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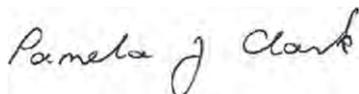
Introduction

This is my final year as Chair following four years served on the Australian Dental Research Foundation (ADRF) Board. I joined the Foundation in 2006, as the President of Australian Dental Industry Association therefore I have been involved in research for 12 years. I am thrilled to present this year's ADRF Special Research Supplement of the Australian Dental Journal. It features all the research work completed by the ADRF funded, or partially funded, grants in this past year. On behalf of the ADRF Board, I commend the researchers and students on their hard work and achievements.

The work of the Foundation is possible by the generous income provided by several streams. We have income from the ADRF Appointment Books, managed and distributed by the Australian Dental Industry Association. This provides significant funding. We also received a large sum from the Continuous Professional Development program of the ADX18 Sydney Dental Exhibition. Perhaps you contributed to ADRF through one of these avenues?

Many companies and organisations give generously each year to ADRF for a named Award within the organisation's area of interest and expertise. If a company or organisation you are involved with would like the Foundation to administer a grant in the organisation's name or you wish to find out more about these opportunities please do not hesitate to contact the Foundation.

On behalf of the Board, I appreciate the opportunity to present the following abstracts to you and are indebted to the *Australian Dental Journal* Editor, Professor Mark Bartold, and Editorial Advisory Board for their continuing support in publishing the ADRF Special Research Supplement.



Pam Clark, AO
Chair

ADRF Special Research Supplement

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ADRF Research Grant Abstracts

***Lactobacillus rhamnosus* capsular polysaccharide modulates neural responses in caries**

C Cahyani,*† RM Farahani,*† D Harty,† N Hunter*†

The aim of the study was to investigate the impact of capsular polysaccharide (CPS) from a clinical isolate of *Lactobacillus rhamnosus* on neurogenic precursors.

L. rhamnosus LRHMDP2 was isolated from the initial stage of dental pulp infection of a carious tooth. CPS of *L. rhamnosus* LRHMDP2 was extracted by autoclaving. As a proxy for neural precursors of dental pulp human neural pericytes (HNPs) were grown in two different media: pericyte medium (PM) or neuro-glial medium comprising DMEM with N2 supplement.

Cultures were challenged with CPS or extract of a probiotic *L. rhamnosus* or PBS as controls and evaluated at 6 hour and 24-hour periods using immunohistochemistry for Notch1 intracellular domain (ICD—an indicator of Notch activation), Notch1 extracellular domain (ECD), and S100+ markers.

Following a 6-hour incubation, no differences of notch expression between CPS-treated and control pericytes were noted. After 24-hour incubation,

Notch1 ICD expression was selectively down regulated by CPS in cultures in PM despite up-regulation of Notch1 ECD.

This result suggested that CPS inhibited proteolytic cleavage of the Notch1 ECD and subsequent activation of Notch signalling by the Notch1 ICD. Possibly, CPS could act as an analogue of the oligosaccharides of glycosylated Notch1 thereby blocking recognition sites for Notch ligands. Notch1 signalling was also suppressed in neuro-glial differentiation medium but with corresponding up-regulation of S100+ glial cell marker.

These findings provide novel evidence regarding the virulence potential of *L. rhamnosus* LRHMDP2 CPS to down-regulate Notch signalling and subsequently drive neural precursors into a glial fate.

Disruption of the homeostatic balance regulating differentiation of neural precursors could perturb formation of protective tertiary dentine and open a pathway to infection of the pulp.

This study was supported by a grant from the Australian Dental Research Foundation. The findings from this research were presented at the 2017 International Association for Dental Research Australian and New Zealand conference ANZ in Adelaide.

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Periodontal regeneration using periodontal ligament cells delivered on a calcium phosphate and lithium chloride-coated polycaprolactone scaffold in a rat model

H (Erica) Dan, S Hamlet, S Ivanovski

Lithium chloride (LiCl) is an activator of the canonical Wnt signaling pathway. Previous studies indicated that systemic delivery of LiCl could enhance bone mass formation in vivo. The aim of this study was to investigate the effect of local injection of LiCl on periodontal regeneration in a rat periodontal defect model.

Fenestration defects were created in Sprague-Dawley rats on the buccal side of the mandible (n = 4 per group). Local daily injection at the defect site was carried out using different concentrations of LiCl

(10 mM, 100 mM, 1000 mM diluted in 0.9% NaCl solution). Defects without any treatment and defects injected with saline alone were used as controls. Three weeks later, the mandible samples were collected and subjected to micro CT and histomorphometric analysis.

Three weeks after the treatment, new bone formation was observed in all of the groups. The animals in the LiCl-10 and LiCl-100 groups had a significantly greater amount of new bone, cementum and periodontal ligament formation ($P < 0.05$ compared to

the blank group), while injection with LiCl-1000 didn't show any significant difference of periodontal tissue formation compared with the control groups.

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Macrophage integrin gene expression in response to titanium surface modification

SM Hamlet,* S Ivanovski*†

Attachment to the surface of a biomaterial is a critical first step, which subsequently influences cellular biological processes. This attachment is effected by transmembrane integrins which facilitate focal adhesions that connect extracellular substrates to actin stress fibers extended from within cells. Resultant cytoskeleton rearrangements activate intracellular signalling pathways, which regulate a wide range of cellular responses, including growth, differentiation, inflammation and apoptosis. Within the immune system, at least thirteen integrin subtypes may be expressed by leukocytes including $\beta 2$, a unique leukocyte-specific integrin.

While studies have shown that the expression of integrins following cell attachment to biomaterials differs depending upon the surface topography and/or chemical composition of the biomaterial, the role of titanium surface properties on integrin expression by specific macrophage phenotypes and their downstream inflammatory and regenerative processes has not been described.

Rat bone marrow-derived macrophages were prepared by culturing bone marrow cells with macrophage colony-stimulating factor. These macrophages (>90% pure) were seeded onto titanium discs (1mm

Our results showed that local injection of LiCl could enhance periodontal regeneration.

This study was funded by Australian Dental Research Foundation.

thick, 15 mm diameter) and cultured for 4 days after which total RNA was collected to examine the expression of 7 integrin genes: ITGAM, ITGA1, ITGA2, ITGB1, ITGB2, ITGA5 and ITGAV. Gene expression by the macrophages on a machined (smooth) titanium surface acted as the control surface to compare gene expression following culture on either a microrough surface (Osseotite[®]) or nanoscale calcium phosphate-modified microrough titanium surface (NanoTite[®], Biomet 3i).

No significant effect of the different titanium surface topographies on either macrophage attachment (after 1, 2 and 4 h culture) or proliferation (after 24 and 72 h culture) was determined. Compared to the machined (smooth) surface after 1 day of culture, the expression of all integrin genes was decreased except ITGB2, which was up regulated on the nanoscale modified surface. By 4 days of culture, ITGA1, ITGA5 and ITGAV gene expression were all significantly upregulated (>10 fold) by the nanoscale modified micro-rough surface.

These results show that titanium surface properties can modulate the expression of receptors important for the adhesion of macrophages. In particular, the α -integrins were significantly upregulated by the nanoscale modified titanium surface. The downstream signalling pathways activated by integrins A1, A5 and AV in macrophages remains to be determined.

This work was supported by an Australian Dental Research Foundation research grant.

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Investigation of osteoinductive and osteoconductive potential of modified titanium using a novel osteogenesis and regeneration model

Y-S Huang,* C Vaquette,† S Saifzadeh,† S Ivanovski*

This pilot study investigated the osteoconductive and osteoinductive properties of surface modified titanium.

Three different titanium surfaces (machined (M), titanium oxide blasted (B), titanium oxide blasted and etched with hydrofluoric acid (B + E)) were used. Osteogenesis was investigated *in vivo* in the mandible of nine sheep with two different models: osseointegration and regeneration. In the osseointegration model, the titanium discs were inserted into the defect with the active surfaces of the titanium disc facing towards the bone marrow.

In the regeneration model, alveolar bone defects were created, and the titanium discs were inserted into the defect with the active surface facing away from the bone marrow, and this was then covered with an occlusive membrane. Sheep were sacrificed at 2, 5, and 10 weeks, and resin-embedded samples were prepared for histomorphometric analysis.

In the osseointegration model, percentage bone-to-disc (BTD) contact was observed as early as 2 weeks post healing on the B + E surface, but not on the B or M surfaces. At 5 weeks BTD was $7.4\% \pm 2.8$ for M, $26\% \pm 3$ for B and $52\% \pm 14$ for B + E surfaces; and at 10 weeks it was $18\% \pm 4.3$ for M, $25\% \pm 1.3$ for B and $70\% \pm 5.8$ for B + E surfaces.

At 5 and 10 weeks BTD was significantly higher for B + E and blasted than machined surfaces. For the regeneration model, at 5 weeks post healing, $6.7\% \pm 0.69$ of bone fill in the open defect was observed on the M surface, compared to $8.1\% \pm 1.1$ on the B surface, while the B + E surface had the highest percentage of bone fill at $15\% \pm 2.5$.

At 10 weeks post healing, $52\% \pm 9.8$ of the defect was filled compared with the B surface of $40\% \pm 8$ and $44\% \pm 8$ for the B + E surface. BTD was also measured from the regeneration model. No BTD was observed at 2 weeks post healing. At 5 weeks, $8.9\% \pm 5.49$ BTD was observed on the B + E only.

At 10 weeks, $9.1\% \pm 4.7$ of BTD was apparent on the M surface compared to $27\% \pm 12$ on the B surface and $33\% \pm 12$ on B + E surface. Two-way ANOVA showed that healing time was the most significant factor influencing the %BF and %BTD in the alveolar bone defect rather than titanium surface characteristics.

In summary, B + E surface is superior for osteogenesis, as demonstrated with the osseointegration model. However, the results from the regeneration model demonstrated that modified titanium discs did not influence the amount of BF in the defect nor the percentage of BTD contact in the defect.

This abstract is based on research that was funded by Australian Dental Research Foundation. The titanium discs were supplied by Dentsply AstraTech.

The findings of this research were presented at the ASP Queensland meeting in Brisbane (2015), and the ANZAP meeting in Perth (2017).

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Novel polymer/mesoporous ceramic composite scaffold: fabrication and evaluation of bone regeneration potential

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

Oro-facial 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. This jaw bone defect can create major problems

for the subsequent placement of dental implants. It is a critical 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.

Mesoporous bioglass (MBG) particles were synthesised at Griffith University by the wet-chemistry

method, and the particles were milled using a cryomill to reduce the size below 40 micrometer. The Particles were characterized using XRD, SEM, BET (surface area) and TEM. The particles were then mixed with medical grade polycaprolactone (PCL) polymer and 3D printed. The prepared 3D scaffolds were physiochemically characterised. These 3D scaffolds showed adequate cell viability and proliferation. These scaffolds were implanted in sheep models and showed enhanced bone regeneration vertically and proved as a good scaffold for the regeneration of bone in the dental field.

The polymer/ceramic biocomposite showed good biocompatibility in terms of cell viability with human

bone cells. The scaffolds were mechanically strong and were able to provide a space for the bone growth. The scaffold showed vertical bone regeneration after 8 weeks. The presence of ceramics assisted the bone formation extra skeletally on the calvarium and hence proved as a great material to use in dental fields.

This study demonstrated the use of a composite scaffold made of polymer and mesoporous bioglass particles. This scaffold can be used as an ideal material to use as an alternative to biomaterials used for dental bone regeneration, especially for vertical bone growth.

The authors are thankful to Griffith University for providing financial support through postdoctoral fellowship to conduct this study. Authors are grateful to Australian Dental Research Foundation for providing a research grant to support this study.

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Osteochondroreticular (OCR) stem cells: stem cells for the periodontium

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Previously, we characterised a clonal population of skeletal stem cells, Osteochondroreticular (OCR) stem cells, considered progenitors to the bone, cartilage and reticular cells¹. The OCR cells have also shown the potential to give rise to α -SMA cells, previously identified as the progenitor cells of PDL fibroblasts, cementoblasts/cementocytes and osteoblasts, further contributing to tissue repair after injury to the periodontium². OCR stem cells do not express the *Acta2* gene that transcripts for α -SMA dental progenitor cells, however, have the ability to give rise to Acta2+ cells.

The Acta2+ cells have a diminished self-renewal ability, which is an important definition of stem cells, suggesting that OCR stem cells may be the origin of the Acta2+ periodontal progenitors. These findings provided a basis for further investigation into the precise role of OCR stem cells in periodontal development, prompting the discovery of potential cellular therapy of predictable regenerative outcomes in periodontal tissues.

The aim of the study was to identify the Gremlin 1 expressing OCR stem cells as the origin of the periodontium using transgenic lineage tracing mice.

- A transgenic mouse colony tracing *Grem1*-expressing cells was established.

- Genotyped transgenic mice were administered tamoxifen to induce tracing in the developmental stage (day 6 of age) and adult stage (6 weeks of age).
- Mandibles of transgenic mice were collected, fixed, decalcified and embedded into cryo-moulds for immunohistochemical and histomorphometric analysis.
- 10 μ m sections were obtained by cryosectioning using cryofilm tapes.
- Identification of dental structures was observed via the co-localisation of the lineage traced OCR cells and staining of ligaments and bone in the mandible using immunofluorescence staining.

After the establishment of the transgenic mouse colony and the consequent tracing of Gremlin 1 expressing cell in the periodontium, we found that the gremlin 1 cells gave rise to odontoblasts, periodontal ligament fibroblasts and dental pulp stem cells in all molars as well as incisors during early tooth development. Tracing in the adult mice found that Gremlin 1 expressing cells gave rise to mainly odontoblasts and dental pulp cells in the incisors, mainly due to the fact that the molars had fully developed at this time point. These results indicate the involvement of Gremlin 1 expressing OCR stem cells in tooth development and the continuous maintenance of incisor growth.

This study confirmed that Gremlin 1 expressing cells are involved in the development of multiple dental structures including the periodontium and dental pulp of mice. The results further propose an opportunity

for identification and characterisation of a different source of stem cells for repair of dental tissues. The promising results obtained in this study require further investigation prior to publication.

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New methacrylated bone-derived extracellular matrix hydrogel for craniofacial tissue engineering

F Obregon-Miano, A Spahr

Extracellular matrix (ECM) plays a significant role in the structural support and cellular processes of organs and tissues. Proteins extracted from the ECM have been shown to stimulate bone host cells. However, their fast degradation and insufficient mechanical strength are obstacles for their application in bone regeneration. The aim of the present study was to extract, characterise and fabricate a new class of hydrogel with proteins isolated from pig bone ECM and blend them with polyethylene glycol diacrylate to promote bone regeneration.

Through smart chemistry, this combination approach was intended to tackle the shortfalls of natural polymers by increasing the material endurance and strength in a simulated physiological environment to create a hydrogel suitable for future clinical applications.

Bone fragments extracted from fresh pig heads were segmented, pulverised, demineralised in 0.5 N HCl for 5 h and defatted in Methanol-Chloroform (1:1 v/v) for 4 h to then be digested with 1% w/v pepsin for 24 h. The resulting product named as Bone Matrix Protein Extract (BMPE) was biologically characterised by electrophoresis and mass spectrometry.

To improve the physicochemical characteristics of the proposed scaffold, a blend of 0.1% w/v BMPE with 20% w/v polyethylene glycol diacrylate (PEGDA) was produced and optimised. Further physicochemical characterisation of the formulation was performed to determine the crosslinking time, compressive strength,

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volume expansion, mass loss and cell proliferation and migration. Finally, injectability by adding ammonium persulphate (APS) (~50 mg/mL) to the BMPE and ascorbic acid (~30 mg/mL) to the PEGDA in a double chamber syringe with a spiral mixer tip was explored and tested (N = 5, statistically analysed ANOVA, Bonferroni, * $P < 0.05$).

The resulting injectable hydrogel scaffold composed of 0.1% w/v BMPE and 20% w/v PEGDA was double sterilised and optimised. The scaffold showed a favourable crosslinking time of approximately ~90 s, a high compressive strength of ~500 Kpa, a controlled volume expansion of 19% and maximum degradation of 8% over 30 days post incubation. Additionally, an interpenetrating polymeric network and porous micro-architecture was demonstrated by scanning electron microscopy (SEM). High cytocompatibility when tested with SaOS-2 and primary human osteoblasts (hOb) was achieved.

The optimised injectable hybrid hydrogel scaffold offers favourable physicochemical properties and high bioactivity in *in vitro* models. In this context, the hydrogel presents potential as a bone regenerative material due to its similarity to the tissue extracellular matrix (ECM).

The authors acknowledge the support of the Australian Dental Research Foundation (ADRF) 40-2015 and School of Dentistry Grant 2015.

The findings from this research were submitted to the Journal of Biomedical Materials Research: Part B - Applied Biomaterials and were presented at the annual conference of the International Association of Dental Research 2017 in San Francisco as well as the Osteology Australasia Symposium 2017 in Melbourne.

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Flexural strength and sensitivity to low temperature degradation of high translucency zirconia

A Oveissian,*† M Guazzato,* I Klineberg*

High translucent zirconia has been recently introduced to improve the optical properties of standard zirconia, but the mechanical properties and sensitivity to aging have not been sufficiently investigated. This study aims to compare the uniaxial flexural strength of four types of zirconia: two high translucent zirconia (Lava Plus, GC Initial HT), and two conventional zirconia (Lava Frame and GC Initial ST), before and after aging.

320 beam-shaped Y-TZP specimens were subjected to two different surface preparation protocols (air-abrasion and polishing) and tested before and after they have been autoclaved for six hours. One sample from each group was tested with XRD to assess the surface monoclinic content. Uniaxial flexural strength was tested by a three-point bend test with an Instron 5655 universal testing machine. A linear regression model and both univariate and multi-variate analyses were performed to analyse the data.

All tested zirconia specimens were sensitive to artificial aging and had an increased monoclinic phase, however, there was no statistical significant difference in sensitivity to aging between the standard and high

translucent groups. The effect of phase transformation on mechanical strength was different in the polished and sandblasted groups. Before artificial aging, sandblasted specimens were stronger than polished specimens, but these results were reversed after four hours of aging. Translucent zirconia demonstrated less flexural strength compared with conventional zirconia in all test conditions.

High translucent zirconia had less flexural strength compared with conventional zirconia. Airborne-particle abrasion with 110 μ Al_2O_3 increased flexural strength before aging and decreased flexural strength after aging.

Based on the data from this in vitro study, improving zirconia translucency degraded the mechanical properties. Air-abrasion improved the strength of zirconia initially, but increased the sensitivity of the material to aging and led to strength degradation. The use of air-abrasion to improve bonding is common practice; however, clinicians should be aware that air-abrasion may lead to strength degradation of the material and should limit the use of such procedure.

Australian Dental Research Foundation Inc. funded this research. The findings of this research were presented in The Journal of Prosthetic Dentistry and presented at the Westmead Hospital Research Day 2015.

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Investigating the response of dental follicle cells to cyclic tension or compression in three-dimensional culture

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Our earlier finite element analysis suggests jaw strain sensed by dental follicle drives tooth eruption through regulation of bone remodelling. We now explore this possibility of studying the response of human dental follicle cells (HDFC) to tension or compression.

Synthetic aliphatic polyester scaffolds coated with 45S5 bioactive glass were seeded with HDFC, and attached to well inserts and magnetic endplates in six well palates. Scaffolds were subjected to either cyclic 10% tensile deformation, or 8% compression, at 1

Hertz and 2 Hertz respectively for 6, 24 or 48 hours, by uniaxial motion of magnetically-coupled endplates.

It was possible to isolate high-quality mRNA from cells in these scaffolds, as demonstrated by high RNA integrity numbers scores, and ability to perform meaningful cRNA microarray analysis, in which 669 and 727 genes were consistently upregulated, and 662 and 518 genes down regulated at all times studied for tensile and compressive loading experiments respectively.

MetaCore analysis revealed the most regulated gene ontogenies under both loading conditions to be for: cytoskeletal remodelling; cell adhesion-chemokines

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and adhesion; cytoskeleton remodelling-TGF WNT and cytoskeletal remodelling pathways. Patterns of gene expression were broadly consistent with our hypothesis.

We conclude that the method here described will be of value for the analysis of the cellular response to cyclic loading, and that further investigation of the role of dental follicle in regulation of bone remodelling for tooth eruption is warranted.

The authors wish to thank the Australian Dental Research Fund for their support of this work. The findings of this research were published in the Journal of the Mechanical Behaviour of Biomedical Materials.

Innovative approach for evaluating the toxicity of medical and dental photopolymers for additive manufacturing: the zebrafish embryo model

F Alifui-Segbaya, R George

In this study, additively manufactured methacrylates for denture bases, orthodontic splints and retainers, and hearing aid devices were scrutinised for biological safety using the innovative zebrafish embryo model adapted to OECD fish embryo test.

Representative materials for experimentation comprised E-Denture ($\geq 75\%$ Ethoxylated bisphenol A dimethacrylate, 30–50% Diurethane dimethacrylate, mixture of isomers and $< 10\%$ 2,4,6-Trimethylbenzoyl Diphenylphosphine oxide); E-Guard ($> 60\%$ Ethoxylated bis-phenol A dimethacrylate, 15–25% Proprietary methacrylic oligomer and $< 2.5\%$ Phenyl bis (2,4,6-trimethylbenzoyl) phosphine oxide)) and E-Shell 450 Clear (60–80% Proprietary methacrylate oligomers, 15–30% Proprietary methacrylate monomers and 1–2% diphenyl and 2,4,6-trimethylbenzoyl).

During experimentation, newly fertilised zebrafish eggs were cultured on non-treated and ethanol-treated methacrylates in glass Petri dishes with ultrapure water, incubated at 28.5°C and assessed for developmental endpoints of toxicity at 24 h intervals until 96 h.

Toxicological data indicate that non-treated methacrylates are extremely toxic (embryonic lethality) in zebrafish bioassays. In ethanol-treated conditions, methacrylates for intraoral devices were generally safe in zebrafish bioassays. Although methacrylate for hearing aid devices showed improved biocompatibility after the applied treatment, it induced cumulative sub-lethal and teratogenic effects in zebrafish bioassays.

By using apposite manufacturing parameters and post-treatment, methacrylates conversion could be increased to improve biocompatibility dependent on the physico-chemical characteristics of the resulting polymer. To extrapolate toxicological data in this study to human responses, a further study will characterise photopolymers for residual monomer and degradation products, and their throughput in zebrafish bioassays.

Experimental work was partly funded by Australian Dental Research Foundation Grant (231- 2017) and Griffith University School of Dentistry and Oral Health Research Grant (2017).

We are grateful to G.J. Lieschke (NHMRC 1044754, 1069284: Australian Regenerative Medicine Institute, Monash University, Australia) for providing zebrafish resources and expertise for our study. We thank EnvisionTec GmbH, Germany for kindly supplying materials for our study.

The emergence of affordable 3D printers means prostheses such as dentures, retainers and hearing aids can be manufactured with speed for patients. However, there are uncertainties surrounding the safety of the materials currently available on the market. To verify this, approved materials were tested by exposing them zebrafish, which has high genetic similarity to humans. Results from the study indicate the need for cautious approach and strict adherence to manufacturing protocols in 3D printing.

The findings from this research were published in Acta Biomaterialia (Elsevier) and Inventions (MDPI) and presented at Griffith University School of Dentistry and Oral Health Research Day (2017).

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Locally delivered bioactive agents as treatment modalities in the management of medication-induced osteonecrosis of the jaws

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Recent literature suggests that suppressed bone healing and angiogenesis contributes significantly to the pathogenesis of Medication induced osteonecrosis of jaws (MRONJ), a complex condition commonly associated with anti-resorptive agents. This in vitro study evaluated the effect of locally delivered angiogenic growth factor (VEGF₁₆₅) with or without osteogenic growth factor (BMP-7) to enhance bone healing, thereby preventing the occurrence of MRONJ.

A chemically defined hydrogel (HyStem[®]-HP, Glycosan Biosciences) containing a combination of thiol-modified hyaluronan, heparin and denatured collagen was crosslinked with thiol-reactive, PEGDA. To establish the in vitro release kinetics, 100 and 200 ng of rat VEGF₁₆₅ (Biovision) or BMP-7 (250 and 500 ng/mL) was loaded into a 500 µL hydrogel prior to crosslinking (Hystem-HP).

The loaded gel was then punched out into 6mm diameter discs and incubated in release buffer under continuous agitation (37°C). The release kinetics of VEGF₁₆₅ and BMP-7 from the hydrogel was determined in triplicate using an ELISA to assess the appropriate loading concentration of both growth factors over 28 days.

The in vitro VEGF₁₆₅ release profile quantified using ELISA showed similar release curves across different doses, proportional to the initial loading dosage. There was a significant burst release (18%–28% of total VEGF) that peaked at 12 hr in both 100 and 200 ng loaded gels. By the end of 3 days about 25%–30% of the VEGF had been released from the 100 ng gel and 38%–42% from the 200 ng gel. After a week, the rate of release was significantly reduced but was sustained between 4 and 8 ng per week over the rest of the observation period of up to 28 days.

A total of 65% of VEGF was released from the 200 ng gel and 67% from the 100 ng gel by the end of 28 days. For BMP-7, regardless of the initial loading dose, the release kinetics were shown to be similar. The cumulative release increased sharply over the first 2 weeks before starting to plateau. By the end of the 28 days of observation, the hydrogels had released approximately 64% (250 ng loaded), and 85% (500 ng loaded) of the initially bound BMP-7.

Based on the above results, sustained-local delivery of VEGF or BMP-7 can be achieved using this chemically defined hydrogel system. This can be a valuable preventive modality in susceptible patients requiring dental manipulation while on anti-resorptive and anti-angiogenic medication.

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The findings of this project were presented as an oral presentation at National Osteology Symposium Australasia on June 2 2017, Melbourne and was awarded the second prize.

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Selenium decoration on polycaprolactone scaffolds for antimicrobial bone and periodontal regeneration

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Bone and periodontal tissue regeneration using scaffolds and occlusive membranes that prevent infiltration of soft tissue is a well-established technique yet one of the challenges is bacterial contamination of the barrier membranes and/or the scaffolds. A local drug delivery approach is promising where the scaffolds and membrane are loaded with antimicrobial agents

to inhibit bacterial contamination and biofilm formation. This project aims to develop a versatile technology of coating antimicrobial selenium nanoparticles on scaffolds and membranes that could be used in bone tissue regeneration.

Polycaprolactone membranes and scaffolds were coated with selenium nanoparticles using in situ

reduction. The scaffolds were chosen to test for antimicrobial activities in vitro and bone regeneration in vivo using a skull defect model in rats. In vitro antimicrobial experiments were conducted with *S. aureus* cultured on the scaffolds for up to 48 hours and bacterial growth and biofilm formation was evaluated using spectrophotometry and crystal violet staining.

In vivo experiments, two circular osseous defects were created on rat's skull and an 8 mm mPCL membrane was placed on top of the exposed dura mater in order to prevent soft tissue infiltration in the defect. The PCL-CaP (5 mm in diameter) with or without Se was placed over each defect. A thin electrospun mPCL membrane covering both defect was used to stabilize the scaffold and to provide soft tissue occlusion. The wound was closed in layers, animals were sacrificed after eight weeks and the implants (n = 6 for each group) were retrieved and used for further analysis with Microcomputed Tomography. Analysis was

undertaken for bone formation within the defect and histology and histomorphometry to determine the area of the new bone formation.

Selenium decoration on polycaprolactone scaffolds provides antimicrobial activities (prohibited bacterial growth and reduced biofilm formation 2 folds compared to PCL-CaP control, $P < 0.05$). New bone volume was 1.3 times higher ($P < 0.05$) in the groups using Se-decorated scaffolds compared to control scaffolds.

Selenium nanoparticles were decorated onto PCL membranes and scaffolds for potential use in guided tissue regeneration for bone and dental applications. The scaffolds with Se nanoparticles showed antimicrobial activities and improved bone regeneration in vivo. This study demonstrated that the technology is promising in creating antimicrobial coatings for applications in bone and periodontal regeneration.

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Fibre guiding melt electrospun scaffold and cell sheet technology for the hierarchical regeneration of the periodontium complex

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The objective of this study was to assess a novel additively manufactured scaffold for periodontal regeneration. This scaffold was hypothesised to provide topographical cues to Periodontal Ligament Fibroblast cell sheets (PDLF) and enhance the insertion of collagen fibres into the root surface.

The scaffold was manufactured by melt electrospinning writing, a technology using molten polymer forced through a high electrical field resulting in the formation of small micrometer scaled fibres. The technology also utilises a programmable stage allowing excellent control over the positioning of the deposited fibres.

We developed a biphasic construct consisting of a bone compartment featuring large pores essential for bone ingrowth and neo-vascularisation, and a periodontal compartment made of aligned channels. The fibre guiding channels were made of polycaprolactone fibres stacked on each other creating a 150 µm deep groove with a 200µm width.

The performance of the scaffolds was assessed in vivo using an ectopic periodontal regeneration model. This consisted in placing a three-layered cell sheet (cultured of ascorbic acid supplemented media) on the periodontal compartment of the biphasic scaffold, which was thereafter positioned over a dentin slice and subcutaneously implanted. The fibre guiding performance along with the percentage of the attachment of the cell sheet on the dentin was quantified by histomorphometry 4 weeks post implantation. As a negative control scaffold without cell sheet was also implanted.

Histology revealed that the biphasic scaffold was capable of space maintenance throughout the experiment, which is crucial for periodontal regeneration and follows the principles of guided tissue regeneration (GTR). However, the cell sheet did not present an extensive degree of adhesion onto the dentin block. In addition, cementogenesis was not observed in any of the samples indicating that cell sheet

preconditioning in mineralisation media is necessary for ectopic cementum deposition. Despite this, the scaffold was capable of displaying fibre guidance

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with fibres of collagen orientated perpendicularly to the dentine, although without attachment.

This work demonstrates that the utilisation of a fibre-guiding scaffold shows some potential in enhancing the functional orientation of newly formed periodontal ligament-like tissue.

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The findings from this research were presented at World Biomaterials Congress 2016.

Oral swirl samples – a robust source of microRNA protected by extracellular vesicles

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MicroRNAs are small non-coding RNAs, which are dysregulated in disease states, such as oral cancer. Extracellular vesicles, a potential source of microRNA, are found in saliva.

To demonstrate that a quantifiable amount of microRNA can be isolated from oral swirl samples. Additionally, we hypothesised that extracellular vesicles may protect contained microRNA from degradation in these samples.

A polyethylene glycol-based precipitation was used for extracellular vesicle enrichment of oral swirl samples. A comparison was made between samples treated with and without RNase. Further, samples from three subjects were exposed to a range of conditions

over 7 days and assessed for the presence of microRNA by reverse-transcription quantitative PCR. Extracellular vesicles from samples were identified under transmission electron microscopy.

An adequate quantity of microRNA for qPCR analysis was extractable from samples despite exposure to conditions under which degradation of RNA would be expected.

A technique was developed to isolate an adequate quantity of microRNA for analysis from oral swirl samples. Extracellular vesicle-associated microRNA may be protected from degradation. This technique moves towards chair-side application of translational microRNA research in the field of oral cancer prognostics.

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Bioactive tissue-engineered constructs for periodontal regeneration

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Recent advances in progenitor cell biology and tissue engineering have enabled the development of cell-based therapies that aim to achieve periodontal tissue regeneration with greater efficacy and predictability. Dental follicle cells (DFCs), acknowledged as the developmental precursor cells of

periodontal tissues, are promising candidates for cementum/periodontal ligament regeneration and bio-root engineering. This project examined the regenerative properties of bioactive tissue-engineered construct, which combined DFCs and a supporting scaffold, and further explored whether predictable

regeneration of damaged periodontal tissues could be achieved.

Nearly a quarter of Australian adults have periodontitis, an inflammation that can degrade specialised supportive tissue and ultimately lead to the loss of teeth. Present treatments have limitations in restoring the periodontium architecture because of the complex structure consisting of soft tissue such as gum and hard tissue such as bone. Recent advances in progenitor cell biology and tissue engineering have enabled the development of cell-based therapies that aim at achieving periodontal regeneration with greater efficacy and predictability, offering a new perspective into the restoration of the functional periodontium. In this project, we have developed a bioactive tissue-engineered construct, which combines dental follicle cell sheet and a supporting scaffold.

The cell-scaffold constructs have shown superior performance and achieved predictable regeneration of damaged periodontal tissues *in vivo*. This study expands our understanding on periodontal tissue repair and regeneration, and provides direct evidence for the use of alternative cell sources in periodontal tissue regeneration. A more detailed understanding of the mechanisms involved in the application of tissue-

engineered constructs in an inflammatory microenvironment is required.

Primary dental follicle cells were harvested from healthy donors and subjected to multi-lineage characterisation. Supporting scaffolds coated with various concentrations of LiCl to activate Wnt signalling pathway were fabricated and optimised. The osteogenic/cementogenic differentiation of DFCs seeded on the scaffolds were assessed by gene and protein expression levels. The tissue-engineered constructs were implanted into periodontal defects of athymic rats and the samples were harvested at four weeks post-surgery to determine the presence of newly formed bone and cementum.

DFCs showed a significant multi-lineage differentiation capacity *in vitro*. Supporting scaffolds with optimised LiCl concentrations were successfully fabricated. The activation of Wnt signalling pathway contributed to the regeneration of cementum-periodontium-like tissues *in vivo*.

Collectively, this study revealed that DFC sheets are promising approaches for periodontal regeneration due to their strong regenerative capacity. The successful approach applied in this study might accelerate the clinical translation although further investigation of the performance of the tissue-engineered constructs in an inflammatory microenvironment is warranted.

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Endodontic fear and anxiety pathways amongst an East Asian population

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Pathways of fear and anxiety in endodontics are reported to differ for different ethnic groups. This can be due to different factors such as cultural differences, religion, education level and social economic status. This study aims to identify the origin and pathways of fear and anxiety of endodontic treatment within an East Asian population.

Patients (18 years and above) of East Asian ethnicity visiting the Griffith University Dental Clinic, Gold Coast, Australia who had root canal treatment or were scheduled to have treatment prior to the survey were invited to participate in this study. Consenting participants were required to complete the “My Endodontic Fear” questionnaire designed by the authors based on Carter *et al.* (2015) “My Dental Fear” Questionnaire (1). Participants with mental disabilities, under the age of 18 years, or who never had

root canal treatment or could not remember having root canal treatment were excluded.

146 participants (18-62 years old) of East Asian descent were included, 106 these participants were of Chinese origin (N=106), while non-Chinese participants were East Asians with backgrounds such as Korean, Japanese and Thai. Both the Chinese population and the non-Chinese population perceived that the cognitive conditioning pathway influenced their fear perception. A significantly greater number of non-Chinese respondents perceived the Verbal Threat pathway as a greater influence on the origin of the dental fear ($P = 0.01$).

This study demonstrates that the primary self-perceived fear and anxiety pathways adopted by an East Asian population were similar. However, the overall fear perception may due to a combination of different

pathways. It is important that the treating dentist is educated on the different pathways when managing

fear and anxiety; allowing for a more patient-centric treatment plan.

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Bone invasive properties of OSCC and its interactions with bone cells via stimulation of cell-adhesion molecules

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Oral squamous cell carcinoma (OSCC) represents about 90% of all head and neck cancers with a high rate of mortality in advanced cases of OSCC most likely due to amplified local invasion and recurrence. The conventional treatment approach; radiation therapy combined with surgery has been static for a number of years. However, research to some extent has enhanced the diagnostic and therapeutic modality to improve the quality of life. Hence, to further reduce the burden of this disease research it is vital to increase prognosis of OSCC and gain profound knowledge of the molecular mechanisms of this disease, with the objective emerging effective therapies to reduce the rate of mortality amplified by this tumour.

Direct co-culture was achieved by culturing OSCC (TCA8113) and primary alveolar bone cell line (ABC). In the indirect co-culture, the conditioned media (CM) of the TCA8113 were collected and used to culture the ABC cells with three control groups. Wound healing, immunostaining and Western blot analysis were performed to detect the migratory ability and expression of cell—adhesion molecule markers EpCAM, E-Cadherin, N-Cadherin, and P-selectin for indirect co-culture. EpCAM and N-Cadherin was used for indirect co-culture group. As well,

the expression of bone invasion proteins, MMP-9 was observed using the same method, in the direct co-cultured group.

Both direct and indirect co-cultured cells showed increased wound healing ability than their control groups. Additionally, intracellular immunofluorescent labelling represented noteworthy expressions of EpCAM, N-Cadherin and E-Cadherin markers in direct and indirect co-cultured cells. Of note, high expressions of EpCAM and N-Cadherin were confirmed with Western blot analysis in direct co-culture cells. Additionally, P-selectin also showed increased protein expression in direct co-culture and TCA8113 cells compared to monoculture ABC cells.

COSCC cells co-cultured with bone cells displayed enhanced expression of cell-adhesion molecules indicating their evident invasive and abnormal proliferative properties. Moreover, this was manifested through greater wound healing potential when the two cells were co-cultured rather than their monoculture controls. The findings of this study shed light on some of the molecular pathways via which OSCC gains its aggressiveness against other oral diseases. Therefore, understanding the precise molecular mechanism of OSCC is essential for the development of biotherapies to improve diagnosis and prognosis.

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Disruption of endodontic biofilms using plasma polymer encapsulated D-amino acids

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Bacteria in infected teeth can withstand dental treatment by forming a protective biofilm. Our previous research found that D-amino acids (DAAs), a component of proteins, can disrupt this biofilm, removing an important protective mechanism of bacteria.

Our aim was to develop particles that produce a slow release of DAAs to improve root canal treatment. We coated DAAs in a hydrophobic polymer to create encapsulated particles (EPs), this process allowed a slow release of the DAAs. The results showed a slow release of the DAAs from EPs when encapsulated for 60 minutes and an antimicrobial effect which increased over time.

Enterococcus faecalis (*Ef*) is a predominant species present in the root canal system of failed endodontically treated teeth as it has the ability to form biofilms. We have shown that a mixture of D-amino acids (DAAs) disrupt established and developing *Ef* biofilms. Our objectives were to investigate the effectiveness of individual DAAs on *Ef* biofilms and to use encapsulation technologies to provide a sustained release of DAAs that may be used to improve the success of root canal therapy.

A mixture and individual DAAs of D-Methionine, D-Tyrosine, D-Leucine and D-Tryptophan were encapsulated with a plasma polymer (1-7 octadecane)

for 10, 20, 40 and 60 minutes to allow for variations in shell thickness of the particles. PPEDAAAs were analysed with scanning electron microscopy. Diffusion of DAAs from the PPEDAAAs was measured over 7 days using HPLC. Micro-titre tray and static biofilm assays were used to compare the effect of DAAs and plasma polymer encapsulated DAAs (PPEDAAAs) on *Ef* biofilms.

Using SEM, the shell thicknesses of the PPEDAAAs ranged between 36-77nm - indicating a substantial coating of plasma polymer and the thickness of polymer coating increased with plasma coating time. HPLC results showed a sustained diffusion of DAAs from the PPEDAAAs over 7 h for most PPEDAAAs and 7 days for 60 minutes encapsulated D-Tyrosine and D-Tryptophan. Visible and statistical biofilm disruption was observed with DAAs and PPEDAAAs with 24, 72 and 144-h biofilms.

Polymer encapsulation is a promising technology to maintain a sustained release of DAAs and may significantly improve the success rates of endodontic treatment.

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An in vitro study comparing the efficacy of dentine desensitising agents

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The aim of this study was to compare the effectiveness of four commercially available desensitising products on the occlusion of dentinal tubules, and their continued effectiveness when challenged by citric acid and phosphate buffered saline.

Extracted human teeth (human ethics approval no. 2003000040) were sectioned to produce dentine discs, whereby each disc was then cut into four pieces. 24 disc sections were randomly assigned to a negative control group, distilled water group and one of the four treatment groups of desensitizing toothpastes (Colgate Sensitive Pro-Relief, Sensodyne Rapid Relief,

Sensodyne Repair and Protect) and mouth rinse (Listerine Advanced Defence Sensitive).

The treatment group samples were all treated with the undiluted product either by being brushed with an electric toothbrush or by being immersed in 5ml of mouth rinse for 2 minutes. Each group was further divided into three sub-groups which were then subjected to (a) no further treatment (for baseline comparison) (b) 6wt% citric acid (pH 2.0) for 1 minute and rinsed with distilled water to simulate dietary acid challenge and (c) phosphate buffered saline (PBS) for 24 h at 37°C and rinsed with distilled water to simulate oral fluids.

All samples were prepared for transverse observation under the scanning electron microscope at 1000x and 5000x. The percentages of occluded dentinal tubules in the micrographs were calculated for comparison between subgroups.

All of the desensitising agents resulted in a 100% tubule occlusion when unchallenged, except for the Sensodyne Repair and Protect toothpaste (84.3%).

Following citric acid challenge, there was a reduction in tubule occlusion in all desensitising agent groups, except the Sensodyne Rapid Relief group, which remained at 100% tubule occlusion. The Colgate Sensitive Pro-Relief toothpaste had the least acid resistance

(5.6%). A decrease in the tubule occlusion was observed in all treatment groups' post-PBS immersion for 24 h, whereby Sensodyne Rapid Relief had the highest percentage of occlusion (93.7%). The desensitising agent with the least tubule occlusion post-PBS immersion was Sensodyne Repair and Protect (67.4%).

Within the limitations of this pilot study, it was concluded that an initial 100% occlusion decreased for several products when challenged with a common dietary acid as well as a saliva-like fluid. Therefore, in vitro testing of desensitizing agents should ideally include other simulated oral challenges to gain a true idea of their long-term effectiveness.

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Selective microRNA expression in molecularly distinct oral cell lines and human oral tissues as biomarkers for early detection of oral squamous cell carcinoma

H El-Sakka,* O Kujan,* S Fox,* CS Farah*†

The objective of this project includes systematically assessing the role of microRNAs in oral potentially malignant disorders (OPMD) as risk stratification biomarkers in the literature; and assessing differentially expressed microRNAs, in both oral cell line and tissue samples of oral squamous cell carcinoma (OSCC) tumour, peri-lesional and disease-free normal surgical margins, discovered in a previous study by Farah et al.

The systematic review collated studies after searching three different electronic databases: PUBMED, Embase and Medline. Forty articles met the inclusion criteria and were assessed regarding how candidate microRNA biomarkers were expressed and validated.

The experimental study used both oral cell lines and human oral tissue samples to validate the expression of selected six microRNA targets from the previous study using RT-qPCR. Cell lines were used to verify that the protocol would be suitable for use on limited tissue samples. MicroRNA expression was assessed using normal (NBI), white light (WL) and tumour (T) oral tissue margins. Experimental results were compared with the literature in the study.

The systematic review examined the expression of microRNAs from human specimens (blood serum/plasma, saliva, tissue) as diagnostic or prognostic

biomarkers in patients with OPMDs. Fifteen of the forty included studies utilised microRNAs as risk stratification biomarkers for malignant transformation (MT) and showed promising findings. However, nine of these fifteen studies did not validate the biomarkers in OPMD MT samples through follow-up.

Only six studies tested the utility of microRNAs in predicting MT in paired OPMD samples. Experimental findings showed paired comparison of OSCC with normal controls expression results, in oral tissue samples were consistent with literature findings for the six selected microRNA targets.

Preliminary results from the experimental study showed potential novel biomarkers (mir-1 and mir-376c) in discriminating potentially malignant oral tissue from normal and tumour controls, respectively.

The systematic review showed that the current evidence to support or refute the prognostic utility of microRNAs in predicting cancer progression in OPMDs is equivocal, warranting further longitudinal prospective studies.

The experimental findings of the paired comparisons between cancerous and non-cancerous margins showed consistent expression results for the selected six microRNA targets when compared with the previous study. Future prospective longitudinal studies are

required to confirm experimental findings, due to some study limitations.

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The effect of Azithromycin containing Polycaprolactone membrane on human oral biofilm: a pilot study

SJ Lim, SM Hamlet, S Ivanovski

Membrane exposure leads to microbial contamination and compromised healing during ‘guided tissue regeneration’ of periodontal defects. The incorporation of antimicrobials into the membrane may reduce the incidence of infection and improve clinical outcomes. The aim of this *in vivo* study was to investigate the effects of a 5% azithromycin (AZM) coated polycaprolactone (PCL) membrane on oral bacterial growth.

In this single-blinded trial, seven participants wore customised splints containing PCL membranes for a total of four weeks. In the first 2 weeks, the participants wore ‘test’ membranes that were coated with AZM, and in the next two weeks, they wore ‘control’ membranes without AZM. Membranes were collected on day 3, day 7 and day 14 of each 2-week period to examine any changes in the quantity and/or quality of oral bacterial growth on the membranes.

The study showed that the samples with AZM had a lower number of bacteria than the samples without AZM. The total number of bacteria between day 3

and day 14 in the control group (day 3 control log₁₀ (6.14) CFU/mL vs. day 14 control log₁₀ (7.15) CFU/mL; $P < 0.01$), and between test and control groups at day 14 showed significant differences (day 14 control log₁₀ (7.15) CFU/mL vs. day 14 test log₁₀ (5.75) CFU/mL; $P < 0.01$). The difference between day 7 and day 14 in the control group was also significant ($P < 0.05$). AZM membranes showed a significant zone of inhibition against gram-positive bacteria until day 14 but did not show any inhibition in total bacteria or gram-negative bacteria after day 3. The individual outcomes of participants showed considerable variation due to issues with maintaining a consistent level of compliance in wearing the splint.

This pilot study showed that AZM-coated membranes decrease the quantity and quality of bacteria in the attached oral biofilm. Further studies with increased numbers of samples and modified sample collection methods are required to improve the reliability of the results.

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Azithromycin inhibits human osteoclast formation and activity in an *in vitro* inflammatory environment

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Periodontal disease is a pathologic condition whereby inflammation is modulated by complex interactions between bacteria and host cells leading to the destruction of the soft and hard tissues of the periodontium. Azithromycin is a macrolide antibiotic with both bacteriostatic and immunomodulatory properties.

Clinically it can be considered a novel adjunct in cases of periodontitis non-responsive to conventional periodontal treatment. The aim of the study was to determine the effect of azithromycin on osteoclast formation and resorptive activity in an *in vitro* inflammatory environment.

Peripheral blood mononuclear cells (PBMC's) were isolated from whole blood of healthy volunteers. The cells were stimulated with Tumor Necrosis Factor Alpha (TNF- α) or *Porphyromonas gingivalis* Lipopolysaccharide (*P. gingivalis* LPS) for 24 h, then differentiated into osteoclasts with macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa B ligand (RANKL). The effects of azithromycin at concentrations of 20 and 40 μ g/mL were tested. Immunohistochemistry was used to determine tartrate-resistant acid phosphatase (TRAP) expression, indicative of osteoclast formation. Dentine resorption assays were used to detect osteoclast activity and the production of key inflammatory cytokines were measured using enzyme-linked immunosorbent assay's (ELISA's).

The results demonstrated that individually, TNF- α and *P. gingivalis* LPS induced an "inflammation-like" milieu with significant increases in monocyte

chemoattractant protein-1 (MCP- 1), interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6) concentrations in cell supernatant compared to unstimulated control cells ($P < 0.05$). In TNF- α or *P. gingivalis* LPS stimulated environments, azithromycin reduced osteoclast formation ($P < 0.05$). Osteoclast activity was significantly suppressed by azithromycin in TNF- α stimulated environments ($P < 0.05$) and a similar trend was observed in *P. gingivalis* LPS stimulated environments.

This study expands our current understanding of azithromycin's effect on osteoclastogenesis and demonstrates that azithromycin reduces osteoclast formation and activity in an *in vitro* environment. This may provide a basis for future targeted and clinically appropriate use of azithromycin in the management of non-responsive periodontitis.

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The findings of this research were presented at the 2017 International Association Dental Research ANZ Divisional Meeting.

The awareness and knowledge of oral cancer amongst adult dental patients attending regional Charles Sturt University Dental and Oral Health Clinics in New South Wales

JJ Zachar, B Huang, E Yates

Greater awareness and knowledge of oral cancer has been shown to increase patient presentation for opportunistic oral cancer screening. Screening is crucial for early detection of oral cancer to reduce morbidity and mortality rates. This study aimed to identify the level of awareness and knowledge of oral cancer amongst adult dental patients attending Charles Sturt University Dental and Oral Health Clinics (CSU-DOHC) in NSW. It was hypothesised that socio-demographic factors would have a significant influence on self-declared awareness and knowledge of oral cancer.

A convenience sample of 444 adult dental patients participated in a self-administered questionnaire at one of five CSU-DOHC between May to July 2017. Data analysis was performed using the chi-squared test and binary logistic regression to compare socio-demographic characteristics and the self-declared awareness and knowledge of oral cancer.

The study revealed 73.8% of patients were aware of oral cancer, however, only 28.8% were aware they

had been previously screened for oral cancer. Being female (OR=2.57, 95% CI [1.66, 3.98]), having an excellent level of oral health (OR=3.34, 95% CI [1.04, 10.73]), previous attendance at a CSU-DOHC (OR=2.89, 95% CI [1.23, 6.75]) and seeing a dental student for treatment (OR=2.49, 95% CI [1.27, 4.87]) significantly influenced overall oral cancer awareness.

It was revealed that 74.6% of participants rated their level of knowledge of oral cancer as either knowing nothing (27.9%) or knowing very little (46.7%). Gender, age, employment status, level of education, previous attendance at the clinics, language and level of oral health; were all significant knowledge predictors when identifying risk factors, signs and symptoms, and treatment modalities of oral cancer.

The findings of this study have broad implications for both clinicians and public health professionals, providing a platform for discussion regarding the socio-demographic factors contributing to reduced

knowledge and awareness amongst patients. This study also identified an essential avenue to allow a more targeted approach in future campaigns to

increase education and understanding of oral cancer in regional areas of NSW and Australia.

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Comparing the periodontal tissue response to non-surgical scaling and root planing alone, adjunctive azithromycin, or adjunctive amoxicillin plus metronidazole in generalised chronic moderate-to-severe periodontitis: a preliminary randomised controlled trial

A Liaw, C Miller, A Nimmo

The administration of systemic antibiotic adjuncts following scaling and root planing to further expedite healing of the periodontal tissues is a topic of considerable interest, given the lack of evidence-based guidelines for their use in periodontal treatment. This study aims to evaluate and compare the clinical and inflammatory cytokine effects between scaling and root planing alone, adjunctive azithromycin, or adjunctive amoxicillin plus metronidazole in the treatment of patients with generalised moderate-to-severe chronic periodontitis.

Thirty-eight patients were randomly assigned to receive scaling and root planing alone, or combined with 500 mg amoxicillin plus 400 mg metronidazole three times per day for 7 days, or 500 mg azithromycin per day for 3 days. Patients were clinically monitored at baseline and two-months post-treatment, and inflammatory cytokine levels were assessed at baseline, one-week, and two-months post-treatment.

All study groups showed significant differences in full-mouth clinical parameters. Compared with scaling

and root planing alone, the amoxicillin plus metronidazole group demonstrated greater clinical reductions for baseline sites with moderate and severe disease.

The treatment of chronic periodontitis is significantly improved by thorough scaling and root planing, irrespective of the use of antibiotic adjuncts. However, the administration of amoxicillin plus metronidazole may provide improved therapeutic outcomes for sites with more severe disease.

This study was supported by two grants from the Australian Dental Research Foundation (ADRF), The Colin Cormie Scholarship and the Dental Student Research Grant. The James Cook University College of Medicine and Dentistry Honours Program also supported this work. The authors declare that there are no conflicts of interest in this study.

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Azithromycin surface-functionalised biodegradable membranes for guided tissue regeneration

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Bacteria contamination on the sites of undergoing tissue regeneration is a significant issue in periodontal regeneration. A local drug delivery approach is an

inherently sound strategy for improving clinical outcomes whereby the GTR membrane is loaded with an antibiotic to prevent or inhibit bacterial contamination.

This strategy would prevent bacterial infiltration during the key early wound healing stages of the regenerative process.

In this project, we developed polycaprolactone (PCL) fibrous membrane for the controlled local delivery of azithromycin to significantly reduce any bacterial infiltration and also studied its effect on bone regeneration.

The PCL scaffold was made via solution electrospinning and was further coated with calcium phosphate (CaP). CaP coated PCL scaffolds were then loaded with azithromycin via a solvent evaporation technique. Azithromycin loaded scaffolds were then tested in-vitro for morphology, release, cell viability, proliferation and antibacterial properties.

The azithromycin scaffold with different doses of azithromycin (100, 500, 1000 µg) were implanted on the calvarium of a rat animal model. The implanted scaffold was harvested after 1, 4 and 8 weeks to assess the bone formation.

PCL membrane surface topography has an effect on the azithromycin encapsulation and efficiency. We have also assessed the in vitro release profile of vari-

ous concentrations of azithromycin (50 to 1000 µg) from the membrane and this demonstrated a dose-dependent release from CaP coated PCL membranes after 7 days of incubation with PBS at 37°C.

Azithromycin loaded scaffolds were active against *Staphylococcus aureus* at all doses (50 -1000 µg) and the antibacterial efficiency was observed even after 7 days of release in PBS. HOB, PDL and GF cells were viable at low doses (50 µg) and was toxic above 100 µg. In-vivo studies revealed increased bone formation after 4 and 8 weeks, especially with 500 µg dose.

Azithromycin was successfully loaded onto PCL/PCL-CaP coated membranes. Calcium phosphate coating on PCL resulted in significant increase in encapsulation efficiency due to its increased surface area. Controlled release of azithromycin was observed over time. Dose dependent viability in-vitro was also observed. Azithromycin loaded scaffolds were active against *Staphylococcus aureus* even at lower doses. Increased new bone formation was observed with azithromycin coated PCL-CaP membranes.

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Preclinical development of a novel porosity indicator for early detection of caries

JE Mangum

Early detection of caries is an important element of minimal intervention dentistry, however, early-stage lesions can be difficult to visualise using current clinical approaches. This project aimed to develop a new indicator for the early detection of caries on the basis of hydroxyapatite porosity.

A hydroxyapatite-binding indicator (dark blue liquid) was synthesised and validated by an in vitro mineral-binding assay. Artificial carious lesions were created in human enamel using acid demineralisation and the ability of the indicator to detect lesions was evaluated by direct application to the tooth surface and imaging densitometry of photographs. Natural

carious lesions were also assessed for detection by the indicator.

The indicator detected porous enamel associated with artificial caries and natural caries. In artificial caries, the indicator bound rapidly and specifically. Natural caries lesions (ICDAS grade 1) were also labelled specifically but only when the lesions were active (i.e. arrested white spot lesions were not detected).

The hydroxyapatite-binding indicator shows promise for early detection of caries and diagnosis of active/arrested lesions.

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Explaining the links between mother's and child's oral health – a prospective cohort study

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Dental caries remains the most prevalent chronic disease in children, exerting significantly impact on children and society. Unhealthy children are less able to learn and develop and are more likely to grow up to become unhealthy adults.

In many cases, unhealthy children will be more likely to have unhealthy offspring themselves leading to intergenerational disadvantages; for instance, mother's poor oral health is associated with poor child oral health. There exists a relationship between a mother's and child's oral health. However, this relationship has not been comprehensively quantified. Furthermore, there are factors that mediate the link between mother and child oral health. Identifying those factors and the magnitudes with which these factors mediate the link between mother and child oral health will be useful to develop effective preventive programs. The study aimed to investigate links between maternal and child oral health and identify factors explaining those links.

The study used data collected in a population-based prospective birth cohort study of newborn children in Adelaide, funded by an NHMRC Project Grant. Questionnaire data was collected at birth and different ages. When children turned 24 months, both the children and their mothers were invited to oral epidemiological examinations to assess dental caries experience. The outcome of this analysis was the

prevalence of Early Childhood Caries (ECC). The main exposure was maternal caries experience in 4 ordinal groups (DMFS=0; 0<DMFS<=4; 4<DMFS<=8 and DMFS>8). Household income at baseline, parental education and country of birth, mother's smoking and soft drink consumption were covariates. An analysis was conducted progressively from bivariate to multivariable log-binomial regression with robust standard error estimation to calculate prevalence ratios (PR) of having ECC at 24 months.

A total of 1040 mother/child dyads had complete data. In the multivariable regression model, mother's caries experience was a significant predictor for ECC (PR=3.24 (95%CI: 1.42, 7.38)). This effect attenuated only marginally when maternal health behaviours and child's tooth brushing were added to the model (PR=3.06 (95% CI: 1.34, 6.96)).

The study has provided evidence of a relationship between maternal and child oral health at an early age. Preventive programs need to reach and benefit children from birth, giving them opportunity to stay healthy and to maximise their life potential. Mother's health and the pregnancy period are important aspects for delivery of successful programs.

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Azithromycin modulation of cytokine production during LPS induced/ chronic inflammation *in vitro*: impact on osteoclastogenesis

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A key feature of chronic periodontal disease (PD) is alveolar bone loss resulting from increased formation and activity of osteoclasts; the cells responsible for bone resorption. As the pathogenesis of PD involves a host immune response to pathogenic bacterium, such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, conventional mechanical periodontal therapy is not satisfactory for all cases of PD. As such, adjuvant treatment with systemic antibiotics such as

azithromycin (AZM) may be beneficial. Osteoclasts are derived from cells of the monocyte/macrophage lineage, and are induced by cytokines/chemokines of the immune response.

Furthermore, previous studies show that AZM under 'normal' (non-inflamed) circumstances inhibit osteoclast differentiation and activity *in vitro* (Gannon et al., 2013). Since periodontitis is a chronic inflammatory condition, the aim of this study was to

determine the effect of azithromycin on osteoclast formation and activity in an induced inflammatory environment *in vitro*.

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood of healthy volunteers (n = 8). The cells were stimulated with *Porphyromonas gingivalis* lipopolysaccharide (*P. gingivalis* LPS) or Tumor Necrosis Factor Alpha (TNF- α) for 24 h. Osteoclastogenesis was induced with macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa B ligand (RANKL) with or without AZM. Osteoclast formation (assessed by tartrate resistant acid phosphatase staining; TRAP) and activity (scanning electron microscopy of resorption pit formation on dentine discs). Cytokines release was measured using enzyme linked immunosorbent assay's (ELISAs).

Monocyte chemoattractant protein-1 (MCP- 1), interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6) concentrations were significantly increased after 24 hrs exposure to *P. gingivalis* LPS or TNF- α (positive control) compared to unstimulated cells ($P < 0.05$). AZM significantly reduced TRAP+ osteo-

clast formation by cells previously exposed to *P. gingivalis* LPS or TNF- α ($P < 0.05$). Osteoclast activity was significantly reduced by AZM treatment of cells previously stimulated by TNF- α ($P < 0.05$) and a similar trend was observed in *P. gingivalis* LPS stimulated environments although not significant.

While the short exposure time of osteoclast precursors to *P. gingivalis* LPS or TNF- α is comparable to an acute inflammatory environment, this study demonstrates that AZM inhibits osteoclast formation and activity in an environment with inflammatory mediators present.

Further studies will be required to mimic the chronic inflammatory state associated with periodontal disease and comprehensive analysis of osteoclast specific gene expression will increase our knowledge of the benefits of the clinically appropriate use of AZM in the management of non-responsive periodontitis.

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