14th European
Oral Microbiology
Workshop 2024

Book of abstracts

GENERALITAT VALENCIANA

Fundació Fisabio

OralMicrobiomeLab

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Welcome

Dear colleagues,

It is a great honor and pleasure to invite you to the 14th edition of the European Oral Microbiology Workshop to be held in Valencia (Spain), in May 2024. It will be 3 years since our last meeting (which had to be celebrated online due to the pandemic) and 7 years since the last face-to-face meeting in Stockholm in 2017. We know that everybody is very excited to meet again in person, present our latest data, discuss the current understanding of the field, and identify future challenges and the corresponding approaches to address them.

We will keep the philosophy of previous EOMW meetings: A reduced number of participants with a **single session** that everybody can attend; talks programmed to allow sufficient time to present and for questions; lunch, dinner, and sessions in the same venue, to allow and facilitate intense **scientific interactions;** a relaxed, out-of-the-crowd venue in the countryside, providing an inspiring atmosphere that allows plenty of social interactions; a relaxed and long enough poster session where everybody can attend; an all-included registration where accommodation, fees, dinner, and special events are included.

We are aware that due to current inflation in most Western countries, the cost of conferences has dramatically increased. We are therefore making an effort to reduce the cost by applying for international (FEMS organization) and national (Valencian Region Congress Funding) grants to help oral microbiologists to attend, and a special fund for early career scientists has been applied for. Contacts with different companies are also being held to apply for support, as well as with local authorities.

A big thank you from all organizing committee members for trusting us to organize this special event, and looking forward to meeting you all again in 2024!

Alex Mira

Oral Microbiome Laboratory, FISABIO Foundation

Organizing Committee

Alex Mira

Maria D. Ferrer

Miguel Carda-Diéguez

Paula Corell

Sandra García

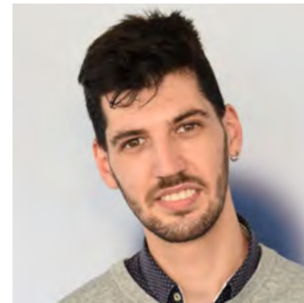
Ana Adrados

Ainhoa Revilla

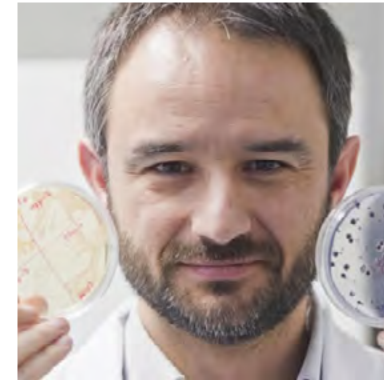
Bob T Rosier

Migle Ziemyte

Elena Buetas


Oscar Climent

Scientific Committee


Alex Mira
FISABIO Foundation, Valencia

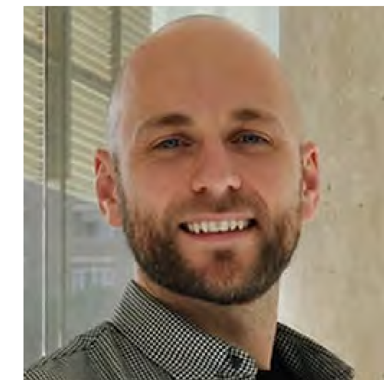
Maria D Ferrer
FISABIO Foundation, Valencia

Miguel Carda-Diéguez
FISABIO Foundation, Valencia

Mike Curtis
Professor at King's College London, London

Georgios Belibasakis
Karolinska Institutet, Stockholm

Egija Zaura
Professor at the Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam


Bob Rosier
FISABIO Foundation, Valencia

Keynote Speakers

Philip D. Marsh
Professor Emeritus at University of Leeds

William Wade
Professor at King's College London

Mike Curtis
Professor at King's College London

Wim Teughels
Professor at Catholic University of Leuven (KU Leuven)

Alex Mira
Group leader of Oral Microbiome Lab, FISABIO Foundation

Bastiaan Krom
Professor at Academic Centre for Dentistry Amsterdam (ACTA)

Egija Zaura
Professor at the Academic Centre for Dentistry Amsterdam (ACTA)

TOPICS FOR DISCUSSION AND MODERATORS

Session 1 - **Oral Biofilms**

Michel Hoogenkamp (ACTA, The Netherlands)
Joseph Aduse-Opoku (Kings College, London)

Session 2 - **Microbial pathogenesis of oral diseases**

Shauna Culshaw (University of Glasgow)
Ana Adrados (FISABIO Foundation, Valencia)

Session 3 - **Modulation of the oral microbiome**

Georg Conrads (Aachen University Hospital, Germany)
Ainhoa Revilla (FISABIO, Valencia)

Session 4 **- Host-Microbial interactions**

Mike Curtis (Kings College, London)
Maria D. Ferrer (FISABIO Foundation, Valencia)

Session 5 **- Systemics effects of oral diseases**

Gary Moran (Trinity College, Dublin)
Bob T Rosier (FISABIO Foundation, Valencia)

Session 6 **- Oral homeostasis and dysbiosis**

Julia Davies (University of Malmö, Sweden)
Wannes van Holm (KU Leuven)

Session 7 **- Novel concepts**

Jessica Mark-Welch (ADA Forsyth Institute, Boston)
Tabita Ramirez-Puebla (ADA Forsyth Institute, Boston)

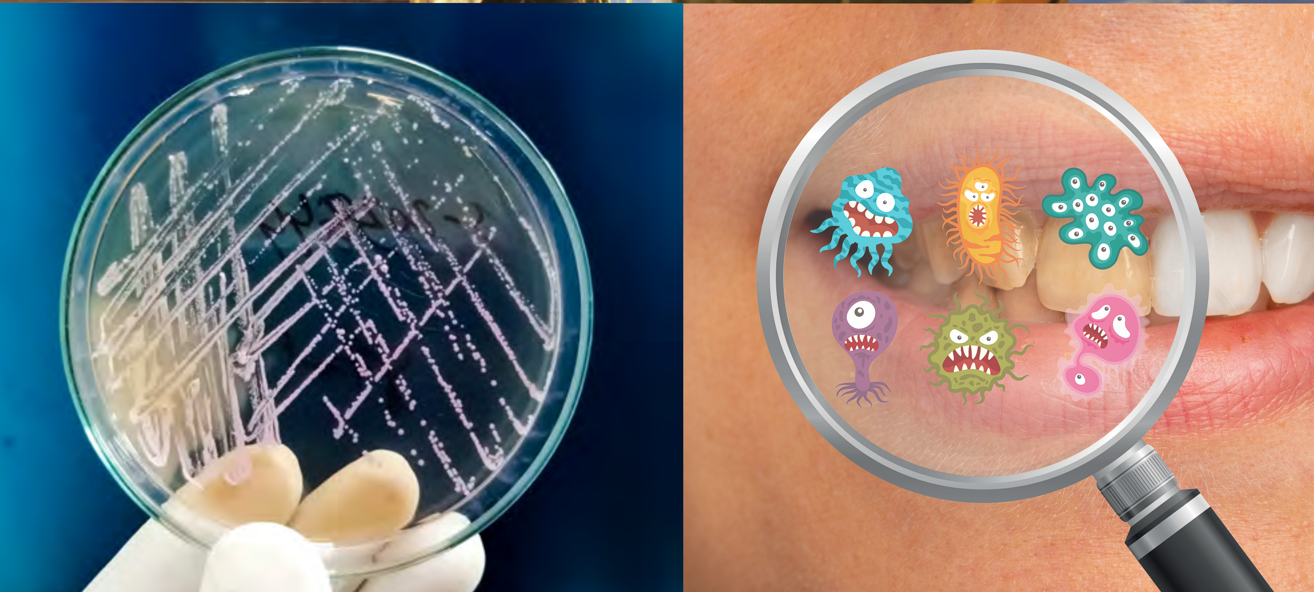
Session 8 **- Novel treatments**

Thuy Do (University of Leeds)
Jessica Neilands (Probi AB, Sweden)

Session 9 **- (Tribute Session)**

Alex Mira (FISABIO Foundation, Valencia)

PROGRAM



Tuesday, 28th May

19:00	Overview of recent advances in Oral Microbiology. **Alex Mira.** ***FISABIO Foundation*** Welcome and general instructions
20:00	**Welcome reception** at Parador El Saler Hotel

Wednesday, 29th May

8:30	**KEYNOTE SPEAKER. Bastiaan Krom.** ***Academic Centre for Dentistry Amsterdam, ACTA.*** Fungi, conductors of oral health and disease.
9:30- 11:00	Session 1 **Oral Biofilms** **Chairs** **Michel Hoogenkamp (ACTA, The Netherlands)** **Joseph Aduse-Opoku (Kings College, London):**
9:30	**Micron-scale spatial patterns in the tongue Dorsum Consortia**. Shamayim Tabita Ramirez Puebla. *ADA Forsyth Institute*
9:45	**Effects of post-surgical rinsing with chlorhexidine mouthwash on the oral microbial composition and the prevalence of antimicrobial resistance genes**. Cieplik Fabian. *University of Freiburg*
10:00	**Have we given up on cultivating new oral bacteria?** Ali Al-Ahmad. *University of Freiburg*
10:15	**Enhanced endodontic infection treatment using chlorhexidine-loaded self-propelled nanoparticles.** Miglė Žiemytė. *FISABIO Foundation*
10:30	**Functional characterization of a sialidase protein from Capnocytophaga ochracea.** Jing He. *The University of Hong Kong*
10:45	**Inhibitory effect of common plant flavonoid cyanidin on dental biofilm.** Viktoriya Shyp. *University of Basel*
11:00	**Coffe break**
11:30 - 13:00	Session 2 **Microbial pathogenesis of oral diseases** **Chairs** **Shauna Culshaw (University of Glasgow)** **Ana Adrados (FISABIO Foundation, Valencia)**
11:30	**Novel regulation of a membrane embedded phosphatase linked to outer membrane vesicle production in Porphyromonas gingivalis.** Yichao Liu. *King's College London*
11:45	**Fusobacterium nucleatum from malignant and potentially malignant oral disease show high levels of genetic variation, recombination and inflammatory microRNA induction**. Claire Crowley. *Trinity College Dublin*

12:00	**Plaque metagenomics on 1,157 dental implant patients reveals new microbial biomarkers of peri-implant diseases.** Vitor Heidrich. *University of Trento*
12:15	**Microbial dysbiosis and increased abundance of oral pathogenic bacteria in the intratumoral environment of oral Squamous Cell Carcinoma.** Hyeunjeong Song. *Seoul National University*
12:30	**Synergies between periodontal pathogens in the oral cavity and beyond.** Elena Buetas Giménez. *FISABIO Foundation*
12:45	**Communication is key – roles of c-di-AMP signalling in Fusobacterium nucleatum virulence.** Sarah Kuehne. *Nottingham Trent university*
13:00	**Lunch**
14:30	**KEYNOTE SPEAKER. Wim Teughels. *Catholic University of Leuven (KU Leuven).*** Interacting with microbiomes: bringing oral microbiology back to the dental chair.
15:30-17:00	Session 3 **Modulation of the oral microbiome Chairs** **Georg Conrads (Aachen University Hospital, Germany)** **Ainhoa Revilla (FISABIO Foundation, Valencia)**
15:30	**Antibiotic resistance genes in the saliva of dental students-A pilot study.** Marwan Mansoor Mohammed. *University of Sharjah*
15:45	**Community metaproteomics to study the functional response of the oral microbiome.** Chandra Lekha Ramalingam Veena. *The University of Sheffield*
16:00	**Oral biofilm composition, dissemination, and inflammation depend on probiotic and synbiotic strain-specificity.** Wannes Van Holm. *KU Leuven*
16:15	**The influence of probiotics on the pH value in oral biofilm formation.** Elisabeth Reichardt. *Aarhus University*
16:30	**Modelling eubiosis and dysbiosis in a multispecies oral consortium.** Madeleine Blomqvist. *Malmö university*
16:45	**Effect of dietary nitrate supplementation on the oral bacterial community in patients with stable angina.** William Wade. *King's College London*
17:00-18:30	**Poster session** + refreshments

20:00 **Dinner** - at Parador El Saler Hotel

Thursday, 30th May

8:30	**KEYNOTE SPEAKER. Mike Curtis. *King's College London.*** The commensal microbiota is required for development of the host chemosensory systems
9:30-11:00	Session 4 **Host-Microbial interactions Chairs** **Mike Curtis (Kings College, London)** **Maria D. Ferrer (FISABIO Foundation, Valencia)**
9:30	**Disrupted gut resolution mechanisms in inflammatory arthritis facilitate pathogenic behaviour of the oral pathobiont Porphyromonas gingivalis.** Magdalena Flak. *King's College London*
9:45	**Microbiome - host inflammatory cell interactions in the subgingival environment.** Julia Davies. *Malmö University*
10:00	**Oral microbiota contribution to the maintenance of essential amino acid balance in human physiology.** Ana Adrados Planell. *FISABIO Foundation*
10:15	**Investigating the association between hypertension and nitrate-reducing activity of oral bacteria.** Amazon Doble. *University of Plymouth*
10:30	**Characterisation of novel murine Streptococcal species.** Joseph Aduse-Opoku. *King's College London*
10:45	**Changes in oral microbiota and markers of inflammation following the use of a polyphenol-containing dentifrice.** Teresa Vinuesa. *University of Sheffield* University of Barcelona
11:00	**Coffe break**
11:30-13:00	Session 5 **Systemics effects of oral diseases Chairs** **Gary Moran (Trinity College, Dublin)** **Bob T Rosier (FISABIO Foundation, Valencia)**
11:30	**Supporting the management of periodontal diseases – probiotics may alleviate inflammation and modify oral microbiota.** Krista Salli. *IFF Health/Danisco Sweeteners Oy*
11:45	**The oral-gut axis: salivary and fecal dysbiosis in inflammatory bowel diseases (IBD).** Georg Conrads. *RWTH Aachen University*
12:00	**Exploring the subgingival microbial signature in individuals at risk of rheumatoid arthritis.** Thuy Do. *University of Leeds*
12:15	**Nitrate reduction capacity of the oral microbiota is impaired in periodontitis: implications for oral and systemic health.** Bob Rosier. *FISABIO Foundation*
12:30	**Aggregatibacter actinomycetemcomitans and its leukotoxin A in collagen-induced arthritis in mice.** Zijian Cheng. *Zhejiang University*
13:00	**Lunch**

14:30	**KEYNOTE SPEAKER. Egija Zaura. *Academic Centre for Dentistry Amsterdam (ACTA).*** Reflection on the current understanding of the oral microbial homeostasis
15:30-17:00	Session 6 **Oral homeostasis and dysbiosis** **Chairs** **Julia Davies (University of Malmö, Sweden)** **Wannes van Holm (KU Leuven)**
15:30	**Comparative analysis of the oral resistome in health and disease: insights from publicly available sequencing data**. Jack Lynch. *University of Leeds*
15:45	**The effect of menopause on the activity of oral nitrate-reducing bacteria.** Katie Muddiman. *University of Plymouth*
16:00	**Examination of the acetaldehyde generating capacity of Rothia mucilaginosa recovered from the oral microbiome of patients with potentially malignant lesions.** Gary Moran. *Dublin Dental Hospital*
16:15	**The effect of different antibacterial compounds on the nitrate-reducing activity of oral bacteria.** Lisa Anne du Toit. *University of Plymouth*
16:30	**Associations between exogenous and intrinsic host factors and tongue microbiota in a healthy population.** Marije Kaan. *ACTA*
16:45	**Evaluating the Impact of Antiseptic Mouth Rinses on Oral Biofilm Resilience.** Naiera Zayed. *KU Leuven*

19:00	Bus departure from Parador El Saler
19:15	Bus departure from Devesa Gardens Resort
19:30	**Albufera Lagoon** **boat ride**
20:00	**Dinner** at Albufera Natural Parc

Friday, 31st May

8:30-10:00	Session 7 **Novel concepts** **Chairs** **Jessica Mark-Welch (ADA Forsyth Institute, Boston)** **Tabita Ramirez-Puebla (ADA Forsyth Institute, Boston)**
8:30	**Standardized disk diffusion method for antimicrobial susceptibility testing of Aggregatibacter actinomycetemcomitans.** Anne Birkeholm Jensen. *Aarhus University*
8:45	**Oral fibroblasts display metabolic changes after exposure to bacterial extracellular vesicles from Porphyromonas gingivalis.** Helene Rygvold Haugsten. *University of Oslo*
9:00	**Together they are stronger. The importance of bacterial clusters.** Miguel Carda Diéguez. *FISABIO Foundation*
9:15	**Strain-level complexity in the oral microbiome at micrometer scales.** Jessica Mark Welch. *ADA Forsyth Institute*
9:30	**The Salivary Microbiome as a Diagnostic Biomarker of Periodontitis: a 16S Multi-Batch Study before and after Removal of Batch Effects.** Alba Regueira Iglesias. *Fundación Instituto de Investigación Sanitaria de Santiago de Compostela (FIDIS)*
9:45	**Developing a 3-dimensional oral epithelium model for evaluating antimicrobial and immune responses to dental implant materials.** Jon Vernon. *University of Leeds*
10:00-11:30	Session 8 **Novel treatments** **Chairs** **Thuy Do (University of Leeds)** **Jessica Neilands (Probi AB, Sweden)**
10:00	**Streptococcus dentisani as a new source of antibiotics to fight the antimicrobial resistance crisis: focus in the probiotic strain 7746.** Ainhoa Revilla. *FISABIO Foundation*
10:15	**Disulfiram as a novel antimicrobial and immune-modulatory agent to treat periodontal diseases via targeting Porphyromonas gingivalis.** Qin Hu. *The University of Hong Kong*
10:30	**The Microbiological Quality of Dental Unit Water is Reaching which Limit?** Michel Hoogenkamp. *ACTA*
10:45	**Personalized antibiotic selection in periodontal treatment improves clinical and microbiological outputs.** María Desamparados Ferrer García. *FISABIO Foundation*
11:00	**Bridging the Gap: Translating Electrolyzed Saline from in vitro Efficacy to Clinical Application in Dysbiosis Prevention.** Haris Munjakovic. *University Medical Centre Ljubljana*
11:15	**Surprises in the clinical, microbiological and inflammatory response to periodontal treatment.** Shauna Culshaw. University of Glasgow
11:30	**Coffe break**
12:00	**KEYNOTE SPEAKERS** **(Tribute Session)** Pending issues in oral microbiology **Phil Marsh. *Professor Emeritus at University of Leeds.*** **William Wade. *Professor at King's College London*** **Chair: Alex Mira (FISABIO Foundation, Valencia)**
13:30	**Farewell** **and final remarks** **Picnic** Lunch Box provided

POSTERS' LIST

1. Oral biofilms

P01. Oral Biofilm Composition and Phenotype in Caries-active and Healthy Children.

Gabriella Boisen, *Malmö University*

P02. Two Oligopeptidase B Homologues Contribute to the Elevated Trypsin-like Peptidolytic Activities of Capnocytophaga gingivalis.

Rory Munro Watt, *The University of Hong Kong*

2. Microbial pathogenesis of oral diseases

P03. Association between Streptococcus mutans detection and caries activity in oral microbiome studies: A systematic review and meta-analysis.

Dongmei Deng, *ACTA*

P04. Investigation of the microbiome of oral leukoplakia (OLK).

Gary Moran, *Dublin Dental Hospital*

P05. New Insights into Evolution and Diversity of Mayor Fimbriae (FimA) of the Oral Pathogen Porphyromonas gingivalis.

Josefa Nuñez-Belmar, *Universidad Mayor*

3. Modulation of the oral microbiome

P06. The effect of chlorhexidine and common antibiotics on the composition and nitrate metabolism of subgingival plaque an in vitro study.

María Nadal Ruiz, *FISABIO*

P07. *Porphyromonas gingivalis* long fimbriae superstructures and their role for targeted transport and release of extracellular vesicles

Georg Conrads, RWTH University Hospital

P08. Effect of a probiotic combination on oral Candida colonization in head and neck cancer patients undergoing radiotherapy: randomized clinical trial.

Tanya Valeria Pereira Riveros, Universidad de Barcelona

4. Host-microbial interactions

P09. 3D oral mucosa model to evaluate bacterial invasion.

Monika Astasov-Frauenhoffer, *University of Basel*

P10. Identification of New Biomarkers in Gingival Crevicular Fluid and Saliva for Diagnosing Periodontitis by SWATH-MS.

Triana Blanco Pintos, *Universidad de Santiago de Compostela*

P11. Immunomodulatory effects of oral streptococci in periodontal disease.

Zita Dinis Lopes da Silva, *Malmö University*

P12. Fitness costs and evolutionary dynamics of Tn916 in oral streptococcal isolates.

Tracy Lunde, *The Arctic University of Norway*

P13. Effects of MUC5B on early oral biofilm glucose metabolism.

Carolina Robertsson, *Malmö University*

P14. The influence of periodontal microbial biomarkers on reaching the endpoints of therapy.

Nils Werner, *University Hospital, LMU*

P15. Determining the Effect of Processed-grain or High-fiber Diets on Microbial and Immunological Markers of Periodontitis.

Lea Sedghi, *University of California*

5. Oral homeostasis and dysbiosis

P16. Citrulline as a potential prebiotic & synbiotic for dental caries.

Oscar Climent Soler, *FISABIO*

P17. Oral and intestinal microbiomes in patients with periodontitis: design and preliminary results of the first national study (Uruguay).

Bruno Manta, *Universidad de la Republica & Institut Pasteur Montevideo*

P18. Rapid Griess Assay a Chair-side Evaluation of Oral Nitrite Production.

Simeon Kosmas Bjørhovde Mavropoulos, *University of Gothenburg*

P19. Salivary microbiome and proteome in kidney transplant recipients up to 24-months after transplantation.

Benedita Sampaio Maia, *i3S-Investigação e Inovação em Saúde*

P20. Interactions of Nitrate-Reducing and Sulphur-Metabolising Bacteria: Implications for Oral Health.

Abish Stephen, *Queen Mary University of London*

6. Systematic effects of oral diseases

P21. Human endothelial cell cytokine and chemokine responses to oral anaerobe infection is species specific.

Antonia Cutts, *The University of Sheffield*

7. Novel concepts/methodologies in oral microbiology

P22. CellTrace - a Tool for Visualisation of Multi-Species Oral Biofilms.

Olivia Aherne, *Malmö University*

P23. Development of a bioinformatic pipeline for archaea detection in dental caries.

Naile Dame-Teixeira, *University of Brasilia*

P24. Comparative characterization analysis of two novel mucin-degrading proteases, MdpL and MdpS, from different oral bacteria.

Fredrik Leo, *Malmö University*

P25. Co-culture of Helicobacter pylori with oral microorganisms in human saliva.

Konstantin Johannes Scholz, *Faculty of Medicine, University of Freiburg*

P26. Oral Species with Highly Similar in-silico 16S rRNA Gene Amplicons: a Reason to Avoid OTU Clustering.

Inmaculada Tomás, *School of Medicine and Dentistry*

8. Novel treatments, including Infection Control in dentistry

P27. Hydrogel-delivered bismuth-doped carbon dots-based nanomedicine treats periodontal disease via antimicrobial & immuno-modulatory approaches.

Xuan Li, *The University of Hong Kong*

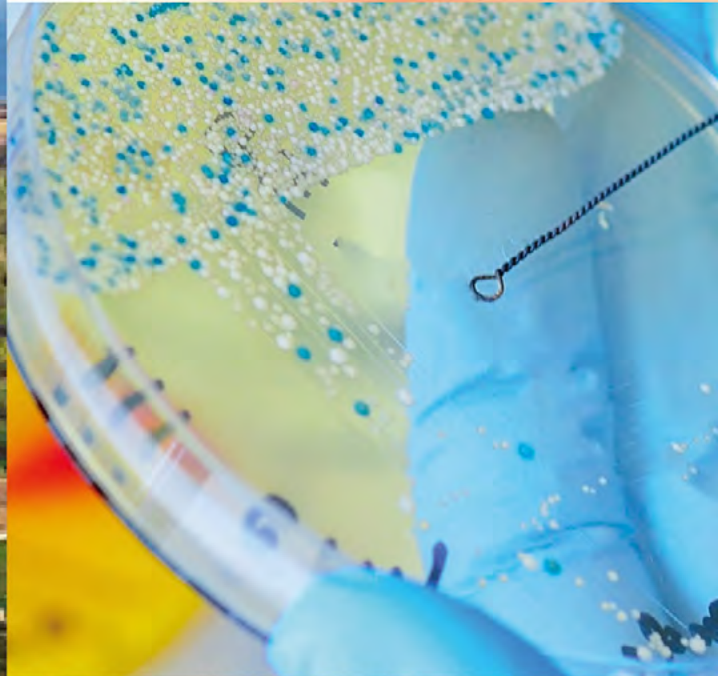
P28. Antimicrobial peptides with a double-glycine leader peptide from Streptococcus oralis subsp. *dentisani* strains.

Ainhoa Revilla, *Fisabio Foundation*

P29. New postbiotic to improve canine oral health.

Adrian Florit, *ADM-Biopolis*

ABSTRACTS



Overview of recent advances in Oral Microbiology

Alex Mira

Division of Head of the Oral Microbiome Laboratory, FISABIO Foundation, Department of Health and Genomics. Valencia, Spain.

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ABSTRACT

It has been three years since the last (on-line) EOMW meeting and seven years since the last face-to-face workshop. During this period, there have been fascinating advances in the field of oral microbiology. These include, among others, novel methodologies in *in vitro* systems that intend to mimic real conditions in the oral cavity; novel concepts about biofilm formation, disease etiology or prevention approaches; development of prebiotic and probiotic strategies to modulate the oral biofilm; identification of new systemic effects of the oral microbiome and some potential underlying mechanisms; and novel antibacterial treatments, such as nanoparticles. I will make a short introduction to some of these fascinating examples that will set up the framework for the different sessions of the conference. Finally, I will present some relevant features about the moment we are currently living in the field, its future prospects and the consequences they may entail for dental practice.

Keywords: *Porphyromonas gingivalis*, oral biofilm formation, *Rothia*, prebiotics, probiotics, systemic effects, nanoparticles, non-communicable disease, dysbiosis, salivary diagnosticS.

“Fungi, conductors of oral health and disease”

Bastiaan P. Krom

Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam

ABSTRACT

Oral fungi in health have remained elusive. With the onset of large-scale sequencing approaches, we are slowly starting to appreciate the function of fungi within the healthy oral ecosystem. Fungi like *Candida albicans* can create micro-environments within oral biofilms by influencing the external milieu. Here I will discuss their ability to actively increase the biofilm pH from acidic to near neutral and they rapidly lower oxygen tension. Through these, and other actions, *C. albicans*, and other fungi, direct the behavior of its bacterial neighbors and ultimately the composition of oral biofilms. Fungi could thus orchestrate oral biogeography affecting health and disease.

Micron-Scale Spatial Patterns in the Tongue Dorsum Consortia

Shamayim Tabita Ramirez-Puebla[1*], Jessica L. Mark Welch[1, 2], Julian Torres-Morales[1], Floyd E. Dewhirst[1], Gary G. Borisy[1]

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[2]Marine Biological Laboratory, Woods Hole, MA, USA
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Bacteria on the tongue dorsum (TD) form consortia tens to hundreds of microns in diameter organized around a core of epithelial cells. Whole mount preparations have been instrumental in revealing their organization and specific microbial associations. However, their thickness and intricate three-dimensional complexity present challenges for a comprehensive spatial analysis. To enhance our understanding of TD consortia we employed a complementary approach: embedding in hydrophilic plastic, followed by sectioning. Samples were labeled by hybridization with multiplexed fluorescent oligonucleotide probes and visualized by spectral imaging and linear unmixing.
Our strategy enabled detection of taxa that were under-observed in whole mount studies, including *Prevotella*, *Fusobacterium*, *Leptotrichia*, *Porphyromonas*, and Lachnospiraceae, and identification of their spatial distribution patterns.
Closely related taxa *Actinomyces* and *Schaalia* formed adjacent yet distinct clusters in the interior of the consortia suggesting similar resource requirements and a degree of mutual exclusion.
Three-dimensional reconstruction revealed taxon abundance changes relative to distance from the surface. *Rothia* dominated the outermost layers and *Actinomyces* the core, suggesting metabolic preferences influencing localization: *Rothia* prefers oxygen while *Actinomyces* thrives in anaerobic environments. Lachnospiraceae filaments and *Leptotrichia* were also located at the core, possibly seeking anaerobic conditions. Interestingly, these two taxa from different phyla frequently formed intermingled clusters at micrometer scales as demonstrated by proximity analysis, suggesting potential cross-feeding interaction.
We observed a crust formed by diverse *Prevotella* species at the periphery of the consortia. Production of exopolysaccharides by *Prevotella* and *Rothia* may provide structural support and enable evasion of the host defenses safeguarding other bacteria within the consortium.
Fusobacterium filaments were dispersed throughout the consortium in the layer below the *Prevotella* crust, indicating the tendency of this taxon to associate with multiple partners.
By demonstrating associations and differential distributions among these health-associated taxa, our results can inform efforts to model and, ultimately, modulate the oral microbiome.

Keywords: Tongue dorsum, microbial consortium, micron-scale spatial organization.

Acknowledgements/Funding (optional): Funding was provided by National Institutes of Health (NIH) grant DE022586 (to GGB and JMW).

Effects of post-surgical rinsing with chlorhexidine digluconate mouthwash on the oral microbial composition and the prevalence of antimicrobial resistance genes

Bartsch S[1*], Kohnert E[2], Kreutz C[2], Wölber JP[3], Burkhardt A-S[1], Anderson A[1], Buchalla W[4], Hellwig E[1], Cieplik F[1,4], Al-Ahmad A[1]

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[3]Policlinic of Operative Dentistry, Periodontology, and Pediatric Dentistry, Medical Faculty Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany
[4]Department of Conservative Dentistry and Periodontology, University Hospital Regensburg, Regensburg, Germany

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Background and Objectives

Chlorhexidine (CHX) is a common antiseptic and widely used in dentistry. However, CHX is considered as a possible factor in the emergence of cross-resistance to antibiotics [1]. The aim of the present study was to investigate the effects of intensive CHX application on the oral microbiota and the prevalence of antimicrobial resistance genes (ARGs).

Methods

Saliva and supragingival plaque samples were collected from 20 patients who applied a 0.2% CHX mouthwash twice daily for four weeks following periodontal surgical procedures. Three time points were investigated: before and directly after four weeks of CHX application and four weeks after discontinuation of CHX. DNA was extracted and a shotgun metagenome analysis was performed.

Results and Conclusions

Microbial diversity was reduced, and oral streptococci dominated the microbiota directly after CHX application, promoting a caries-associated bacterial community. Although no significant changes of ARGs could be detected, the prevalence of two genes involved in tetracycline efflux, *tet*B(60) in the oral biofilm and *tet*(B) in saliva, tended to increase sharply during CHX treatment. The *tet*B(60) gene (with *tet*A(60)) additionally confers resistance to the last resort antibiotic tigecycline. The *tet*(B) gene originates from Gram-negative species and was detected for the first time in 2011 in the Gram-positive *Streptococcus suis* isolated from pigs. In 2019 it was found in *Streptococcus oralis* from the human oral cavity [2, 3]. Correlations of these genes with oral isolates and CHX resistances are the subject of current studies.

Keywords: chlorhexidine, resistance, streptococci, tetracycline

Acknowledgements/Funding (optional): We thank Annette Wittmer and Bettina Spitzmüller for their excellent technical assistance. This research was funded by the Deutsche Forschungsgemeinschaft (DFG), grant numbers AL 1179/4-1 and CI 263/3-1.

Literature references

[1] Cieplik, F., et al., Front Microbiol, 2019. **10**: p. 587.;
[2] Arredondo, A., et al., J Oral Microbiol, 2019. **11**(1): p. 1643204.;
[3] Chander, Y., et al., Vet J, 2011. **189**(3): p. 359-360.

Have we given up on cultivating new oral bacteria?

A. Al-Ahmad[1*], A. Wittmer[2], A.-K. Schulz[1], C. Tennert[3], G. Häcker[2], F. Cieplik[1], S. Bartsch[1]

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2Institute of Medical Microbiology and Hygiene, Faculty of Medicine, University of Freiburg, Germany
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Introduction*:* The increasing application of NGS in recent years has led to the identification of a large number of new bacteria from different niches, including the oral cavity. This gave rise to the so-called microbial dark matter, which consists of bacteria that can be identified but have not yet been cultivated [1,2]. To date, very few studies have been carried out on the isolation of oral bacteria using intensive culture techniques (culturomics). The largest group of uncultivated bacteria belongs to the so-called Candidate Phyla Radiation (CPR) [3]. Nevertheless, new species and genera are still being isolated even in the already known phyla.

Objective: This study aimed to characterize a new genus isolated from a tooth with secondary endodontic infection and endodontic-periodontal lesion.

Methods: A sample was taken from the infected root canal and spread on HCB agar plate and on FAA agar. The bacterium was isolated as a single colony and examined using various culture methods (Gram staining, sequencing of the 16S rRNA gene, whole genome sequencing, quinone and lipid analysis, scanning electron microscopy).

Results and conclusions: A Gram-negative, rod-shaped and translucent bacterium was isolated that grows anaerobically preferably with a so-called nurse (*Capnocytophaga* sp. or *Prevotella intermedia*). The most closely related species have been found in the human oral cavity and in skin swabs, while other closely related species have been detected in oral samples from dogs and cats. However, none of these species have been cultured to date.

We propose the name *Radixoralia hellwigii* gen. nov., sp. nov. for our new genus, named after the place of isolation (radix - root, os - mouth) and in honor of Prof. Dr. Elmar Hellwig, recently retired Professor and Head of the Department of Conservative Dentistry and Periodontology in Freiburg, in recognition of his work in oral microbiology.

Keywords: uncultivated oral bacteria, oral infections, novel bacteria, microbial dark matter

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Enhanced Endodontic Infection Treatment Using Chlorhexidine-Loaded self-propelled nanoparticles

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According to the World Health Organization (WHO), endodontic infections rank as the fourth most expensive pathologies in direct treatment costs. Treatment of these biofilm-related infections involves removing vital and necrotic tissues and microorganisms from the pulp chamber, followed by irrigation with antimicrobial agents like sodium hypochlorite, chlorhexidine (CHX), or hydrogen peroxide. However, 25-30% of cases end in treatment failure due to persistent microorganisms, emphasizing the need for innovative therapeutic strategies to eradicate these biofilm infections [1].

To address this growing issue, we developed novel self-propelled nanoparticles. These nanoparticles are anchored with a fig tree protease, ficin, which effectively breaks down the bacterial biofilm matrix [2]. Additionally, they are loaded with chlorhexidine (CHX) and contain a pH-dependent molecular gate in order to enable controlled CHX release within the biofilm backbone. The findings indicate that self-propelled nanoparticles were capable of effectively eradicating pre-formed endodontic biofilms and mature 24-hour biofilms derived from saliva *in vitro*. Notably, their efficacy surpassed that of CHX when used alone. To validate their performance in a clinically relevant setting we further tested on mature 7-day *Streptococcus mutants* biofilms grown on dentin blocks. Viable colony counts revealed that self-propelled nanoparticles completely eliminated all bacterial cells in less than five minutes. Furthermore, its efficacy in disrupting and eliminating established biofilms in root canals was verified through confocal microscopy of dentinal tubules. Additionally, tolerability tests conducted in animal models confirmed the safety of CHX-loaded nanoparticles *in vivo*. Therefore, we propose that the use of these novel nanocarriers could offer an effective treatment of endodontic infections.

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Functional Characterization of a Sialidase Protein From *Capnocytophaga ochracea*

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Capnocytophaga ochracea is a commensal oral bacterium that is a common component of human dental plaque. *C. ochracea* coaggregates with multiple bacterial species and plays important roles in dental biofilm formation and maturation. Unlike *Capnocytophaga gingivalis*, *C. ochracea* exhibits sialidase activities. The biological significance of sialidase activities in *C. ochracea* remains unknown. *C. ochracea* encodes one sialidase homologue (Coch_0016, Co-NanH) containing an N-terminal signal peptide, whose activities remain unstudied.

Methods. The Co-NanH protein (without signal peptide) from *C. ochracea* DSM 7271(T) was cloned, expressed and purified using standard procedures. Its activities against 4-methylumbelliferyl-N-acetylneuraminic acid (MUNANA), a commonly-used model sialidase substrate, were characterized. The *nanH* gene in *C. ochracea* DSM 7271 was deleted by allelic replacement with an erythromycin resistance cassette. The growth rate, sialidase activities, biofilm formation capacities and coaggregation activities of the wild type and D*nanH* mutant strains were compared.

Results. Co-NanH showed optimal sialidase activities under acidic conditions (*ca*. pH 4.0 - 6.5). Kinetic analyses of MUNANA hydrolysis demonstrated that Co-NanH had high catalytic efficiency (K_M = 0.03 ± 0.01 mM, k_{cat} = 26.1 ± 0.9 s^{-1}). Size-exclusion chromatography analysis indicated that Co-NanH was monomeric in solution. The *C. ochracea* D*nanH* mutant had undetectable sialidase activities, confirming its cellular biochemical functions. Compared with the wild type, no obvious growth defect was detected in the D*nanH* mutant in replete culture medium. The *C. ochracea* DSM 7271 wild type and D*nanH* mutant strains had comparable coaggregation activities against *Fusobacterium nucleatum* ATCC 25586*.* However, crystal violet staining assays revealed the D*nanH* mutant had significantly lower biofilm-forming capabilities than the wild type.

Conclusions. Co-NanH is responsible for *C. ochracea* sialidase activities, is not essential for growth in replete media, and plays a role in biofilm formation. Investigations into its biological and ecological activities are ongoing.

Keywords: Enzyme biochemistry, sialidase, Capnocytophaga, biofilm formation

Acknowledgements/Funding: This work was supported by the Research Grants Council (RGC) of Hong Kong through a General Research Fund (GRF) grant #17121820 to RMW.

Inhibitory effect of common plant flavonoid cyanidin on dental biofilm

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Prevalence of mutans streptococci such as *Streptococcus mutans* and *Streptococcus sobrinus* in a dental biofilm plays a discriminatory role in oral microbiome disbalance and dental caries progression. *S. mutans and S. sobrinus* virulence strongly depends on their biofilm formation and enamel demineralization abilities due to the production of surface adhesins, exopolysaccharides, and acid in the presence of dietary sugars. Plant flavonoids have recently gained a growing interest as antimicrobial alternatives with potent antibiofilm and anti-cariogenic properties.

In this study, the effect of a key pigment component present in red barriers and fruits, cyanidin, has been studied on oral streptococci including the main cariogenic pathogens, *S. mutans and S. sobrinus.* Biofilm inhibitory assay combined with microscopic analysis revealed a strong antibiofilm activity of cyanidin against both *S. mutans and S. sobrinus,* while it remains non-toxic for their cell viability. Essentially, cyanidin does not kill commensal streptococcal species such as *Streptococcus sanguinis*, *Streptococcus oralis*, *Streptococcus gordonii*, and *Streptococcus mitis*, the first colonisers of the tooth surfaces and the main antagonists of cariogenic bacteria. At the same time, dual-species biofilm of *S. mutans* and *S. sanguinis,* as well as mixed multispecies streptococcal biofilm revealed to be more susceptible to cyanidin treatment and less acidogenic in comparison to monospecies *S. mutans* biofilm.

These observations provide promising insights into the antibiofilm properties of common plant flavonoid cyanidin, while laying out a framework for future therapeutic strategies targeting virulence factors of complex dental biofilms.

Keywords: Dental biofilm, *Streptococcus mutans*, flavonoids, caries, lactic acid.

Novel regulation of a membrane embedded phosphatase linked to outer membrane vesicle production in *Porphyromonas gingivalis*

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The Gram-negative oral pathogen *Porphyromonas gingivalis* (*Pg*) uses the type-IX secretion system (T9SS) and outer-membrane vesicles (OMVs) to export virulence factors (e.g. gingipains R and K) [1]. This is dependent on a conserved C-terminal domain within the cargo proteins, which are covalently attached to the cell surface via A-LPS (anionic-lipopolysaccharides, mediated by lipid A) or become subsequently sorted into OMVs via blebbing from the bacterial surface [2-3]. We have shown the production of OMVs is associated with modifications to lipid A structure [1] and this appears to be linked to the T9SS [4]. LpxE is a lipid A 1-phosphatase broadly distributed in Gram-negative bacteria which modulates the phosphorylation status of lipid A. In *P. gingivalis*, $LpxE_{PG}$ is twice the size of other LpxEs with a long C-terminal extension of unknown function(s). $LpxE_{PG}$ was expressed as fusion protein His_{10}-MBP-$LpxE_{PG}$ (100 kDa) in *E. coli*. The purified protein was confirmed by LC/MS-MS (liquid chromatography/tandem mass spectrometry). LpxEs are typically monomeric, but negative-stain TEM (transmission electron microscopy) and cryo-TEM studies indicate that MBP-$LpxE_{PG}$ forms a unique trimer-like compact complex of 120Å in length. This is consistent with the suggested molecular weight of 300-500 kDa from size exclusion chromatography with multi-angle light scattering (SEC-MALS). The C-terminal region of $LpxE_{PG}$ forms a small domain that sit on top of the inner membrane. This mediates trimerization, while also inserting within the inner-membrane and modifying the arrangement of the embedded helices within the N-terminal phosphatase. Different conformations of cryo-TEM maps indicate the C-terminus may regulate the structure and activity of the phosphatase. We are currently investigating point mutations and truncations in $LpxE_{PG}$ to elucidate the role of the C-terminal region. This work presents new insights into bacterial secretion and outer membrane vesicle production and may have wider implications in other organisms with T9SS.

Keywords: Lipid A 1-Phosphatase (LpxE), Cyro-TEM, Outer Membrane Vesicles, Type IX Secretion System, *Porphyromonas gingivalis*

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Fusobacterium nucleatum from malignant and potentially malignant oral disease show high levels of genetic variation, recombination and inflammatory microRNA induction

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Fusobacterium nucleatum is an anaerobic commensal of the oral cavity, associated with periodontitis and extra-oral diseases, including colorectal cancer [1]. Previously, our studies have shown an increased relative abundance of this bacterium on potentially malignant oral leukoplakia (OLK) [2].

Using direct culture, we recovered isolates from healthy oral sites, OLK sites and carcinomas and found that 79% of Fusobacterium species isolated were *F. nucleatum* subspecies *polymorphum*. To characterise these isolates, we carried out whole genome sequencing (Illumina and MinION) and pangenome analysis with Panaroo. 76 isolate genomes, including 60 genomes sequenced in this study and 16 genomes recovered from GenBank, were included.

Analysis of the pangenome has shown that these isolates possess a relatively small core genome, compared to the larger accessory genome. A phylogenetic tree based on the alignment of the core genome shows that isolates from healthy and OLK sites of the same patient are genetically closely related suggesting that there is no malignancy-associated genotype. A large repertoire of adhesins was identified and copy number of major adhesins, including FadA and Fap2, was shown to vary greatly between isolates. fastGEAR was used to investigate recombination at adhesin encoding loci and we detected evidence of recombination, not only between strains of *F. nucleatum* subspecies *polymorphum,* but also between subspecies *nucleatum*, *animalis* and *vincentii* genomes. This heterogeneity among isolates in genotype is also seen in phenotypic assays such as hemagglutination, serum resistance and adhesion to different cell lines. *F. nucleatum* subspecies *polymorphum* was also shown to induce inflammatory and pro-tumorigenic responses in oral keratinocytes. Induction of the inflammatory microRNA miR-21, which is overexpressed in a variety of cancers, was also demonstrated.

Overall, it appears that *F. nucleatum* subspecies *polymorphum* isolates exhibit great variability genotypically and phenotypically, indicating potential variation in virulence and ability to modulate the tumour microenvironment.

Keywords: *F. nucleatum*, oral leukoplakia, pangenome, recombination, miR-21

Literature references: [1] Kostic *et al.*, 2013; [2] Amer *et al.*, 2017

Plaque metagenomics on 1,157 dental implant patients reveals new microbial biomarkers of peri-implant diseases

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Millions of dental implants are placed each year to rehabilitate completely or partially edentulous patients [1]. At the same time, it is estimated more than half of new dental implant patients will develop one of the two implant-associated conditions (peri-implant mucositis and peri-implantitis), highlighting the growing burden of peri-implant diseases that accompany wider dental implant use [2]. Peri-implant mucositis and peri-implantitis have both been linked with plaque bacteria in recent investigations, but the small sample size and low taxonomic resolution of such studies preclude solid conclusions with translational potential. In this study, we analyzed the PreBiomics Beta Program cohort, which comprises 1,157 dental implant patients from 52 Italian dental clinics. We sampled implants that were either healthy (n = 331), associated with peri-implant mucositis (n = 463), or associated with peri-implantitis (n = 363). Then used shotgun metagenomic sequencing to profile the peri-implant microbiome at species-level genome bin (SGB) resolution [3]. We found the peri-implant microbiome was strongly associated with the clinical condition of the implant, such that it was possible to predict the condition from microbiome data alone (AUC = 0.67–0.89). This was linked to a list of bacterial biomarker species, which included Neisseria sp. oral taxon 014 (SGB9463), Prevotella oris (SGB1525), and Porphyromonas gingivalis (SGB2057) as the top biomarker for health, peri-implant mucositis, and peri-implantitis, respectively. The three clinical conditions were also associated with unknown SGBs (representing species with no cultured representative) and with phenotypically diverse species (e.g., multiple distinct Fusobacterium nucleatum clades) showing association in different directions at SGB-level. Our study reveals the potential of peri-implant metagenomic sequencing as a tool to aid in the diagnosis and prognosis of peri-implant diseases and point to novel microbes with a possible role in peri-implant disease pathophysiology and treatment.

Keywords: metagenomics, plaque microbiome, dental implant

Funding: This project was developed within PreBiomics S.r.l (Trento, Italy) and has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant agreement No. 957533).

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Microbial Dysbiosis and Increased Abundance of Oral Pathogenic Bacteria in the Intratumoral Environment of Oral Squamous Cell Carcinoma

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Oral squamous cell carcinoma (OSCC) is highly prevalent cancer in worldwide, known for its malignancy. Although precise mechanisms remain incompletely understood, dysbiosis has been suggested as a contributing factor predisposing individuals to cancer development. Numerous studies have examined the oral microbiome profiles in OSCC patients to understand the microbial environment in tumorigenesis, but there is still insufficient evidence to clearly identify microbial factors contributing to the initiation or progression of cancer. In this study, we collected tumor and adjacent normal tissue samples from 40 OSCC patients to elucidate microbial differences between these two closely located tissues. Microbial signatures within the samples were examined using 16S rRNA sequencing amplified with the V3-V4 region. We identified noticeable discrepancy in evenness within tumor tissues suggesting occurrence of dysbiosis. Moreover, the bacterial community between tumor and adjacent normal tissues exhibited significant differences with higher abundance of species from the *Fusobacterium*, *Treponema*, *Parvimonas*, *Selenomonas* genera observed in the OSCC tissues. Additionally, the colonization of this bacterial community within the intratumoral microenvironment was examined by in situ hybridization. Predicted functional profiling of the microbiota indicated a decrease in metabolic pathways within the tumor tissue, while pathways associated with bacterial chemotaxis and flagella assembly, proposed as virulence factors enhances progression of cancer, were notably enriched. In this study, we have identified specific bacterial genera abundantly present in intratumoral environment of OSCC, providing compelling evidence of microbial dysbiosis. These findings may be employed as potential biomarkers and serve as fundamental data for understanding the mechanism of the tumorigenic process.

Keywords: OSCC, cancer, microbiome, bacteria, intratumor

Synergies between periodontal pathogens in the oral cavity and beyond.

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More than 25 years ago, Socransky proposed that different bacterial complexes are involved in periodontal disease [1]. Although it is widely accepted that periodontitis does not depend on a single pathogen, the interactions and synergies that occur between the bacteria of these complexes are not yet clearly defined. This is especially relevant in the case of orange complex bacteria, since their co-occurrence has also been observed in other extra-oral diseases such as colorectal cancer. Co-culture studies have shown how *Parvimonas micra* promotes the growth of *Porphyromonas gingivalis* and stimulates the production of gingipains [2]. Likewise, greater biofilm production has been observed in cultures of *Fusobacterium nucleatum* with *P. micra* [3]. In this work, the synergistic effects of three members of the orange complex (*F. nucletum, P. micra*, and *Peptostreptococcus stomatis*) were studied using three different approaches. Firstly, changes in growth dynamics were studied through co-cultures. A higher growth rate of *P. micra* and *P. stomatis* was observed when grown in combination with *F. nucleatum*. Secondly, transmission electron microscopy and co-aggregation assays showed a physical interaction between *F. nucleatum* and *P. micra* but non between *F. nucleatum* and *P. stomatis*. Finally, when Caco-2 cells (colorectal cancer cell line) were exposed to combinations of the bacteria higher cell proliferation was detected than when grown individually. Additionally, after 4h of exposure, both *F. nucleatum* and *P. micra* were able to invade Caco-2 cells and co-invasion was detected through fluorescence microscopy suggesting a potential interaction towards pathogenesis.

Taking together our work indicates that studying oral microbes as consortia could be key to elucidate their pathogenic potential in the oral cavity, but also in other extra-oral niches.

Keywords: Periodontopathogens, co-culture, consortia, colorectal cancer

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Communication is key – roles of c-di-AMP signalling in *Fusobacterium nucleatum* virulence

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A B S T R A C T

Fusobacterium nucleatum is pivotal in the formation of oral biofilms and in particular the shift from a healthy oral microbiome to a disease-associated state. Despite this recognition, little is known about the molecular mechanisms of *F. nucleatum*, including its interaction with other oral bacteria. Second messengers like cyclic dimeric adenosine monophosphate (c-di-AMP) control signalling pathways involved in virulence and communication in many bacteria.
The aim of this study was to investigate the role of c-di-AMP in virulence traits of *F. nucleatum*.
Hypothesis
C-di-AMP is involved in co-aggregation, biofilm formation and antimicrobial susceptibility of *F. nucleatum*.

Methodology

The wildtype strain *F. nucleatum* ATCC23726, a c-di-AMP synthesis mutant and a c-di-AMP hydrolysis mutant were used in the experiments. Co-aggregation was measured using photo-spectrometry and included two further oral species: *Streptococcus oralis* and *Porphyromonas gingivalis*. Mono- and multi-species biofilm biomass was determined using a crystal violet (CV) assay. Biofilm viability after 2 days of anaerobic growth and 1 day of metronidazole treatment was quantified using an MTT assay. The minimum biofilm inhibition concentration (MBIC) was determined using broth dilution assays and subsequent CV staining.
Results
The accumulation of c-di-AMP increased the co-aggregation of *F. nucleatum* with *P. gingivalis* and *S. oralis*. Results from this study show that both the reduction and accumulation of this second messenger reduced biofilm formation and increased the susceptibility of *F. nucleatum* biofilms to metronidazole. The accumulation of c-di-AMP also resulted in a decreased MBIC value.

Conclusion

Results suggest a role for c-di-AMP in auto-aggregation, as well as in co-aggregation and biofilm formation. Susceptibility to metronidazole has also been affected, suggesting a role for c-di-AMP in virulence of *F. nucleatum.*

Keywords: *Fusobacterium nucleatum,* cyclic di nucleotides, bacterial communication

Interacting with microbiomes: bringing oral microbiology back to the dental chair

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ABSTRACT

Oral microbiology has experienced tremendous growth and change over the last decade, and the future of the discipline appears even more auspicious. There are multiple factors that contribute to this sense of exhilaration. One such concept is the growing evidence that the oral microbiome not only plays a significant role in disease development, but also significantly influences oral health. This results in a shift away from the dogmatic goal of completely eliminating bacteria from the oral cavity and towards prioritizing the preservation and enhancement of the oral microbiome in relation to health. There is a growing acceptance and adoption of this novel promicrobial concept among patients, oral health professionals, and the oral health industry. However, this also necessitates a greater understanding of the oral microbiome and reinstates its inclusion in preventative and therapeutic strategies. From the bench to the dental chair, this translation lecture will emphasize the significance and consequences of interacting with oral microbiomes, particularly in periodontitis.

Keywords: probiotics, prebiotics, biofilm, antiseptics, promicrobials, periodontitis

Antibiotic resistance genes in the saliva of dental students-A pilot study

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ABSTRACT

Antimicrobial resistance (AMR) is a major global healthcare challenge. Due to the decline in new antibiotics, treatment options are limited [1]. The human oral cavity, home to diverse bacteria, plays a key role in maintaining oral and systemic health. Recent studies have found antibiotic resistance (AMR) genes in saliva, indicating that the oral cavity may serve as a reservoir for these genes [2]. No studies have been conducted on this important topic in the UAE or in most Middle Eastern countries.

Materials & Methods: In this study, we investigated the presence of antimicrobial resistance genes in saliva obtained from forty 5th year dental students and compared it with the same number of students who are not working on patients (1st year dental students). Demographic characteristics and participants information about health parameters were obtained by a structured questionnaire composed of 28 questions. Real Time PCR was used to detect a panel of preselected AMR genes in the bacterial DNA extracted from saliva samples.

Results: The participants' ages ranged from 20-31, with 41 females and 39 males. The percentage of positive genes among samples were varied. blaCTX-M grp 1; 29%, blaCTX-M grp 9; 85%, blaCTX-M grp 8; 39%, blaOXA-48;69%, blaKPC-1; 6%, blaVIM; 49%, DHA; 53%, ACC; 25%, MOX; 59%, armA 83%, rmtB;63%.

There were no statistical differences in the prevalence of the positive AMR genes between 5th year and 1st year dental students or between male and female students.

Conclusion: Results revealed a high occurrence of AMR genes in the oral microbiome. A comprehensive metagenomic analysis may be required to evaluate the prevalence and relative amount of antibiotic resistance genes in the oral biofilm samples of the UAE population. This could help establish a database or reference point for these ARGs, enabling effective monitoring in the future.

Keywords: Antibiotic resistance genes, antimicrobial resistance, oral resistome
Acknowledgements/Funding (optional): This research was supported by Research Funding Department, University of Sharjah, UAE.
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Community metaproteomics to study the functional response of the oral microbiome

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ABSTRACT

The oral microbiome is a complex community of commensals, symbionts, and pathogens. While previous research has primarily focused on taxonomic classification in both healthy and diseased states, there remains a significant knowledge gap regarding any functional dynamics. This study will investigate the functional response of the oral microbiome to sub-lethal levels of antimicrobials, particularly Chlorhexidine (CHX). Utilizing biofilms derived from healthy human saliva and an 8-species bacterial consortium, modified Nanopore pipelines were employed to characterize microbial composition. Viability assessments using LIVE/DEAD staining and scanning electron microscopy (SEM) imaging were conducted to examine the impact of CHX on biofilm integrity. Individual species within the biofilms were exposed to varying concentrations of CHX, and resistance was monitored through passaging on FAA+CHX plates. Disk diffusion assays (DDA) were employed to evaluate the sensitivity of CHX-treated species to common antibiotics. Furthermore, a novel compound IPMP at 0.1% was tested on biofilm models. A protocol for protein extraction was established for subsequent LC-MS analysis. Salivary biofilms subjected to 16S DNA sequencing revealed the presence of over 40 bacterial genera. SEM imaging illustrated the spatial organization of biofilms and the adverse effects of CHX. Minimum inhibitory concentrations of CHX varied between 0.02% and 0.002% across individual species. Passaging on FAA+CHX plates indicated induction of tolerance, and we are currently assessing alterations to other antimicrobials as well as the heritability of this trait. Interestingly, biofilms treated with varying CHX concentrations exhibited altered levels of specific oral commensals. Proteomics data will provide insights into protein expression and improve understanding of metabolic changes. This research aims to contribute to knowledge of how the oral microbiome responds to routine antimicrobial use, informing the development of pre- and pro-biotics and advancing understanding of potential risks associated with antimicrobial resistance.

Oral biofilm composition, dissemination, and inflammation depend on probiotic and synbiotic strain-specificity

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ABSTRACT

Objectives: A number of inflammatory diseases are characterized by a disruption in the equilibrium between the host and its microbiome. Due to the increase in resistance, the use of antibiotics for the widespread, nonspecific killing of microorganisms is at risk. Pro-microbial approaches focussed on stimulating or introducing beneficial species as opposed to pathobionts may be a viable alternative for restoring the host-microbiome equilibrium. Unfortunately, not all potential probiotic/synbiotic species and even subspecies (as strains) are equally effective for the designated pathology, resulting in conflicting accounts of their efficacy.

Methods: To determine the magnitude of these species- and strain-specific effects, 13 probiotic candidates were evaluated for their probiotic and synbiotic potential (with glycerol) on *in vitro* oral biofilms, dissemination from biofilms to keratinocytes, and anti-inflammatory activity.

Results: Species- and strain-specific effects and efficacies were observed in how they functioned as probiotics or synbiotics by influencing oral pathobionts and commensals within biofilms and affected the dissemination of pathobionts to keratinocytes, ranging from ineffective strains to strains that reduced pathobionts by 3+ log. All the *Limosilactobacillus reuteri* candidates required glycerol as a synbiotic to be antimicrobial and showed a distinctive effect from other candidates. Additionally, a minority of the candidates exhibited the ability to mitigate the inflammatory response of LPS-stimulated monocytes through inducing the production of IL-10.

Conclusions: For a rigorous assessment of probiotic therapy for oral health, a judicious selection of a fully characterized probiotic strain for the designated pathology is required to renounce the current "form over function" perception of probiotics.

Keywords: Periodontitis, Biofilm, Probiotic, Synbiotic, Inflammation

The influence of probiotics on the pH value in oral biofilm formation

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ABSTRACT

Aim: The present experimental *in vitro* study was performed to determine the effect of *Streptococcus salivarius* K12 on the pH value in cariogenic oral biofilm formation.

Methods: Anaerobic suspensions of a five species cariogenic culture including *Streptococcus mutans* ATCC 25175 were co-cultivated with or without 0.5% sucrose supplementation at 37°C for two weeks on bovine enamel specimens. The co-culture was compared to a co-culture including the probiotic strain *S. salivarius* K12 and a control group without bacteria. The pH of the culture media (InLab Expert, Mettler Toledo, Columbus OH, USA) and the CFU/ml (Difco™ Mitis Salivarius Agar, BD, Allschwil, Switzerland) were determined. Microsurface hardness loss was quantified at the beginning and the end of the experiment using an enamel surface microhardness measurement (Zwick-Roell, ZHU 0.2, Fa. Zwick, Ulm, Germany).

Results: In the presence of *S. salivarius* K12, the co-cultivation showed an increase in the pH (5,1 ± 0.26) in comparison to co-cultivation without *S. salivarius* K12 (4,4 ± 0.06). Loss of enamel hardness was highest in the cariogenic group and was significant less in the *S. salivarius* K12 and the control group (p <0.05) after 14 days of co-cultivation. Data analysis was performed by a two-way analysis of variance (ANOVA) with a multiple comparison test (GraphPad Prism, 9.3.1 for Windows, GraphPad Software, San Diego California, USA).

Conclusions: *S. salivarius* K12 probiotics are able to increase pH values during the cariogenic oral biofilm formation and reduce enamel hardness loss throughout a long-term intervention. Thus, the use of *S. salivarius* K12 could be an efficient prophylactic measure to prevent the carious processes on enamel.

Keywords: probiotics, pH, multispecies biofilm, enamel surface microhardness

Modelling eubiosis and dysbiosis in a multispecies oral consortium

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ABSTRACT

Periodontitis is a multifactorial condition where the subgingival microbiota is central to disease progression. In health, a complex balance with the host is responsible for maintaining a healthy (eubiotic) microbiota but in disease, this equilibrium is changed leading to dysbiosis in microbial communities. One factor influencing this ecological shift is a change in nutrient availability at the gum margin due to increased flow of gingival crevicular fluid (GCF) in gingivitis [1]. To investigate mechanisms underlying development of dysbiosis, we have developed a model to study the effects of saliva and serum (GCF) on growth and activity of a multispecies consortium.
The consortium [*Streptococcus dentisani* CW, *Streptococcus constellatus* NCTC10714, *Actinomyces naeslundii* CW, *Actinomyces odontolyticus* NCTC9985, *Parvimonas micra* EME, *Fusobacterium nucleatum* BK:0, *Veillonella parvula* 10BB and *Porphorymonas gingivalis* W50-d] was grown anaerobically at 37°C for 48 hours in growth medium supplemented with 20% saliva or 20% serum. Growth was monitored using OD_{600nm} and culturing. General proteolytic and gingipain activities were measured with spectrophometry using fluorescent substrates. All experiments were conducted in triplicate using independent biological replicates.
After 48 hours, a small increase in growth was seen with saliva-supplemented medium compared to control, while 20% serum gave a 3-fold increase in community growth compared to saliva. Saliva promoted growth of *S. dentisani* and Actinomyces species whereas serum promoted growth of anaerobic species such as *P. gingivalis* and *F. nucleatum*. Compared to saliva-supplemented media, general proteolytic activity after 48 hours was 4-fold higher in consortia grown in serum-supplemented media and high gingipain-specific activity was also observed.
Thus, saliva facilitated growth of a health-associated community with low proteolytic activity enriched in streptococci and Actinomyces, whereas serum promoted growth of proteolytically active, anaerobic bacteria associated with periodontal disease.

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Keywords: Multispecies consortium, dysbiosis, periodontitis, proteolytic activity
Funding: Research programme FORESIGHT, Malmö University

Effect of dietary nitrate supplementation on the oral bacterial community in patients with stable angina

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ABSTRACT

Dietary supplementation with nitrate leads to raised levels of nitric oxide (NO) via the entero-salivary circuit which improves endothelial function and vascular remodelling post vascular damage. In this investigation, part of a larger study (NITRATE-OCT) investigating the effect of dietary nitrate supplementation on restenosis, the effect of once-a-day inorganic nitrate administration for 6 months on oral nitrate reduction and salivary bacterial composition was investigated in patients with stable angina.

The study population comprised 178 patients with stable angina undergoing elective percutaneous coronary intervention. Patients were randomised to receive beetroot juice (4-5 mmol nitrate in 70 ml) or placebo (nitrate-depleted beetroot juice) daily for 6 months. Salivary and circulating nitrate and nitrite levels were measured using ozone chemiluminescence. Salivary microbiome community profiling was conducted on samples collected at baseline and 6 months. The V1-V2 region of the 16S rRNA gene was sequenced using the Illumina MiSeq platform and the data was analysed using the mothur pipeline.

Circulating nitrite levels were significantly raised in the treatment versus placebo arm with an associated increase in circulating nitrate. There were also significant increases in salivary nitrite and nitrate levels in the treatment group. There was no difference in salivary bacterial composition between the treatment and placebo groups at baseline (p=0.47, AMOVA) but a significant difference was seen after 6 months (p=0.005). The taxa responsible for the difference in composition at 6 months were the genera *Neisseria* (LefSe, LDA=3.4; p<0.01) and *Rothia* (LefSe, LDA=3.1; p<0.01), which include nitrate- and nitrite-reducing species.

The main study showed that a sustained elevation of nitrite using a dietary nitrate intervention caused a decrease in coronary restenosis following stent implantation. The results presented here demonstrate that this clinical improvement is associated with modification of salivary microbiome composition mediated by nitrate, specifically an increase in the relative abundance of nitrate-reducing genera.

Keywords: Saliva, microbiome, nitrate, angina

The commensal microbiota is required for development of the host chemosensory systems

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ABSTRACT

The detection of chemical signals in the outside world is fundamental to the survival of all animals. In mammals the ability to respond to chemical cues in the external environment is important for the detection of food, social behaviour including mate selection and avoidance of toxic compounds and predators. Chemo-sensing of odours, pheromones and tastes is achieved largely through specialised chemoreceptors expressed in sensory neurons within the main olfactory epithelium (MOE) and vomeronasal organ (VNO) respectively in the nasal cavity and the taste buds in the mouth. The sensory neurons of the MOE and VNO are the only neurons in mammals that are in direct contact with the external environment and are therefore permanently exposed to oxidative stress and xenobiotics as well as the cells and metabolites of the distinct and specialised microbial communities of the nasal and oral tissues.

In recent comparative meta-transcriptomic studies using germ free (GF) and conventionally reared (SPF) mice we demonstrate that the presence of a host associated microbiota is required for normal expression of a significant repertoire of olfactory and vomeronasal chemoreceptors of the MOE and VNO. In addition, the VNOs in adult GF mice are significantly smaller with disrupted non-sensory and sensory epithelium when compared to SPF mice, consistent with a role for the microbiota in development and homeostasis of this chemosensory organ. These differences in the chemosensory systems of GF and SPF animals are associated with altered behavioural responses of these animals to odours and pheromones indicating that microbially-mediated development of chemosensory detection systems has a direct influence on animal behaviour.

The findings add to the significant evidence that the host microbiome can play a fundamental role in host development and biology and, more specifically, that chemical sensing of the outside world is controlled by the commensal microbiota.

Keywords: Chemo-sensing, development, commensal microbiota

Acknowledgements/Funding Medical Research Council UKRI

Disrupted gut resolution mechanisms in inflammatory arthritis facilitate pathogenic behaviour of the oral pathobiont *Porphyromonas gingivalis*

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ABSTRACT

Changes to the host environment of pathobionts facilitate their switch from commensalism/symbiosis to pathogenesis [1]. Oral pathobiont *Porphyromonas gingivalis* has been linked to rheumatoid arthritis [2]. Which host environment changes initiate its pathogenic actions remains unclear. We found in pre-clinical models that during inflammatory arthritis resolution mechanisms are disrupted in the intestine. Specifically, decreases in pro-resolving and gut-protective omega-3-fatty acid-derived lipid mediator Resolvin (Rv)D5_{n-3} DPA resulted in impaired IL-10/IL-10R expression in lamina propria macrophages, gut inflammation and gut barrier weakening including decreased numbers of goblet cells and downregulation of epithelial tight junction proteins [3]. In this altered host environment, but not in the intestines of non-arthritic mice, *P. gingivalis* was able to induce further loss of intestinal barrier function, barrier breach by gut luminal bacteria and exacerbated joint inflammation. Inversely, addition of RvD5n-3 DPA augmented IL-10 and IL-10R expression in macrophages *via* the upregulation of aryl-hydrocarbon receptor agonist L-kynurenine. Administration of RvD5n-3 DPA to arthritic *P. gingivalis*-inoculated mice increased intestinal *Il-10* expression, restored gut barrier function and reduced joint inflammation. Together, these findings uncover novel mechanisms in the pathogenesis of rheumatoid arthritis, where disruption of the gut-RvD5n-3 DPA–IL-10 axis weakens the gut barrier, facilitating the switch of pathobiont *P. gingivalis* from benign to pathogenic behaviour.

Keywords: Rheumatoid arthritis, *Porphyromonas gingivalis*, pathobiont, Resolvin D5_{n-3} DPA, gut barrier

Acknowledgements/Funding (optional):

This work was supported by funding from Versus Arthritis (prev. Arthritis Research UK); European Research Council and Marie Skłodowska Curie Individual Fellowship (both: EU's Horizon 2020 research and innovation program); Wellcome Trust &Royal Society; Barts Charity.

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Microbiome - host inflammatory cell interactions in the subgingival environment

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ABSTRACT

In health (eubiosis), the resident oral microbiome is in a balanced relationship with the host but, in response to environmental perturbations such as increased flow of protein-rich gingival crevicular fluid, biofilms can become dysbiotic leading to disease [1]. Keratinocytes, neutrophils and monocytes form the first line of host defence in the sub-gingival tissues but most studies aimed at investigating their role in oral disease have focused on single bacterial species or endotoxins derived from them. We have used a co-culture model to study the responses of keratinocytes and host inflammatory cells to *multi-species oral biofilms* related to health and disease.

Biofilms representative of eubiosis: *Streptococcus oralis, Streptococcus gordonii, Streptococcus intermedius, Veillonella parvula, Actinomyces odontolyticus* and *Capnocytophaga sputigena* or subgingival dysbiosis: *Streptococcus constellatus, Parvimonas micra, Fusobacterium nucleatum* and *Porphyromonas gingivalis* were grown on nitrocellulose membranes for 7 days. They were then transferred to transwell inserts and co-cultured in six-well plates with immortalized oral keratinocytes for 6 hours. The cell supernatants were subjected to multiplex cytokine analysis and tested for their ability to activate neutrophils, monocytes and platelets.

Both eubiotic and dysbiotic biofilms elicited a cytokine response from the keratinocytes and 14 cytokines with variable importance of projection (VIP) ≥ 1.5 were identified. In addition to the anti-inflammatory cytokine, IL-10, those that were positively associated with the dysbiotic biofilms included pro-inflammatory species with neutrophil and monocyte chemoattractant properties such as ENA-78, MIP-3a, IL-8, MCP-1 and IL-1b. Supernatants also showed differential effects on attraction and activation of the inflammatory cells.

These data suggest that multi-species biofilms associated with health and periodontal disease elicit different responses from oral keratinocytes leading to differential recruitment and activation of inflammatory cells, thus highlighting the role of keratinocytes in orchestrating the host response in the periodontal tissues.

Keywords: Multi-species biofilm, Cytokines, Host response, Dysbiosis, Eubiosis
Funding: The Foresight Programme at Malmö University
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Oral Microbiota Contribution to the Maintenance of Essential Amino Acid Balance in Human Physiology

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ABSTRACT

Humans need 9 essential amino acids (EAAs) for optimal growth and function [1]. Diet is the main strategy for EAAS incorporation, as opposed to alternatives like rumination or coprophagy in other mammals, which enable absorption of EAAs produced by intestinal microorganisms. This becomes relevant in the current context of processed food abuse in developed countries, as many EAAs are precursors of neurotransmitters, whose altered levels are associated with neurological disorders. Likewise, those pathologies have been associated with dysbiosis of host microbiota and its interactions [2], particularly in the context of ageing, excessive antibiotic use, and changes in parenting behaviour. The oral cavity serves as a major gateway for pathogens and environmental factors, and changes in its microbial composition have a systemic impact in human health [3]. We aimed to assess the contribution of oral bacteria to EAAs balance under physiological conditions, considering saliva as vehicle for systemic distribution of microbial metabolites. Bioinformatics analysis of EAAs biosynthesis pathways in common oral bacteria, showed greater prevalence in health-associated genera like *Neisseria*, *Streptococcus*, or *Rothia*. Conversely, disease-associated genera like *Porphyromonas* or *Fusobacterium* lacked key genes for EAAs biosynthesis. EAAs measurements in saliva samples from healthy individuals showed that total salivary EAAs, with the assumption of swallowing 1L of saliva daily, contribute 5-10% to adults' daily EAA requirements. Additionally, we observed in *S. salivarius* strains that EAAs biosynthesis pathways increase the expression of its key genes in the absence of amino acids, leading to the extracellular release of those compounds in a free form. The rationale behind the mechanisms driving EAAs release by oral bacteria is yet to be understood. However, it marks an initial effort to comprehend the influence of EAAs derived from oral microbiota on human physiology and their potential connection to systemic diseases associated with the gut-brain axis.

Keywords: essential amino acids, neurotransmitter, oral microbiota, dybiosis, systemic.

Acknowledgements/Funding (optional): This work was supported by grant from Conselleria de Sanitat Universal i Salut Pública Plan GenT Generació Talent 2019 Anex II (CDPT-02/20-B), in collaboration with Darwin Bioprospecting Excellence SL.

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Investigating the association between hypertension and nitrate-reducing activity of oral bacteria

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ABSTRACT

Objectives: Nitrate reducing (NR) oral bacteria can facilitate the reduction of nitrate into nitrite. Once nitrite is swallowed with saliva, it is reduced to nitric oxide (NO) in the acidic gastric environment[1] and contributes to reduced blood-pressure[2]. There is limited knowledge about the potential association between the nitrate-reducing activity (NRA) of oral bacteria in patients with hypertension. Thus, this study aimed to address this gap in the literature by analysing the NRA of oral bacteria in patients with normal and high blood pressure (hypertension) attending a primary care dental school in the UK. **Methods**: NRA was measured in 29 participants (n=18 normotensive [NORM] (SBP≤129 and/or DBP≤79) and n=11 hypertensive [HYPT] (SBP≥140 and/or DBP≥90). All participants rinsed their mouth with a mouthwash (10mL) for 5 min, containing water and $NaNO_3$ (10mmol). The mouthwash was collected and centrifuged at 13,000rpm and 4°C for 10min. The supernatant was collected to analyse the concentration of nitrite using a high-performance liquid chromatography device (ENO-30, EICOM, USA). Unstimulated saliva was collected to analyse ammonia, pH, buffering capacity, nitrate, nitrite, lactate and glucose. Normal distribution of data was assessed using Shapiro-Wilk test. Differences between BP groups were analysed using independent t-tests (normally distributed) or Mann-Whitney test (non-normally distributed). **Results**: NRA was significantly higher in the HYPT group(11.1±20.0μM), compared to NORM group(1.7±1.6μM;p=0.009). Salivary ammonia (NORM=58.1±43.7mg/L; HYPT = 27.4±14.6mg/L;p=0.013) and pH (NORM=7.2±0.2;HYPT=7.0±0.3;p=0.036) were significantly lower in the HYPT group compared to the NORM group. **Conclusions**: NRA was significantly higher in hypertensive individuals compared to normotensive, suggesting increased nitrate reducing capacity of oral bacteria in hypertension, contrary to our initial hypothesis. The lower levels of salivary ammonia and pH that accompanied this, could also influence the activity of the oral microbiome. Further studies will determine the oral microbiome composition in hypertensive patients, to interpret these findings more fully.

Keywords: nitrate reducing bacteria, hypertension, dentistry, pH, ammonia

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Characterisation of novel murine *Streptococcal* species.

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ABSTRACT

Rodent models of diseases are popular and invaluable preliminary steps in the study of infectious disease. *In vivo* studies of periodontitis frequently rely upon mouse models using oral gavage with a human periodontal bacterium and / or ligature-induced pathology to examine the deregulation of the host immune and inflammatory response by a dysbiotic microbiota. In both instances, the contribution of the mouse oral commensal microbiota appears to be critical.
The murine oral microbiome is relatively simple in community structure and most of the bacteria are culturable. *Streptococcus danieliae* predominates in laboratory housed animals where it may comprise >90% of the total community and is also a prominent member of the oral microbiota in wild mice populations. Other *Streptococcus sp* are also present at lower abundance. Our murine oral database (MOMD): https://momd.org/ consisting of 16S ribosomal RNA gene sequences and draft genome sequences of representative strains, has been instrumental in our analysis of meta transcriptomic data in health and disease, and identification of isolates from a variety of mouse strains and different models. We have recently added the complete genomes of murine oral *Streptococcus* sp.
The *S. danieliae* genome is similar in size and %GC composition to human *Streptococcus sp*. Whole genome phylogeny indicates that *S. pneumoniae, S. mitis and S. oralis,* are nearest neighbors and more related than murine *Streptococcus sp.* including *S. acidominimus* and *S. cuniculi*. Over half the proteins (1052) are shared with *S. pneumoniae and S. mitis* but over 400 are unique: hypothetical and mobile genetic elements. Furthermore, *S. danieliae* encodes several very large multi-domain proteins with numerous repeats, a characteristic feature of adhesins/surface-located proteins involved in molecular interactions. We are optimizing methods for the genetic manipulation of *S. danieliae* to facilitate analysis of the properties of this organism and other *Streptococcus sp* in health and disease.

Keywords: MOMD, *oral Streptococcus sp*, *S. danieliae*, whole genome sequencing

Changes in oral microbiota and markers of inflammation following the use of a polyphenol-containing dentifrice.

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ABSTRACT

Balance in the oral microbiota is necessary for proper oral Health and adequate oral hygiene can help to it. The aim of our study is to evaluate the effects of a toothpaste for the protection of oral microbiota in patients attending our Dental Hospital (HOUB).
Material and methods: Randomised, double-blind, clinical trial in patients treated at the HOUB. One-month study: value of 0 (baseline) and 1 at the end. The study group [SG] used a specific toothpaste containing olive oil. The control group [CG] used a placebo paste without active ingredients. All patients underwent an oral examination with measurement of salivary pH and flow, cytokine counts (TNF, IL-1 and IL-4) and qPCR 16S total bacterial load, *Porphyromonas gingivalis* and *Fusobacterium nucleatum* (cfu/ml).
Results: 22 patients were included, 15 women and 7 men (mean age 48.5 years). The groups were similar in terms of age and sex. In relation to the inflammatory markers, no significant differences were observed between the groups at baseline. Similar with respect to bacterial counts. In the one-month control there was no significant change in the concentration of inflammatory and bacterial markers. But if we analyze the data by groups, it could be seen that the intervention was effective in reducing TNF levels. The same trends were observed for IL-1 concentration, which showed a significant reduction in the intervention group ($p=0.03$). In contrast, no differences were observed for IL-4 levels. Regarding bacterial concentration, the intervention was effective in reducing the total bacterial load ($p=0.02$). No statistically significant differences were found between groups for *P. gingivalis* and *F. nucleatum* levels.
Conclusions: The product tested was well accepted by patients reducing both oral inflammation and overall bacterial load. A study with a larger sample size and with cases of active periodontal pathology would be desirable to evaluate the efficiency of the product.

Keywords: Oral microbiota, inflammatory markers, oral hygiene

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Supporting the management of periodontal diseases – probiotics may alleviate inflammation and modify oral microbiota

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ABSTRACT

The literature reinforcing the connection between oral and systemic health is expanding. Therefore, new, holistic and more effective care approaches are needed to support periodontal health. *Bifidobacterium animalis* subsp. *lactis* HN019 (*B. lactis* HN019) has been clinically shown to function as a good adjunct to conventional treatment of periodontal diseases [1-3]. In generalized periodontitis, *B. lactis* HN019 promoted prolonged reduction in pocket probing depth and clinical attachment gain [1,2]. In generalized gingivitis, *B. lactis* HN019 decreased bleeding on probing and improved gingival index scores versus placebo [3]. The use of *B. lactis* HN019 induced reduction of selected pro-inflammatory mediators and increased both anti-inflammatory cytokines and beta-defensins in gingival crevicular fluid [1-3] and in gingival tissues. In pre-clinical studies using experimental periodontitis (EP), reduced RANKL (receptor activator of nuclear kappa-B ligand) level and RANKL/OPG (osteoprotegerin) ratio were found. Combined, these results indicate that *B. lactis* HN019 promotes gingival health supporting immunomodulation. *In vitro* studies have shown that *B. lactis* HN019 inhibits several periodontal pathogens. Similarly, clinical biofilm samples [1] and samples from EP showed increased levels of oral commensals and reductions of periodontal pathogens. These results suggest that *B. lactis* HN019 may modify the oral microbiota by enhancing health associated and reducing disease associated bacteria. More recently, the effects of *B. lactis* HN019 have been studied both on EP alone and EP in combination with systemic diseases like rheumatoid arthritis and metabolic syndrome. Benefits were demonstrated in serum anti-citrullinated protein antibodies, blood lipid levels, expression of lipogenic genes in white adipose tissue, intestinal morphology and in stool microbiota composition in EP. While demonstrating local effects in the oral cavity supporting periodontal health, *B. lactis* HN019 may also alleviate some symptoms of systemic diseases providing a holistic adjunct for gingivitis and periodontitis treatment.

Keywords: Probiotics, Gingivitis, Periodontitis, Systemic diseases
Acknowledgements/Funding (optional):
Danisco Sweeteners Oy, a subsidiary of IFF Health (formerly DuPont) has donated the probiotics for the clinical trials. K.S. and A.C.O are employed Danisco Sweeteners Oy/IFF Health.

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The oral-gut axis: salivary and fecal dysbiosis in inflammatory bowel diseases (IBD)

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ABSTRACT

Inflammatory bowel disease (IBD) is a group of chronic inflammatory disorders of the gastrointestinal (GI) tract. The GI traverses from mouth to anus and harbors diverse bacterial communities. Several sequencing-based studies have identified intestinal enrichment of oral-associated bacteria and their ability to induce gut inflammation in mice, suggesting that intestinal pathobionts originate from the oral cavity. This study aimed to investigate the composition of the salivary and fecal microbiome of IBD patients (n = 14) compared to healthy controls (HC, n = 12) and to determine the abundance of common bacterial taxa in both niches. Metagenomic DNA was extracted from saliva and fecal samples, and the 16S rRNA gene was targeted for V3-V4 sequencing [1] as well as for absolute abundance quantification via RT-qPCR [2]. Four taxa namely, *Streptococcus* spp., *Veillonella* spp., *Prevotella* spp., and *P. salivae* as a single (under-investigated) species were found to play a possible key-role in the mouth-gut cross talk [2]. Therefore, 407 (IBD) and 168 (HC) *Veillonella* strains, 173 (IBD) and 90 (HC) *Prevotella* strains (including *P. salivae*, very rarely isolated before), as well as 122 (IBD) and 97 (HC) *Streptococcus* strains were isolated, identified to species level and investigated in depths. Streptococcal strains underwent genome-sequencing. In the case of Streptococci, the oral cavity seems to be a reservoir for intestinal strains. This presentation combines the results of two published major studies [1, 2] done on the same samples but will provide updates since then also.

Keywords: oral-gut axis, *Streptococcus* spp., *Veillonella* spp., *Prevotella* spp., *Prevotella salivae*, inflammatory bowel disease, real-time quantitative PCR, 16S rRNA amplicon sequencing

Acknowledgements/Funding (optional): This study was funded by the START Program (STREPTORANTES #109/19) as well as the Interdisciplinary Centre for Clinical Research (IZKF OC1-06) of the RWTH Aachen University Hospital. Ethical approvals No. EK 069/19 for IBD patients and No. EK206/09 for healthy control subjects.

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Exploring The Subgingival Microbial Signature in Individuals At Risk of Rheumatoid Arthritis

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ABSTRACT

Individuals who test positive for anti-cyclic citrullinated peptide/protein (CCP+) and experience musculoskeletal symptoms but do not exhibit clinical synovitis are at an elevated risk of developing rheumatoid arthritis (RA). This CCP+ at-risk population has evidence of increased prevalence of periodontal disease (PD). This study investigates the association between the subgingival microbiome and RA susceptibility.
A UK cohort of 86 CCP+ at-risk individuals, 34 new-onset RA (NORA) patients, and 38 control subjects (HC), with and without periodontal disease, were recruited. All participants underwent a comprehensive periodontal assessment by a dentist. Subgingival dental plaque samples from both healthy and diseased periodontal sites were used for DNA metagenomic sequencing using the Illumina NovaSeq platform. Taxonomic profiling was performed using Metaphlan4.
A total of 744 bacterial species of 16 phyla were detected. At both healthy and diseased sites, there was no significant difference in bacterial alpha- and beta-diversity among HC, CCP+ at-risk individuals and NORA patients. However, bacterial differences at the phylum and species levels were observed. At species level, keystone periodontal pathogens associated with RA were found to be enriched in both CCP+ at-risk individuals and NORA patients. Compared to control subjects with periodontal disease (having both healthy and diseased sites), *Porphyromonas gingivalis* (*P.g.*) was more abundant at healthy sites and *Aggregatibacter actinomycetemcomitans* (*A.a.*) was significantly increased at diseased sites in CCP+ at-risk individuals. Additionally, *P.g.* was less abundant and *A.a.* was more abundant at diseased sites in NORA patients than in CCP+ at-risk individuals.
Our findings demonstrate a distinct subgingival microbiome profile in CCP+ at-risk individuals and NORA patients compared to control subjects. There was an increased abundance of *A.a.*, particularly in diseased sites, suggesting the possible role of the oral microbiome in RA development. Further research is needed to confirm these findings.

Keywords: Subgingival microbiome, CCP+, *P. gingivalis*, *A. actinomycetemcomitans.*

Nitrate reduction capacity of the oral microbiota is impaired in periodontitis: implications for oral and systemic health

Bob T. Rosier

ABSTRACT

In the last decade, nitrate has gained popularity in biomedical research because oral bacteria can convert this molecule into nitrite and nitric oxide, which can improve oral and cardiometabolic health. Some studies indicate that nitrate can prevent dysbiosis from a periodontitis perspective. However, these studies focussed on individuals without periodontitis, while in this periodontal disease there is a clear decrease in nitrate-reducing bacteria. We therefore tested the effect of nitrate and a nitrate-reducing *Rothia aeria strain (Ra9) on subgingival biofilms of periodontitis patients in vitro.* The addition of nitrate limited periodontal biofilm growth, decreased the levels of periodontitis-associated species, and lowered the dysbiosis index. Five millimolar nitrate (which can be found in saliva after vegetable intake) was sufficient, while increasing this concentration to 50 mM (which could be achieved by topical applications such as a periodontal gel) increased the positive effects. Ra9 increased the nitrate metabolism of periodontitis communities and should be tested in vivo. In a second study, we observed that the nitrate-reduction capacity (NRC) of patients with periodontitis was impaired, which could contribute to the relationship between periodontitis and systemic diseases. Periodontal treatment led to an increase in the levels of nitrate-reducing bacteria and recovery of the NRC. To test if periodontitis could limit the beneficial effects of nitrate on systemic health, beetroot juice was consumed by periodontally healthy individuals and periodontitis patients before and after treatment. In healthy individuals, the beetroot juice lowered the blood pressure significantly as expected, but not in patients with periodontitis. However, after treatment, blood pressure lowering effects of beetroot juice were recovered. Our data indicates that nitrate-reducing bacteria and their metabolism decrease in periodontitis, while stimulating these bacteria with periodontal treatment or nitrate could improve oral and systemic health.

Aggregatibacter actinomycetemcomitans and its leukotoxin A in collagen-induced arthritis in mice

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ABSTRACT

Aggregatibacter actinomycetemcomitans (*A. actinomycetemcomitans*) is the major pathogen in periodontitis and the highly leukotoxic JP2 genotype of *A. actinomycetemcomitans* may boost its ability to interfere with the innate immune defense [1]. Recent studies have shown that leukotoxin A (LtxA) produced by *A. actinomycetemcomitans* may cause hypercitrullination of neutrophils, leading to the production of anti-citrullinated protein antibodies (ACPA) associated with rheumatoid arthritis (RA) [2]. However, direct evidence linking *A. actinomycetemcomitans* and RA is still lacking. In present study, an RA model was established in mice by tail injection of type II bovine collagen, and the mice were infected with JP2 or non-JP2 genotype of *A. actinomycetemcomitans*. The progression and severity of arthritis were observed, and serum levels of inflammatory factors and antibodies were measured for intergroup comparisons to clarify the role of *A. actinomycetemcomitans* and LtxA in the development of RA. Furthermore, proteomic sequencing of mouse joint tissues was performed to further investigate the mechanisms of *A. actinomycetemcomitans* in the development of RA. Our results showed that both genotypes of *A. actinomycetemcomitans* accelerated and aggravated RA in mice. The JP2 genotype exhibited increased production of RA-associated autoantibody ACPA in mice, compared with the non-JP2 genotype. Proteomic sequencing analysis of mouse joint samples revealed that the promotion of RA by *A. actinomycetemcomitans* may be associated with differential expression of transforming growth factor-2. This study provides the first evidence in an animal model confirming the role of periodontal pathogen *A. actinomycetemcomitans* in the development of RA, offering new insights for the diagnosis, prevention, and treatment of RA.

Keywords: *Aggregatibacter actinomycetemcomitans*, leukotoxin A, rheumatoid arthritis, periodontal pathogen, anti-citrullinated protein antibodies
Funding: This study was supported by the National Natural Foundation of China (No. 82001048) and Young Elite Scientist Support Program by CSA (No. 2020PYRC001).

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Reflection on the current understanding of the oral microbial homeostasis

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ABSTRACT

Once established, the oral microbial ecosystem emerges as the most resilient among human microbial habitats. This resilience, termed 'oral microbial homeostasis', denotes its ability to maintain stability in the face of various stressors. Within this equilibrium, the impact of stressors is effectively counteracted by the ecosystem, preventing long-term damage. However, when the ecosystem becomes overwhelmed by stressors, it may transition into a state of dysbiosis. Dysbiotic microbiota signifies a loss of balance with its host, potentially exacerbating processes leading to disease. Importantly, responses to specific stressors vary significantly among individuals. This presentation will delve into the gaps in our understanding of homeostasis and resilience towards dysbiosis, shedding light on current knowledge and areas for further exploration.

Comparative analysis of the oral resistome in health and disease: insights from publicly available sequencing data.

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A B S T R A C T

Antimicrobial resistance (AMR) infections are projected to cause over 10 million deaths annually by 2050 [1]. Biofilms, like dental plaque, promote horizontal gene transfer, exacerbating AMR [2]. Oral antibiotic resistance contributes to systemic infections, such as endocarditis linked to oral streptococci [3]. This study examines the resistomes of oral biofilm samples collected from healthy individuals and those with caries and periodontitis. We aimed to elucidate the differential composition and dynamics of resistance genes within the oral microbiome, shedding light on the potential role of the resistome for directing oral health treatment.
To explore the oral resistome, publicly available shotgun metagenomic datasets were analysed from 11 previously unexamined studies for antibiotic resistance genes (ARGs) using the Comprehensive Antibiotic Resistance Database (CARD). The cohorts included healthy adolescents (n=77), adults (n=80), individuals with caries (n=117), and periodontitis (n=80). Differential analysis was performed using the DESeq2 R package, and changes in diversity were assessed using phyloseq along with other R and Python packages.
Differential abundance analysis revealed significant differences in ARGs between caries and periodontitis samples compared to healthy samples (padj<0.05, Log2FoldChange>1). *lsaC*, a *Streptococcus spp.*-associated gene conferring resistance to lincomycin, clindamycin, and dalfopristin, was more abundant in caries, while *pgpB*, a gene encoding polymyxin B resistance exclusively found in *Porphyromonas gingivalis*, was enriched in periodontitis. Furthermore, specific ARGs displayed strong positive correlations with oral disease-associated genera (p<0.005). One such genus is *Porphyromonas*, which exhibited correlations with genes like *ErmF*, responsible for the macrolide, lincosamide, and streptogramin B (MLSb) resistance phenotype. Euclidean distance analysis classified samples into distinct 'resistotypes', demonstrating a unique resistome profile in periodontitis distinct from health and caries.
The oral resistome varies significantly between health and disease, potentially hindering the effectiveness of dental infection treatments.

Keywords: Resistome, Caries, Periodontitis, Resistotype.

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The effect of menopause on the activity of oral nitrate-reducing bacteria

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ABSTRACT

Menopause is commonly associated with dry mouth (xerostomia), a physiological phenomenon that can modify the composition and activity of the oral microbiome. Nitrate-reducing species within the oral microbiome are important to maintain oral and cardiovascular health, however, the impact of menopause on these bacteria is poorly understood. The aim of this study, therefore, was to investigate the activity of oral nitrate-reducing bacteria in women. Saliva samples were taken from healthy women and grouped according to age: Control (20-32 years, n=16), 40-49 years i.e. perimenopause (n=7), 50-59 years i.e. menopause (n=19), and 60+ years i.e. post-menopause (n=13). The results did not demonstrate a significant change in either salivary nitrate (control = 573 µM, 40-49 years = 124 µM, 50-59 years = 212 µM, 60+ years = 183 µM) or nitrite (control = 211 µM, 40-49 years = 107 µM, 50-59 years = 168 µM, 60+ years = 269 µM) levels, in any menopausal stage compared to controls. However, there was significant increase in nitrate reducing (NR) capacity in both the 50-59 years and the 60+ years groups, compared with the control (Kruskal-Wallis with Dunn's multiple comparisons test, H (4) = 18.65 P = <0.0003). In conclusion, during menopause, there appears to be an increase in the activity of oral nitrate-reducing capacity of oral bacteria. Further studies will directly measure xerostomia and the composition of oral microbiome communities, to determine whether there is a concurrent dysbiosis of the oral microbiome that occurs in menopausal women.

Keywords: Oral microbiome, menopause, dysbiosis, nitrate, nitrite
Acknowledgements/Funding (optional):
University of Plymouth Enterprise Solutions – R&D Solutions Fund
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Examination of the acetaldehyde generating capacity of *Rothia mucilaginosa* recovered from the oral microbiome of patients with potentially malignant lesions

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ABSTRACT

Oral cancer is a malignant neoplasia that usually develops in squamous cells of the oral cavity or lip. Many oral cancers develop from potentially malignant disorders such as oral leukoplakia (OLK). Alcohol consumption and smoking are the main risk factors for OLK and oral cancer development. An increased abundance of *Rothia mucilaginosa* has been detected in OLK patients [1]. In addition, individuals with a high acetaldehyde producing microbiome have also been shown to have increased abundance of *R. mucilaginosa* [2]. We have also previously shown that *R. mucilaginosa* isolates recovered from OLK patients may produce acetaldehyde from ethanol on vitro [3]. Consequently, we hypothesise that oral *R. mucilaginosa* could accelerate oral cancer development by converting alcohol to acetaldehyde.
In this study, whole genome sequencing of a collection of *R. mucilaginosa isolates* (n=38) was performed, including isolates from OLK patients and healthy individuals. The core genome alignment identified a genetically related cluster of *R. mucilaginosa* isolates (n=9) with the highest levels of acetaldehyde generating ability. When habits of the patients were analysed, a positive association was observed between the presence of acetaldehyde-producing strains of *R. mucilaginosa* and alcohol consumption and current smoking (*P* 0.0012; OR 21.7). Analysis of the pangenome shows that *R. mucilaginosa* does not encode aldehyde dehydrogenase (AdhE) but no correlation with alchohol dehydrogenase (Adh) coding capacity was detected. However, expression of alcohol dehydrogenase 1 (Adh1), which converts ethanol to acetaldehyde, is significantly higher in acetaldehyde producing strains.
These data suggest possible selection of the oral microbiota by smoking and alcohol consumption for strains of *Rothia mucilaginosa* that can metabolise ethanol to generate acetaldehyde and could represent a novel risk for the development of oral cancer.

Keywords: acetaldehyde, oral cancer, alcohol dehydrogenase
Acknowledgements/Funding (optional): This study was funded by the Health Research Board, Ireland Grant no. ILP-POR-2019-30
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"The effect of different antibacterial compounds on the nitrate-reducing activity of oral bacteria"

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ABSTRACT

Objectives: Antibiotics and antiseptics are used for their antimicrobial properties to manage oral disease, their effects on microbial activity are less well known. The main aim of this study was to analyze and compare the effect of chlorhexidine, propolis, amoxicillin and metronidazole on the activity of oral nitrate-reducing bacteria (ONRB), which are important for maintaining oral and cardiovascular health.

Methods: 15 healthy participants (7M:9F) provided an unstimulated saliva sample (5 mL). Then, 0.5 mL of saliva were mixed with:

1) 4.5 mL ultrapure water
2) 3.5 mL water + 1 mL sodium nitrate ($NaNO_3^{-}$) (10mmol/L)
3) 3.5 mL chlorhexidine (0.2% v/v) + 1 mL $NaNO_3$-
4) 3.5 mL bee propolis (2,5% v/v) + 1 mL $NaNO_3$-
5) 2.5 mL water + 1 mL amoxicillin (500 mg/L) + 1 mL $NaNO_3$-
6) 2.5 mL water + 1 mL metronidazole (500 mg/L) + 1 mL $NaNO_3$-

Samples were incubated at 37˚C for 24 hours under aerobic and anaerobic conditions (only metronidazole). At baseline, 6h and 24h, 0.5 mL of each solution was collected to measure nitrate and nitrite levels using a high-performance liquid chromatography device (ENO-30, EICOM, USA). A two-way repeated measures ANOVA was used to analyze differences between time and treatment.

Results: The activity of ONRB was significantly higher at 6h and 24h when samples were mixed with metronidazole compared to baseline ($p < 0.001$), and compared to other treatments ($p < 0.001$). At 24h, activity of ONRB increased with amoxicillin while it did not change with propolis and chlorhexidine ($p > 0.05$).

Conclusion: Metronidazole did not inhibit ONRB in saliva and amoxicillin had a much stronger inhibitory effect than metronidazole. Chlorhexidine and propolis inhibited ONRB more than metronidazole and amoxicillin. Future research should confirm these results in *in-vivo* conditions and the impact of antibacterial compounds on oral and cardiovascular health.

Associations between exogenous and intrinsic host factors and tongue microbiota in a healthy population.

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ABSTRACT

Objective Although associations between tongue-coating microbiota and systemic disease have been suggested, our understanding of factors shaping tongue microbiota in health is limited. This study identified exogenous and intrinsic host factors in relation to tongue microbiota in a healthy population.

Methods Systemically and orally healthy Dutch adults (n=264, mean age±SD: 22.59±2.6; 148 males, 118 females) were included. An extensive clinical oral examination was performed. Multiple oral parameters were assessed including ICDAS, radiographs for caries detection, bleeding on periodontal probing, tongue coating thickness and discoloration, plaque index, oral malodor (organoleptic score and levels of volatile sulphur compounds, hydrogen sulfide, methyl mercaptan and dimethyl sulfide). Bacterial composition of the anterior one-thirds (AT) and posterior two-thirds (PT) of the tongue dorsum were assessed using 16S rRNA gene amplicon sequencing (Illumina). The pH and metabolic activity of supragingival plaque and biochemical components of unstimulated saliva were measured *ex vivo*. Oral hygiene habits and dietary preferences were assessed using questionnaires. Associations between 86 factors and tongue microbiota were analysed in R (v.4.3.1) with the envfit function (vegan v2.6-4) using Aitchison distance. P-values were FDR corrected and deemed significant at $p<0.05$.

Results Twenty factors were significantly associated with AT and 28 with PT. Nine factors were shared (AT; PT respectively): tongue coating thickness (r^2=0.19, p=5.2e-5; r^2=0.23, p=2.9e-5) and discoloration (r^2=0.05, p=9.8e-5; r^2=0.07, p=2.9e-5), hydrogen sulfide levels (r^2=0.14, p=1.87e-3; r^2=0.09, p=3.53e-2), alcohol ingestion (r^2=0.11, p=1.47e-2; r^2=0.16, p=1.89e-4), salivary pH (r^2=0.13, p=5.67e-3; r^2=0.09, p=4.76e-2) salivary concentrations of lactoferrin (r^2=0.11, p=2.11e-2; r^2=0.10, p=1.54e-2) and albumin (r^2=0.14, p=1.51e-3; r^2=0.11, p=9.68e-3), metabolic activity of supragingival plaque (e.g., resting concentration of propionate (r^2=0.13, p=4.52e-3; r^2=0.12, p=4.79e-3) and lactate concentration after stimulation (r^2=0.10, p=4.39e-2; r^2=0.09, p=4.05e-2)).

Conclusion Our study revealed significant associations between the tongue microbiota and various exogenic and intrinsic factors in healthy individuals.

Keywords: Microbiota, tongue, oral ecosystem,16S rRNA gene.

Evaluating the Impact of Antiseptic Mouth Rinses on Oral Biofilm Resilience

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A B S T R A C T

Background: Periodontal diseases are associated with dysbiosis in the oral microbial communities. Managing oral biofilms is therefore key for preventing these diseases. Management protocols often include over-the-counter antimicrobial mouth rinses, which lack enough data on their effects on the oral microbiome's ecology, bacterial composition, metabolic activity, and dysbiosis resilience. This study examined the efficacy of antimicrobial mouth rinses to halt dysbiosis in in-vitro oral biofilms under periodontitis-simulating conditions.
Methods: Multi-species oral biofilms were grown on hydroxyapatite discs (HADs) and rinsed daily with one of six mouth rinses [1]. Positive and negative controls were included. After 3 rinses, biofilms were analyzed with viability quantitative polymerase chain reaction and visualized using scanning electron microscopy. Supernatants of rinsed biofilms were used for metabolic activity analysis. In addition, human oral keratinocytes were exposed to rinsed biofilms to assess their inflammatory response. All outputs were analyzed for correlation using Spearman coefficient.
Results: Product-related changes were observed in the rinsed biofilms. Three of the 6 tested mouth rinses could significantly increase biofilm resilience and prevent dysbiosis with ≥30% reduction in the pathobionts abundance relative to the control. These biofilms had lower metabolic activity and the exposed human oral keratinocyte produced less interleukin-8. Interleukin-8 production correlated to both pathobiont quantity and the metabolic activity of the biofilms.
Conclusion: Some mouth rinses could support biofilm's resilience and stop dysbiosis evolution in the biofilm model, with a clear product-related effect. Such mouth rinses can be considered for patients under maintenance/supportive periodontal therapy to prevent/delay disease recurrence. Others are more useful for different periodontal therapy stages.

Keywords: Antiseptic mouth rinse, dysbiosis, resilience, prevention, oral biofilm.
Acknowledgements/Funding (optional): This study was supported by grants from the Fund for Scientific Research Belgium (FWO G0B2719N) and KU Leuven (C24/17/086).
Links (optional)
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Standardized disk diffusion method for antimicrobial susceptibility testing of *Aggregatibacter actinomycetemcomitans*

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ABSTRACT

Aim: *Aggregatibacter actinomycetemcomitans* is an oral facultative gram-negative rod associated with severe periodontitis in addition to extra-oral infections in the human body, and treatment may include antimicrobial agents, e.g., -lactams [1]. At present, there are no criteria for antimicrobial susceptibility testing (AST) of *A. actinomycetemcomitans* available from the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The present study aimed to evaluate the disk diffusion method for *A. actinomycetemcomitans*.

Material and Methods: Twenty-nine *A. actinomycetemcomitans* strains were tested with the EUCAST disk diffusion method for AST [2] using Fastidious Anaerobe Agar with 5% defibrinated horse blood (FAA-HB) plates prepared from agar from three different manufacturers, the inoculum of 0.5 McFarland prepared in sterile saline (0.9 %), and ampicillin 2µg, azithromycin 15µg, and metronidazole 5µg disks. Plates were incubated in air with 5 % CO_2 at 35±1˚C and anaerobically at 35±1˚C in parallel for comparison. Inhibition zone diameters were read using a calibrated ruler after 20- and 44-hours incubation.

Results: Twenty-seven of 29 strains grew sufficiently after 20 hours of incubation in air with 5 % CO_2 at 35±1˚C, whereas anaerobic incubation needed 44 hours to reach sufficient growth. The FAA-HB media from the different manufacturers resulted in comparable inhibition zone diameters. The two modes of incubation (air with 5 % CO_2 and anaerobically) did not result in comparable inhibition zone diameters for all strains. Overall, anaerobic incubation resulted in poorer growth over 20 hours and less reproducible zone diameters. The metronidazole disk required anaerobic atmosphere.

Conclusion: The EUCAST disk diffusion method for AST can be applied to *A. actinomycetemcomitans.* Reading of inhibition zone diameters was possible after 20 hours on FAA-HB, one of the EUCAST recommended media, in 5 % CO_2 (excepting metronidazole) for the absolute majority of the strains.

Keywords: Antimicrobial resistance, AST, oral microbiota, periodontal pathogen, antibiotic resistance.
Acknowledgements: The project was funded by the Riisfort Foundation. We thank laboratory technician Anette Aakjær for laboratory assistance.

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Oral fibroblasts display metabolic changes after exposure to bacterial extracellular vesicles from *Porphyromonas gingivalis*

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ABSTRACT

Porphyromonas gingivalis (*P. gingivalis*) is a keystone pathogen in periodontitis. It releases bacterial extracellular vesicles (bEVs) by outward budding of the bacterial membrane, and these bEVs have a content similar to the membrane and cytosol of the bacteria itself, such as nucleic acids, lipids, proteins, metabolites, and virulence factors [1]. The bEVs are taken up by nearby host tissue cells, such as oral fibroblasts in the periodontal tissue [2], yet their effects on human cells remain incompletely understood. Our study aimed to analyse the metabolic content of bEVs from three *P. gingivalis* strains with different pathogenicity [3]: ATCC 33277, A7A1-28, and W83, and to explore the metabolic changes in oral fibroblasts after exposure to bEVs from these strains.

In this study, targeted LC-MS was used to assess changes in the metabolome of the oral fibroblasts following exposure to the bEVs from three strains of *P. gingivalis* for 10 minutes and 24 hours. Metabolite content of the bEVs from each strain was also characterized. Statistical and functional analyses were performed using Metaboanlyst.

Preliminary analyses revealed common metabolites between bEVs from the three strains, but also strain-specific metabolites. Following exposure to bEVs, the fibroblasts exhibited a distinct metabolic shift. This shift differed between the two more pathogenic strains (A7A1-28 and W83) with the less pathogenic one (ATCC 33277). Notably, there was an enrichment of glutathione metabolism in fibroblasts after 24 hours, indicating a cellular stress response. Moreover, we observed changes associated with several metabolic pathways, such as biosynthesis of unsaturated fatty acid and lysine metabolism, potentially providing essential nutrients for the bacteria. Additionally, alterations in purine metabolism, crucial in replication, were evident. Our findings suggest changes in cellular processes induced by the bEVs and this may contribute to more knowledge about the role of bEVs in the pathogenesis of periodontitis.

Keywords: Periodontitis, Bacterial extracellular vesicles, *P. gingivalis*, Pathogenesis.

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Together they are stronger. The importance of bacterial clusters

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A B S T R A C T

For more than 100 years the studies in microbiology have been focused on the isolation and study of single colonies, and this included the microbiome field. However, when the NGS technologies arrived, the point of view of microbiologists changed, and single bacteria studies became microbiota or community studies. As assumed for a long time, these studies proved that bacteria behave differently when they are in pure culture compared to when they are living as part of a complex community. These communities have multiple interactions in which bacteria interchange metabolites, information or genetic material.
This is especially interesting for the oral microbiota where bacterial biofilms are very consistent and stable. Thanks to the sequencing of the oral microbiota through different stages and locations, we have observed that oral microbes form clusters that are also consistent not only in the oral cavity but also when these microbes are located in other parts of our body.
We believe it is important to start studying these clusters and the interactions between the microbes in order to comprehend the influence of it over the development of oral (and not oral) diseases. We have been studying *Fusobacterium nucleatum*, *Parvimonas micra* and *Peptostreptococcus stomatis* and their relevance in periodontal disease and colorectal cancer as an example of the importance of clustering for the development of inflammation.

Keywords: oral microbiome, clusters, colorectal cancer, periodontitis

Strain-Level Complexity in the Oral Microbiome at Micrometer Scales

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ABSTRACT

Most bacterial cells in the mouth are from oral bacterial species with near-100% prevalence and high abundance. Strain-level complexity within these species is high; however, *in vitro* studies are usually done with single strains. A fundamental question is whether oral bacterial populations require multiple strains to function in the complex environment of the mouth or whether single strains are sufficient. Here we use metagenomic sequencing of individual consortia isolated from the tongue dorsum to address the question: in micron-scale neighbourhoods of interacting taxa in the oral microbiome, does each species exist as a clone or a complex mixture of strains?

Our imaging studies show that the tongue biofilm is organized into individual consortia, built around a core of host epithelial cells that project from filiform papillae into saliva. We isolated, imaged, and analysed these biologically relevant units, individual consortia 100-200 microns in diameter, to determine whether strain-level differences exist within consortia or between consortia from the same person. We isolated 12 consortia from each of three subjects by microdissection and performed Illumina metagenomic shotgun sequencing of each consortium separately.

Results showed that the ~4 million bacterial cells per consortium are divided among more than two dozen taxa in every consortium, comparable to the complexity in swabs across a much larger area of the tongue. Metagenomic read mapping to reference genomes showed that some taxa have low diversity while others show many polymorphisms that differ in proportions between the sequenced consortia, indicating a more complex population.

Analysis of whether bacteria thrive clonally in the human microbiome or require more complex population structures could impact the development of probiotics and therapeutic modulation of the microbiome. Our findings demonstrate that clonality may be assessed species by species within each subject, and that most species are represented by multiple strains within each micrometer-scale consortium.

Keywords: biofilm, health, metagenomics, strain, tongue dorsum

Acknowledgements/Funding: Funding was provided by National Institutes of Health (NIH) NIDCR grants DE022586 (to JMW and GGB) and DE030136 (to GGB).

The Salivary Microbiome as a Diagnostic Biomarker of Periodontitis: a 16S Multi-Batch Study before and after Removal of Batch Effects.

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ABSTRACT

One of the focuses of interest in precision medicine is developing salivary microbiome-based tools to improve the diagnosis of oral diseases [1]. To date, the scarce research on this topic shows important methodological shortcomings and none of it evaluates the impact of batch effects (BEs) [2]. This is the first 16S multi-batch study to analyse the salivary microbiome of periodontally healthy and periodontitis patients at the amplicon sequence variant (ASV) level in terms of differential abundance and predictive models. This is done before and after removing BEs.

Saliva was collected from 124 patients (50 healthy, 74 periodontitis) in our setting. Sequencing of the V3-V4 16S rRNA gene region was performed in Illumina MiSeq. In parallel, searches were conducted on four databases to identify previous Illumina V3-V4 sequencing studies on the salivary microbiome. Investigations that met predefined criteria were included in the analysis and sequences were processed under the same bioinformatics protocol. The statistical analysis was performed in the R-Bioconductor environment [3].

The elimination of BEs reduces the number of ASVs with differential abundance between the groups by approximately one-third (265 before *vs.* 190 after). The model built using two-thirds of the specimens (training=531) comprised 35 ASVs (0.36%) and had an AUC of 0.955, sensitivity of 86.54%, and specificity of 90.06% after being validated in the remaining one-third (test=265). After removing BEs, models required more ASVs (training=100-1.01%) to obtain slightly lower AUC value (test=0.947) and lower sensitivity and similar specificity (test= 78.85% and 90.68%, respectively).

The removal of BEs controls false positive ASVs in the differential abundance analysis. However, their elimination implies a significantly larger number of predictor taxa to achieve optimal performance, creating less robust classifiers. Given all our models can accurately discriminate the two conditions, the salivary microbiome demonstrates potential clinical applicability as a precision diagnostic tool for periodontitis.

Keywords: Saliva, microbiome, 16S rRNA gene, batch effects, predictive modelling.

Acknowledgements/Funding (optional): This study has been funded by Instituto de Salud Carlos III (ISCIII) through the project PI21/00588 and co-funded by the European Union (UE) and the Galician Innovation Agency (A. Regueira-Iglesias support, IN606B-2023/005).

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Developing a 3-dimensional oral epithelium model for evaluating antimicrobial and immune responses to dental implant materials

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ABSTRACT

Peri-implantitis is the major cause of dental implant failure and is on the rise. Reducing the microbial burden on implant surfaces is an important strategy to counteract peri-implant inflammation. By modelling the oral epithelium in three dimensions, a more reflective *in vitro* assessment of the influence of putatively antimicrobial implant materials can be determined.

A novel agar-mould procedure was used to construct rat tail collagen scaffolds (3%) embedded with G292 fibroblast cells, which were overlaid with dysplastic oral keratinocytes (D.O.K.) and airlifted to achieve epithelial differentiation. Laser-sintered titanium alloy disks (Ti-6Al-4V) were inoculated with pooled saliva, plaque and tongue scrapings and cultured for three weeks to generate complex oral biofilms. These implant biofilms were assessed for biomass (crystal violet assay), population dynamics (16S rRNA gene sequencing) and co-cultured for 24 hours with D.O.K. monolayers and 3-dimensional epithelial models. Cell viability (MTT assay) and cytokine responses (ELISA) were assessed.

Optimal seeding ratios were determined, with $5x10^5$ G292 and $1x10^5$ D.O.K. cells demonstrating the most favourable live (93.1%) / dead (6.9%) ratios by confocal laser scanning microscopy, with a clear surface layer observed. The effect of the airlifting process was documented through light microscopy, with epithelial formation observed in airlifted scaffolds and minimal differentiation exhibited by non-airlifted constructs. IL-8 production for unstimulated scaffolds exhibited slightly elevated levels (83.4 pg mL^{-1}) compared to monolayers (73.3 pg mL^{-1}). This low baseline concentration is useful to the model, allowing for stimulated cell responses (>10-fold increases) to be clearly distinguished. In preliminary material testing, rougher implant surfaces promoted greater microbial biomasses and elicited increased cytokine responses.

This model represents a relatively simple procedure to generate 3-dimensional organotypic oral structures. By assessing implant material, microbial load and cellular responses in one model, the impact of dental implant surface modifications can be evaluated in a more reflective manner.

Keywords: Peri-implantitis, Biofilms, Co-culture, Dental Implants, Antibacterial

Streptococcus dentisani as a new source of antibiotics to fight the antimicrobial resistance crisis: focus in the probiotic strain 7746

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ABSTRACT

Background: The probiotic *Streptococcus oralis* subsp. *dentisani* 7746 produces antimicrobial peptides (bacteriocins) exhibiting activity against Gram-positive and Gram-negative pathogens [1]. Previous research identified up to 11 bacteriocins in the genome of 7746 [2].

Objectives: This study aimed to comprehensively explore the bacteriocin profile of *S. dentisani* 7746 through *in silico* and *in vitro* approaches. The objectives included: *(i)* Characterizing all bacteriocin biosynthetic gene clusters (BGCs) in the genome through informatics analysis, *(ii)* Assessing bacteriocin production via transcriptome analysis, and *(iii)* Testing antimicrobial activity of chemically synthetized peptides. Additionally, the conservation of these bacteriocins within the *S. dentisani* group was investigated *(iv).*

Methods: The genome of strain CECT 7746 was sequenced, closed, and analyzed to identify BGCs. Bacteriocin genome mining searches were performed using AntiSMASH to identify core peptides. Structural and functional predictions were made using SMART and InterPro. Peptides of interest were chemically synthesized, and their *in vitro* activity against human pathogens was determined.

Results: Our analyses expanded the known repertoire of bacteriocins encoded by *S. dentisani* CECT 7746 to 14, confirming their expression for the first time. We propose a novel model for competence and bacteriocin regulation in this strain. This information is crucial for optimizing bacterial growth conditions to maximize bacteriocin production, potentially aiding in peptide purification and the development of post-biotic products with antimicrobial activity. Bioinformatics suggested activity against *Listeria*, which was experimentally verified with chemically synthesized peptides.

Conclusion: In the context of the antibiotic resistance crisis, the significance of bacteriocins produced by GRAS and probiotics has increased. The probiotic strain 7746, along with its diverse bacteriocins, holds considerable potential for various applications (disease prevention, treatment, and utilization in the food industry). Comparative studies with other *S. dentisani* strains indicate that strain 7746 possesses one of the largest bacteriocin repertoires known to date.

Keywords: antimicrobial peptide (AMP), bacteriocins, two-component system, ABC transporter, quorum sensing, oral microbiota, oral health, human pathogens

Acknowledgements:

This project has received funding from the European Union's Horizon 2020 Research and Innovation Program under the Marie Sklodowska-Curie grant agreement No 101026278, project SMILES; and from FISABIO Foundation for the project BACTIDENT (UGP-20-108).

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Disulfiram as a novel antimicrobial and immune-modulatory agent to treat periodontal diseases *via* targeting *Porphyromonas gingivalis*

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ABSTRACT

Periodontal disease, as a chronic inflammatory disease associated with several systemic diseases, remain a major global health burden with marked socio-economic impacts [1]. Notably, severe periodontitis can result in adult tooth loss and edentulism, while *Porphyromonas gingivalis* is the keystone pathogen playing an essential role to the periodontal pathogenesis. As *P. gingivalis* develops various strategies and diversified approaches to invading the host and concurrently sabotaging the immune systems [2], it is highly warranted to develop effective approaches to tackling this noxious pathogen and concurrently modulating host immune responses. In this study, we re-purpose disulfiram as a new strategy targeting the *P. gingivalis* and its biofilms and reversing *Pg*-perturbed host inflammatory responses in periodontal diseases. We evaluated the antimicrobial effect of disulfiram using the minimum inhibitory concentration assay on periodontal pathogens including *P. gingivalis, Fusobacterium nucleatum, and Aggregatibacter actinomycetemcomitans* compared with the metronidazole as the positive control. For the biofilms, crystal violet staining, 2,3,5-triphenyl tetrazolium chloride colorimetric analysis, and colony-forming units enumeration experiments were performed to evaluate the anti-biofilm adhesion and biofilm eradication ability of disulfiram against *P. gingivalis*. Meanwhile, the live and dead staining using SYTO 9 and propidium iodide was performed on the biofilms and the fluorescent intensity was determined using a confocal scanning laser microscope confocal scanning laser microscope equipped with 543 nm HeNe laser and 488 nm Argon laser. The primary human gingival epithelial cells and gingival fibroblast cells were primed with IL-1 followed by incubation with *P. gingivalis* with or without disulfiram pretreatment, and the supernatants were collected to examine the concentration of pro-inflammatory cytokines using ELISA. The results showed that disulfiram could specifically inhibit planktonic *P. gingivalis* at 0.078 ug/mL with potent anti-biofilm activity, while disulfiram could also reverse Pg-perturbed IL-1-induced host inflammatory responses. Our results demonstrate the potential of disulfiram in the treatment of periodontal diseases.

Keywords: periodontal diseases, disulfiram, antimicrobial activity, modulating inflammatory responses

Acknowledgements/Funding: This work was supported by the Health and Medical Research Fund from Hong Kong Food and Health Bureau [20190682] to X. Li, and Health and Medical Research Fund [17181701] to G. Pelekos

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Periodontal disease, as a inflammatory disease associated with several systemic diseases, remain a major global health burden. Notably, severe periodontitis can result in adult tooth loss and edentulism, while Porphyromonas gingivalis is the keystone pathogen in the periodontal pathogenesis. As Pg develops various strategies and diversified approaches to invade the host and concurrently sabotaging the immune systems. It is highly warranted to develop effective approaches to tackling this noxious pathogen. We re-purpose disulfiram as a new strategy targeting the Pg and its biofilms and reversing Pg-perturbed host inflammatory responses in periodontal diseases. We evaluated the antimicrobial effect of disulfiram using the minimum inhibitory concentration assay on periodontal pathogens including Pg, Fusobacterium nucleatum, and Aggregatibacter actinomycetemcomitans compared with the metronidazole as the positive control. For the biofilms, crystal violet staining, 2,3,5-triphenyl tetrazolium chloride colorimetric analysis, and colony-forming units enumeration experiments were performed to evaluate the anti-biofilm adhesion and biofilm eradication ability of disulfiram against Pg. Meanwhile, the live and dead staining using SYTO 9 and propidium iodide was performed on the biofilms and the fluorescent intensity was determined using a confocal scanning laser microscope confocal scanning laser microscope. The primary human gingival epithelial cells and gingival fibroblast cells were primed with IL-1 followed by incubation with Pg with or without disulfiram pretreatment, and the supernatants were collected to examine the concentration of pro-inflammatory cytokines using ELISA. The results showed that disulfiram could specifically inhibit planktonic Pg at 0.078 ug/mL with potent anti-biofilm activity, while disulfiram could also reverse Pg-perturbed IL-1-induced host inflammatory responses. Our results demonstrate the potential of disulfiram in the treatment of periodontal diseases.

The Microbiological Quality of Dental Unit Water is Reaching which Limit?

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ABSTRACT

During treatment the dental unit water system (DUWS) supplies water to the instrumentation. This water can get aerosolized while using the high-speed burs and scalers or can get in contact with the mucosa through irrigation of the treatment site. The DUWS is known to be susceptible to multi-kingdom biofilm formation and water expelled from the DUWS could pose a risk to staff or immunocompromised patients. To ensure safety the DUWS water needs to meet certain microbiological parameters.
National guidelines for the microbiological quality of dental unit water are in mostly linked to drinking water standards. Within Europe the quality of the dental unit water must meet the drinking water standards being 100 CFU/ml of heterotrophic water bacteria grown at 22°C. Some national guidelines also describe the testing for *Pseudomonas* spp. and *Legionella* spp. All these parameters are based on the European Council directive 98/83/EC on the quality of water intended for human consumption. In 2020 however, this directive was revised and the HPC at 22°C, 37°C as the *Pseudomonas* counts were removed as these are not considered of particular significance for health in relation to the consumption of the water. In conclusion, there doesn't seem to be a limit to adhere to anymore with regards to the microbiological quality of dental unit water. Luckily, the determination of *Legionella* is still described, but this is not a standard test in most dental guidelines. Therefore, the aim of this presentation is to stimulate discussion on which microbiological parameters should be included in forthcoming guidelines or if there should be a European guideline to ensure that the health risks of contaminated dental unit water is kept at bay?

Keywords: Dental unit water, Biofilm, Guidelines, European unity

Personalized antibiotic selection in periodontal treatment improves clinical and microbiological outputs.

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ABSTRACT

Periodontitis is usually treated by non-surgical biofilm elimination with or without antibiotics. Antibiotic treatment is typically selected empirically or using qPCR or DNA hybridization methods by which different periopathogens are quantified. However, these approaches do not consider the antibiotic susceptibility of the whole subgingival biofilm. In this work we wanted to evaluate the xCELLigence system for the selection of the most effective antibiotic treatment for patients with periodontal disease. This method based on impedance measurements [1] allows the growth of subgingival biofilm in real time and evaluate the *in vitro* antibiotic effect on periodontal biofilm growth dynamics in less than 4 hours. To test its efficacy, a double-blind, randomized clinical trial was performed (n = 64), where half of the patients were treated with the antibiotic suggested by this system, while the other half were treated with the antibiotic selected by the standard hybridization methodology. The results of the patients' evolution two months after treatment showed that although both groups improved in both clinical and microbiological parameters, this improvement was significantly greater in the group treated with the antibiotic selected with the xCELLigence system [2]. Thus, we concluded that the Real-Time methodology could enhance the personalized antibiotic treatment selection for periodontal patients compared to standard tools and improve disease prognosis.

Keywords: periodontitis, antibiotic treatment, xCELLigence, subgingival plaque, oral biofilm, personalized medicine.
Acknowledgements/Funding (optional): This work was funding from Agilent Technologies.

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Bridging the Gap: Translating Electrolyzed Saline from *in vitro* Efficacy to Clinical Application in Dysbiosis Prevention

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ABSTRACT

Periodontitis often persists despite conventional measures, so targeted antimicrobial strategies are required. Our *in vitro* study focused on investigating the efficacy of electrolyzed oxidizing saline (EOS) as a novel irrigant to control microbial recolonization in oral biofilms. EOS, a chlorine-based antimicrobial agent with the primary active component HOCl, acts as a strong oxidizing agent with a broad-spectrum antibacterial activity. Subgingival multispecies biofilms were rinsed daily with EOS, demonstrating its efficacy in preventing dysbiosis by inhibiting the overgrowth of pathobionts and, more importantly, by ensuring the dominance of *Streptococci* as commensal species within the biofilms while being safe for human oral keratinocytes [1].
Building on these promising *in vitro* results, we investigated the plaque-inhibiting effect of EOS mouthrinse on *de novo* plaque formation, gingival inflammation and subgingival microbial composition in healthy volunteers. In a double-blind, randomized, placebo-controlled, cross-over model with three mouthrinse arms, subjects refrained from oral hygiene for four days to test the efficacy of EOS (free chlorine=200 ppm, pH 7.0), the positive control (0.12% chlorhexidine digluconate; CHX) and placebo. Volumetric analysis of the plaque showed that the inhibitory effect of EOS was significant, but lower than that of CHX. However, as the main role of EOS is to positively alter the microbial community within oral biofilms rather than affecting the overall quantity, EOS showed promising results in preventing dysbiosis – absolute qPCR quantification of 15 periodontal species revealed that EOS balanced the quality of plaque composition by controlling selected key pathobionts and restoring health-associated commensals.
In summary, the *in vitro* efficacy of EOS in controlling biofilm dysbiosis is confirmed by the clinical study, highlighting its potential as an alternative to conventional treatments. However, further clinical studies in periodontitis patients are needed to confirm the role of EOS as a microbiome-friendly adjunct in non-surgical periodontal treatment.

Keywords: Periodontal disease, Microbial Ecology, Electrolyzed Saline, Mouth rinse, Plaque
Acknowledgments/Funding (optional): This study was supported by grants from the Ministry of Higher Education, Science and Innovation, Republic of Slovenia (Grant 363 P3-0293) and Fund for Scientific Research Belgium (FWO G0B2719N), KU Leuven (C24/17/086).

Literature reference: [1] Zayed, N., Munjaković, H., Aktan, M. K., Simoens, K., Bernaerts, K., Boon, N., Braem, A., Pamuk, F., Saghi, M., van Holm, W., Fidler, A., Gašperšič, R., & Teughels, W. (2024). Electrolyzed Saline Targets Biofilm Periodontal Pathogens In Vitro. *Journal of Dental Research*. https://doi.org/10.1177/00220345231216660

KEYNOTE SPEAKER

Pending issues in oral microbiology

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ABSTRACT

Huge advances in oral microbiology have been made over recent decades, often following the adoption of novel technologies. Our knowledge of what constitutes the human oral microbiome increased initially with the introduction of procedures to preserve and cultivate fastidious and obligately anaerobic micro-organisms to the subsequent widespread application of culture-independent approaches to sequence, classify and identify the most demanding species while also permitting insights into their activity and functions. Advances in modelling and imaging demonstrated that the oral microbiome exists not as a collection of individual organisms but as highly structured and functionally-organised biofilm communities that continue to defy our attempts to control and remove them.

Most research has inevitably focussed on disease but there is now a greater appreciation of the role of the oral microbiome in promoting health. Indeed, it is now widely accepted that the aetiology of the main oral diseases is not due to the action of single species but is a consequence of disruption (dysbiosis) of this beneficial microbiome, which can also increase the risk of disease elsewhere in the body.

Despite these advances, dental disease remains at alarmingly high levels. Opportunities exist for working with industry to develop new tools for the diagnosis, treatment and prevention of oral diseases. Oral biologists have had some of their greatest impact when working collaboratively, often with other medical specialities and investigating the role of the oral microbiome and inflammation in systemic disease. COVID emphasised the issues arising from the presence of overt pathogens in the mouth and their potential distribution in dental surgeries via aerosols. Under-explored areas include the role of the mycobiome and virome in health and disease, and approaches to actively promote a healthy oral microbiome.

The aim of the session will be to generate debate and discussion of research priorities for the future.

Keywords: Oral microbiome, dysbiosis, mycobiome, virome, oral health

P O S T E R S

P.01

Oral Biofilm Composition and Phenotype in Caries-active and Healthy Children

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OBJECTIVE

During development of dental caries, oral biofilms undergo changes in microbial composition and phenotypical traits. The aim of this study was to compare the acid tolerance (AT) and metabolic profile of oral biofilms in relation to their microbial composition in two groups of children: one with severe caries and one with no caries experience.

METHOD

Dental plaque samples were collected from 40 children aged 2-5 years, including 20 children with severe caries (CA) and 20 children who were caries-free (CF). The AT was analyzed by viability assessment after exposure to an acid challenge (pH 3.5), using LIVE/DEAD® BacLightä stain and confocal microscopy. Levels of acid tolerance (AT) were evaluated using a scoring system ranging from 1 (no/low AT), to 5 (high/all AT). Metabolic profiles were investigated following a 20mM glucose pulse for one hour through Nuclear Magnetic Resonance (NMR). Microbial composition was characterized by 16S rRNA Illumina sequencing.

RESULTS

The bacterial characterization of the samples revealed twenty-five species significantly more abundant in the CA samples, including species of Streptococcus, Prevotella Leptotricia and Veillonella ($p<0.05$). When comparing the end-products of glucose metabolism detected after a glucose-pulse, the CA samples showed a significantly higher ratio of lactate to acetate, formate, succinate and ethanol than the CF samples ($p<0.05$). The mean AT score of the CA group (4.1) was significantly higher than the mean AT score of the CF group (2.6, $p<0.05$).

CONCLUSION

Our results show that AT in oral biofilms is increased in children with severe caries compared to healthy subjects and that this can be related to differences observed in the metabolic activity and microbial composition of the biofilms.

Keywords: Oral Biofilm, Acid Tolerance, Microbiome, Metabolomics, NMR, Dental Caries.

Acknowledgements: A special thanks to Anders Bay Nord at the Swedish NMR Centre, University of Gothenburg, Gothenburg, Sweden, for performing the analysis of bacterial metabolites by NMR.

P.02

Two Oligopeptidase B Homologues Contribute to the Elevated 'Trypsin-like' Peptidolytic Activities of *Capnocytophaga gingivalis*.

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OBJECTIVE

Capnocytophaga are abundant within oral niches. They are considered commensal, although moderate evidence associates *Capnocytophaga gingivalis* (Cg) with periodontal disease. Uniquely, Cg encodes two oligopeptidase B (OpdB) homologues (CgOpdB1, CgOpdB2), putatively responsible for its 'trypsin-like' activities.

Objectives. 1) Compare and contrast the trypsin-like activities of OpdB homologues encoded by Cg, *Capnocytophaga ochracea* (Co), and select periodontal pathogens. 2) Characterize the peptidolytic profiles and cellular phenotypes of diverse oral *Capnocytophaga*, including a *C. ochracea* OpdB deletion mutant.

METHOD

OpdB homologues from *C. gingivalis* CCUG9715, *C. ochracea* CCUG 9716 (CoOpdB), *Treponema denticola* (Td) ATCC35405 (TdOpdB), *Tannerella forsythia* (Tf) CCUG21028 (TfOpdB) and *Porphyromonas catoniae* (Pc) CCUG41358 (PcOpdB) were cloned, expressed and purified using established procedures. Strains were generally cultivated anaerobically in modified ATCC medium 2722 (planktonic and biofilm mode). Peptidolytic activities were quantified using commercial chromogenic/fluorogenic peptide substrates, including: BApNA, Z-Arg-Arg-AMC, Z-Gly-Phe-AMC, Ala-Phe-Lys-AMC) (Z=benzyloxycarbonyl; pNA=p-nitroanilide; AMC=aminomethylcoumarin). The CoOpdB gene was deleted by allelic replacement with an erythromycin resistance cassette.

RESULTS

All recombinant OpdB proteins had 'trypsin-like' activities, preferentially cleaving polypeptide chains after Arg compared to Lys residues, However, there were notable differences in substrate specificity. TdOpdB, TfOpdB, PcOpdB and CgOpdB2 had the most potent 'trypsin-like' activities. CoOpdB consistently had the weakest activities, CgOpdB1 was intermediate. CgOpdB1, which contains a Bacteroides/Flavobacterium lipoprotein export signal (LES) peptide that targets it to the cell surface, was atypical being dimeric, with its proteolytic activities stimulated ca. 3-fold by calcium ions. Growth rates and biofilm formation capabilities of Co wild-type and ΔopdB strains were similar. Deletion of *opdB* abrogated BApNA activities, and reduced Z-Arg-Arg-AMC hydrolysis rates 80%, indicating other peptidases (putatively CoDPP4) have partial redundancy with OpdB. Further biological investigations are ongoing.

CONCLUSION

Two OpdB homologues contribute to the overall 'trypsin-like' peptidolytic activities of *C. gingivalis*, which are comparable to those of Td, Tf and Pc.

Keywords: Bacteria, peptidase, enzyme, oral biofilm, periodontitis
Acknowledgements/Funding (optional): RMW acknowledges financial support from the Research Grants Council (RGC) of Hong Kong via GRF grant #17121820.

P.03

Association between Streptococcus mutans detection and caries activity in oral microbiome studies: A systematic review and meta-analysis

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ABSTRACT

It has been questioned whether *Streptococcus mutans* can still be considered the major etiological agent for caries. The main argument is that most evidence has been based on single-species identification. The composition of oral microbiome was not analyzed. This systemic review aims to assess the detection frequency and abundance of *S. mutans* in caries-active and caries-free subjects based on clinical studies where microbiome was investigated. Three databases (PubMed, Cochrane, Embase) were searched until May 22, 2023, for eligible publications which included caries-active (CA) and caries-free (CF) subjects and reported the detection of both *S. mutans* and the oral microbial community, using DNA-based methods. The clinical and microbial outcomes were summarized and further analysed using a random-effects model. Of 22 eligible studies, 3 were excluded due to the high risk of bias. In the remaining 19 studies, 4 reported the mean detection frequency of *S. mutans* only, 3 reported its mean relative abundance only, 9 reported both parameters, and 3 reported a significant difference only. The detection frequency of *S. mutans* in CA was either similar to (n = 4) or higher than (n = 9) CF group. The reported relative abundance in CA was higher than CF in all studies (n = 11), though the values of both groups varied from 0.001 to 5%. Meta-analysis confirmed the significance of these findings. The summary of microbial community data did not reveal other caries-associated bacterial genera/species than *S. mutans*. In conclusion, the collected evidence based on oral microbiome studies suggests a strong association between the detection frequency and abundance of *S. mutans* and high caries activity, even though the relative abundance of *S. mutans* in oral microbiota was generally low.

Keywords: Dental caries, Caries susceptibility, Case-control studies, Detection frequency, Relative abundance

P.04

Investigation of the microbiome of oral leukoplakia (OLK)

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A B S T R A C T

Aims: The aims of this study were 1) Investigate the microbiome of OLK, the most common of the 11 recognised oral potentially malignant disorders (OPMDs) [1], and establish if it differs from that of normal healthy mucosa and 2) Examine whether the degree of dysplasia on biopsy, currently the most accurate predictor of malignant transformation risk [2], influences the microbial community of OLK.

Methods: Mucosal swabs of disease and contralateral normal sites were collected from patients with OLK attending Oral Medicine and Dysplasia clinics in the Dublin Dental University Hospital. Exclusion criteria included no available histology report, certain systemic diseases, and any immunosuppressant, antibiotic or steroid use in the previous two months. DNA was extracted from the swab samples, the V1-V3 region of the 16S rRNA gene was sequenced using the Illumina MiSeq and the data analysed using RStudio.

Results: 216 disease and 202 contralateral normal site samples from 178 patients were available for analysis. Five taxa, Bergeyella sp. HMT322, Leptotrichia sp. HMT215, Streptococcus australis infantis clade 431, Gemella morbillorum, and Leptotrichia sp. HMT392, consistently showed increased abundance in OLK after adjusting for multiple variables, including smoking and oral site. Two species, Gemella morbillorum and Mogibacterium diversum were associated with no and mild dysplasia while three taxa, Bergeyella sp. HMT322, Streptococcus australis infantis clade 431 and Leptotrichia sp. HMT215 were associated with moderate and severe dysplasia, with Bergeyella sp. HMT322 appearing to be an independent marker for severe dysplasia.

Conclusion: In the largest oral microbiome swab study to date, we have identified microbiome changes consistently associated with OLK. We have also demonstrated that OLKs with different degrees of dysplasia display different microbial communities, suggesting that the microbiome may play a role in the pathogenesis of OLK.

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Keywords: Oral microbiome, oral leukoplakia, oral epithelial dysplasia
Acknowledgements/Funding: This work was funded by the Health Research Board, Ireland

P.05

New Insights into the Impact of Major Fimbriae (FimA) Genotypes of Porphyromonas gingivalis on the Periodontitis Phenotypes

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A B S T R A C T

Periodontitis is a chronic, immuno-inflammatory disease characterized by the loss of the supporting tissues leading to tooth loss [1]. *Porphyoromonas gingivalis* plays a crucial role in causing dysbiosis, disrupting host immunity, and perpetuating inflammation in periodontitis. The first step of host infection is the initial fimbriae (FimA) adhesion to gingival epithelial cells. FimA are filamentous structures anchored to the external bacterial membrane [2]. Previous studies have shown a correlation between the phenotypes of FimA and the adhesion capacity to the host epithelium. Type I has been related to health and type IV to periodontitis [3]. Currently, there are few diversity and structural studies of FimA genotypes that link it to the disease phenotype. The aim of this study is to determine the diversity and evolutionary properties of FimA of *P. gingivalis* to gain a better understanding of their role in infection and their host specificity.
We obtained nucleotide and amino acid sequences from 84 high-quality *P. gingivalis* genomes and conducted phylogenetic analyses using IQTREE. Genetic variability was assessed using Tajima's D value and Datamonkey. The 3D structure of FimA genotypes was obtained from the PDB database, and the structural comparisons were conducted using PyMOL.
The phylogenetic analysis of FimA sequences showed clustering based on fimbrial genotype. Tajima's D value suggests that there is no excessive allele diversity in the population of *fimA* genes. However, the Datamonkey analysis reveals five positively selected sites, indicating hotspots of non-synonymous substitutions.
This study provides a novel approach to investigating the initial adhesion processes of the key pathogen *P. gingivalis* in periodontitis, by exploring de evolution and mechanical properties of FimA This comparative genomics study allowed us to describe the differences between the sequences and structure of FimA; and contributes to our understanding of the genetic diversity of FimA and its potential implications for periodontitis.

Keywords: *Porphyromonas gingivali*s, periodontitis, comparative genomics, FimA
Acknowledgements/Funding: This work was supported by ANID through FONDECYT projects 11200209 (JPC) and 1221064 (JARP). JNB is supported by the Genomics Integrative PhD Program at Universidad Mayor
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P.06

The effect of chlorhexidine and common antibiotics on the composition and nitrate metabolism of subgingival plaque – an *in vitro* study

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A B S T R A C T

Chlorhexidine and antibiotics are commonly recommended as adjunct treatments for patients with periodontitis to eliminate oral bacteria that cause inflammation. However, these antimicrobial compounds often target a broad spectrum of bacteria and can lead to microbial resistance. Additionally, recent evidence indicates that chlorhexidine-containing mouth rinses can impair nitrate metabolism, leading to a decrease in systemic nitric oxide levels and the associated adverse effects on human health. The aim of this study was to test effect of chlorhexidine (CHX) and two common antibiotics, namely metronidazole (MET) and amoxicillin (AMX), on the composition and nitrate metabolism of subgingival plaque. For this, subgingival plaque of 12 patients with periodontitis was incubated for 8 hours under eight different conditions: control, 8 mM nitrate (NO_3-), MET, MET+NO3-, AMX, AMX+NO3-, CHX and CHX+NO3-. Gingival crevicular fluid (GCF) levels of MET were used (16 µg/ml), but GCF levels of AMX (8 µg/ml) and commonly studied levels of CHX (0.02%) killed all bacteria and had to be diluted 1:12 to determine their effects on bacterial metabolism. Supernatant samples were taken for nitrate, nitrite and pH measurements, and biofilm samples for 16S rRNA gene Illumina sequencing. Due to the metabolism of the subgingival communities, the pH decreased significantly in all conditions ($p < 0.05$), except AMX. Nitrate reduction was limited by CHX and AMX, but not by MET. The different conditions had different significant effects on bacterial species associated with periodontal health and disease. In conclusion, CHX and AMX can limit nitrate metabolism and future research should determine how this can affect nitric oxide availability and systemic health.

Keywords: Nitrate, antibiotics, chlorhexidine, subgingival microbiota, periodontitis.

P.07

Porphyromonas gingivalis long fimbriae superstructures and their role for targeted transport and release of extracellular vesicles

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The fimbriae of *P. gingivalis* are lamellar appendages on the cell surface composed of various proteins. Fimbriae play a critical role in biofilm formation, auto- (self-)aggregation, co-aggregation with other bacteria, adhesion to host molecules and, subsequently, host cell invasion. A direct role of fimbriae in osteoclastogenesis and thus alveolar bone resorption as well as in the activation of the Toll-like receptor-2 (TLR2) and TLR4/NF-B signaling pathway was assumed. PG expresses long Fim-fimbriae of 0.3-1.6 microns length, composed of FimA (stalk), FimB (anchor) and accessory FimCDE, as well as short Mfa-fimbriae, side by side. Long (major) fimbriae can be arranged in superstructures including bundling, cell-cell knotting and brick-wall formation, as recently demonstrated by our group[1].

Here the interplay between long fimbriae and release/transport of PGs extracellular vesicles (ECV) was investigated by applying transmission- (TEM) and raster- (REM) electron microscopy. ECV are known to contain a cocktail of more than 20 proteins, among them gingipains, hemagglutinin and the fimbrial tip-complex FimCDE. It is further known that FimCDE is required for i) invasive activity of ECV on gingival fibroblasts[2], i) TLR2/CR3-mediated and PPAD-dependent entry and persistence in macrophages[3], and iii) adherence to type I collagen/fibronectin[2].

Taken together we hypothesize that the FimCDE-tip of long fimbriae may function as ECV outlet/syringe and that long fimbriae and ECV co-act as virulence and invasion mechanism. We will show some pictures underlining the role of bundled PG fimbriae on biofilm formation and this targeted transport and release of ECV.

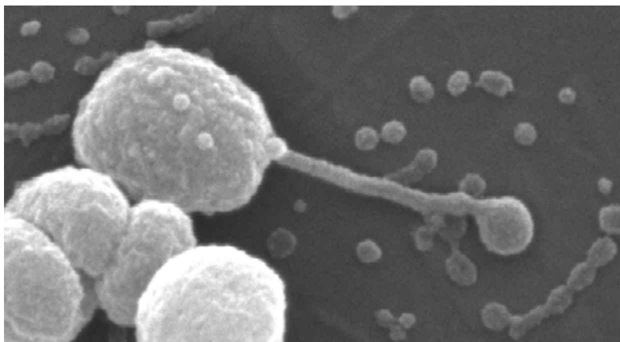

Fig. 1: ECV flow along fimbriae

Keywords: *Porphyromonas gingivalis*, long fimbriae, FimCDE, extracellular vesicles, virulence.

Acknowledgements/Funding (optional): This study was funded by the Interdisciplinary Centre for Clinical Research (IZKF OC1-06) of the RWTH Aachen University Hospital.

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P.08

Effect of a probiotic combination on oral Candida colonization in head and neck cancer patients undergoing radiotherapy: randomized clinical trial.

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A B S T R A C T

The adverse effects of antineoplastic therapies in the head and neck region can significantly impair the quality of life of patients. Among these effects, oral complications, such as candida infections, stand out [1], [2], [3]. The aim of this study is to evaluate the effect of a combination of probiotics on the presence of oral Candida spp. in patients with head and neck cancer undergoing radiotherapy treated in the radiotherapy service at the Catalan Institute of Oncology (ICO) Hospitalet.
A randomized, double-blind clinical trial was conducted in patients at the Catalan Institute of Oncology (ICO) Hospitalet. The probiotic group received a combination that included *L. rhamnosus, L. casei, S. thermophilus, B. breve, L. acidophilus, B. infantis and L. bulgaricus*. One sachet dissolved in water was administered once daily for 30 days. The placebo group received sachets identical in appearance, but without the active ingredient. Unstimulated saliva samples were taken before and after the intervention, and cultures were performed in Brilliant Green chromogenic medium to quantify and identify the type of Candida present.
A total of 30 patients were recruited, consisting of 11 women and 19 men, with a mean age of 56 years. An effort was made to ensure an equal distribution in terms of age and sex in the studied population. A change in Candida count (CFU/ml) was recorded in both groups, with a higher prevalence of Candida albicans. In addition, other species were identified, including *Candida glabrata, Candida tropicalis, Candida krusei, Candida parapsilosis and Candida guilliermondii.*

Keywords: Oral cancer, probiotics, Candida

Literature references.

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P.09

3D oral mucosa model to evaluate bacterial invasion

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ABSTRACT

In recent years, significant progress has been made to develop physiologically relevant models of the oral environment mimicking hard and soft tissues, starting from simple 2D to also more complex 3D models and from the application of planktonic bacteria to multi-species biofilms. This study aims to establish a 3D oral mucosa model consisting of primary human gingival fibroblasts in collagen matrix covered with primary human oral keratinocytes. Collagen type I (rat tail, Ibidi) was diluted to 3 mg/mL and pH was adjusted to 7 before human gingival fibroblasts (ScienCell) were added. The cells with the matrix were incubated in CnT-Prime Fibroblast Proliferation Medium (CellnTec) for 7–10 days (medium change every 2-3 days) before human oral keratinocytes (ScienCell) were added and medium exchanged to CnT-Prime Co-culture medium (CellnTec) for another 3 days. After that the samples were airlifted and medium was exchanged to CnT-Prime Full Thickness 3D Airlift medium (CellnTec) for 14 days (medium change every 2-3 days). In order to see the structure, 3D models were stained using anticytokeratin-19-488 (Biolegend #628508) for keratinocytes, anti-phalloidin-TRITC (Sigma #P-1951) for fibroblasts and DAPI (Sigma #D9542) for cell nuclei or prepared for histological staining. At the same time culturing conditions for *Fusobacterium nucleatum* and *Porphyromonas gingivalis* were optimized to allow oral mucosa model infection. The oral 3D mucosa model showed some limitations regarding the contraction of collagen matrix; however, this could be overcome by adding agents (e.g fibrinogen) to improve elasticity of the structure. Moreover, both anaerobic pathogens were able to survive in 3D mucosa model setting. This model allows to test bacterial infection and, in the future, screen different approaches for soft tissue regeneration.

Keywords: oral mucosa model, human oral keratinocytes, human gingival fibroblasts, periodontal pathogens
Acknowledgements/Funding: This study is funded by Geistlich-Stucki-Stiftung in Basel, Switzerland.

P.10

Identification of New Biomarkers in Gingival Crevicular Fluid and Saliva for Diagnosing Periodontitis by SWATH-MS

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A B S T R A C T

The objective of this study was to discover new gingival crevicular fluid (GCF) and salivary biomarkers to diagnose periodontitis. GCF and unstimulated saliva samples were collected from 44 healthy periodontal individuals and 41 with untreated periodontitis (stages III-IV). Samples were analysed by sequential window acquisition of all theoretical mass spectra (SWATH-MS), and proteins were identified employing the UniProt human-specific database [1]. Data are available via ProteomeXchange with identifiers PXD043474 (GCF) and PXD043491 (saliva). The diagnostic capacity of the proteins was determined with generalised additive models (GAM).

In GCF, three proteins showed an outstanding bias-corrected area under the curve (bc-AUC) >0.930 [2]. The bc-accuracy (ACC) (bc-sensitivity/bc-specificity) of these proteins were: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with 92.4% (91.3%/91.3%), zymogen granule protein 16 homolog B (ZG16B) with 89.4% (91.8%/87.2%), and carbonic anhydrase 1 with 89.3% (83.5%/94.6%). Two-protein combinations increased the predictive capability as GADPH with matrix metalloproteinase-9 (MMP9) and ZG16B with cornulin showed a bc-AUC of 0.999 with a bc-ACC of 99.9% (100%/99.8% for both combinations).

Three salivary proteins achieved excellent bc-AUC values >0.840 [2]. The bc-ACC (bc-sensitivity/bc-specificity) of these molecules were: isoform 2 of tropomyosin alpha-3 chain with 86.8% (70.5%/98.1%), resistin with 85.0% (81.7%/88.1%), and beta-2-microglobulin with 84.0% (67.7%/96.3%). Again, bivariate modelling increased these values, as beta-2-microglobulin with profilin-1 and lysozyme C with ZG16B obtained a bc-AUC >0.985 with a bc-ACC of 96.3% (96.0%/96.7%) and 92.8% (93.4%/92.3%), respectively.

Consequently, predictive modelling of GCF and salivary proteins uncovered unprecedented biomarkers with excellent performance in distinguishing periodontitis from periodontal health. In both fluids, diagnostic accuracy improved by two-protein combination. As expected, GCF proteins show better values than salivary molecules. However, the proteins with the highest diagnostic ability in each sample type differ, which would justify the use of different protein biomarkers to identify periodontitis in each medium. Future research must validate these findings.

Keywords: Diagnostic accuracy, molecular biomarkers, periodontitis, proteomics, oral fluids

Funding: This study has been funded by Instituto de Salud Carlos III (ISCIII) through the project PI21/00588 and co-funded by the European Union (UE).

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P.11

Immunomodulatory effects of oral streptococci in periodontal disease

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ABSTRACT

The oral microbiome is a critical component of oral health and disease. Bacteria exist in communities, in close communication with one another and the human host. Within these communities *Streptococcus spp.* play an important role in health but also in response to changes in environmental conditions that can lead to dysbiosis. Periodontitis is a significant oral inflammatory disease of the supporting tissue of the teeth that is triggered by dysbiosis. The inflammatory response is coordinated by intercommunicating immune cells and inflammatory mediators that respond to bacterial stimuli leading to an overstimulation of the immune response and consequently tissue damage. The critical components involved in this transition to disease have not been delineated. Platelets recognize bacteria and participate in inflammation by release of mediators, binding to neutrophils and monocytes, modifying their immune function [1]. We propose that platelets may contribute to the pathogenesis of periodontitis. We are therefore investigating the effect of different *Streptococcus* spp. on innate immune cell activation in blood: platelets, neutrophils, and monocytes using flow cytometry. Our preliminary results have demonstrated pathogen-specific profiles of innate immune cell activation. In particular, distinct patterns of activation have been observed for neutrophils and monocytes exposed to *Streptococcus gordonii* and *Streptococcus constellatus.* This highlights the need to investigate the immunomodulatory effects of distinct species of *Streptococcus* associated with oral health and disease.

Keywords: *Streptococcus* spp., immune response, immune cells, inflammation, periodontitis
Acknowledgements/Funding: The Crafoord foundation. Biofilms Research Center for Biointerfaces.

[1] DOI: 10.1016/j.tmrv.2020.09.005

P.12

Fitness costs and evolutionary dynamics of Tn916 in oral streptococcal isolates

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ABSTRACT

The oral microbiome draws increasing scrutiny within the health sector due to the presence of antimicrobial resistance determinants and Mobile Genetic Elements (MGEs), which significantly aid the dissemination of antimicrobial resistance genes. Our investigation aimed to explore the prevalence, diversity, and evolutionary patterns of the Integrative Conjugative Element (ICE) belonging to the Tn*916*-Tn*1545* family in oral streptococci. Analyzing a sample pool of 100 oral streptococcal isolates from Norwegian hospitals revealed the predominant member as the wild-type Tn*916*. Additionally, our study uncovered two novel ICE family members, named Tn*6815* and Tn*6816*, alongside Tn*916*. These elements demonstrated high transferability to various oral streptococcal isolates at relatively high frequencies, despite incurring considerable fitness costs upon acquisition. However, their sustained presence in bacterial populations and potential impact on host survival remain unclear. To address this, we traced the evolutionary path of Tn*916* within a naïve host, monitoring growth and metabolic rates across 1000 generations. Upon Tn*916* integration into the naïve *Streptococcus oralis* host, we observed an initial fitness cost, resulting in reduced growth and maximum metabolic activity. Interestingly, within 500 generations, these costs were mitigated, with evolved populations outperforming their non-evolved counterparts by 1000 generations. Despite restored fitness post-Tn*916* acquisition, a lower metabolic rate persisted. This strategic shift suggests energy efficiency, facilitating heightened growth rates while conserving cellular resources. These findings underscore the significant role Tn*916* elements play in propelling antibiotic resistance among oral streptococci and how Tn*916* acquisition influences cellular functions beyond growth rates. Such factors highlight the importance of monitoring bacterial evolutionary paths to comprehensively understand the impact of horizontal gene transfer, MGEs, and ICEs on bacterial populations.

Keywords: Bacterial evolution, Antibiotic resistance, Fitness cost, Metabolic rate, Integrative Conjugative Elements (ICEs), Tn*916*, *Streptococcus oralis*

P.13

Effects of MUC5B on early oral biofilm glucose metabolism

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ABSTRACT

In this study, nuclear magnetic resonance was used to study biofilm metabolomic profiles. Oral biofilms contain metabolic networks that are highly integrated, regulated by factors of bacterial (intra- and interspecies), host (oral surfaces, fluids and immune components) as well as external (dietary, pharmaceutical and other) origin. Metabolic events in establishing biofilms play essential roles in modulating succession and determine the conditions for eubiosis. The salivary mucin MUC5B seems to promote homeostasis between the host and the oral biofilms by promoting commensal colonization and proteomic and metabolomic responses associated with eubiotic phenotypes in oral bacteria such as *Streptococcus gordonii*. The current study examined the regulatory effects of human salivary MUC5B on glucose metabolism in dual species oral biofilms from clinical isolates of *S. gordonii* and *Actinomyces naeslundii*. Preliminary results indicate that the presence of MUC5B had modulatory effects on the glucose metabolism in the early oral biofilms. Studying host-mediated regulation of early oral biofilm metabolism and microbial metabolic interactions is essential for understanding mechanisms for oral biofilm maturation and may contribute to the development of novel predictive biomarkers or preventative treatment strategies for biofilm-related oral health.

Keywords: Oral biofilms, salivary MUC5B, glucose metabolism, oral microbiology

P.14

The influence of periodontal microbial biomarkers on reaching the endpoints of therapy

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A B S T R A C T

Periodontitis is elicited by the manifestation of a dysbiotic microbiota, that induces an immune-inflammatory infiltration within the periodontium [1, 2]. In the current classification, Tonetti et al. suggested various additional parameters, i.e. the phenotype or biomarkers for periodontal grading [3]. In this context, the overwhelming majority of biomarkers comprise cytokines and chemokines particularly expressed by the diseased periodontal tissue in response to a dysbiotic subgingival microbiome [1, 2].

Therefore, this study aimed to analyse the influence of microbial biomarkers collected from the gingival crevicular fluid (GCF) on non-surgical periodontal therapy (NST). NST was performed in 222 patients with stage III periodontitis. Prior to NST Bacterial DNA was isolated from GCF samples and quantitatively analysed for the microbial biomarkers (Porphyromonas gingivalis (PG), Aggregatibacter actinomycetemcomitans (AA), Prevotella intermedia (PI), Fusobacterium nucleatum (FN), Treponema denticola (TD), and Tannerella forsythia (TF)). The identification and detection of the bacteria were carried out using ELISA-based assays, following the manufacturer's instructions. The quantity of microbial biomarkers was assessed as relative optical density and correlated with the therapy endpoint (TE) defined as ≤4 sites with PD≥5mm. Following to completion of NST 28% of the patients achieved TE. Patients not achieving TE after NST had higher PG, FN, and TF concentrations at baseline (PG: p=0.010, FN: p=0.008 TF: p=0.004). Multivariate binary logistic regression analysis identified TF (OR 2.766, p=0.009) as an independent modulator of treatment outcome after NST.

In conclusion, an increased microbial load leads to a higher risk of failing the TE after NST. The findings suggest that specifically baseline TF levels are correlated with poorer treatment outcomes and might improve the accuracy of grading of periodontitis.

Keywords: Periodontal therapy, periodontitis, microbiota

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P.15

Determining the Effect of Processed-grain or High-fiber Diets on Microbial and Immunological Markers of Periodontitis.

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ABSTRACT

Objectives. Microbial dysbiosis and inflammation are well-recognized drivers of periodontal disease etiology and progression. However, the role of diet in these processes is poorly understood. Consumption of ultra-processed carbohydrates has been associated with increased incidence of periodontitis. Conversely, high fiber consumption typifying pre-agricultural diets is linked to periodontal health. Here, we sought to determine the effect of processed carbohydrate or high-fiber diets on periodontal disease severity via morphological, immunological, and microbiological outcomes.

Methods. BALB/cByJ mice were assigned to whole grain, processed grain, fiber bar, or placebo bar diets (n=16) for four weeks. Mice were subsequently split into infection and non-infection groups (n=8); infected mice received a polymicrobial inoculum of periodontal pathogens P. gingivalis, F. nucleatum, T. denticola, and T. forsythia for four weeks while control groups received a sham inoculum. Hemi-mandibles and maxillae were harvested to assess alveolar bone loss via micro-computed tomography, periodontal inflammation via cytokine analysis, and the taxonomic profile of the periodontal microbiome by 16S rRNA sequencing and analysis.

Results. Significant increases in alveolar bone loss were observed in processed compared to whole grain groups ($p<0.05$). Significant differences in inflammatory cytokine profiles were observed among processed vs whole grain groups ($p<0.05$). Microbiota composition significantly related to diet among uninfected groups (Unweighted UniFrac; $R^2=0.31$; $p<0.05$). Significant differences were identified for processed vs whole grain groups among the infection cohort (Unweighted UniFrac; $R^2=0.32$; $p<0.05$) and for fiber vs placebo bar groups in the uninfected groups (Unweighted UniFrac; $R^2=0.32$; $p<0.05$).

Conclusions. The present study suggests immunological and microbially mediated mechanisms by which diet influences periodontitis phenotypes. Processed grain diets induce significant increases in alveolar bone loss and inflammatory cytokine profiles compared to whole grain groups. Diet prompts significant differences in oral microbiota composition in both processed vs whole grain and fiber vs placebo bar diet groups.

P.16

Citrulline as a potential prebiotic & synbiotic for dental caries

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A B S T R A C T

Oral diseases such as caries, periodontitis or halitosis are the result of dysbiosis in the oral microbiome. Cavities develop due to an increase in acidogenic bacteria that metabolize carbohydrates from food, producing organic acids that lead to enamel demineralization. Therefore, among several strategies to address this dysbiosis, such as the use of arginine as a prebiotic neutralizing acids through ammonium production [1]. During the arginine metabolism through the arginine-deiminase system (ADS) by some oral bacteria, two molecules of ammonium are released. The ammonium produced increases dental biofilm pH, reducing the acidification and inhibiting demineralization. In the first stage of ADS, arginine is hydrolyzed to citrulline, releasing the first ammonium molecule, and during the subsequent metabolism of citrulline, the second ammonium is generated [2]. In this study we wanted to evaluate the *in vitro* effect of citrulline as a potential prebiotic on oral biofilms derived from saliva samples. Furthermore, a possible synergistic effect between citrulline and the probiotic *Streptococcus dentisani* (ADS–positive bacteria) on oral biofilms was studied. Results show that in both conditions (prebiotic and symbiotic), citrulline treatment produced a significant increase in the pH of the biofilms supernatant attributed to an increase in ammonium concentration. Likewise, a synergistic effect was observed in the improvement of both parameters in the combination with the probiotic, where citrulline significantly improved beneficial effects of *S. dentisani*. Regarding biofilm bacterial composition, no significant differences were observed after prebiotic or synbiotic administration, suggesting that the effect may be related with changes at the transcriptional level. In conclusion, although the ammonium-induced alkalization was lower than that observed with arginine, these results demonstrate the prebiotic potential effect of citrulline against dental caries.

Keywords: Citrulline, prebiotic, caries disease, arginine, *Streptococcus dentisani*.

Funding: This study has been funded by Sunstar Group.

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P.17

Oral and intestinal microbiomes in patients with periodontitis: design and preliminary results of the first national study (Uruguay)

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A B S T R A C T

Periodontitis is a chronic immunoinflammatory disease that results from deregulation of homeostasis between the oral microbiota (dysbiosis) and the host's defense mechanisms that cause damage to the tooth-surrounding tissues. In addition to its impact on oral health and quality of life, periodontitis has a significant impact on other organs and constitutes a relevant comorbidity for diseases such as diabetes, rheumatoid arthritis or gastrointestinal dysbiosis. The connection between periodontitis and systemic health occurs as a consequence of an altered oral microbiota and an inflammatory process, which affects other bacterial communities such as the gut microbiota. Non-surgical periodontal treatment involves control of the supra and subgingival bacterial biofilm, and has been shown to affect the oral and intestinal microbiota. In this project, we will determine which are the most relevant microbiome changes induced by non-surgical periodontal treatment in Uruguayan patients with periodontitis. In addition, we're collecting clinical, biometric, sociodemographic and nutritional data of patients, aiming to develop an algorithm to improve diagnosis and management based on a limited number of oral bacterial species combined with metadata. While the core idea of this project is common ground in the field, it is the first of its kind on the Uruguayan population and, due to the type and complexity of data collected we look for species that can serve as prognostic or diagnostic biomarkers for periodontal disease, or even discover species with therapeutic potential (e.g. probiotics). This work will presents the methodology and preliminary results obtained with the first cohort.

Keywords: periodontitis, nutrition, oral microbiome, gut microbiome, sequencing

Acknowledgements/Funding (optional): This project is sponsored by Comision Sectorial de Investigacion Cientifica, Universidad de la Republica (ID#22520220100160UD) grant to BM and EA, Banco Santander Uruguay small grants to BM and Comision Academica de Postgrado to MC.

P.18

Rapid griess assay – a chair-side evaluation of oral nitrite production

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OBJECTIVE

Nitrite (NO_2-) is formed in the oral cavity through the enzymatic reduction of dietary nitrate by certain bacteria in the resident oral microbiota. High oral nitrite concentrations are strongly linked to cardiovascular health. Recent studies pose a possible relationship between oral nitrite production and oral health. However, due to the complexity of nitrite quantification and lack of simple chair-side methodologies, clinical studies become time consuming and difficult to perform. We therefore set out to develop and evaluate such a method, as well as to investigate the long-term stability of oral nitrite production in healthy subjects.

METHOD

Nitrite concentration in samples were evaluated using a colorimetric chair-side method: the Rapid Griess Assay (RGA). The colorimetric results were controlled against measurements of optical density. Samples from 12 healthy individuals were collected from the dorsum the tongue, whole saliva, and supragingival dental plaque on four separate occasions and with one week spacing. Bacterial densities from the different sites were controlled for by total viable counts.

RESULTS

The RGA was found to provide reproducible and stable results for chair-side nitrite concentration estimation from *ex vivo* samples, with a detection level of 0.64 µM. Tongue and salivary samples showed good long-term intra-individual stability, whereas plaque samples varied greatly. Inter-individual nitrite concentrations showed substantial variation for all sample sites. The dorsum of the tongue showed the greatest nitrite concentrations, whilst salivary samples showed the lowest.

CONCLUSION

The RGA is a viable option for chair-side estimation on oral nitrite production capacities. Healthy individuals differ in nitrite production abilities, but tongue samples systematically show the strongest and most consistent levels.

Keywords: Oral nitrite, Nitrate reduction, Griess test, Oral health, Chair-side test.

P.19

Salivary microbiome and proteome in kidney transplant recipients up to 24-months after transplantation

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ABSTRACT

Kidney transplantation is the gold-standard renal replacement therapy for end-stage kidney disease (ESKD) patients. Despite oral dysbiosis and altered saliva composition has been described in ESKD, the relationship between oral microbiome (OM) and oral proteome (OP) is currently unexplored. This study aimed to investigate the interplay between OM and OR in ESKD receiving a kidney transplantation (KTR) from live kidney donors (LKD) and dissect any association with renal function recovery, assessed by estimated glomerular filtration rate (eGFR), or with organ donation.
The salivary OM and OP from KTR/LKD pairs was evaluated by throughput sequencing (16S rRNA gene) and proteomics analysis, respectively, before kidney transplant/donation (T0) and at 6 (T6), 12 (T12), and 24 (T24) months after.
Oral microbiome of ESKD differed significantly from healthy donors (T0; $p=0.04$), however these differences fade away progressively post-transplant, following renal function recovery, presenting only a slight difference at T6 ($p=0.046$) but no differences at T12 and T24. Also, 21 salivary proteins were found dysregulated in ESKD, falling into two functional clusters: one mainly composed by cysteine protease inhibitors and the other mainly composed by metabolism-related enzymes (lactate dehydrogenase, catalase, and arginase-1). Almost all differences between LKD and KTR became elusive at 6 months. An inverse correlation was found between *Streptococcus* and peptidyl-prolyl-cis-trans-isomerase-A (cyclophilin A), a proinflammatory cytokine with a strong chemotactic effect on leukocytes, and a positive correlation between *Bergeyella* and cystatin-D (CST5), a cysteine proteases-protective protein. With the exception of CST5, all remaining secreted cystatins (CST1/2/4) were significantly correlated with GFR, suggesting that renal function recovery is paralleled with an increase in oral cystatins. Also, an inverse relationship between *Bergeyella* and GFR suggests that renal function recovery has implications in oral microbiota composition. This study provides relevant clues about the oral cavity-kidney axis dynamics and pinpoint potential markers of kidney function impairment/recovery.

Keywords: Chronic Kidney Disease, End-Stage Kidney Disease, Oral Microbiome, Oral Peptidome

Acknowledgements/Funding: This work has been supported by EPIC-XS, project number 823839, funded by the Horizon 2020 programme of the European Union. Alencastre I.S. was supported by FCT/MCTES contract DL 57/2016/CP1360/CT0007 and Ricardo Araujo by Individual Call to Scientific Employment Stimulus—Second Edition (grant number CEECIND/01070/2018). The PhD fellowship of Maria João Maia Azevedo was supported by an FCT/MCTES scholarship with the reference SFRH/BD/144982/2019.

P.20

Interactions of Nitrate-reducing and Sulphur-metabolising bacteria: Implications for oral health

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ABSTRACT

The beneficial impact of nitrate-reducing bacteria (NRB) on oral health has been shown previously, however the effects of these bacteria in competition with other pathogenic oral bacteria are unclear. In this study, the dynamics of the oral microbiota were examined, focusing on the interplay between NRB (*Veillonella dispar* and *Actinomyces naeslundii*), proteolytic sulphur catabolizing species closely linked to periodontitis (*Porphyromonas gingivalis*), and cariogenic species (*Streptococcus mutans*). This study examined their interactions, as regards sulphur and nitrate metabolism which are crucial in oral microbial ecology.
Combinations of *P. gingivalis, V. dispar*, *A. naeslundii*, and *S. mutans* were cultured in the presence of excess nitrate or cysteine and their interactions analysed with biochemical assays to measure nitrate reduction and H_{2S} production. Gene expression of nitrate reductases *narG* and *narH* in *A. naeslundii* and *V. dispar* was also measured. The study revealed synergistic interactions between H2S production and nitrate reductase activity, dependent upon the bacterial combinations present. Co-cultures of *P. gingivalis, A. naeslundii* and *V. dispar* synergised to produce H2S with cysteine incubation and were inhibited by *S. mutans*. Synergism was observed in the nitrate reductase activity of *A. naeslundii* and *V. dispar* under nitrate supplementation. *P. gingivalis* or *S. mutans* in co-cultures with the NRB significantly enhanced or inhibited this activity respectively. Gene expression data indicated that *narG/narH* expression was elevated in co-cultures compared to monocultures, however the expression was comparable across different combinations. In conclusion, this study highlights the potential of nitrate supplementation to enhance nitrate reductase activity in NRB and suggests a potential role for *P. gingivalis* and *S. mutans* in affecting nitrate reductase activity. Further studies could unravel the complex mechanisms at play in oral microbiota interactions and their implications for oral health.

Keywords: synergism, antagonism, ecology, homeostasis

P.21

Human endothelial cell cytokine and chemokine responses to oral anaerobe infection is species specific

ABSTRACT

Periodontitis is a chronic inflammatory disease associated with inappropriate inflammatory reactions to host oral microbiota, favouring disease progression and resulting in extensive tissue damage. Atherosclerosis is a cardiovascular disease, also associated with chronic inflammation, where increased expression and production of proinflammatory cytokines by vascular cells drives plaque formation and progression. During periodontitis, oral bacteria can invade the bloodstream and interact with endothelial cells, where they can contribute to a plaque-favouring, proinflammatory environment. This study aimed to examine the inflammatory response of endothelial cells in response to a number of oral pathobionts.

Human microvascular endothelial cells (HMEC-1) were incubated with oral bacteria (*Porphyromonas gingivalis (Pg), Fusobacterium nucleatum (Fn), Aggregatibacter actinomycetemcomits (Aa), Tannerella forsythia (Tf) and Streptococcus mitis (Sm)* at increasing MOI (0.1 - 1000) for 4 hours, followed by conditioned medium collection and RNA isolation. RT-qPCR was used to measure fold-change in cytokine gene expression, while ELISA was used to measure protein secretion levels.

Fn caused a significant ($p<0.05$) MOI-dependent fold-change increase in gene expression and protein release of pro-inflammatory cytokines compared to uninfected controls at MOI 100 and 1000. Aa and Tf showed a significant increase at MOI 1000 only. In contrast, Pg and Sm did not cause any significant difference in gene expression or cytokine production.

These data suggests that endothelial cell inflammatory responses toward planktonic-grown oral bacteria is species-dependent, and these may drive an environment that favours atherosclerotic plaque development. Current work examines the interaction of multispecies biofilm fragments on endothelial cells.

P.22

CellTraceä - a Tool for Visualisation of Multi-Species Oral Biofilms

Olivia Aherne, Martina Bardina Mørch, Yashuan Chao, Oonagh Shannon & Julia R. Davies

ABSTRACT

Expanding our understanding of oral diseases requires the use of multi-species microbial biofilm models to better replicate *in vivo* settings. However, identification of individual species and examination of spatial relationships within these models can be difficult and time-consuming using methodologies such as fluorescence *in situ* hybridisation. CellTraceä dyes were originally developed to measure proliferation in eukaryotic cells using flow cytometry. In this study we assess their suitability for visualization of bacterial species in multi-species oral biofilms using confocal microscopy.

CellTraceä fluorescent proliferation dyes [CFSE - green, far red, yellow or violet] were incubated with oral bacterial species (*Actinomyces naeslundii*, *Actinomyces odontolyticus*, *Fusobacterium nucleatum*, *Lactobacillus paracasei*, *Streptococcus mutans*, *Streptococcus gordonii*, *Parvimonas micra*, *Porphyromonas gingivalis* and *Veillonella parvula*) to create single- (SS) and multi-species (MS) biofilms in multi-channel flow-cells. Biofilms were then analysed using confocal microscopy and flow cytometry.

Flow cytometry confimed dyes were taken up effectively into both Gram-positive and Gram-negative bacteria and confocal microscopy confirmed good visualisation and clear identification of the different species in early SS and MS biofilms. Dyes remained stable up to 96 hrs within viable MS biofilms and could be used to determine biofilm parameters such as composition, % coverage and spatial relationships between different species. Staining was independent of cell viability. As proof of principle, microscopy was combined with flow cytometry to study the rate of detachment of bacteria from biofilms over time.

In conclusion, CellTraceä dyes appear to be a simple and effective identification tool for different bacterial species within SS and MS biofilms. Being non-toxic and stable, they allow for continuous monitoring and analysis of complex biofilm development. Finally, their compatibility with both confocal microscopy and flow cytometry highlight the potential for numerous applications.

P.23

Development of a bioinformatic pipeline for archaea detection in dental caries

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A B S T R A C T

The difficulty of establishing a relationship between archaea and oral diseases such as dental caries stems from the challenges of detecting, identifying, and isolating these microorganisms. This study aimed to develop a rigorous bioinformatic pipeline to identify archaea in shotgun sequencing datasets related to dental caries.

A comprehensive systematic search was performed on PubMed to identify studies utilising DNA or cDNA shotgun metagenomic on samples obtained from individuals with dental caries (including dental biofilms, carious dentin, and saliva). Two independent reviewers meticulously selected studies based on predetermined eligibility criteria. Sequencing and metadata from each study were retrieved from their SRA Bioproject. A count table was generated for each database by mapping reads against an archaea genome database, specifically tailored for this study, using stringent filtering parameters of above 95% similarity and 90% query coverage. Archaeal prevalence was determined using an arbitrary cut-off point, considering the proportion of samples with over 500 archaeal reads in total.

A thorough PubMed search yielded 154 titles, from which a collection of 11 datasets from 9 studies was obtained. Despite a low number of reads assigned to archaea within these studies, a remarkable range of archaeal prevalence was observed, spanning from 0 to 82.5% of samples containing archaea. Notably one dataset revealed 100% of archaeal prevalence in saliva samples. Various archaeal phyla were identified including Euryarchaeota, Thermoplasmatota, and Nitrosphaeria. Data suggest the presence of methanogens, more specifically *Methanobrevibacter* spp., *Methanosarcina* and *Methanosphaera*. Genes frequently identified across all databases included ATP synthase subunit A, PFL family protein, ribulose-bisphosphate carboxylase, and ATP-dependent chaperone ClpB. In conclusion, this stringent analysis, designed to minimise false-positive outcomes, uncovered compelling evidence of archaea in dental caries-associated samples. Furthermore, saliva emerged as a notable site of archaeal abundance, surpassing that of dental biofilms and dentin.

Keywords: *Archaea* domain, Oral Archaeome, Methanogens, Oral microbiome, Dental Caries, bioinformatic analysis.**Acknowledgements/Funding (optional):** ND-T and TD acknowledge the UK's Academy of Medical Sciences Newton International Fellowship (NIF \R5\242). JAC acknowledges CAPES (Coordination for the Improvement of Higher Education Personnel) and DPG/DPI/UnB.

P.24

Comparative characterization analysis of two novel mucin-degrading proteases, MdpL and MdpS, from different oral bacteria

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A B S T R A C T

While several studies have contributed to our understanding of the microbial composition within an eubiotic oral flora, the pivotal role of enzymes and their interactions with salivary glycoproteins has mainly been disregarded. Recently, two novel proteases, MdpL and MdpS (Mucin Degrading Protease from *Limosilactobacillus fermentum* and *Streptococcus oralis* respectively) were characterized in regards of their sequence homology, physicochemical properties, substrate and amino acid specificity, cellular localization, and their hydrolytic interactions with MUC5B [1]. Both enzymes exhibit a remarkable conservation of their protein backbone within their respective species and share similarities with streptococcal species that rely on mucins for attachment and nutrition. These enzymes operate extracellularly, degrading MUC5B into smaller protein fragments, albeit with differing levels of efficiency. Additionally, they hydrolyze other O-glycoproteins independently of the O-glycan presence but lacks activity towards non-glycosylated substrates. MdpL functions optimally under reducing conditions and showcases tolerance across various temperatures, salt concentrations, and pH values. It exhibits an amino acid preference for C-terminally located hydrophobic residues, suggesting a limited sequence preference. Conversely, MdpS acts as a serine protease with strict physicochemical properties, displaying sensitivity to increased sodium chloride and reducing agent concentrations, and operates within a narrow pH window. Uniquely, MdpS demonstrates activity towards IgA1/2 and IgM, indicating potential immunomodulatory effects. Its hydrolytic preference appears to be around Ser/Thr residues, elucidating its specificity for O-glycoproteins. In comparison, MdpL and MdpS offer distinct insights into mucin degradation, mirroring the diverse biofilm environments of *L. fermentum* and *S. oralis*. While MdpL showcases broader physicochemical preferences, MdpS exhibits selectivity while significantly enhancing MUC5B degradation. Understanding the intricate interplay between *L. fermentum*, *S. oralis* and MUC5B holds significant implications for managing a healthy eubiotic oral microenvironment. This comprehension offers potential targets for intervention aimed at modulating the composition and succession of oral biofilms.

Keywords: MUC5B, mucin degradation, protease, *Limosilactobacillus fermentum*, *Streptococcus oralis*

Acknowledgements/Funding (optional): FL is part of the Combine Graduate Research School at Malmö University funded by the KK foundation, Grant No. 202100-4920, and FORESIGHT multidisciplinary research programme at Malmö University, Grant No. LED1.4-2017/414

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Co-culture of *Helicobacter pylori* with oral microorganisms in human saliva

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Helicobacter pylori is known for colonizing the gastric mucosa and instigating severe upper gastrointestinal diseases such as gastritis, gastroduodenal ulcers, and gastric cancer [1,2]. To date, there is no data available on the oral cavity as transmission pathway, whether *H. pylori* can survive in the oral cavity and whether *H. pylori* can be cultured in saliva [3].

OBJECTIVE

To investigate the influence of oral microorganisms on the survival of *H. pylori* in human saliva.

METHOD

H. pylori strains KE and SS1 were grown in pooled human saliva (0.22 µm filter-sterilized) or in BBF (Brucella browth formula; control) either as mono-cultures or in co-culture with *Streptococcus mutans*, *Streptococcus oralis*, *Actinomyces naeslundii*, *Lactobacillus casei* and *Candida dubliniensis*. Bacterial growth of *H. pylori* and the oral microorganisms were investigated using colony forming units (CFU) assay at baseline and after 24, 48 and 168 hours.

RESULTS

In saliva, *H. pylori* KE demonstrated enhanced growth in co-culture with *S. mutans*, *A. naeslundii*, and *C. dubliniensis*, enduring for at least 48 hours. In contrast, *L. casei* showed a slightly inhibitory effect on *H. pylori* CFU. *S. oralis* inhibited *H. pylori* KE. Notably, *H. pylori* KE could not be cultured after 168 hours in saliva, either in mono- or co-culture. In contrast, *H. pylori* SS1 could be cultured after 168 hours in co-culture with *S. mutans* and *C. dubliniensis*, but not in mono-culture. All other co-cultured microorganisms survived at high CFU numbers similar to those of the inoculated values.

CONCLUSION

The study suggests that *H. pylori* can transiently survive in human saliva and even with presence of certain oral microorganisms. However, it may not be a permanent resident of the oral microbiota. The co-survival with oral microorganisms emphasizes the necessity for studying the role of oral microbiota in the infectious cycle of *H. pylori*.

Keywords: *Helicobacter pylori*, Human saliva, Oral microrganisms, Co-culture Experiments, Microbial Survival

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P.26

Oral Species with Highly Similar *in-silico* 16S rRNA Gene Amplicons: a Reason to Avoid OTU Clustering

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ABSTRACT

The problems associated with clustering 16S rRNA gene sequences into operational taxonomic units (OTU) are well-known [1]. However, no research to date has evaluated the extent of these limitations in the oral microbiome. The goal of this study was to evaluate the *in-silico* coverage of a set of pre-selected primer pairs to detect oral species having 16S sequence segments with ≥97% similarity.
Thirty-nine primer pairs [2] were employed to obtain *in-silico* amplicons from the complete genomes of 186 oral-bacteria and 135 oral-archaea species. Each fasta file for the same primer was inserted as subject and query in BLASTN [3] for obtaining the similarity percentage between amplicons belonging to different oral species. We selected amplicons with 100% alignment coverage of the query sequences and an amplicon similarity value ≥97% (ASI97). The species coverage with no ASI97 (SC-NASI97) was calculated for each primer.
The primer pairs with the best SC-NASI97 values were OP_F053-KP_R020 for bacteria (region V1-V3; primer position for *Escherichia coli* J01859.1: 9-356), KP_F018-KP_R002 for archaea (V4; undefined-532), and OP_F114-KP_R031 for both domains (V3-V5; 340-801). Approximately 80% of the bacterial and archaeal species tested had an ASI97 with at least one other. These highly-similar species have different roles in the oral environment and belong to bacterial genera such as *Campylobacter*, *Rothia*, *Streptococcus*, *Tannerella*, and archaeal genera such as *Halovivax*, *Methanosarcina*, and *Methanosalsum*. It is worth noting that ~20% and ~30% of the two-by-two similarity relationships were among species from different bacterial and archaeal genera, respectively. Even taxa from distinct families, orders, and classes can be grouped in the same OTU.
Sequence clustering with 97% similarity provides an inaccurate description of oral-bacterial and oral-archaeal species, irrespective of the primer pair used. This can greatly impact the microbial diversity parameters and condition the credibility of associations between specific species and certain health/disease conditions.

Keywords: oral microbiome, 16S rRNA gene, operational taxonomic unit, primer.
Acknowledgements/Funding (optional): This study has been funded by Instituto de Salud Carlos III (ISCIII) through the projects PI17/01722 and PI21/00588 and co-funded by the European Union (UE).

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P.27

Hydrogel-delivered bismuth-doped carbon dots-based nanomedicine treats periodontal disease *via* antimicrobial & immuno-modulatory approaches

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A B S T R A C T

Porphyromonas gingivalis (Pg) as the keystone pathogen in periodontal disease could produce different virulence factors to subvert immuno-inflammatory responses *via* various mechanisms and effector molecules. We previously report that metronidazole-treated Pg maintains the capabilities to invade host cells and perturb the innate responses [1], while the bismuth drug could inhibit Pg and reverse Pg-perturbed host immuno-inflammatory responses [2]. Meanwhile, the nanoparticle-based system, like carbon dots (CDs), can efficiently enhance treatment outcome in oral healthcare [3]. Herein, we report a bismuth-doped CD-based nanomedicine delivered by hydrogel to inhibit Pg and modulate Pg-perturbed host immuno-inflammatory responses *in vitro* and *in vivo*. The bismuth-doped CDs (BiCD) are synthesized *via* hydrothermal reactions and then conjugated with amine-modified berberine derivative (Ber-NH2) for BiCD-Ber. The as-synthesized nanomedicines could eliminate planktonic Pg and its biofilms, and BiCD-Ber show stronger antimicrobial effect than BiCD, resulting from the synergistic effect of conjugated Ber-NH2. Moreover, both BiCD and BiCD-Ber can significantly decrease the extracellular and intracellular Pg. Importantly, after eliminating the extracellular Pg using antibiotics, BiCD-Ber at 100 μg/mL effectively eradicate 97.5% and 97.8% intracellular Pg in primary human gingival fibroblasts (pHGFs) and gingival epithelial cells. BiCD and BiCD-Ber at 50 μg/mL partially reverse Pg-perturbed immune-inflammatory responses in pHGFs by restoring the IL-1-stimulated expression of IL-6 and IL-8. This might be due to that the as-synthesized BiCD and BiCD-Ber could neutralize the gingipains and prevent the degradation of the cytokines. Afterwards, a functionalized hyaluronic acid (HA) is photo-crosslinked into the injectable gel to encapsulate BiCD-Ber and treat the ligature-induced and Pg-inoculated rat periodontitis by reducing the inflammation at the gingival compartment and bone absorption. Taken together, the bismuth-doped nanomedicines have demonstrated promising therapeutic effects in periodontal treatment by inhibiting the growth of Pg and modulating the innate immuno-responses.

Keywords: periodontal disease, bismuth carbon dots, anti-*Porphyromonas gingivalis*, immuno-modulation

Acknowledgements/Funding: This work was supported by the Health and Medical Research Fund from Hong Kong Food and Health Bureau [20190682] to X. Li

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P.28

Antimicrobial peptides with a double-glycine leader peptide from *Streptococcus oralis* subsp. *dentisani* strains

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A B S T R A C T

The antimicrobial resistance (AMR) crisis as a consequence of the misuse of antibiotics urges for the development of novel treatments. Antimicrobial peptides (AMPs) stand out as promising alternatives to classical antibiotics. *S. oralis* subsp. *dentisani* strains are found in the oral cavity and some of them are known to have antimicrobial activity. In this work we investigate the double glycine leader peptide-like AMPs encoded by different *S. dentisani* strains, as a means to characterize the antimicrobial potential of this species. For that we searched all the AMPs they encode by using the bacterial version of antiSMASH and BAGEL softwares, followed by manual curation of the results. We analyzed AMPs amino acid sequence conservation, and predicted their 3D structures. Finally, we tested *in vitro* the activity of some AMPs against oral pathogens. Our results show that some of the peptides are conserved between different strains, but some seem to be strain-specific. *In vitro* testing shows promising results with one of the AMPs having activity against *Porphyromonas gingivalis*. This highlights the therapeutic potential of *S. dentisani*-derived AMPs in oral health maintenance and warrants further investigation into their efficacy and mechanism of action.

Keywords: antimicrobial peptide (AMP), bacteriocins, oral microbiota, oral health, human pathogens

Acknowledgements/Funding:

This project has received funding from the European Union's Horizon 2020 Research and Innovation Program under the Marie Sklodowska-Curie grant agreement No 101026278, project SMILES; and from FISABIO Foundation for the project BACTIDENT (UGP-20-108).

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New postbiotic to improve canine oral health

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A B S T R A C T

Oral health is a key factor in the health and well-being of domestic dogs, especially in adult, elderly, and small breed dogs [1]. Domestic dogs, due to a diet rich in proteins and lack of oral hygiene, can develop dysbiosis. The aim of the following work is to identify a product (probiotic or tyndallized postbiotic) to support canine oral health.

Postbiotics, or “a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host” as defined by the International Scientific Association of Probiotics and Prebiotics [2], are increasingly appreciated as potentially effective interventions within the microbiome space.

In this study, an *in vitro* screening pipeline was established following a funnel strategy to identify the most effective bacterial strain targeting inhibition of the growth and formation of biofilm of pathogens related to periodontitis. Moreover, these results were validated in a pre-clinical model using dynamic biofilm simulator (Real Time Cell Analysis xCELLigence system), in which canine saliva samples were used as inocula for multiple-species biofilm analysis [3]. Statistically significant inhibition of biofilm growth (average 55%; $p<0.05$) was observed when the postbiotic was added with respect to the control. This was corroborated by the quantification of DNA extracted from the biofilms formed on the bottom of each well. The metagenomic analysis showed the capability of the selected candidate to modulate the oral microbiota, increasing the potential probiotics such as *Bifidobacterium breve*.

The postbiotic additionally showed anti-inflammatory properties in an oral epithelium model (TR146 cells), being able to inhibit the secretion of interleukins Il-8 and CXCL-10.

In conclusion, the selected bacterial-derived postbiotic may support aspects related to oral health in healthy dogs based on pre-clinical and *in vitro* data.

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Keywords: Canine oral health, Postbiotic, Oral biofilm,Cell culture.

ORGANIZERS

SPONSORS

Actividad financiada por la Conselleria d'Educació, Cultura, Universitats i Ocupació mediante la subvenciones para la organización y difusión de congresos, jornadas y reuniones científicas, tecnológicas, humanísticas o artísticas de carácter internacional (CIAORG/2023/63)

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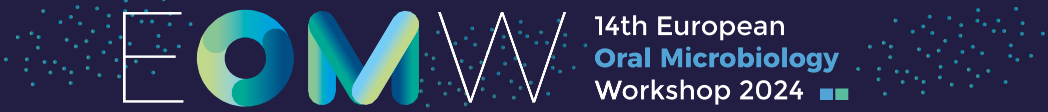

ISBN 978-84-482-6975-3