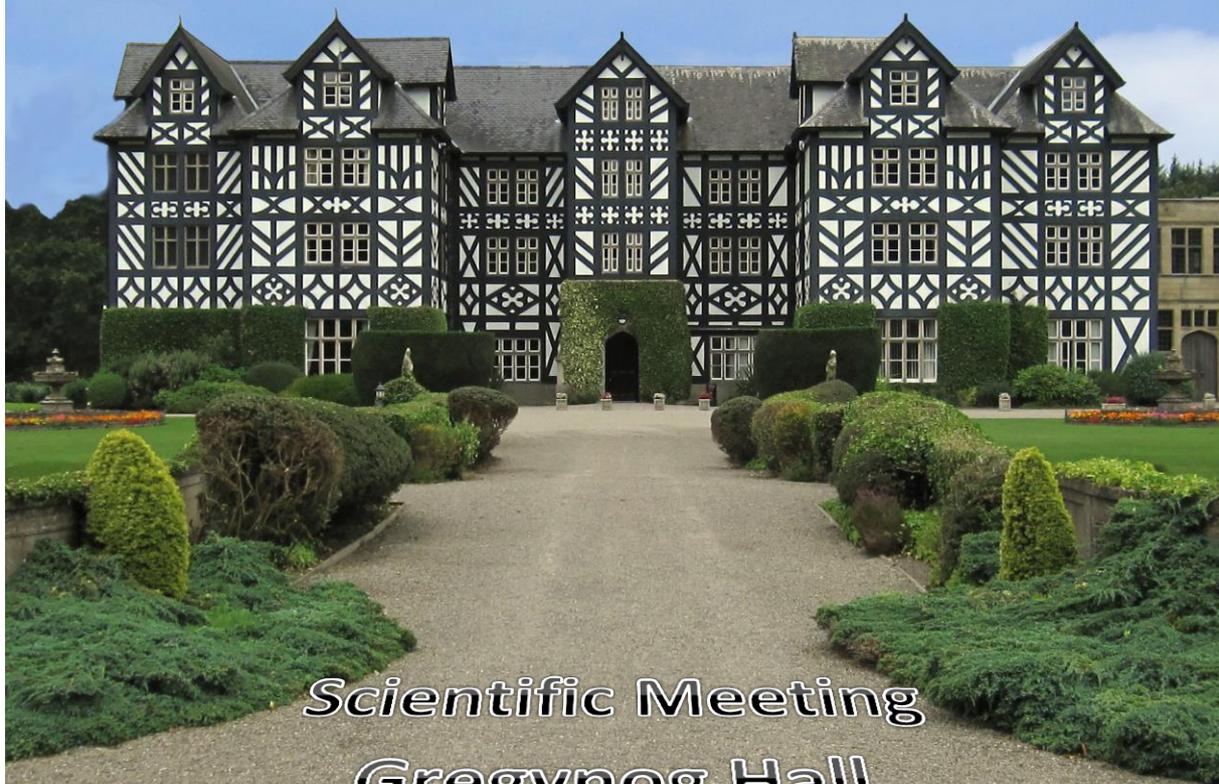


OMIG Oral microbiology and immunology group



Scientific Meeting
Gregynog Hall
Tregynon, Wales
28th Feb – 2nd March 2018



OMIG Scientific Meeting
Gregynog Hall, Tregynon,
Wales
28th Feb – 2nd March 2018



PROGRAMME

Wednesday 28th February

Afternoon: Delegates arrive

18.00: *Interacting with industry* – Q&A session (**Jon Pratten & Dave Bradshaw, GSK**)

19.00: Dinner

Thursday 1st March

09.00: WELCOME: Marcello Riggio (Secretary/Treasurer, OMIG)

09.10: KEYNOTE LECTURE: *The oral microbiome – friend or foe?* (**Phil Marsh, School of Dentistry, University of Leeds**)

Session 1: Host-pathogen interactions (Chair: Joey Shepherd)

10.10: *The relationship between periodontitis, the periodontal microbiome and risk of developing rheumatoid arthritis* (**Deirdre Devine, School of Dentistry, University of Leeds**)

10.45: TEA / COFFEE

11.10: *Activation of epithelial immunity by fungal and bacterial toxins* (**Olivia Hepworth, King's College London Dental Institute**)

11.45: *Porphyromonas gingivalis-mediates vascular damage in-vitro and in a novel in-vivo zebrafish systemic infection model* (**Magdalena Widziolek*, School of Clinical Dentistry, University of Sheffield**)

12.10: LUNCH

Session 2: Understanding biofilms 1 (Chair: Wim Crielaard)

13.30: *Age related variations in the oral microbiome and periodontal bone levels in the wild wood mouse *Apodemus sylvaticus* compared to laboratory mouse models* (**Susan Joseph, King's College London Dental Institute**)

14.05: *Role of the *Streptococcus gordonii* extracellular deoxyribonuclease SsnA in dental plaque* (**Nadia Rostami, School of Dental Sciences, Newcastle University**)

14.40: *Omics approaches to studying microbial biofilms in oral health* (**Christopher Delaney*, Dental School, University of Glasgow**)

15.05: TEA / COFFEE

Session 3: Understanding biofilms 2 (Chair: Chris Hope)

15.30: *Peri-implantitis: investigating the attachment of key pathogens to titanium surfaces* (**Clotilde Haury*, School of Dentistry, Cardiff University**)

15.55: *Developing three dimensional oral mucosal models for denture stomatitis* (**Samantha Gould*, Plymouth University Peninsula Schools of Medicine and Dentistry**)

16.20: *An early colonising, multispecies interproximal biofilm model* (**Rebecca Jones*, School of Dental Sciences, Newcastle University**)

16.45: SESSION ENDS

Three-minute thesis competition

17.00: *A multispecies model representative of the microbial community within periodontal pockets: initial pilot work* (**Katherine Ansbro*, School of Clinical Dentistry, University of Sheffield**)

17.05: *An investigation into the role of WFDC2 in the oral cavity and respiratory tract* (**Hannah Armes*, School of Clinical Dentistry, University of Sheffield**)

17.10: *Investigation of novel virulence factors and receptors for *P. gingivalis* host-pathogen interactions* (**Ashley Gains*, School of Clinical Dentistry, University of Sheffield**)

17.15: *A scoping review on bio-aerosols in healthcare and the dental environment* (**Charifa Zemouri*, ACTA, Amsterdam**)

17.20: *Enrichment of innate lymphoid cell populations in murine gingival tissue* (**Jason Brown*, Dental School, University of Glasgow**)

17.25: END OF COMPETITION

Prize for the best presentation: £100 Amazon gift voucher.

19.00: CONFERENCE DINNER

Friday 2nd March

Session 4: Understanding biofilms 3 (Chair: Sarah Kuehne)

09.00: *Environments mimicking the periodontal pocket select pathogenic bacterial communities* (**Thuy Do, School of Dentistry, University of Leeds**)

09.35: *Beneficial influence of microcosm biofilm on reconstructed human gingiva* (**Lin Shang*, ACTA, Amsterdam**)

10.00: *Development of an adaptable, reproducible model for dental plaque* (**Mehmet Davrandi*, UCL Eastman Dental Institute, London**)

10.25: TEA / COFFEE

Session 5: Antimicrobials (Chair: Jon Pratten)

10.50: *Development of a three-dimensional collagen model for the in vitro evaluation of osteogenesis for dental prostheses* (**Rebecca Yuan, Barts & The London School of Medicine and Dentistry, QMUL, London**)

11.25: *Silica particles as a drug delivery system for antimicrobial agents in dental applications* (**Menisha Manhota*, School of Chemistry, University of Birmingham**)

11.50: SESSION ENDS

Prizes for the best oral presentations by a PGR student: winner (£100 Amazon gift voucher), runner-up (£50 Amazon gift voucher).

11.50: PRESENTATION OF PRIZES AND CLOSE OF MEETING: Dave Spratt (Chair, OMIG)

12.00 END OF MEETING (packed lunch available)

* Denotes a PGR student presenter.
All other presenters are staff.

Keynote Lecture, oral presentation prizes and three-minute thesis competition are sponsored by GSK.

Delegates are invited to visit the trade stand of Johnson & Johnson during the meeting.



OMIG wishes to thank our sponsors for their generous financial support and the oral presenters and delegates who have made this meeting possible.

Connect with OMIG:

Website: www.bsodr.org.uk/omig



<https://www.facebook.com/OMIGUK>



https://twitter.com/OMIG_BSODR_UK



OMIG is a research group of the British Society for Oral & Dental Research (BSODR)

ABSTRACTS

OMIG RESEARCH MEETING 2018

GREGYNOG HALL, TREGYNON, WALES

28TH FEBRUARY – 2ND MARCH 2018



do more
feel better
live longer

Johnson & Johnson
LIMITED

THE MAKERS OF

LISTERINE
ADVANCED DEFENCE

BRITISH SOCIETY FOR
Oral and Dental
RESEARCH



KEYNOTE LECTURE

The oral microbiome – friend or foe?

Phil Marsh

School of Dentistry, University of Leeds, UK

The oral microbiome is diverse and exists as multi-species microbial communities on oral surfaces in structurally- and functionally-organised biofilms. The oral microbiome is natural and has a symbiotic relationship with the host. The mouth is a warm and nutritious habitat for microbial growth, while the microbiome delivers important benefits, which include pathogen exclusion, immunomodulation, and regulation of important physiological functions.

In oral health, a balance is maintained between the host, the environment and the microbiome. However, this balance is dynamic and susceptible to perturbation which can increase the risk of disease [dysbiosis]. The frequent intake of sugar and/or a reduction in saliva flow results in extended periods of low pH in the biofilm. This inhibits the growth of beneficial species and drives the selection of bacteria with an acid-producing/acid-tolerating phenotype, thereby increasing the risk of caries. Inflammation can occur if plaque accumulates around the gingival margin beyond levels compatible with oral health. If the inflammatory response fails to remove the microbial insult, then an inflammo-philic microbial community is selected which can lead to a subverted and exaggerated host response, resulting in tissue destruction. An increased understanding of the relationship between the host and the oral microbiome can provide new insights and fresh opportunities to promote symbiosis and prevent dysbiosis. These include: modifying the oral microbiome (e.g. with prebiotics and probiotics), manipulating the oral environment to selectively favour the growth of beneficial species, and moderating the growth and metabolism of the biofilm to reduce the likelihood of dysbiosis. Evidence will be provided to suggest that the regular provision of interventions that deliver small but relevant benefits, consistently over prolonged periods, can support the maintenance of a symbiotic oral microbiome.

SESSION 1

Host-pathogen interactions

The relationship between periodontitis, the periodontal microbiome and risk of developing rheumatoid arthritis

Cheng, Z.¹, Do, T.¹, Mankia, K.², Meade, J.L.¹, Kang, J.¹, Hunt, L.², Nam, J.², Tugnait, A.³, Speirs, A.⁴, Clerehugh, V.³, Emery, P.², **Devine, D.**¹

1. Division of Oral Biology, University of Leeds, School of Dentistry, UK.

2. Leeds Musculoskeletal Biomedical Research Centre, University of Leeds, School of Medicine, UK

3. Division of Restorative Dentistry, University of Leeds, School of Dentistry, UK.

4. Leeds Dental Institute, Leeds Teaching Hospitals Trust, UK

Background. Rheumatoid arthritis (RA) is associated with increased prevalence of periodontal disease. *Porphyromonas gingivalis* (*Pg*) and *Aggregatibacter actinomycetemcomitans* (*Aa*) are hypothesised to contribute to the formation of RA-associated autoantibodies. Thus, periodontitis may be a key initiator of RA-related autoimmunity and it is important to assess its role in seropositive individuals at risk of developing RA.

Methods. Anti-cyclic citrullinated protein antibody positive individuals with no synovitis (CCP+; n=48), healthy controls (HC; n=32) and new-onset RA patients (NORA; n=26) were matched for age, sex and smoking. DNA, isolated from subgingival plaque from diseased and healthy periodontal sites, was paired-end sequenced (Illumina HiSeq3000). Taxonomic and functional profiles were obtained and significant differences between groups studied using DESeq2. Mann-Whitney U tests were used to compare groups and Spearman Rho for correlations.

Results. 73% of CCP+, 38% of HC (p=0.02) and 54% NORA had periodontitis. The % of sites with clinical attachment loss, probing depth, bleeding on probing and active disease were greater in CCP+ compared with HC (p<0.05). CCP+ microbiomes contained higher proportions of Bacteroidetes than HC. The relative abundance of both *Pg* and *Aa* was higher in CCP+ compared to HC and NORA (p<0.001). *Pg*, but not *Aa*, was more abundant in individuals with periodontitis. Abundance of *Pg*, but not of *Aa*, was associated with the % of periodontal sites with active disease in CCP+ (p=0.05) and HC (p=0.04). Variations were seen in sequences encoding the active site of PPAD (*Pg* peptidyl arginine deiminase) and PAD-like sequences were also frequently detected, particularly in *Prevotella* spp.

Conclusions. An increased prevalence of periodontitis was seen in individuals at risk of developing RA; this risk was also associated with increased abundances of *Pg* and *Aa*. This supports the hypothesis that periodontitis and periodontal pathogens are involved in the initiation of RA-related autoimmunity.

Activation of Epithelial Immunity by Fungal and Bacterial Toxins

Olivia Hepworth, Jonathan Richardson, Julian Naglik

King's College London

Introduction: *Candida albicans* is a medically important human fungal pathogen, which can cause severe morbidity and mortality in the context of systemic, life-threatening infections. Recently, we discovered that *C. albicans* produces a cytolytic peptide toxin, Candidalysin, which has the ability to cause damage and activate epithelial immunity via MAPK signalling. We have now established that there is a family of Candidalysins produced by various *Candida* species. The aim of this project is to compare the epithelial mechanisms activated by the Candidalysins and whether similar mechanisms are also induced by bacterial toxins.

Methods: TR146 buccal epithelial cells were stimulated with the Candidalysins and various bacterial toxins (Pneumolysin, Streptolysin O, α -hemolysin, δ -hemolysin, Cholera Toxin) *in vitro* in order to assess effects on epithelial damage and immunity. A lactate dehydrogenase (LDH) assay was used to determine the degree of epithelial cell damage, and the activation of MAPK signalling via MKP1 phosphorylation (p-MKP1) and c-Fos activation was determined by Western blotting. Early innate cytokine responses were assessed using a Magnetic Luminex Performance assay.

Results: The Candidalysins were all found to induce epithelial damage, stimulate epithelial MAPK signalling via c-Fos and p-MKP1, and induce cytokine release. Preliminary data suggest that bacterial toxins stimulate similar epithelial mechanisms. However, some toxins have shown to be much more potent in inducing epithelial cell damage and subsequent signalling activity than Candidalysin.

Conclusion: The Candidalysins and bacterial toxins appear to stimulate similar epithelial mechanisms, suggesting a common mechanism through which all microbial toxins signal.

***Porphyromonas gingivalis*-mediates vascular damage *in-vitro* and in a novel *in-vivo* zebrafish systemic infection model**

Magdalena Widziolek^{1,3}, Robert Wilkinson², Jan Potempa³, Graham P Stafford¹, Craig Murdoch¹

¹*School of Clinical Dentistry and* ²*Dept. Infection, Immunity & Cardiovascular Disease, University of Sheffield, Sheffield, UK.* ³*Department of Microbiology, Jagiellonian University, Kraków, Poland*

Introduction: *Porphyromonas gingivalis* (*Pg*) is the main pathogen involved in the development of periodontitis. Reports show that *Pg* also enters the bloodstream contributing to cardiovascular diseases. The cysteine protease gingipains are a main virulence factor that are secreted through the type IX secretion system (T9SS) and may contribute to pathogenesis. We recently established a novel zebrafish larvae systemic infection model for *Pg* that allows real-time visualisation of host-pathogen interactions. In this study we use this *in-vivo* model alongside monolayers of human dermal microvascular endothelial cells (hDMEC) to investigate the mechanism of *Pg* systemic dissemination and interaction with host cells.

Methods: Fluorescently labelled wild-type *Pg* W83 and mutants lacking *rgp*, *kgp* gingipains or elements of the T9SS were injected into transgenic zebrafish containing a fluorescently-tagged vasculature. Bacterial dissemination and interaction with host cells was visualised using light-sheet microscopy. The efficiency of phagocytosis by immune cells was assessed using a pH sensitive dye. The ability of *Pg* to increase endothelial permeability was evaluated using fluorescent dextrans both *in-vivo* and *in-vitro*.

Results: *Pg* penetrated the zebrafish vasculature into surrounding tissues causing pericardial oedemas and cardiac damage in a gingipain-dependent manner. Zebrafish injected with wild-type *Pg* displayed significantly reduced phagocytosis and increased survival *in-vivo* compared to those injected with mutants lacking *rgp*, *kgp* gingipains or elements of the T9SS. *Pg* reduced expression of the endothelial cell junction protein CD31 and increased the permeability of hDMEC monolayers *in-vitro* and preliminary data suggest that intra-vascular *Pg* also increases zebrafish endothelium permeability.

Conclusions: *Pg* markedly affects the zebrafish vascular system causing decreased systemic immune cell clearance, increased endothelial damage and severe oedema. This evidence supports the idea that *Pg* may be a significant contributor for initiating oral microbe-related cardiovascular damage leading to long-term systemic disease.

SESSION 2

Understanding biofilms 1

Age related variations in the oral microbiome and periodontal bone levels in the wild wood mouse *Apodemus sylvaticus* compared to laboratory mouse models

Susan Joseph¹, Asil Alsam¹, William Wade², Mike Curtis¹

¹*Division of Mucosal and Salivary Biology, Kings College London Dental Institute, Tower Wing, Guy's Hospital, London SE1 9RT*

²*Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AD*

Introduction: Laboratory mouse strains such as C57/BL6 and C3H/He among others, have been traditionally used for periodontal research models. However, it is known that these have much “cleaner” microbiomes and immunology compared to animals in the wild which have greater evolutionary pressures.

Methods: Oral and fecal samples of wild wood mice, *Apodemus sylvaticus*, of varying ages were collected from two different locations in UK and compared with laboratory mice by culturing as well as NGS analysis of the V1-V2 region of the 16S rRNA gene. Periodontal bone loss was estimated by measuring the CEJ-ABC distance on defleshed jaws.

Results: Total oral microbial counts were found to be significantly elevated (> 10 fold; $p < 0.0001$) in the wild mice compared to laboratory mice, even though no significant variations were observed with the progression of age. Increased taxonomic diversity was also observed in the wild mice by both culturing and NGS methods, predominated by members of the Pasteurella, Lactobacillus, Neisseria and Streptococcus genera in the oral microbiome and Lactobacillus and Bacteroidetes in the gut microbiome. Variations in microbial diversity were also observed between the two wild mice populations. Alveolar bone loss analysis of the healthy wild mice revealed elevated bone loss ($p < 0.001$) at the onset of 29 weeks age, comparable to that of a lab mouse with acute periodontal disease, which continued to significantly increase with advancing age.

Conclusion: The observed increase in microbial richness and abundance as well as alveolar bone loss levels in the wild wood mice indicate the possibility of a unique immune mechanism for oral health in the natural environment. Investigations are ongoing to further understand this using immunohistochemistry methods.

Role of the *Streptococcus gordonii* extracellular deoxyribonuclease SsnA in dental plaque

Nadia Rostami, Nicholas Jakubovics

School of Dental Sciences, Centre for Oral Health Research, Newcastle University, Newcastle upon Tyne, United Kingdom

Introduction

Extracellular DNA (eDNA) has recently been identified as a key component of the matrix of natural dental plaque. Many oral bacteria produce DNase enzymes, including the pioneer coloniser *Streptococcus gordonii*. Here, we aimed to characterize the *S. gordonii* DNase enzyme, SsnA (Streptococcal Surface Nuclease A), including its regulation, enzyme activity and ability to inhibit biofilm formation by the eDNA-dependent strain *S. mutans* GS5.

Methods

An *S. gordonii ccpA* null mutant was constructed by PCR overlap extension mutagenesis, and a complemented strain was produced by expression of the gene from a plasmid. Expression of SsnA was determined in the presence of different sugars in the wild type *S. gordonii* versus *ccpA* mutant and complement. In addition, a knockout strain of the maltose-responsive regulator gene *malR* was assessed. The enzymatic activity of recombinant SsnA was measured in a fluorescence-based assay under varying conditions including pH and metal availability. Inhibition of *S. mutans* biofilms was assessed by staining with crystal violet.

Results

The presence of sucrose, glucose or maltose during growth inhibited SsnA expression. This inhibition was overcome by disruption of *ccpA*, but not *malR* and was restored by complementation of *ccpA*. Recombinant SsnA exhibited the highest activity at approximately pH 9.5 and was almost completely inhibited below pH 6. SsnA exhibited preference for divalent cations and Mg²⁺ in particular. SsnA inhibited the formation of biofilms by *S. mutans*.

Conclusions

S. gordonii SsnA is an extracellular DNase that potentially plays a role in shaping of plaque structure through remodelling the eDNA component of the biofilm matrix. SsnA may mediate competition with other species, particularly at higher pH. Further studies will aim to investigate the impact of SsnA on the structure and microbial composition of dental plaque, with a view to developing new approaches for controlling plaque by targeting the extracellular matrix.

Omics approaches to studying microbial biofilms in oral health

Christopher Delaney¹, Karl Burgess¹, Chris Nile¹, Angela Nobbs², Gordon Ramage¹

¹College of Medical, Veterinary and Life Sciences, Dental School, University of Glasgow.

²Bristol Dental School, University of Bristol.
Email: c.delaney.1@research.gla.ac.uk

Biofilm formation by *Candida* spp. has been implicated in numerous oral diseases, such as periodontal disease, caries and denture stomatitis. The ability for the organism to successfully colonise and form biofilms is highly heterogeneous and dependent on numerous factors including nutrient availability, co-infection with other microbes, and host interactions.

The aim of this work is elucidating the mechanisms that cause a shift from a healthy to disease state using omics technologies. It is our hypothesis that metabolic adaptation leads to more pathogenic biofilms, so the aim of this work is to utilise bioinformatics approaches to categorise these mechanisms under differing stimuli.

Transcriptomic analysis by RNA-Seq and metabolomics by Liquid Chromatography Mass Spectrometry were carried out using two clinical *C. albicans* strains with distinct biofilm forming phenotypes grown in the presence and absence of foetal calf serum (FCS). RNA-Seq data was also generated for *C. albicans* grown with and without the presence of the periodontopathogen *Porphyromonas gingivalis*.

Pathway analysis using KEGG and GO databases revealed significant changes involving fatty acid and amino acid metabolism, and also MAPK pathways to be differentially regulated in the presence of FCS. Additionally, pathway analysis revealed distinct changes in peroxisome and arginine biosynthesis in the presence of *P. gingivalis*.

Through use of correlative and pathway analysis, it is possible to infer key pathways involved with regards to the biofilm forming phenotype of *C. albicans*. The use of omics technologies as substantial analytical tools provides a powerful methodology to interrogate the biological basis of pathogenic biofilms. These pipelines and informatics approaches can be used to investigate more complex polymicrobial biofilms, and the impacts of therapeutic intervention.

SESSION 3

Understanding biofilms 2

Peri-implantitis: Investigating the attachment of key pathogens to titanium surfaces

Clotilde Hauray^{1,2}, Andrew Wescott³, David Beeby³, Bryan Austin³, Simon Q. Jones¹, Wayne N. Ayre^{1,2}, Rachel J. Waddington^{1,2}, Alastair J. Sloan^{1,2}

¹Mineralised Tissue Group, School of Dentistry, Cardiff University, Wales, UK.

²Cardiff Institute of Tissue Engineering and Repair, Cardiff University, Wales, UK.

³Renishaw PLC, Gloucester, England, UK.

Objectives

Peri-implantitis (PI) is an infectious disease associated with inflammatory destruction of implant-supporting tissues, which can lead to implant failure. The inflammatory host response is stimulated by the accumulation of anaerobic pathogens in implant-associated biofilms. Previous research showed that 'late colonisers' can attach directly to metal surfaces. This finding challenges the conventional paradigm of successive colonisation, with the requirement that 'early colonisers' facilitate subsequent attachment of anaerobic PI-associated pathogens.

This study aimed to assess the direct attachment of PI pathogens to laser-sintered titanium alloy (Ti).

Methods

Physicochemical characterisations were performed on Ti, including chemical composition, profilometry, surface charge and grain boundaries.

The PI-associated pathogens *Fusobacterium nucleatum* and *Porphyromonas gingivalis* were anaerobically cultured until mid-log phase. Ti discs were incubated for up to 2h in bacterial suspensions. Attachment and viability were quantified by microbiological culture counts and image analysis following fluorescence microscopy. Studies were performed on plain discs and discs pre-treated with artificial saliva.

Results

The discs presented smooth and hydrophilic surfaces with $R_a=0.056\mu\text{m}$, and contact angle $\theta=49^\circ$. Pre-treatment with artificial saliva increased the hydrophilicity ($\theta=37^\circ$). The pathogens attached directly to Ti. A modulation in attachment was observed to correlate with surface hydrophilicity. *F. nucleatum* attachment decreased with surface hydrophilicity, with 42% attachment on plain Ti and 17% on Ti pre-treated with artificial saliva. The opposite was observed for *P. gingivalis* with 14% attachment on plain Ti and 43% on pre-treated Ti. *F. nucleatum* formed patterns on the discs surfaces that correspond to the Ti grain boundaries produced during the laser-sintering process.

Conclusion

Direct adherence of PI pathogens to metallic surfaces in the absence of 'early colonisers' has been shown, which challenges conventional theory. Of clinical importance, the ability of Gram negative pathogenic bacteria to bind to metals has potential to increase the probability and severity of peri-implantitis in susceptible patients.

Developing three dimensional oral mucosal models for denture stomatitis

Gould S, Belfield L, Upton M, Salih V

Plymouth University, PUPSMD, Dental School

Introduction:

Denture stomatitis (DS) is a multifactorial disease, which causes symptoms ranging from mild discomfort to severe nodule formation. The disease aetiology is not fully understood, although, the dimorphic fungus *Candida albicans* is known to be involved. Most DS infection studies use the SC5314 *C.albicans* (non-oral) strain, due to its unique ability to form hyphae in the presence of serum. The bacterium *Staphylococcus aureus* is alleged to be involved, however its role is undetermined. Animal models have been used to model DS infection, however they are often considered unethical and not fully applicable to DS. This study aims: to assess the efficacy of Three Dimensional Oral Mucosal Models (3DOMMs), in the context of infection (DS) modelling; and to obtain relevant clinical microbiological isolates for infection modelling.

Methods:

3DOMMs were created using a collagen gel based method. Employing human gingival fibroblast (HuGF) and keratinocyte (HaCaT) cell lines, grown for up to 21 days. Structural comparisons were made with native tissue using histological techniques. Constitutively expressed and inducible immune system components (TLR's and pro-inflammatory cytokines) were provisionally assessed via ELISA and western blot techniques. Clinical isolates were obtained by swabbing DS and control patient dentures.

Results:

3DOMMs appear morphologically similar to native oral tissue (multi-layered epithelium displaying differentiation and keratinisation). Analysis of pattern recognition receptors (PRRs) expressed within the 3DOMMs (TLR's-2,4) indicate they are capable of recognising pathogenic challenge. Provisional data suggests constitutive expression of the pro-inflammatory cytokines IL-6 and IL-8 without pathogenic challenge.

Conclusion:

3DOMMs are morphologically similar to the oral mucosa. The expression of PRR's warrants further development of models to include an immune system, however, first the constitutive expression of pro-inflammatory cytokines needs to be elucidated. Further work using DS clinical isolates and 3DOMMs will improve the understanding of *S.aureus* and *C.albicans* interactions, and their role in DS.

An early colonising, multispecies interproximal biofilm model

Rebecca Jones¹, Owen Darch² and Nicholas Jakubovics¹

¹*Centre for Oral Health Research, Newcastle University, UK.*

²*Philips Research, UK*

r.s.jones2@newcastle.ac.uk

owen.darch@philips.com

nick.jakubovics@newcastle.ac.uk

KEYWORDS: interproximal, modelling, multiplex qPCR, immunofluorescence

Introduction

The build-up of bacterial biofilm (dental plaque) on tooth surfaces is the main contributor to the most common oral diseases of humans, dental caries and periodontitis. Plaque accumulates in 'sheltered sites' such as the subgingival margins and interproximal spaces, where it is difficult to remove. Bacterial adhesion in the mouth is somewhat hierarchical; with certain species able to adhere to the acquired enamel pellicle (early colonising bacteria); while others adhere better to established bacterial biofilms. Later colonising bacteria are more closely associated with oral disease, yet without initial bacterial adhesion couldn't develop into mature plaque.

Aim

This project aims to develop tools for establishing and analysing a three-species biofilm model of early colonising bacteria in interproximal spaces.

Methodology and results

A multiplex qPCR assay was developed and validated for absolute quantification of *Streptococcus gordonii*, *Actinomyces oris* and *Veillonella parvula* in mixed cultures. Polyclonal antibodies were generated against whole bacterial cells to study the spatial distribution of the species within biofilms. Antibodies were validated by Western blotting and immunofluorescence microscopy. A mixed-species 3D Typodont model for biofilm growth on polymethylmethacrylate substrates was established to mimic interproximal spaces. Addition of sterile natural saliva before application of microorganisms qualitatively affected the biofilm structure but did not impact cell numbers.

Conclusion

Methods have been developed to culture and characterise a three-species biofilm model of early interproximal dental plaque. This model can now be used for screening of anti-biofilm compounds and to judge their effectiveness in conjunction with mechanical removal forces.

THREE-MINUTE THESIS

A Multispecies Model Representative of the Microbial Community within Periodontal Pockets: initial pilot work

K. Ansbro¹, K. Moharamdzeh¹, J. Shepherd¹, W.G. Wade², D. J. Bradshaw³, J. Pratten³, G. P. Stafford¹

1. School of Clinical Dentistry, University of Sheffield, UK

2. Centre for Immunobiology, Blizard Institute, Queen Mary University of London, UK

3. GlaxoSmithKline Consumer Healthcare, Weybridge, UK

Introduction:

Chronic periodontitis affects over 700 million people worldwide, developing as the result of inappropriate host response to the normal microbiota in susceptible individuals. Periodontitis causes a change in periodontal microbiota composition to a dysbiotic one where particular Gram-negative anaerobes may provoke further host inflammation. *In-vitro* oral multi-species biofilm models are published to better understand the disease but almost all are inoculated with ten or fewer bacterial species and often lack fastidious oral and other spp. The aim of this project is to create an *in-vitro* biofilm model more representative of the *in-vivo* periodontal pocket microbial community that we can then use to address the question of how microbial glycobiology influences microbial community structure.

Methods: To address this, a literature search of 63 papers was conducted to determine the oral bacteria and potential media relevant to periodontitis development.

Results & Conclusion: 18 bacterial strains were selected for designing a model community which included early colonisers such as *S. gordonii* and *A. oris*, as well as the anaerobes: *P. gingivalis*, *T. denticola* and *T. forsythia*, alongside less often considered spp. such as *Filifactor alocis* and several *Campylobacter* and *Prevotella* spp.

Putative media for the model and primers specific for each bacterial strain have also been established and begun to be tested with initial data presented here. These 18 bacterial strains are being grown together in a multi-species biofilm model and qPCR assays run to quantitatively detect the strains present in biofilms harvested from the model. This work is a prelude to biofilms directly grown from sub-gingival periodontal plaque, for comparing perturbations to this model and the 'wild' community to establish robustness of findings.

Consequently, this project will provide a tool for better understanding the community dynamics occurring in biofilm maturation that could lead to novel oral disease interventions.

An investigation into the role of WFDC2 in the oral cavity and respiratory tract

HJ Armes¹, CD Bingle² and L Bingle¹.

¹ *Academic Unit of Oral and Maxillofacial Pathology, School of Clinical Dentistry, the University of Sheffield.*

² *Department of Infection, Immunity & Cardiovascular Disease, the Medical School, the University of Sheffield.*

Introduction

WAP four-disulfide core protein 2 (WFDC2) is a small secretory glycoprotein that is used clinically as a biomarker for ovarian cancer. The expression of WFDC2 is also elevated in other malignancies and benign conditions including cystic fibrosis. In healthy tissues WFDC2 is most abundant within the oral cavity and respiratory tract. Little is known about the functions of WFDC2 in healthy or diseased tissues, however structural similarities to other family members suggests that it may play a role in innate immunity. Our aim is to further elucidate the role of WFDC2 in healthy and malignant tissues of the oral cavity and respiratory tract.

Methods

We have cloned human and murine WFDC2 and produced recombinant proteins. To determine whether WFDC2 exhibits antimicrobial activity we used several standard bacterial assays to analyse the binding, bactericidal and agglutination capacity of WFDC2 against oral (*S.gordonii*, *S.mutans*) and respiratory (*S.pneumoniae*) pathogens. Western blotting and ELISA were used to quantify WFDC2 in human secretions. Standard protease inhibitory assays were used to determine whether WFDC2 is capable of inhibiting serine proteases.

Results

Our results show that, although WFDC2 is secreted into saliva and bronchoalveolar lavage fluid (BALF), neither human or mouse orthologues can kill the bacteria found in the oral cavity/respiratory tract. Murine WFDC2 can adhere to *Streptococcus* strains via its O-glycans. Interestingly, *S.gordonii* seems to harvest N-glycans from human WFDC2. Additionally, WFDC2 does not inhibit serine protease activity.

Conclusion

These studies show that WFDC2 is secreted into saliva and BALF but is not involved in bacterial killing at these sites. Murine WFDC2 is capable of binding to *Streptococci* however the outcome of this interaction is unclear. WFDC2 is also unable to inhibit serine proteases. Our data suggests that although related proteins SLPI and elafin have antimicrobial and anti-protease activity, WFDC2 appears to exhibit distinct and, as yet, unknown functions.

Investigation of novel virulence factors and receptors for *P. gingivalis* host-pathogen interactions

Ashley Gains, Prachi Stafford, Daniel Lambert, Graham Stafford

School of Clinical Dentistry, University of Sheffield, UK

Introduction: Periodontitis is a chronic inflammatory disease that affects the supporting structures of the tooth, and is linked to cardiovascular disease and arthritis. It is a polymicrobial disease, characterised by the growth of a dysbiotic pathogenic community. Within this community *Porphyromonas gingivalis* is considered a keystone pathogen influencing dysbiosis. Previous studies in the lab identified a set of genes implicated in interactions with oral epithelial cells. One of these is the outer membrane OmpA2 protein, which we showed plays a significant role in the virulence of *P. gingivalis*. Specifically, one of four loops on the outer surface of OmpA2 is involved in *P. gingivalis* host-pathogen interactions with oral epithelial cells. The objective of this study is to identify the receptor(s) that the protein loop binds to on the cell surface, in order to investigate the mechanism further. In parallel we will also investigate the influence of several more of the invasive gene set.

Methods: The receptor will be isolated and purified by a biotin pulldown assay using biotinylated OmpA2 peptide loops; the receptor-peptide complex will then be purified using streptavidin resin and isolated using SDS-PAGE before identification through mass spectrometry fingerprinting. In parallel, a set of candidate invasive genes are being targeted by knockout mutagenesis.

Results & conclusions: Preliminary work with the peptides and their recombinant production will be presented alongside preliminary experiments with newly created invasive gene set mutants of *P. gingivalis*. Once identified, the work will shed new light on the mechanisms involved in host-pathogen interactions of *P. gingivalis*. The role of the receptor in the invasion mechanism of *P. gingivalis* will be further investigated, e.g. to determine the effect of inhibiting the receptor during *P. gingivalis* invasion. This could potentially provide a future therapeutic treatment for patients suffering with periodontitis.

A scoping review on bio-aerosols in healthcare and the dental environment

Charifa Zemouri, Hans de Soet, Wim Crielaard, Alexa Laheij.

Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam, University of Amsterdam & Vrije Universiteit Amsterdam, Amsterdam, The Netherlands.

Introduction

Bio-aerosols originate from different sources and their potentially pathogenic nature may form a hazard to healthcare workers and patients. This study aimed to review the bio-aerosol generating sources, the microbial load and composition, the potential hazard posed by pathogenic micro-organisms.

Methods

Systematic scoping review design. Searched in PubMed and EMBASE from inception to 09-03-2016. References were screened and selected based on abstract and full text according to eligibility criteria. Full text articles were assessed for inclusion and summarized. The results are presented in three separate objectives and summarized for an overview of evidence.

Results

The search yielded 5,823 studies, of which 62 were included. Dental hand pieces were found to generate aerosols in the dental settings. Another 30 sources from human activities, interventions and daily cleaning performances in the hospital also generate aerosols. Fifty-five bacterial species, 45 fungi genera and ten viruses were identified in a hospital setting and 16 bacterial and 23 fungal species in the dental environment. Patients with certain risk factors had a higher chance to acquire *Legionella* in hospitals. Such infections can lead to irreversible septic shock and death. Only a few studies found that bio-aerosol generating procedures resulted in transmission of infectious diseases or allergic reactions.

Conclusion

Bio-aerosols are generated via multiple sources such as different interventions, instruments and human activity. Bio-aerosols compositions reported are heterogeneous in their microbiological composition dependent on the setting and methodology. *Legionella* species were found to be a bio-aerosol dependent hazard to elderly and patients with respiratory complaints. But all aerosols can be hazardous to both patients and healthcare workers.

Enrichment of innate lymphoid cell populations in murine gingival tissue

Jason L Brown^{1,2}, Lauren Campbell², Jennifer Malcolm², Ana Adrados Planell², John Butcher^{1,3}, Shauna Culshaw²

¹ *Institute of Biomedical and Environmental Health Research, University, School of Science and Sport, University of the West of Scotland, Paisley, UK, PA1 2BE*

² *Institute of Infection, Immunity, and Inflammation and Oral Sciences Research Group, Glasgow Dental School, School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK, G12 8TA*

³ *Department of Life Sciences, School of Health and Life Sciences, Glasgow Caledonian University, Glasgow, UK, G4 0BA*

Email Address; jason.brown@uws.ac.uk

Introduction

Innate lymphoid cells (ILCs) are lymphocytes that act as the first line of immunological defence at mucosal surfaces such as the gut, lungs and the skin. ILCs are divided into three subsets (ILC1s, ILC2s, ILC3s), each with different functions and cytokine profiles. Interferon- γ producing ILCs (ILC1s) have been identified in the human gingivae, but an identification of ILCs in the murine oral mucosa, to our knowledge, is restricted to IL-17+ ILC3s. Here, we provide a detailed appraisal of the whole ILC population (group 1, 2 and 3 subsets) in the murine gingivae and the regional lymph nodes (dLNs) draining the oral cavity.

Methods

Oral dLNs and gingivae were harvested from mice and processed/digested to obtain single cell suspensions, which were subsequently stained with antibodies for identification of ILCs by flow cytometry. For cytokine profiling of ILCs, cells were stimulated with phorbol-myristate acetate and ionomycin/GolgiPlug prior to staining.

Results

We show that ILCs made up a greater percentage of the whole CD45+ lymphocyte population in the murine gingivae than in the oral dLNs ($0.356 \pm 0.039\%$ vs. $0.158 \pm 0.005\%$, $p < 0.001$). The gingivae-resident ILCs were more diverse than the oral dLNs, with a significantly greater proportion of CD117+, NKp46- ILCs (35.19 ± 3.84 compared to $5.03 \pm 0.69\%$, $p < 0.0001$). The cytokine profile of ILCs in the gingivae also differed from the oral dLNs; there was a relatively similar proportion of IFN- γ + and IL-5+ ILCs in the murine gingivae, whereas IL-5+ ILCs predominately populated the oral dLNs.

Conclusion

The function of ILCs in the oral cavity is currently unknown; here, we demonstrate that the ILC compartment is enriched, more diverse, and has a different cytokine profile at the gingival mucosal surface compared to the oral dLNs. Future work investigating inflammatory oral diseases using mouse models may merit consideration of these ILC populations.

SESSION 4

Understanding biofilms 3

Environments mimicking the periodontal pocket select pathogenic bacterial communities

Monika Naginyte, **Thuy Do**, Josephine Meade, Deirdre Devine and Phillip Marsh

Division of Oral Biology, School of Dentistry, University of Leeds, Leeds, U.K.

Background: Periodontal disease occurs as a consequence of changes in the resident oral microbiota and host responses to them. Environmental changes in the periodontal pocket arise through increased inflammation and are commonly associated with the enrichment of pathogenic bacteria.

Methods: Pooled saliva, supragingival and tongue plaque samples were inoculated into Calgary biofilm devices, grown in basal media ± serum and biofilms were harvested after one and three weeks. DNA was isolated and metagenomes were sequenced using Illumina HiSeq3000. Taxonomy and functional analyses were carried out. Alpha diversity and weighted and unweighted UniFrac distances were compared. Significantly abundant species and their genes were evaluated using DESeq2. Variance stabilizing transformation was applied to compare relative abundance of species.

Results: After three weeks of enrichment, there was a major shift from an inoculum containing high proportions of *Streptococcus*, *Haemophilus*, *Neisseria*, *Veillonella* and *Prevotella* species to biofilms harbouring taxa implicated in periodontitis, such as *Porphyromonas gingivalis*, *Fretibacterium fastidiosum*, *Filifactor alocis*, *Tannerella forsythia*, and several *Peptostreptococcus* and *Treponema* spp., with a concomitant decrease in health associated species. Sixty-five species were found in biofilms after enrichment that could not be detected in the inoculum, including *Jonquetella anthropi*, *Desulfovibrio desulfuricans* and *Dialister invisus*. Some species were enriched preferentially in the presence (e.g. *P. gingivalis* and *F. alocis*) or absence (e.g. *F. fastidiosum*) of serum. Following enrichment, the abundance of genes representing carbohydrate metabolism decreased while genes implicated in virulence and amino acid metabolism were increased.

Conclusion: While richness and diversity decreased over culturing time, phylogenetic variation between communities was conserved. Biofilms cultured in medium with serum became enriched with periodontitis associated bacteria.

Beneficial influence of microcosm biofilm on reconstructed human gingiva

Lin Shang¹, Dongmei Deng¹, Jeroen Kees Buskermolen², Marleen Marga Janus¹, Bastiaan Philip Krom¹, Sanne Roffel², Taco Waaijman³, Cor van Loveren¹, Wim Crielaard¹, Susan Gibbs^{2,3}

1) *Department of Preventive Dentistry, 2) Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, Amsterdam, The Netherlands*

3) *Department of Dermatology, VU University Medical Centre, Amsterdam, The Netherlands*

Introduction: Oral mucosa actively recognizes and responds to microbes. Microbial exposure can modulate a broad diversity of protective responses which are beneficial to the host. However, conventional *in vitro* models cannot provide sufficient evidence as they lack some representative native features. Therefore, to represent the complexity of the *in vivo* situation, we investigated the influence of a multi-species microcosm biofilm on a reconstructed human gingiva (RHG) model during a seven day co-culture period.

Methods: The following parameters were compared between microcosm-exposed and unexposed RHGs (Day 1, 2, 4 or 7). Epithelium thickness, cellular proliferation (PCNA, Ki-67) and antimicrobial peptide Elafin were investigated by immunohistochemical staining. The secretion of cytokines was assessed by ELISA. The presence and viability of microcosm was detected by FISH staining and viable bacterial cell counts.

Results: In the microcosm-exposed RHG, a epithelium which more closely represented native tissue was developed. The epithelium was thickened, and highly proliferative as shown by PCNA and Ki-67. Elafin was strongly expressed in upper epithelium where host-microbe interactions begin. While unexposed RHG remained thin, readily senesced and expressed low Elafin. Increased secretion of IL-6, CXCL1, CXCL8, CCL20 was observed in microcosm-exposed RHGs. Although FISH showed the presence of microcosm, a rapid decrease of viable microbes was revealed by counting.

Conclusion: We showed that the exposure to microcosm beneficially stimulates the RHG to become more representative of healthy native gingiva and contributes to regulating its inflammatory and antimicrobial properties, thus increasing the resistance of gingiva to potential pathogens.

Development of an Adaptable, Reproducible Model for Dental Plaque

M. Davrandi¹, J. Pattem², L. Bozec², E. Allan¹, D. Spratt¹

¹Department of Microbial Diseases, UCL Eastman Dental Institute, London, UK

²Department of Biomaterials and Tissue Engineering, UCL Eastman Dental Institute, London, UK

Objectives: The aim of this study was to develop a high throughput microcosm dental plaque biofilm model to investigate the bacterial composition and matrix constituents.

Methods: A fed batch culture approach utilising 96-well MBEC™ plates, with hydroxyapatite coated pegs, was developed to grow dental plaque biofilms. Pooled human saliva was inoculated in artificial saliva, and brain heart infusion broth containing 2.5 g/L mucin (BHIM). Plates were incubated at 37°C in 5% CO₂ for 120 h, and pulsed with 5% sucrose in respective growth medium 3 times daily for 30 min. pH of the spent media was recorded following every media change. Biofilm development was assessed by viable counts over 120 h, and 16S rRNA sequencing (MiSeq) was used to characterise the biofilm community. Matrix composition was analysed by determination of water-soluble carbohydrate (WSC) and total protein (TP).

Results: The total viable counts of aerobes and anaerobes increased then subsequently plateaued over time in all biofilm samples. By 120 h, the number of viable bacteria ranged between 7.9 - 8.0 log₁₀CFU, and there was no significant difference between two growth media. The pH of the artificial saliva was maintained above critical pH (5.5) for 96h, while the pH of BHIM dropped below 5.5 on day 2. The 16S analysis was used to determine the richness and diversity of the community and its relevance to human plaque. The amount of biofilm matrix constituents, WSC and TP, per viable cell in all biofilm samples increased with time and with increasing carbon source.

Conclusions: Here we show that 96-well MBEC™ plates can be successfully employed to produce dental plaque biofilms *in vitro*, and to quantify matrix components, in a reproducible manner. Although, growth conditions require further optimisation to make biofilm communities more representative of real plaque.

SESSION 5

Antimicrobials

Development of a Three-Dimensional Collagen Model for the *In Vitro* Evaluation of Osteogenesis for Dental Prostheses

Rebecca Zhiyu Yuan¹, Kaveh Memarzadeh¹, Abish Stephen¹, Robert Allaker¹ and Jie Huang²

1. *Barts & The London School of Medicine and Dentistry, Queen Mary University of London*

2. *Department of Mechanical Engineering, University College London*

Introduction: An ideal dental implant/prosthesis should allow bone growth on its surface without development of infection and immune / inflammatory responses. For early bone formation to occur, the conditions surrounding the implant should encourage osteoblast attachment and proliferation. In this study, osteoblasts and magnetic iron oxide nanoparticles (IONPs) which are known to be antimicrobial, were incorporated into plastic compressed (PC) collagen gels to produce a range of cell-laden models to study the effect of magnetic stimulation (SMFs) on osteogenesis. The influence of a magnetic field on cell proliferation, differentiation, mineralization and gene expression was evaluated.

Methods: A 3D collagen model was constructed by using plastic compression. IONPs and MG-63 cell line (Homo sapiens, osteosarcoma, ATCC) were seeded inside the collagen matrix with a density of 10,000 cells/ml. A magnetic bio-reactor was designed by ANSYS Maxwell and 3D printed. The alamar blue assay, alkaline phosphatase production and alizarin red s (ARS) staining and extraction were used to measure cell proliferation, differentiation, and mineralization, respectively. PCR was conducted to evaluate the expression of Runx2, BMP-2 and BMP-4 genes. TEM and histology were used to investigate the microstructure of the 3D model.

Results: Results demonstrated that SMFs and IONPs can stimulate the expression of Runx-2, BMP-2 and BMP-4 genes in collagen matrix ($p < 0.01$; day 14), hence accelerating cell proliferation ($p < 0.0001$; day 14), differentiation ($p < 0.0001$; day 21) and mineralization ($p < 0.001$, day 21). Histology and TEM images demonstrated the microstructure of the cell-laded 3D model, with fibrous, lamella collagen structure and healthy attached cells. Results from current study indicate that the combination SMFs and IONPs can enhance the osteogenesis process of MG-63 cells when embedded in 3D collagen matrix without an unwanted inflammatory response.

Conclusions: Plastic compressed collagen hydrogels were used as 3D bio-mimetic models with dense, cellular and mechanically strong native structures to study the effects of magnetic stimulation on osteogenesis, which paves the way for further applications in dentistry.

Silica particles as a Drug Delivery System for Antimicrobial Agents in Dental Applications

M. Manhota^{1,2}, N. Vyas^{1,3}, S. Claire^{1,2}, G. Li², Z. Pikramenou^{1,2}, A.D. Walmsley^{1,3}, S.A. Kuehne³, R.L. Sammons³ and Hamid Dehghani^{1,4}

1. *EPSRC Centre for Doctoral Training in Physical Sciences for Health, University of Birmingham*
2. *School of Chemistry, University of Birmingham*
3. *School of Dentistry, University of Birmingham*
4. *School of Computer Science, University of Birmingham*

Introduction: Dental caries is a global health problem, affecting an estimated 90 % of the world population at least once in their lifetime. The disease develops from the natural build-up of plaque to form biofilms that adhere to the non-shedding tooth surface. The plaque contains cariogenic bacteria, most commonly *Streptococcus mutants* and *Lactobacillus species*, responsible for fermenting dietary carbohydrates into organic acids. This results in the demineralisation and destruction of hard tissue of the tooth, enamel and dentine.

The tooth structures contain dentinal tubules, approximately 1 to 4 microns in size that can become exposed, allowing bacteria to invade. Thus, leading to infection, pain and potential tooth loss. The elimination of bacteria from dentinal tubules is therefore essential to treat caries and can be achieved with antimicrobial agents.

The aim of this study is to investigate different sizes of silica particles for occlusion and penetration of dentinal tubules and their use to deliver antimicrobial agents with controlled release.

Method: 1) Functionalised silica sub-micron particles were synthesised and encapsulated with ruthenium luminescent complex and coated with different surfactants and applied to a dentine slice surface. 2) The particles were delivered using cavitation from ultrasonic scalars and examined using imaging techniques: scanning electron microscopy (SEM) and confocal fluorescence microscopy. 3) Mesoporous silica nanoparticles (MSN's) were synthesised with an antibacterial agent and evaluated for their bactericidal activity against *Streptococcus sanguinis*.

Results: Qualitative observations from different imaging techniques showed the particles to occlude the dentinal tubules and penetrate inside the tubules up to twice the distance than without cavitation. Antimicrobial agent release from MSN's was shown to be controlled in both analytical and bacterial studies.

Conclusion:

The ability to occlude tubules can be achieved with silica sub-micron particles, which is dependent on surface coating. Additionally, ultrasonic scalar produces cavitation bubbles, which have been shown to deliver these particles to the dentinal tubules and have the potential for deeper penetration. Finally, the antimicrobial agent showed a controlled release from MSN's and was effective killing all the bacteria. Therefore, delivery of an antimicrobial agent to the dentinal tubules looks promising.