a panel of OSCC-associated microRNA in oral swirls from individuals with OSCC and oral potentially malignant disorders (OPMDs).

An OSCC-associated panel of five microRNAs (miR-24, miR-21, miR-99a, let-7c, miR-100) was quantified

by quantitative polymerase chain reaction in 190 individuals with and without mucosal abnormalities, including OSCC (n = 53) and OPMDs (n = 74). Each sample was analysed using a developed dysregulation score (dSCORE) and risk classification algorithm, allocating a LOW-RISK or HIGH-RISK score. The influence of demographic, systemic, oral health, and mucosal disease factors on the developed test were analysed.

MicroRNA for analysis can be predictably isolated from oral swirls sourced from individuals with a range of demographic, systemic, and oral health

findings. The HIGH-RISK classification identified OSCC patients with 86.8% sensitivity and 81.5% specificity. Older age and female gender were associated with higher dSCOREs and higher proportions of HIGH-RISK classification among individuals with no

mucosal abnormalities. The dSCOREs for all subgroups of OPMDs were significantly different from those of the OSCC group.

This is the first study to compare microRNA sourced from oral swabs from individuals with OPMDs and individuals with and without OSCC. A HIGH-RISK dysregulation signature was found to be accurate for indicating the presence of OSCC and demonstrated to parallel malignant transformation.

This research was funded by grants from the Australian Dental Research Foundation and *the Australia and New Zealand Head and Neck Cancer Society Research Foundation*.

The findings of this research were published in Oral Oncology and presented at the Australia and New Zealand Head and Neck Society Annual Meeting and the European Academy of Oral Medicine Biennial Meeting.

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ADRF Dental Student Research Grant Abstracts

Motor impairment and oral health-related quality of life (OHRQoL) of children with cerebral palsy

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The study was conducted to investigate the association between motor impairment and oral health-related quality of life (OHRQoL) among children with cerebral palsy (CP) in rural Bangladesh, a low-resource setting.

A total of 90 children with CP aged 2–17 years (median age 10 years; 37.8% female and 62.2% male) were selected from the Bangladesh cerebral palsy register (BCPR) for the study. Motor impairment data was extracted from the BCPR using the Gross Motor Function Classification System (GMFCS). The Child Perceptions Questionnaire (CPQ) and Family Impact Scale (FIS) were used to assess the impact on

OHRQoL. Statistical analysis was performed using SPSS version 21.0. Data was analysed using chi-square test and binary logistic regression. A P value of <0.05 was considered statistically significant.

The CPQ and FIS both showed that motor impairment exerted a significant impact on OHRQoL, but the CPQ in particular highlighted children with CP who had difficulty in saying words ($P = 0.05$); trouble sleeping ($P = 0.05$); difficulty in eating, drinking, or chewing firm foods ($P = 0.01$); had taken longer to eat a meal ($P = 0.01$); and felt terrible or frustrated ($P = 0.01$) (Table II). X^2 tests using GMFCS IV–V revealed statistically significant differences ($P = 0.001$) in children's experience of feeling upset or shy, and avoiding smiling or laughing (Table IV). The FIS showed the significant impact of motor impairment in parents who reported blaming themselves or others in the family ($P = 0.05$), the child requiring more attention from themselves or others in the family ($P = 0.05$), and had less time for the family ($P = 0.01$). After adjusting for age and gender, binary logistic regression analysis showed that motor impairment was significantly associated with CPQ and FIS

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scores among children with CP in Bangladesh, particularly children with CP who had difficulty in eating, drinking, or chewing firm foods (rate ratio 5.20, $P = 0.02$); felt upset (rate ratio 9.06, $P = 0.04$); felt shy (rate ratio 6.06, $P = 0.02$); and parents who had less time for the family (rate ratio 4.2, $P = 0.01$).

Motor impairment is associated with a negative impact on OHRQoL of children in terms of perceptions of both children and their parents. Oral health promotion programs should be implemented with a

focus on adaptive care and education for families and children with CP with motor impairment of GMFCS IV and V.

This research was funded by a grant from the Australian Dental Research Foundation.

The findings of this research were published in Research in Developmental Disabilities. Julia Allyn won the 2018 Colgate poster competition and presented the research findings at IADR ANZ 2018.

Morphometric and histological craniofacial features in a murine model of craniofrontonasal dysplasia

S Bereza,* R Yong,* S Ranjitkar,* A Arthur,†‡ PJ Anderson*†§

Ephrin B1 (EFNB1) gene mutation causes craniofrontonasal dysplasia (CFND), presenting clinically with craniosynostosis, orbital hypertelorism, nasal dysplasia, and maxillary hypoplasia, as well as joint and limb abnormalities. Murine models have been used extensively to better understand craniofacial syndromes and how individual genes may regulate development of the craniomaxillofacial complex; however, a reliable murine model for CFND has not yet been developed. The aim of this study was to conduct extensive morphometric and preliminary histological analysis of the craniomaxillofacial (CMF) features observed in mice with *EFNB1*-mutated osteoprogenitor cells.

The Cre recombination system was utilised to breed mice with targeted deletion of *EFNB1* in osteoprogenitor cells, under the control of the *Osterix* promoter. Skulls of 24 8-week old samples were obtained, representing two groups: *EfnB1* mutants (males are hemizygotes and females are homozygotes) and *Osterix-Cre* controls (males and females) ($n = 6$ in each group). Cranium, midface, and mandible morphology was analysed and compared between groups by measuring landmark-based linear dimensions from volume rendered, three dimensional micro-computed tomography reconstructions. Sections obtained from coronal and sagittal suture areas were stained with haema-

toxylin and eosin (H&E) and examined via light microscopy.

Compared to the control samples, *EfnB1* mutant craniums displayed significantly increased anterior cranial width ($P = 0.013$), posterior cranial width ($P = 0.020$), and cranial height ($P < 0.001$). *EFNB1*-mutant mice also displayed significantly increased interorbital distance ($P = 0.001$) and nasal bone width ($P < 0.001$) compared to the control samples. There were no significant differences in measurements between maxilla or mandible dimensions. One *EfnB1* homozygote displayed craniosynostosis of the coronal suture. Visual assessment of the H&E stained cranial sections demonstrate an increased eosinophilic tissue volume in the coronal suture and increased quantity of bone marrow and greater parietal bone thickness in the sagittal suture area of *EfnB1* mutated mice when compared to controls.

Increased interorbital width and nasal bone width without changes in maxilla and mandibular morphology promote this population as a reliable model for CFND patients. The phenomic data obtained contributes to an understanding of the role of *EFNB1* in CMF morphogenesis and in addition to clinical data, to improve patient management. Further instigation of cranial suture histology may further elucidate the role *EFNB1* plays in regulating suture homeostasis.

This research was funded by a grant from the Australian Dental Research Foundation. The author acknowledges the assistance of Adelaide Microscopy with Micro-CT analysis and the staff at the South Australian Health and Medical Research Institute (SAHMRI), for assistance with histological analysis, as well as breeding and housing the animal colony.

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Orthodontic mechanotherapies and their influence on external root resorption: A systematic review

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This systematic review assesses the literature regarding the association between orthodontic tooth movement and external root resorption. A determination of the evidence level supporting the association could provide clinical evidence for minimizing the deleterious effect of orthodontic tooth movement.

Electronic databases, including MEDLINE, PubMed, Embase, Scopus, CINAHL, the Cochrane Library, and LILACS, were searched up to February 2018, with hand searching of selected orthodontic journals undertaken to identify any pre-electronic publications. Searches were undertaken with no restrictions on year, publication status, or language. Selection criteria included randomized controlled trials conducted with the use of fixed orthodontic appliances or sequential thermoplastic aligners on human patients. The quality of included studies was assessed with the use of the Cochrane Risk of Bias Tool and the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach.

Inter-rater agreement of the review authors was used for the inclusion of primary articles, risk of bias assessment, and evaluation of the quality of evidence (GRADE), and it was calculated with the use of the Cohen kappa statistic.

A total of 654 articles were retrieved in the initial search. After review, 25 articles describing 24 individual trials met the inclusion criteria. Sample sizes ranged from 6 to 154 patients. Most articles were classified as having unclear risks of bias and very low to low quality of evidence.

There is very low to low evidence to support positive associations between root resorption and increased force levels, force continuity, intrusive forces, and treatment duration. Moreover, by including a pause in treatment for patients experiencing root resorption, it may be possible for the clinician to reduce the severity of the condition. The most common methodological flaws in the included studies were the lack of a control group, appropriate randomization strategy, or adequate examinations before and after treatment.

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The effectiveness of the Caries Management System in lowering caries risk at Westmead Centre for Oral Health

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The traditional clinical solution to caries has been to remove tooth structure and place restorative material, despite growing evidence that non-cavitated lesions can be arrested and remineralised. An alternative to restorative dentistry is a preventive protocol called the Caries Management System (CMS), which aims to arrest caries and remineralise teeth, as well as reduce a patient's future risk of caries. The CMS has been successfully trialed in private clinics and General Practice at Westmead Centre for Oral Health (WCOH), producing positive clinical outcomes for high-risk patients. However, the CMS has been used for less

than 6 months at WCOH and has not been implemented in student clinics. Therefore, we monitored the progress of patients attending WCOH student clinics from 2017–2019 to assess the effectiveness of the CMS in lowering caries incidence and risk in patients.

Data was collected from the dental records and bitewing radiographs of 100 high or medium caries risk patients who were treated by University of Sydney students at WCOH under CMS guidelines. Data documented at the first appointment was compared to data at the recall appointments, with changes in risk

status, caries incidence, and oral hygiene behaviour analysed using SPSS version 23.0.

According to the CMS guidelines, 70% of patients attending student clinics had a reduction in caries risk. In addition, 84% (162) of non-cavitated lesions (ICDAS 1–3) arrested without restorative intervention and 85% of patients had no new lesions by 2019. Furthermore, a significant ($P < 0.05$) reduction was noted in the number of clinical and radiographic carious lesions using paired t -tests. Multivariate ANOVA revealed that patients taking regular medication experienced higher gingival bleeding, and decayed, missing, and filled teeth scores. High-risk

patients were associated with more missing teeth. Patients born overseas produced higher gingival bleeding scores. Thirty-eight patients increased brushing to twice a day and 44 reduced their sugary snacking scores.

Implementation of the CMS was effective in reducing caries risk and incidence, arresting early caries, and modifying the oral health behaviour of high and medium-risk patients at WCOH. These results indicate that preventative dentistry should be taught in student clinics and continued in practice by all general practitioners at WCOH. This study may serve as a baseline for long-term monitoring of these patients to see if they maintain good oral health.

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The efficacy of interventions to improve oral health outcomes for people with serious mental illness: A systematized review

A Huang, B Christian, M Masood, A Kenny

The review was conducted to systematically map the existing literature on the efficacy of interventions to improve oral health outcomes for people with serious mental illness (SMI); to describe the interventions and the commonly measured oral health outcomes; and to identify gaps in the research on this topic.

This paper is a systematized review, as it includes elements of a systematic review but without a quality assessment of the included studies (Grant MJ, Booth A. A typology of reviews: an analysis of 14 review types and associated methodologies. *Health Info Libr J* 2009;26(2): 91-108). This review was guided by Arksey and O'Malley's five-stage review framework, and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement. (Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol* 2009;62(10): 1006-1012). Data were primarily sourced from MEDLINE, supplemented with data from other databases, including CINAHL, Scopus, and EMBASE. The search was limited to the English language. The reference lists of papers selected for inclusion in the review were also searched. Covidence

was used to manage the search and screening processes.

The results show that in the last 30 years only seven studies reported interventions to improve oral health among people with SMI. Except for one study in a child population, most of the research on this topic has focused on adults. While definitions of SMI differed across studies, schizophrenia was observed to be the dominant definition. The most commonly reported study design was pre-post intervention testing on a cohort of subjects. Most interventions were related to oral health capacity building and in these studies the primary outcomes were changes in oral health-related knowledge and clinical indicators of oral hygiene, such as the Plaque Index. Except for one study, the results of the included studies showed significant improvements in the reported outcome measures as a result of the intervention.

The literature on this topic is very limited. People with SMI are a high-risk group for oral diseases and urgent action is required at the individual, family, and community level to ensure their health is protected.

This research was funded by a grant from the Australian Dental Research Foundation.

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Oral Medicine Academy of Australasian Award

Expression of BRCA1/2 and associated genes in oral epithelial dysplasia

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We hypothesize that the expression of BRCA1/2 will differ significantly at both gene and protein levels between normal tissues, grades of oral epithelial dysplasia (OED), and oral squamous cell carcinoma (OSCC).

Thirty-three formalin-fixed paraffin-embedded (FFPE) specimens were retrieved from tissue archives including samples classified histopathologically as normal, mildly dysplastic, moderate to severely dysplastic, or OSCC. Immunohistochemistry was performed using heat-induced antigen retrieval in BORG buffer and overnight incubation at 4°C with either BRCA1 (1:600) or BRCA2 primary antibody (1:500). Specifically bound antibodies were detected using the MACH1 Universal Polymer Detection kit, the nuclei briefly counterstained with Mayer's haematoxylin, and slides dehydrated and mounted with dibutylphthalate polystyrene xylene. Immunoreactivity was assessed as described in our previous study (Chaw *et al.*, 2012, *Oral Oncol.* 2012 Oct;48(10):997-1006) with two independent reviewers assessing staining intensity and positivity. Differences between groups were assessed using one-way ANOVA and Tukey's multiple comparison test. RNA was extracted from frozen samples of normal, dysplastic, and OSCC tissues and *BRCA1* and *BRCA2* gene expression was quantified using quantitative polymerase chain reaction.

BRCA1 staining was significantly reduced in OSCC compared to normal tissue ($P < 0.0001$). Hyperplastic and dysplastic tissue displayed BRCA1 staining similar to normal tissue. BRCA2 staining was significantly reduced in both OED and OSCC compared to normal tissue ($P < 0.0001$). When OED was divided into mild and severe, mild OED had significantly higher BRCA2 expression than severe OED. This suggests that OSCC is associated with a loss of BRCA1 and BRCA2 protein expression and that in the case of BRCA2, this loss is present in severely dysplastic tissue. We confirmed this result in a different cohort of samples demonstrating a significant decrease in both *BRCA1* and *BRCA2* gene expression by quantitative polymerase chain reaction from normal to dysplastic and OSCC tissues.

Progression to oral cancer is associated with a loss of BRCA1 and BRCA2 expression at both the gene and protein level.

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The findings of this research were published in Oral Oncology and presented at IADR ANZ 2015, APIAP 2015 and IAOO 2015.

Exome sequencing of oral leukoplakia and oral squamous cell carcinoma implicates DNA damage repair gene defects in malignant transformation.

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In honour of the memory of Dr Nathan Cochrane Grant

The effects of proanthocyanidin application when applying silver diammine fluoride to treat artificial root caries lesions

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As the population ages, the increase in root caries-affected teeth is becoming a new challenge for the profession. Current methods of managing early

lesions have not been overly successful, especially where protection and preservation of the collagen fibre matrix is desired. Laboratory studies have

shown that plant-based cross-linking agents such as proanthocyanidin (PA) may stabilise the collagen that may enhance treatment methods using fluorides such as silver diammine fluoride. The aim of this study was to investigate the effects of the combination of silver diammine fluoride/potassium iodide (SDF/KI) solution with adjunctive application of PA on artificial root caries.

After ethical approval was granted to collect human teeth, 60 root dentine blocks adjacent to the cemento-enamel junction had a window (4×3 mm) painted on the surface and were immersed in a pH 5 demineralizing solution (120 h) to create artificial root carious lesions. Four groups were analysed: (i) deionised water (DW, control); (ii) 6.5% PA; (iii) SDF/KI (Riva Star); and (iv) 6.5% PA+SDF/KI. After storage in DW for 24 h, specimens were subjected to a Knoop hardness test, nanoindentation, polarised light microscopy (PLM), and micro-computed tomography (micro-CT) analyses. Surface microhardness (SMH) data were analysed with one-way ANOVA, while nanoindentation, cross-sectional microhardness (CSMH), and mineral content (Z) were analysed with two-way ANOVA and Tukey's post hoc test ($P < 0.05$).

PA did not affect SMH, CSMH, and Z of demineralised root dentine ($P > 0.05$). SDF/KI significantly improved SMH, CSMH, and Z up to a 60 μm depth ($P < 0.05$). PA+SDF/KI achieved a more significant incremental increase in SMH, CSMH, and Z up to a depth of 60–90 μm . Micro-CT images demonstrated that adjunctive use of PA contributed to more homogeneous mineral distribution throughout artificial carious lesions. PLM analysis showed penetration of PA along dentinal tubules with surface precipitation; PA+SDF/KI generated a stained band with a visible tubular structure.

It was concluded that PA could diffuse into demineralised dentine but did not affect the mechanical properties and mineral content of the dentine. Application of SDF/KI on artificial root carious lesions contributed to the recovery of the nano-mechanical properties of the demineralised tissues and an incremental increase in mineral. Adjunctive use of PA seemed to enhance these effects, with incremental increases in SMH, CSMH, and Er observed, especially in the subsurface zone. Combined use of PA and SDF/KI contributed to a higher mineral content in deeper regions of the demineralised lesion with more homogenous distribution of SDF/KI.

This research was funded by a grant from the Australian Dental Research Foundation in honour of the memory of Dr Nathan Cochrane.

The findings of this research were published in Acta Biomaterialia and presented at IADR 2018.

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International College of Dentists, Australasian Section Award

Oral care capacity, oral health, and cognitive function in late adulthood

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Research indicates that cognitive decline is related to poorer oral health in late adulthood, however the relative contributions of specific cognitive domains to oral health outcomes remain unclear. In particular, although prospective memory has been identified as a particularly important determinant of the capacity to live autonomously, it remains unclear whether it is specifically related to oral health. The aim of the current study was therefore to investigate the association between cognitive functions with oral health outcomes in older adults, with a particular focus on the potential role of prospective memory.

The study assessed the cognitive and dental status of healthy older adults either living independently in the community or in a retirement village in Brisbane. A total of 16 healthy older adults (at least 65 years old) completed the study, 13 of whom were living in a retirement village in Brisbane and three of whom were dwelling in the community while regularly accessing services at a multidisciplinary clinic that specialised in promoting healthy ageing for older adults. Two participants only completed part of the study – one was not able to complete measures requiring reading or writing due to poor vision and another

ended participation prematurely due to the onset of tinnitus. Their data was included in the study and pairwise deletions were used to address missing data. The final sample included two men and 14 women of Caucasian ethnicity, ranging in age from 72 to 98 years old ($M = 80.25$, $SD = 6.17$), with years of full-time education ranging from 6 to 18 years ($M = 11.87$, $SD = 3.44$).

Prospective memory, attention, verbal fluency, and retrospective memory were significantly correlated with oral health outcomes. Hierarchical linear regression analysis revealed that prospective memory

remained a significant predictor of oral health even after controlling for other cognitive functions.

Cognitive functions were broadly associated with oral health outcomes. Specifically, it was found that prospective memory predicted oral health outcomes over and beyond other cognitive functions. However, given the study limitations of small sample size and cross-sectional nature, an important avenue for future research will be to establish whether similar findings are also identified in larger cohorts and longitudinal studies.

This research was funded by grants from the Australian Dental Research Foundation and the International College of Dentists, Australasian Section.

The findings of this research were published in Gerontology and presented at 2018 CERA & ADA NSW Conference on the Oral Health of Older Australians.

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Regionald and Pamela Hession Award

The influence of titanium surface characteristics on protein adsorption in diabetes: A pilot study

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As impaired healing of bone in subjects with diabetes mellitus may significantly increase the risk of implant failure, the aim of this study was to determine whether titanium surface characteristics, such as topography and chemistry, could promote the adsorption of serum proteins onto the titanium surface that may subsequently enhance downstream osteogenic cell adhesion and ultimately osseointegration in a diabetic animal model.

Proteomic analysis of material adsorbed onto sandblasted acid etched (SLA) and hydrophilic-modified SLA (modSLA) titanium discs covering a critical sized calvarial defect was assessed in healthy Sprague Dawley and Type 2 diabetic Goto-Kakizaki rats using liquid chromatography-coupled tandem mass spectrometry (MS). Sequential window acquisition of all theoretical spectra (SWATH) MS of the wound exudate beneath these discs in the diabetic animals was also assessed. Protein data obtained were cross-

referenced against the “Swissprot Rat” database to assess their biological functions.

Proteins associated with both wound healing and immune response regulation, such as fibrinogen, the osteoblast integrin ligand fibronectin, Ig gamma-1 chain C region, and T-kininogen 1, were all differentially adsorbed onto the modSLA surface in both healthy and diabetic animals. In the wound exudate collected from the healing defect beneath the modSLA surface in diabetic animals, fibronectin levels were significantly down-regulated compared to those in the SLA exudate. Anti-inflammatory proteins, such as serine proteinase inhibitor (SPA) A3N, SPA3K, annexin A1, and S10A9 were also all significantly up-regulated within the modSLA exudate.

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

This research was funded by a grant from the Australian Dental Research Foundation. The authors

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The findings of this research were presented at IADR 2016.

Colin Cormie Scholarships

Dentists' and dental students' knowledge and adherence to the Australian Therapeutic Guidelines for the prevention of infective endocarditis

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Infective endocarditis is a rare but potentially fatal condition. To exercise antibiotic stewardship, dental professionals must have adequate knowledge of and comply with the relevant guidelines. Currently there is no published literature on the knowledge, compliance, or attitudes of Australian dentists or dental students with reference to the Australian Therapeutic Guidelines (ATG) for the prevention of infective endocarditis; therefore this study aimed to evaluate these factors at a Cairns University and associated dental clinics. Variables considered to affect knowledge, compliance, and attitudes were analysed.

A sequential mixed-method exploratory study was employed, including focus groups and a subsequent survey of dentists and students from a Cairns University and Cairns University dental clinic. The survey was distributed to 248 participants. A retrospective chart audit of patients identified as requiring antibiotic prophylaxis for the prevention of infective endocarditis who attended the Cairns and Townsville University dental clinics was undertaken. A demographic questionnaire was also distributed to potential supervising dentists in order to determine factors that affected the dentists' compliance with the guidelines.

Focus groups were conducted with 5 dentists and 29 students. Six organisational themes formed a thematic network with two global themes: clarity in practice and ambiguity in care. A total of 178 surveys were returned (response rate: 84% dentists, 70% students). Mean knowledge scores for cardiac conditions based on the percentage of correct answers of dentists and students were 73% and 68%, respectively. Mean knowledge scores for dental procedures of dentists and students were 69% and 67%, respectively. Amoxicillin was chosen correctly as the first line drug in 100% of responses.

Two hundred and four incidents were audited during the specified time period. The prevalence of compliance of the supervising dentists to the guidelines was 86%. There were 28 incidents of non-compliance; no supervising dentist or patient factors were associated with compliance.

Results from this multi-method study show that there are clear adequacies and deficiencies in dentists' and students' knowledge of and compliance with the ATG. There are also varying attitudes among participants toward the guidelines and prescribing antibiotic prophylaxis.

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University student's awareness and knowledge of dental erosion and some of the beverages that may cause it

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Dental erosion (DE) is the irreversible loss of tooth structure due to acids. One key source of extrinsic acids is the beverages we consume. Of significance is the pH of these beverages at the time of consumption, and the frequency, duration, and timing that these acidic beverages are consumed. Determining the aetiology of DE and implementing preventative measures is key to arresting progression and applying successful restorative treatment. Limited data is available of pH and the erosive potential of beverages in Australia. The interrelationship between sociodemographics, awareness, and knowledge of DE; the ability to identify acidic beverages; and the consumption of such beverages have not been investigated. This study aims to investigate these shortcomings in the literature.

The erosive potential of 177 non-alcoholic, commercial beverages pH were tested in triplicate at room temperature using a calibrated benchtop metre and probe to determine their erosive potential. An online questionnaire was completed by a convenience sample of 421 Charles Sturt University students to assess awareness and knowledge of DE, the ability to identify acidic beverages, and the methods in which such beverages were consumed. Data analysis was subse-

quently carried out to investigate significant relationships.

This study revealed that most (87.47%) commercially available, non-alcoholic beverages tested were erosive to some degree. Significantly, each year increase in age was associated with 8% lower awareness ($P < 0.001$) and 1% knowledge ($P = 0.005$) of DE, and consumption of 4% more acidic drinks per day ($P < 0.001$) via a higher risk method (without food for extended periods; $P < 0.001$). Degree influenced awareness ($P < 0.001$), knowledge ($P < 0.001$), the ability to identify acidic beverages ($P < 0.001$), the frequency of acidic beverage consumption ($P < 0.001$) and the method in which beverages were consumed ($P < 0.001$). Those aware of DE were 15% more likely to correctly identify acidic beverages ($P = 0.011$) and consumed 66% less acidic beverages per day ($P = 0.006$). Those knowledgeable of DE were 100% more likely to correctly identify acidic beverages ($P < 0.001$) and consumed 42% less acidic beverages per day ($P < 0.001$).

Older, lay individuals should be educated on the reduction in frequency, duration, and timing of exposure to prevent DE. Broader research extending to lay populations would complement these results and offer greater insight to better develop health promotion strategies to prevent DE.

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Incorporation of silver nanoclusters into a denture base resin to reduce adherence of *Candida albicans* on denture surfaces

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Denture stomatitis is prevalent among denture wearers due to its multifactorial aetiology, strong association with poor denture hygiene, and adaptability of *Candida albicans*. Recurrence is common and with antifungal resistance on the rise, there is a need for a denture prosthesis that is antimicrobial and biocompatible. The aim of this study was to investigate the use of silver nanoclusters (AgNC) for inhibiting the

growth of *C. albicans*, in comparison to silver nanoparticles (AgNP), whose use has been well documented. The second aim was to explore their incorporation into a denture base resin, then test for reduction in adherence of *C. albicans* on the modified-polymethyl methacrylate (PMMA) surfaces.

Glutathione (GSH) ligated AgNC and AgNP were synthesized, freeze-dried, and resuspended in ultrapure

water or a culture media (tryptone soya broth, TSB). Inhibitory tests were carried out via disk diffusion and minimum inhibitory concentration (MIC) measured using absorption spectroscopy. The AgNP and AgNC were incorporated into PMMA during mixing stage, and a 24 h *C. albicans* biofilm was then grown on the control and modified PMMA samples, followed by scanning electron microscopy (SEM) analysis.

The disk diffusion studies showed larger zones of inhibition around AgNC pellets compared to AgNP, and resuspension of the AgNC in TSB did not hinder the antifungal effect. No definitive MIC was achieved with AgNC and AgNP, however a significant inhibitory effect was obtained at the highest concentrations; 68% growth reduction at 4.9 mM for AgNC,

and 42% and 69% reduction at 1.96 mM, respectively. The inhibitory effect of AgNC showed a dose-dependent relationship, however the colour change in the wells indicated instability of the nanoclusters. SEM analysis revealed dense populations of *C. albicans* on all control PMMA pieces. Fifty percent of the AgNC-modified PMMA surfaces exhibited sparse *Candida* cell attachment, while one-third of the AgNP-modified PMMA surfaces showed such reduction in adherence.

This study demonstrated the significant growth inhibitory effects of both AgNC and AgNP on *C. albicans*, although the concentrations tested were not high enough to reach fungicidal levels. Published articles have documented very low concentration MIC in comparison to the concentrations used in this study, and the instability of the nanoclusters or use of glutathione ligand may have contributed to this. As such, further biochemical analysis is required to understand the interactions of AgNC.

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