

SCHOOL OF DENTISTRY

Research Day

2025

Tuesday, March 4
Robertson Life Science Building, Portland, OR



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DENTISTRY

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8:00 am Poster setup in RLSB Atrium
8:30 am Poster Session #1 (odd numbers)
10:10 am Poster Session #2 (even numbers)
12 noon Keynote and Awards
In-person RLSB 3A003A/B
or online via Webex

A special thank you to

Drs. Barry Taylor
OREGON DENTAL ASSOCIATION

Drs. Cyrus Lee and Dan Philstrom
PERMANENTE DENTAL ASSOCIATES

School of Dentistry Presents the 2025 Research Day Keynote



Cariology and Cardiology

Robert H. Lustig, M.D., M.S.L.
Emeritus Professor,
Department of Pediatrics, UCSF

About Dr. Robert Lustig

Robert H. Lustig, M.D., M.S.L. is Emeritus Professor of Pediatrics in the Division of Endocrinology, and Member of the Institute for Health Policy Studies at UCSF. Dr. Lustig is a neuroendocrinologist, with expertise in obesity, diabetes, metabolism, and nutrition. He is one of the leaders of the current "anti-sugar" movement that is changing the food industry. He has dedicated his retirement from clinical medicine to help to fix the food supply any way he can, to reduce human suffering and to salvage the environment, by interacting with all stakeholders to bring them together around a common vision of metabolic health: protect the liver, feed the gut, support the brain. Dr. Lustig graduated from MIT in 1976, and received his M.D. from Cornell University Medical College in 1980. He also received his Masters of Studies in Law (MSL) degree at University of California, Hastings College of the Law in 2013. He is the author of the popular books *Fat Chance* (2012), *The Hacking of the American Mind* (2017), and *Metabological* (2021). He is the Chief Science Officer of the non-profit Eat REAL, and is a member of the Nutrition Task Force of the American Dental Association.

Learning objectives

- Understand the effect of fluoride on cariogenesis, and its role as adjunct vs. primary prevention.
- Understand how subcellular energy overload drives insulin resistance.
- Be familiar with the differences and similarities between hepatic glucose vs. ethanol vs. fructose metabolism.
- Be familiar with classes of lipids, their role in cardiovascular disease, and which diet components are involved.
- Explain the changes in the composition of the American diet specifically as it pertains to ultraprocessed foods.

Please visit our Learning Stream page for our continuing dental education course calendar.



This course has been approved for 1 CDE credit.

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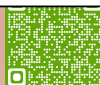
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Research Day

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Session 1

Presenter	#	Category	Title
Ana Caroline Lima Colombino	27	PhD STUDENT	Evaluation of effect NNGH inhibitor and collagenase enzyme activity on dentin disks and RTT collagen: a comparative study.
Andrew Allen	75	DMD/ CASECat	Stability of Posterior Intrusion using Skeletal Anchorage for Correction of Anterior Open Bite
Angela Hung and Crystal Ly	65	DMD/ CASECat	Rebuilding Roots: Regenerative Endodontics for Immature Permanent Teeth with Pulpal Necrosis
Bao Huynh	3	STAFF	Assessment of polymeric properties and self-healing kinetics of dental resin composites containing chemically modified PUF microcapsules.
Celyna Becerra	57	DMD/ Research	Dental students' attitudes and beliefs about caring for people living with Human Immunodeficiency Virus (HIV)
Christina Borland	11	STAFF	Mouse model of oral microbial community remodeling
Christopher Yoon	79	DMD/ Research	Performance Comparison of AI Chatbots and Prosthodontic Residents on the National Prosthodontic Resident Examination (NPRE)
Cristiane Miranda Franca	39	FACULTY	Microengineering the oral carcinoma environment to understand the role of hybrid cells on tumor progression.
Daniel Chen	53	DMD/ CASECat	Bone morphogenic proteins as graft material
Daniela Roth	23	POSTDOC	A Novel Organ-On-a-chip Model of Multiple Myeloma Interactions With Bone
Diyar Dezay, Andie Jamison, Daniel Stratte, Hussain Awadh, and Kellen Olsen	61	DMD/ CASECat	Does AI assistance in bitewing radiographs improve diagnostic accuracy and sensitivity?
Dohyun Kim, Graham Kang	49	DMD/ CASECat	The Efficacy of Zygomatic Implants for the Atrophic Maxilla
Dustin Higashi	7	STAFF	Parvimonas micra manipulation of the host innate immune response results in the promotion of a pro-inflammatory feedback cycle.
Emily Tran	69	DMD/ CASECat	Efficacy of exercise on reducing prevalence of periodontitis
Felipe Fabrício Farias da Silva	25	PhD STUDENT	The impact of fatty acid and phospholipid synthesis on Streptococcus mutans stress response, virulence, and bacteriocin production
Gregg Smith	59	DMD/ CASECat	The Effects of Orthodontic Extrusion on Preserving Biomechanical Function and Crown/Root Ratio
Jack Klar	55	DMD/ CASECat	Coronectomy: A Safer Alternative to Complete Extraction for High-Risk mandibular Third Molars
Jade Wong	13	STAFF	Acquired Pellicle Alpha amylase Abundance in Hydrophilic/Hydrophobic Dental Materials
Jinci Yan	19	POSTDOC	The T7SS mediate the Interaction between Streptococcus parasanguinis and pathogenic cohabitant Aggregatibacter actinomycetemcomitans
Jonah Tang	1	STAFF	The salivary virome during childhood dental caries



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Session 1 continued...

Presenter	#	Category	Title
Jonah Tang	1	STAFF	The salivary virome during childhood dental caries
Jonathan Cha	63	DMD/ CASECat	Treatment of maxillary skeletal transverse deficiency in non-growing patients.
Jonathan Nguyen	67	DMD/ Research	Photocrosslinkable Bone-Derived ECM Hydrogels: Enabling Bioprinting of Scaffolds for Tissue Engineering and Regenerative Medicine
Krishna Kumar Kungumaraj	17	POSTDOC	Synthesis, characterization of Vit-D-PEG (A-B-A) tri-block polymer as a new drug delivery platform for treating periodontitis
Laura Iwasaki	37	FACULTY	Temporomandibular Disorders and Anatomic-Psychologic Score
Lisa Greene	33	FACULTY	Evaluation of Caries Risk Assessment Implementation, Risk Factors and Outcomes in a Predoctoral Dental Clinic
Mauricio Sousa	5	STAFF	High-Fidelity Bone-on-a-Chip Platform Recapitulates Bone Physiology and Oral Cancer Invasion
Mingzhe Guo	21	POSTDOC	Unsaturated fatty acids are a critical determinant of ComCDE signaling in <i>Streptococcus mutans</i>
Mohammed Kadem	29	RESIDENT	Assessing Noise Levels of Endodontic Dental Equipment During Microscope-Assisted Procedures: Implications for Occupational Health and Safety of Dental Professionals
Molly McCoy and Narita Narkhede	73	DMD/ CASECat	Fluoride varnish vs. resin infiltration for post-orthodontic white spot lesions
Nicole O'Dierno, Finn Peck, Chloe Zhou	45	DMD/ CASECat	Ozone as an alternative to Chlorhexidine in non-surgical periodontal treatment
Peter Nguyen	51	DMD/ Research	Inhibition of Dentinal Collagen Degradation by Matrix Metalloproteinases Using Quaternary Ammonium Methacrylates
Ronn Leon	81		OHSU Innovates: Supporting the advancement of OHSU research, innovation, and entrepreneurship for the benefit of society
Samyia Chaudhry	35	FACULTY	Haptics: A Modern Technology to Enhance Dental Anesthesia Training in Dental Education
Seon Young Min	31	RESIDENT	Assessing the Validity of Chatbot Responses in Endodontic Diagnosis and Treatment Plans with Clinical Vignettes
Stephanie Momeni	41	FACULTY	Biosynthetic Gene Cluster significantly alters <i>Streptococcus mutans</i> gene expression
Tapas Ghosh	15	POSTDOC	Smart Enzyme-Responsive Nanomicelles for Controlled and Sustained Delivery of Metalloproteinase Inhibitors
Taylor Carpenter	43	DMD/ Research	Nanoparticles For Combatting Inflammation in Chronic Periodontal Disease
Taylor Maestas and Luke George	71	DMD/ CASECat	Endo Comparison between Gentlewave System and Passive Ultrasonic Irrigation
Tiana Pham	47	DMD/ Research	QAM Inhibition of MMP Activity and Biofilm Formation in Composite Restorations
Valerie Truong	77	DMD/ Research	Temporomandibular Joint Loads and Mandibular Length in Children
Zhengzhong Zou	9	STAFF	Mechanism Underlying the Synergistic Interaction Between <i>Veillonella</i> and <i>Prevotella</i>



Dental students' attitudes and beliefs about caring for people living with Human Immunodeficiency Virus (HIV)

Celyna Becerra

DMD Student and SoD Prematriculation Research Program Fellow, OHSU School of Dentistry

Mentor: Lyndie Foster Page

Co-Authors: Bilal Manzer - DS4; Maria Ayad - Portland State University; Lyndie Foster Page

Introduction

Despite advancements in HIV treatment and a broader understanding of the virus, stigma remains a pervasive barrier in healthcare and especially oral health care, influencing both patient outcomes and provider attitudes.

Aim: To evaluate first, second, third-, and fourth-year dental students' attitudes towards treating HIV+ patients, focusing on aspects of prejudice, stereotyping, and discrimination.

Methods

In 2024/2025 academic year dental students' perceptions of their knowledge and or preconceived attitudes to treating HIV+ patients were evaluated using the 30-item Healthcare Provider HIV/AIDS Stigma Scale (HPASS). Students' sociodemographic details (age, sex, and race/ethnicity) were also collected. Data were analyzed using descriptive statistics, measures of central tendency, and frequency of distribution to survey questions. Internal consistency was tested using Cronbach's alpha (SPSS 30).

Results

Overall, 85% of dental students completed the HPASS survey. There were slightly more males (51%) than females (48%) that completed the survey with most students identifying as White (45%) or Asian (38%). Internal consistency was high for the overall HPASS and its domains; discrimination, stereotype and prejudice (0.95, 0.93, 0.92 and 0.92, respectively). A students' race, sex, age and DS year group significantly influenced their knowledge and or attitudes to treating HIV+ patients.

Conclusions

Dental students' attitudes and beliefs towards treating HIV patients were influenced by sex, race, age, and year attending dental school. Future research is needed to understand if there are strategies that will improve how dental students prior to graduating can improve their attitudes to treating HIV+ patients.

Mouse model of oral microbial community remodeling

Christina Borland

Research Associate, Merritt Lab, Dept of Oral Rehabilitation and Biosciences,
OHSU School of Dentistry

Co-Authors: Madeline Krieger, Mona Sivaneri, Justin Merritt

Introduction

Dysbiosis of the oral microbiota is associated with increased incidence of inflammatory disease. Under healthy conditions, a combination of pressure from the immune system and the diverse microbiota prevents potential pathogens from establishing residency in the oral cavity. The oral cavity is particularly rich in immune components, offering commensal microbes an opportunity to train against and evolve ways to evade the immune system. Here, we use serial passage through a mouse abscess model to identify commensal species with immune evasion capabilities.

Methods

- Serial passage through a mouse abscess model and 16S sequencing to select for oral microbes that have the capability of evading the immune system.
- Bacteriological techniques to isolate and culture individual species
- qPCR and differential plating to determine the frequency of individual species in the community
- Flow cytometry to identify the immune cells responding to the infection.

Results

- Over four rounds of serial challenge, the mouse immune system selects for a limited number of species
- As the community becomes increasingly dysbiotic, the severity of the infection increases
- The dysbiotic group is enriched for species that have previously been identified as associated with inflammatory disease
- Individual species from the dysbiotic group can be cultured independently
- Immune and cytokine expression phenotypes have been identified which distinguish between infections caused by healthy and dysbiotic microbial communities

Conclusions

A healthy mouth contains a diverse commensal microbial community. Persistent inflammatory conditions select for a dysbiotic microbial community that is capable of immune evasion, and includes species known to correlate with inflammatory disease. This dysbiotic community elicits an immune response that is fundamentally different from that elicited by the original healthy community. This suggests a mechanism by which inflammation in the oral cavity can increase the likelihood of systemic inflammatory disease.

Nanoparticles for Combatting Inflammation in Chronic Periodontal Disease

Taylor Carpenter

DMD Student and SoD Prematriculation Research Program Fellow, OHSU School of Dentistry

Mentor: Ana Paula Fugolin

Co-Authors: Tapas Ghosh (OHSU); Dr. Ana Fugolin

Introduction

The goal was to synthesize a small, positively charged, Zinc Hydroxyapatite (HAp) Nanoparticle (NP) that can adhere to a negatively charged Cell Free DNA (cfDNA) molecule to stop an inflammatory cytokine pathway similar to that found in periodontal disease. Meanwhile, the coating of the HAp NP with a polyamidoamine dendrimer (PAMAM) poses a potential toxicity issue that needs to be overcome.

Methods

The goal was to first learn about nanoparticles and how they are synthesized. As a subcomponent, Zinc was selected for its biological role in inflammation and hydroxyapatite due to its ability to not be toxic in cells (biocompatibility). The ZNHA NPs are naturally negatively charged, and thus not compatible with the negatively charged DNA it needs to bind to. The ZN HAp NPs had to be coated with a large branched dendrimer containing positively charged amine groups called Poly(amidoamine) or (PAMAM). Effectively this changes the charge of the NPs from negative to something that the DNA will bind to.

Results

Aptly sized nanoparticles were made, and were successfully coated with Zinc and PAMAM due to a long incubation time of 15 hours. TEM imaging data showed that the NPs adhered to all three of the generations of the PAMAM and had no statistically significant difference in DNA binding efficiency.

Conclusions

Increasing the incubation time of the NPs led to a smaller size that allowed for all the Zn HAp NPs to successfully bind to DNA. Future challenges include, but are not limited to, potentially cellular toxicity. Effects of toxicity will be tested using a hydrogel to mimic the structure of the extracellular matrix and to create a favorable microenvironment for periodontal regeneration using saliva from patients with periodontal disease.

Haptics: A Modern Technology to Enhance Dental Anesthesia Training in Dental Education

Samyia Chaudhry

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Co-Authors: Juliana da Costa; Erinne Lubisich, Ana Paula Fugolin

Introduction

Effective administration of dental anesthesia is fundamental to dental practice, ensuring patient comfort. However, mastering techniques like the inferior alveolar nerve block (IANB) poses challenges for students due to the need for precision, anatomical knowledge, and tactile sensitivity. Traditionally, hands-on training involved student-to-student practice, but ethical concerns have led to its discontinuation. Consequently, students enter clinical settings with anxiety and reduced confidence. Haptic-based simulators, widely used in aeronautics, have emerged as promising educational tools in dentistry. Therefore, this study aimed to validate a dental simulator as a learning tool and assess its ability to create a clinically accurate scenario.

Methods

Seventy dental students were recruited for this study (OHSU - IRB #STUDY00027597), including 35 second-year students learning the inferior alveolar nerve block (IANB) technique and 35 third- and fourth-year students with prior clinical IANB experience. Each participant completed a 30-minute session using the simulator (SIMtoCARE, Vreeland, the Netherlands). For the second-year group, the first 10 minutes covered simulator setup and capabilities, followed by a theoretical review and a demonstration of a IANB technique by a trained dentist. Students then practiced the technique as many times as possible for 15 minutes, exploring the simulator's features. In the final 5 minutes, they performed a last IANB injection and completed an anonymous survey with eight multiple-choice questions assessing the simulator's value as a learning tool. For the third- and fourth-year students, the first 10 minutes focused on demonstrating the simulator's functionality. The remaining 20 minutes followed the same structure as the first group; however, their survey evaluated the simulator's ability to replicate clinical conditions accurately. Survey responses were collected via Smartsheet software, averaged, and analyzed using GraphPad.

Results

Regarding the assessment of the haptic simulator as a learning tool, 93.75% of participants found the equipment user-friendly, while 83.8% reported increased confidence in performing IANB procedures after the training session. Additionally, 93.8% considered the simulator a valuable tool for improving their understanding of the technique, and the same percentage felt better prepared to perform an IANB procedure in a clinical setting following the simulation training. In terms of the simulator's ability to mimic a clinical scenario, 77.5% of participants considered it accurate, particularly regarding bone and soft tissue resistance (90.5% and 77.5%, respectively). The primary limitation identified was the syringe design, which did not accurately replicate a carpule syringe.

Conclusions

The findings of this study support the effectiveness of haptic-based simulation as a valuable educational tool for teaching dental anesthesia. The majority of participants found the simulator user-friendly and reported increased confidence and preparedness for clinical practice following the training session. Additionally, the simulator was perceived as highly effective in replicating

Chaudhry (cont'd)

bone and soft tissue resistance, though improvements are needed in syringe design to better mimic clinical conditions. These results highlight the potential of haptic simulators to enhance dental anesthesia training, reducing students' anxiety and improving their competence before performing procedures on patients.

Evaluation of effect NNGH inhibitor and collagenase enzyme activity on dentin discs and RTT collagen: a comparative study

Ana Caroline Lima Colombino
Graduate (PhD) Student, Division of Biomaterials and Biomechanics,
OHSU School of Dentistry

Mentor: Carmem Pfeifer

Co-Authors: Wong J; Lucena FS; Pfeifer CS

Introduction

The purpose of study was to compare the efficiency NNGH as inhibitor of collagenase on dentin discs and RTT collagen at different times.

Methods

A total of 12 samples of collagen RTT were prepared with collagen type I (Clostridium histolyticum) from rat tail diluted in acetic acid and mixed with DMEM, 10X PBS and NaOH. All the reagents were prepared on ice slowly to prevent bubbles. The samples were incubated for 2h at 37°C. The dentin discs (n=9) were prepared in 0.6 mm thickness to place in a Falcon tube with 10% phosphoric acid for 24h at 4°C. The demineralized discs were cut with punch with 5mm diameter. All the samples (RTT collagens and dentin discs) were put in a 48 well plate to undergo degradation with collagenase and the effect of NNGH as inhibitor. The storage modulus was tested on rheometer following timepoints: baseline, 2h, 4h and 6h and kinetic activity was performed in the plate reader. The statistical analysis was performed by ANOVA/Tukey's test ($\alpha=0.05$).

Results

The results showed NNGH was not capable of inhibiting collagenase action in both samples and the storage modulus values were decreased according to timepoints.

Conclusions

NNGH was not capable of inhibiting collagenase.

The impact of fatty acid and phospholipid synthesis on *Streptococcus mutans* stress response, virulence, and bacteriocin production

Felipe Farias da Silva

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Mentor: Jonathon Baker

Co-Authors: Jonathon L. Baker.

Introduction

Streptococcus mutans is considered a primary etiologic agent of dental caries. It has the ability to outcompete other oral microflora, exhibit an adaptive response to oxidative conditions, and survive in an acidic pH environment. Therefore, this study aimed to examine the impact of fatty acid and phospholipid synthesis on *S. mutans* strains in terms of their virulence, stress response, and bacteriocin production.

Methods

Growth curves were conducted to assess the stress adaptation of 15 *S. mutans* strains under different growth conditions. Growth was monitored using a Tecan Infinite Nano by measuring the optical density at 600 nm (OD600) every hour for 17 hours at 30°C, 37°C or 42°C. To simulate acid and oxidative stress, different media were used: Todd Hewitt broth (THB), THB with a pH of 5.4, THB + 1 mM hydrogen peroxide (H₂O₂), or THB + 0.5 mM H₂O₂. The *S. mutans* strain UA159 was used as a positive control, as it is well established that this strain can survive in acidic conditions, tolerate moderate oxidative stress, and outcompete commensal bacteria. A deferred-antagonism assay was performed to evaluate the ability of different strains to inhibit other *Streptococcus* species (*Streptococcus sanguinis* and *Streptococcus salivarius*) through bacteriocin production. Cultures of *S. mutans* strains were spotted onto THB agar and incubated overnight. Cultures of *S. sanguinis* and *S. salivarius* were added to 5 mL of soft THB agar, overlaid onto the plates containing the *S. mutans* strains, and incubated overnight. Zones of inhibition were measured 24 hours later.

Results

All strains were capable of inhibiting the growth of the secondary colonizers *S. salivarius* and *S. sanguinis*. The Δ plsX and Δ fabM strains showed the smallest zones of inhibition ($P < 0.0001$) compared to the UA159 strain, which exhibited the greatest inhibition. There was no statistically significant difference between Δ plsX and Δ fabM. Δ plsX, Δ fabM and Δ tesS/ Δ plsX did not adapt well to acidic conditions, unlike UA159. Under oxidative stress, Δ plsX and Δ fabM exhibited the lowest growth, while the other strains showed similar growth patterns.

Conclusions

The comparative analyses showed that the Δ plsX, Δ fabM, and Δ tesS/ Δ plsX strains do not have an acid-adaptive response like the control strain, UA159, and that Δ plsX and Δ fabM exhibited the lowest ability to outcompete commensal *Streptococcus* species.

Microengineering the oral carcinoma environment to understand the role of hybrid cells on tumor progression

Cristiane Miranda Franca

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Introduction

Oral squamous cell carcinoma (OSCC) poor outcomes are primarily due to metastatic disease. A critical mechanism by which cancer cells gain metastatic potential is through fusion with immune cells, forming hybrid cells, which retain characteristics of both parent cells (tumor and immune), exhibiting altered function, protein expression, and gene regulation. Hybrid cells have been detected in the blood of OSCC patients and are associated with lymph node metastasis. However, their presence in the primary tumor is elusive, and how they interact with the extracellular matrix (ECM) to disseminate is unclear. Our goal is to investigate the presence of hybrids in OSCC, their interaction with the ECM during migration and ability to intravasate into circulation.

Methods

We evaluated OSCC incisional biopsies to investigate the presence of hybrid cells in primary tumors. Next, we tested whether ECM stiffness promotes hybridization by assembling collagen I at different temperatures to achieve distinct mechanical and structural properties. Additionally, we engineered capillaries on-a-chip to assess whether hybrid cells migrated more into the vasculature compared to parental cells.

Results

All primary OSCC samples contained hybrid cells, with counts ranging from 1.6 to 10 hybrid cells per 50,000 epithelial cells. Elevated ECM stiffness (27.7 kPa) significantly increased cell fusion compared to softer reticular collagen (4 kPa) ($p < 0.05$). Furthermore, hybrid cells in the organ-on-a-chip model exhibited twice the intravasation rate into the vasculature compared to parental cancer cells ($p < 0.05$).

Conclusions

Hybrid cells are present in primary OSCC and are significantly influenced by ECM's mechanical properties. They exhibit greater migratory potential and an enhanced ability to intravasate compared to parent cells, indicating their role as metastasis effectors in OSCC.

Smart Enzyme-Responsive Nanomicelles for Controlled and Sustained Delivery of Metalloproteinase Inhibitors

Tapas Ghosh

PostDoc, Dept of Oral Rehabilitation and Biosciences, OHSU School of Dentistry

Mentor: Ana Paula Fugolin

Co-Authors: Bao Huynh, Sivashankari Rajasekaran, Ana Paula Fugolin

Introduction

Metalloproteinases (MMPs) play a crucial role in the degradation of soft and hard oral tissues in periodontal disease and at the adhesive interface. Although potent MMP inhibitors have been identified over the years, their delivery remains a challenge, making clinical translation impractical. Therefore, the aim of this study is to synthesize and validate the use of MMP-responsive nanomicelles as carriers for delivering MMP inhibitors to oral tissues.

Methods

MMP-2/9 and MMP-8 enzyme-responsive nanomicelles were synthesized by conjugating peptide sequences (D and L versions) recognized and cleaved by the targeted enzymes with a block-copolymer based on norbornene. The process was followed by micellization with simultaneous encapsulation of the inhibitors quercetin or epigallocatechin gallate (EGCG). Bare nanomicelles were synthesized as a control. The nanomicelles were morphologically characterized by transmission electron microscopy (TEM) (n=5) and dynamic light scattering (DLS) (n=5) before and after incubation with the targeted MMPs for 24 hours. The encapsulation of quercetin and EGCG was assessed by high-performance liquid chromatography (HPLC) (n=3). The critical micellar concentration (CMC) was assessed using pyrene fluorescence assay, and micelle stability was evaluated by zeta potential (n=5). Finally, biocompatibility was determined by MTT assay on dental pulp stem cells (DPSC) treated with serial concentrations of nanomicelles for 24 hours (n=6). The data were statistically analyzed with one-way ANOVA and Tukey's tests ($\alpha=0.05$).

Results

The successful synthesis of the newly synthesized nanomicelles was confirmed by TEM microscopy, showing the formation of spherical particles with an average diameter of 20 ± 0.5 nm. The responsiveness of the nanomicelles to their respective target MMPs was confirmed by TEM and DLS, showing dramatic morphological changes from spherical to worm-like connected structures, along with an increase in hydrodynamic diameter from 27.1 ± 0.5 nm to 334.3 ± 14.3 nm. The successful encapsulation of EGCG and quercetin was confirmed by HPLC, showing strong and sharp peaks at 5.35 and 9.58 min, respectively. The CMC of the synthesized MMP-2/9 and MMP-8 responsive peptide-block copolymers were 40.5 and 24.8 $\mu\text{g/mL}$, respectively. The encapsulation of the inhibitors did not compromise the stability of the nanomicelles, as indicated by the zeta potential, which averaged -33.9 ± 1.3 mV for quercetin-containing nanomicelles and -40.2 ± 2.8 mV for EGCG + quercetin-containing nanomicelles, compared to -41.9 ± 1.2 mV for bare micelles. Regarding cell viability, metabolic activity ranged from $75.1\pm6.1\%$ to $91.2\pm9.0\%$, indicating excellent biocompatibility with no statistically significant difference compared to quercetin and EGCG dissolved in the cell culture media.

Conclusions

The newly developed nanomicelles demonstrate strong potential as a responsive platform for the targeted delivery and on-demand release of MMP inhibitors with high specificity.

Evaluation of Caries Risk Assessment Implementation, Risk Factors and Outcomes in a Predoctoral Dental Clinic

Lisa Greene

Assistant Professor, Division of Restorative Dentistry, OHSU School of Dentistry

Co-Authors: Rita Patterson, Despoina Bompolaki, Lenny Supnet

Introduction

Caries risk assessment (CRA) is a core component of CAMBRA (Caries Management by Risk Assessment) and is used to determine the susceptibility of the dental patient to caries disease. The primary objective of this study was to discover if CRA is performed consistently in the Oregon Health and Sciences University (OHSU) dental clinic, and whether the resulting treatment interventions are being effective in reducing the patient's caries risk. The secondary objective was to evaluate the most common risk factors in the high caries risk patient population at OHSU.

Methods

The study protocol was reviewed by the IRB and determined to be IRB-exempt. Deidentified data from 22,128 patient examinations completed by student practitioners were extracted from the electronic health record (EHR) from 2019 to 2023 and analyzed for CRA completion. Risk factors for high caries risk patients and intervention recommendations were extracted as well. Lastly, EHR was examined to determine whether intervention recommendations were followed through by the patient.

Results

50% of the comprehensive and periodic examinations had a CRA code completed. In the patient population that had a CRA code completed, only 37.7% had completed the actual caries risk form.

When interventions were analyzed, we found that 14.7% of high caries risk patients lived in a fluoridated community and high fluoride prescription toothpaste was given to 22.8% of high-risk patients.

The most common risk factor for high caries risk patient was plaque (56.7%). The next most common risk factors were exposed roots (52.9%) and intra-oral appliances (24.8%).

Conclusions

Despite CRA being required for all comprehensive and periodic exams, our results showed that the assessment was often incomplete. These findings emphasize the need for improved CRA education and implementation to optimize patient care and caries prevention.

Unsaturated fatty acids are a critical determinant of ComCDE signaling in *Streptococcus mutans*

Mingzhe Guo

PostDoc, Dept of Oral Rehabilitation and Biosciences, OHSU School of Dentistry

Mentor: Jonathon Baker

Co-Authors: Mingzhe Guo, Jonathon Baker

Introduction

Streptococcus mutans is currently considered a primary etiological agent of dental caries, which relies on its bacteriocin arsenal to compete with other oral microbes. Unsaturated fatty acid (UFA) production in *S. mutans* is dependent on *fabM* and its caries forming capability is severely impaired in a *fabM* deletion mutant. We found that loss of *fabM* and exogenous UFA treatment could both shut down bacteriocin production in *S. mutans*. We hypothesized that intrinsic UFA production and exogenous UFA impact ComCDE activation, and therefore bacteriocin production, through different mechanisms.

Methods

Transcription fusion of promoters of bacteriocin/competence related genes with fluorescent protein or luciferase reporter.

Deferred antagonism assay

Transformation assay

Gene deletion and complementation

RNAseq

Results

Bacteriocin production and competence development was abolished in $\Delta fabM$ *S. mutans* during growth in complex liquid and solid media.

Competence development is abolished in *S. mutans* treated with monounsaturated oleic acid (OA) or polyunsaturated linoleic acid (LA), but not with saturated stearic acid during growth in complex liquid media.

ComRS can still mediate competence activation in a $\Delta fabM$ background and the in presence of OA/ LA during growth in chemically defined media.

Conclusions

UFAs impact *S. mutans* bacteriocin production and competence through ComCDE circuitry, which will affect the competitive fitness of *S. mutans*, and consequently its ability to cause disease.

Parvimonas micra manipulation of the host innate immune response results in the promotion of a pro-inflammatory feedback cycle.

Dustin Higashi

Research Associate, Merritt Lab, Dept of Oral Rehabilitation and Biosciences,
OHSU School of Dentistry

Co-Authors: Dustin L. Higashi, Hua Qin, David Anderson, Christina Borland, Elizabeth A. Palmer, Jens Kreth and Justin Merritt

Introduction

Parvimonas micra is a pathobiont from the oral cavity that is strongly associated with mucosal dysbiotic disease, as well as multiple types of cancer. Inflammation is a hallmark of a number of oral diseases such as periodontitis, apical abscesses, and peri-implantitis. Despite a persistent inflammatory state at the sites of these oral infections, the host is unable to clear the infection. In these chronic and persistent infections, inflammophilic (loving or attracted to inflammation) microbes such as P. micra are enriched. We hypothesize that P. micra is a driver of inflammation through its manipulation of the host innate immune system.

Methods

Neutrophils (PMNs) and serum were isolated from the peripheral blood of human subjects and in vitro studies with P. micra were performed.

Results

P. micra was able to bind and activate human serum complement proteins involved in inflammation. In PMNs, P. micra infection induced degranulation, leading to the release of cellular proteases which were able to cleave the extracellular matrix protein collagen. P. micra significantly induced the expression of the cytokine IL-8 in PMNs and was able to induce the cellular migration of PMNs across transwell membranes.

Conclusions

Successful pathogens have the ability to manipulate host defenses to cause disease. The ability of inflammophiles to promote disease through manipulation of the human complement system as well as neutrophils, may reflect their success as drivers of chronic infections. Our results indicate that the inflammophile P. micra is especially adept at manipulating host inflammatory control mechanisms, which is critical for promoting an inflammatory positive feedback cycle. Studies exploring the nature of P. micra interactions with the host innate immune system will allow for a better understanding of the development and persistence of inflammatory diseases such as periodontitis, peri-implantitis, and endodontic abscesses.

Assessment of polymeric properties and self-healing kinetics of dental resin composites containing chemically modified PUF microcapsules

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Introduction

Resin composite fractures, caused by the accumulation and propagation of microcracks within the resin matrix due to thermal and masticatory stresses, are a leading cause of dental restoration failure. One solution is incorporating polymeric microcapsules loaded with a healing agent into the resin matrix. However, the lack of chemical adhesion between microcapsules and the organic matrix can reduce composite's mechanical properties or prevent capsule rupture. This issue may be addressed by functionalizing microcapsule shells with a silane agent, promoting physicochemical interlocking with the resin matrix. This study investigates the performance of chemically functionalized microcapsules incorporated into a dental resin composite formulation.

Methods

PUF (poly-urea formaldehyde) microcapsules without or with melamine-modification at 5% (PUMF), were synthesized via a double emulsion reaction using mechanical stirring at 400 rpm. Microcapsules were loaded with a healing agent mixture of 80% triethylene glycol dimethacrylate (TEGDMA) and 20% N,N-Dimethylacrylamide (DMAM), with N,N-Bis(2-hydroxyethyl)-p-toluidine (DHEPT) as a chemical catalyst. After synthesis, microcapsule surfaces were functionalized with 3-(Trimethoxysilyl)propyl methacrylate (TMSPM) by dispersing microcapsules in a 4% wt. solution of TMSPM (pH=4), followed by characterization using optical microscopy, scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), and thermogravimetric analysis (TGA) (n=3). The size distribution of microcapsules was analyzed using ImageJ (n=50). Microcapsules were incorporated at 10 or 15 wt% into a resin composite formulation consisting of bisphenol A-glycidyl methacrylate (BisGMA), ethoxylated bisphenol-A dimethacrylate (BisEMA), urethane dimethacrylate (UDMA), and TEGDMA in a 2:2:2:1 weight ratio, along with 50 wt% barium inorganic particles. Benzoyl peroxide (BPO) and phenylbis (2,4,6-trimethylbenzoyl)phosphine oxide (BAPO) were used as chemical initiator for the healing agent and photoinitiator for the composite, respectively. Resin composite specimens were prepared by photocuring with LED light (1000 mW/cm²) and tested for degree of conversion (DC) (n=3), formaldehyde release (n=3), and dynamic mechanical analysis (n=3). Healing kinetics were assessed in real-time via a compression test using cylinders (4 x 8 mm) at 0.25 mm/min, monitored with a high-definition camera. Data were analyzed using two-way ANOVA and Tukey's test.

Results

Successful chemical modification of PUF microcapsules was confirmed via EDS, showing peaks at 1.8 KeV, indicating Si at 0.72% wt.. Morphological changes observed in SEM were increased surface roughness, due to accumulation of silane aggregates. Modification of PUF

microcapsules with melamine and/or TMSPM enhanced thermal stability, evidenced by a rightward shift in the TGA thermograms. Formaldehyde release ranged from 1.89 to 5.65 μM , with 15% PUMF + TMSPM showing the highest and 15% PUF the lowest values. Regarding healing kinetics, the inclusion of microcapsules in compressive cylinders reduced in $\sim 39\%$ the load required for first fracture. In non-capsule-loaded controls, the first fracture caused catastrophic failure. Microcapsule-loaded cylinders demonstrated load recovery, as seen in the strain vs. stress curve, likely due to the release and polymerization of the healing agent. At 15% microcapsule inclusion, load required for first fracture was on average lower than at 10% microcapsule inclusion.

Conclusions

The functionalization of the microcapsules proved to be a promising approach for increasing the microcapsule loading ratio in resin composites without compromising the bulk properties of the composite. The newly developed method for assessing healing kinetics demonstrated sensitivity to small changes in load and effectively monitored real-time healing of the polymeric networks.

Temporomandibular Disorders and Anatomic-Psychologic Score

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Introduction

Currently, it is not possible to accurately predict the prognosis of temporomandibular disorders (TMDs). The objective of this study was to test two hypotheses concerning predicting longitudinal changes in the temporomandibular joint (TMJ) of human subjects. Hypothesis 1: To test if Anatomic-Psychologic Score (APS), an instrument which utilizes easily measured variables, predicted longitudinal changes in TMJ tissue integrity. Hypothesis 2: To test if Machine Learning Models accurately rank ordered anatomic and psychosocial variables that were associated with longitudinal changes in TMJ tissue integrity.

Methods

According to Institutional Review Board oversight, subjects ≥ 18 years-of-age were recruited. Baseline and >5-year follow-up data were obtained for Axis I (physical assessment) and Axis II (psychosocial status) of Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). Cone-beam computed tomography (CBCT) and magnetic resonance imaging (MRI) were used to determine whether or not there were changes in TMJ integrity. A calibrated radiologist characterized TMJ integrity and created 3 diagnostic groups based on if the combined hard and soft tissue diagnoses had no change (Group A), got better (Group B), or got worse (Group C). Baseline variables used in APS were from two domains. Firstly, from the psychosocial domain of DC/TMD Axis II, numeric data was derived from the Patient Health Questionnaire-15 (PHQ-15) and 7-Question Behavior Score (7QBS). Secondly, CBCT images which were used to derive anatomic domain measures of i) sagittal occlusal plane angle and anteroposterior position, ii) mandibular ramus length, and iii) variables associated with the axial plane geometry of the mandibular condyle. This included condyle loading area and aspect ratio, and angle of the condyle relative to the midsagittal plane. Means and standard deviations of APS and its component variables at baseline were calculated. To test Hypothesis 1, ANOVA was used to test for significant differences ($p < 0.05$) in APS scores amongst the 3 diagnostic groups. Hypothesis 2 was tested using three machine learning models, which rank ordered variables of importance in predicting TMJ integrity changes and were evaluated according to accuracy of prediction (> 0.90), and sensitivity and specificity ($0.70 - 0.90$).

Results

Thirty-one subjects (18 females and 13 males) met the inclusion criteria and right and left condyles were included for each subject resulting in a total of 62 TMJs. MRI and CBCT TMD diagnoses by the radiologist showed that 36 TMJs (58%) had no change from T1 to T2, 11 TMJs (18%) got better, 10 TMJs (16%) got worse, and 5 TMJs (8%) had no diagnoses. APS was not significantly different amongst TMJ integrity groups A, B, or C. Gradient Boosting Machine modeling had a 74% predictive accuracy (sensitivity = 0.66, specificity = 0.81) of TMJ integrity change and had condylar area as the variable of highest importance. Classification Tree modeling had a 61% predictive accuracy (sensitivity = 0.53, specificity = 0.77) and also had condylar area as the variable of highest importance. Support Vector Machine modeling had a 60% predictive accuracy (sensitivity = 0.45, specificity = 0.70) and had major axis as the variable of highest importance. Classification Tree modeling identified condylar area $\geq 90 \text{ mm}^2$,

Iwasaki (cont'd)

PHQ-15 < 6, and major axis < 19 mm predicted TMJ integrity changes, and were 83-100% predictive of TMJs that had no change, 54% predictive of TMJs that got better, and 54% predictive of TMJs that got worse. Sample sizes of 88 to 180 TMJs are needed to produce a 95% confidence interval with a width of no more than 0.2 for sensitivity and specificity of 0.7 to 0.9, assuming equal prevalence.

Conclusions

The proposed novel APS equation was not predictive of longitudinal changes in TMJ integrity. Machine learning models reported that condylar area and variables associated with the geometry of mandibular condylar area (major axis, minor axis, aspect ratio) have high relative importance in the predictive accuracy of TMJ changes.

Assessing Noise Levels of Endodontic Dental Equipment During Microscope-Assisted Procedures: Implications for Occupational Health and Safety of Dental Professionals

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Introduction

Dental clinicians are routinely exposed to noise from various endodontic devices, which may contribute to hearing impairment over time. This study seeks to quantify and compare noise levels generated by endodontic instruments and assess whether these levels exceed the exposure limits established by the National Institute for Occupational Safety and Health (NIOSH).

Methods

Noise levels were measured for an air-driven high-speed handpiece, a piezoelectric ultrasonic handpiece under load, the Rispisonic® irrigation activation device, and the GentleWave console. Measurements were conducted with and without high-volume suction (HVS) in a clinical setting. Sound pressure levels (dB) were recorded continuously over 30-second intervals, with each measurement repeated three times. The sound level meter was positioned 12 cm from the operator's ear, capturing readings on both the left and right sides. Mean noise levels were analyzed via ANOVA, with Tukey pairwise comparisons across devices.

Results

Average noise levels ranged from 60.7 to 82.2 dB. Pairwise comparisons indicated statistically significant differences between most devices, except for GentleWave and Rispisonic ($P = 0.061$). The presence of HVS significantly impacted noise levels for all devices. Peak noise levels demonstrated notable differences between the Rispisonic and both the air-driven high-speed handpiece ($P = 0.002$) and ultrasonic handpiece ($P = 0.002$), including when HVS was used.

Conclusions

Noise levels recorded in this study remained within NIOSH-recommended exposure limits for occupational safety.

Synthesis, characterization of Vit-D-PEG (A-B-A) tri-block polymer as a new drug delivery platform for treating periodontitis

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Introduction

Chronic periodontal disease (PD) is an inflammatory condition characterized by the accumulation of pathogenic biofilm, which triggers an unbalanced immune response and leads to the destruction of both soft and hard oral tissues. Vitamin D has been associated with several beneficial therapeutic effects in PD, including anti-inflammatory, antimicrobial, immune response modulation, and bone metabolism regulation. However, its delivery remains a significant challenge. Therefore, this study aims to develop an injectable, biodegradable platform that can be applied directly into the periodontal pocket immediately after scaling and root planning, delivering Vitamin D locally in a sustained, on-demand manner.

Methods

An innovative system inspired by a hydrogel platform used to treat colorectal cancer is based on vitamin D and polyethylene glycol (PEG). The hydrogel precursors were synthesized via organocatalytic ring-opening polymerization of the MTC-VD monomer (vitamin D-functionalized polycarbonate) using PEG as the macroinitiator, in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) and N-(3,5-trifluoromethyl)phenyl-N'-cyclohexylthiourea (TU) as catalysts. Successful synthesis was confirmed by proton nuclear magnetic resonance (¹H-NMR), with a resonance peak observed at 0.5 to 0.9 ppm. For the hydrogelation reaction, the precursor was at concentrations ranging from 4 to 5 wt.% in HPLC-grade water, and gelation and physical properties were monitored. The rheological properties of the hydrogels, which are important for determining their viscoelastic responses and their ability to be injected, were assessed using a rotational rheometer equipped with plate-plate geometry (Ø=8 mm), a 1-mm gap, and at 25°C. Degradation kinetics were evaluated by incubating 250 µL (11.25 mg of polymer) of the hydrogel in 250 µL of cell growth media (α-MEM complete media) at 37°C. Data were analyzed statistically using one-way ANOVA and Tukey's test (p < 0.05).

Results

The results demonstrated the successful formation of hydrogels at all tested concentrations. Gelation times ranged from 30 minutes to 6 hours, increasing as the precursor concentration decreased. The hydrogel network became cloudier at precursor concentrations above 5.5 wt.%, with the clear formation of non-dissolved precursor clusters, indicating the maximum concentration was 5 wt.%. Rheological testing revealed higher storage modulus and less loss modulus, confirming the hydrogel's injectability. In the stability test, all formulations showed rapid degradation after 30 minutes, with complete degradation after 3h. These findings highlighted the need to modulate the degradation kinetics, which was achieved by adding diamine-alkyl PEG (10 kDa) as an additive at 5 wt.%. The bi-PEG strategy successfully modulated degradation kinetics, leading to gradual hydrolysis and enhanced stability, suggesting potential for sustained local delivery.

Conclusions

In conclusion, the developed bi-PEG hydrogel system effectively modulates degradation kinetics and enhances stability, making it a promising candidate for sustained, localized delivery of vitamin D in periodontal treatment. Future steps will involve in vitro and in vivo characterization to investigate the hydrogel's anti-inflammatory properties and bone-conductive potential.

OHSU Innovates: Supporting the advancement of OHSU research, innovation, and entrepreneurship for the benefit of society

Ronn Leon

Alliance Manager, OHSU Collaborations and Entrepreneurship

OHSU Innovates is a collaborative network that supports the innovation and entrepreneurial ecosystems at OHSU and beyond. Services we provide include Alliance management for strategic partnerships, evaluation, management and marketing of OHSU innovations and intellectual property, development and implementation of strategies to establish partnerships, commercialization and intellectual property agreement management, guidance and assistance for new startup company formation and entrepreneurial education programs

Assessing the Validity of Chatbot Responses in Endodontic Diagnosis and Treatment Plans with Clinical Vignettes

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Introduction

To assess the accuracy and comprehensiveness of chatbot-generated responses for endodontic diagnoses and treatment plans using clinical vignettes.

Methods

GPT-4o, Copilot, Gemini, and Gemini Advanced provided endodontic diagnosis and recommended treatment plans for 7 clinical vignettes from the AAE Colleagues for Excellence (2013). Data were collected twice, 10 days apart, resulting in 56 responses. Two faculty endodontists independently evaluated the responses, resolving scoring disagreements through evidence-based discussion. Responses were graded based on: (1) binary accuracy of the pulpal and periapical diagnosis (correct/incorrect) and (2) descriptive accuracy (6-point Likert scale, with 1 being completely incorrect and 6 being completely correct) and completeness (3-point Likert scale, with 1 being incomplete and 3 being complete with additional context). Scores were analyzed using mixed-effects models. The study, including data analysis, was conducted from June to Oct 2024.

Results

Overall binary accuracy for pulpal diagnosis was 87.5%, with no significant differences among the chatbots ($p=0.100$). For periapical diagnosis, it was 76.7%, with a significant difference ($p=0.026$): Gemini Advanced at 100%, ChatGPT-4o at 85.7%, Copilot at 64.3%, and Gemini at 57.1%. Descriptive accuracy differed significantly among chatbots ($p=0.001$), with Gemini Advanced scoring 6 on the 6-point Likert scale in 92.9% of responses, followed by ChatGPT-4o at 64.3%, and both Copilot and Gemini at 42.9%.

Conclusions

Chatbots demonstrated high accuracy in pulpal diagnosis; however, their performance in periapical diagnosis and descriptive accuracy varied significantly across models, with Gemini Advanced achieving the highest validity.

Biosynthetic Gene Cluster significantly alters *Streptococcus mutans* gene expression

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Introduction

Streptococcus mutans is a key organism in dental caries. Key virulence traits include the ability to attach to tooth surfaces and form biofilms. The butyrolactone-ladderane biosynthetic gene cluster (BL-BGC) has been reported in a prevalent *S. mutans* strain type (G18). Previously, deletion of the BL-BGC resulted in significant biofilm reduction. The purpose of this study was to determine the impact of the BL-BGC on key genes related to biofilm fitness and virulence with RNA sequencing.

Methods

Subcultures of *S. mutans* UAB-10 (parent strain) and BL-BGC (whole cluster knockout mutant) in Todd Hewitt Broth with 1% sucrose were used to prepare overnight biofilms (16 hours). All cells (biofilms and planktonic) were collected, washed and RNA extraction performed. RNA Sequencing was performed by Genewiz and transcriptomic analysis was performed using DESeq2. All experiments were repeated in at least triplicate. The top 100 p-adjusted significantly differential transcripts were evaluated for differential gene clusters.

Results

A total of 2,037 transcripts were reported, 66% were significantly differential with 8% showing differential expression within the <-1 or >1 fold cut-off. Transcripts for BL-BGC genes were the significantly downregulated for BL-BGC mutant. Transcripts associated with other bacteriocins clusters, mutacin synthesis, quorum sensing, ribosome synthesis and DNA alkylation repair were significantly upregulated. Sugar transport, small molecule phosphatases, glucosyltransferase C (gtfC), and cell surface antigen I/II (SpaP) were significantly downregulated.

Conclusions

Removal of the BL-BGC resulted in global transcriptional shifts in *S. mutans* UAB-10. The significant downregulation of biofilm attachment antigen I/II (SpaP) and the primary glucosyltransferase (gtfC) in BL-BGC mutant may be responsible for the significant reduction in biomass and increase of planktonic cells reported previously for BL-BGC. *S. mutans* adapts to the loss of the BL-BGC through increased expression of other mutacins. These findings indicate that the BL-BGC is fundamental to the cariogenic potential of clinically prevalent *S. mutans* UAB-10.

Photocrosslinkable Bone-Derived ECM Hydrogels: Enabling Bioprinting of Scaffolds for Tissue Engineering and Regenerative Medicine

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Introduction

Strategies to mimic native bone tissue in vitro are gaining traction in the bioengineering community, particularly due to the limitations of bone autografting, which include heightened infection risk, limited donor supply, and donor-site morbidity. This study explores the potential of bioprinted, micron-sized hydrogels made from methacrylated extracellular matrix (ECM)-derived materials called BoneMA.

Methods

Derived from native bone, BoneMA exhibits a unique composition that includes both collagen and non-collagenous proteins. The photopolymerizable nature of BoneMA allows for superior printability and precise mechanical property control, distinguishing it from existing ECM-derived biomaterials. Notably, the collagen backbone facilitates the incorporation of hydroxyapatite nanocrystals via a biomimetic mineralization process previously established by our team, closely resembling native bone structure and composition.

Results

Using a 10% BoneMA solution combined with 1% Lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate (LAP), we observed a significant increase in mineral deposition, confirmed through Von Kossa and Alizarin Red S staining techniques. This enhancement was notable compared to Gelatin Methacryloyl (GelMA) microgels (10% and 1% LAP) and non-mineralized controls. Raman spectroscopy further validated the findings, revealing distinct phosphate and amide peaks indicative of robust hydroxyapatite formation. Scanning electron microscopy confirmed the nanoscale mineralization within BoneMA ECM. Preliminary trials assessing the injectability of these microgels indicated promising potential for minimally invasive surgical applications.

Conclusions

These results suggest that BoneMA not only effectively promotes mineralization but also surpasses GelMA in performance, paving the way for significant advancements in bone therapies.

Inhibition of Dentinal Collagen Degradation by Matrix Metalloproteinases Using Quaternary Ammonium Methacrylates

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Mentors: Fernanda Lucena, Matthew Logan, Steven Lewis, Carmem Pfeifer

Co-Authors: Fernanda Lucena, Matthew Logan, Steven Lewis, Carmem Pfeifer

Introduction

Dentin's organic matrix consists primarily of type I collagen, which is degraded by matrix metalloproteinases (MMPs) activated during caries progression or the adhesive bonding process. Chlorhexidine (CHX) effectively inhibits MMPs but leaches out rapidly, limiting its long-term efficacy. Quaternary ammonium methacrylate (QAM)-based adhesives provide antimicrobial benefits and could sustain MMP inhibition. This study evaluates the antimicrobial and anti-enzymatic properties of a QAM-based dental adhesive. The null hypotheses tested were that the QAM-based adhesive does not significantly differ from the control adhesive or the 2% CHX-based adhesive in preserving the collagen matrix or exhibiting antimicrobial activity.

Methods

MMP inhibition was assessed using a fluorescence-based assay with recombinant MMP-2, incubated with varying concentrations of CHX or QAM. Fluorescence intensity indicated enzymatic activity. The shear storage modulus (G') of demineralized dentin slices was measured using a rheometer after incubation in treatment solutions. Collagen degradation was evaluated by hydroxyproline quantification after incubation in artificial saliva. Two-step, total-etch adhesives were formulated with BisGMA/HEMA, with 10 wt% DMAHDM added for the QAM-modified adhesive. The degree of conversion was analyzed via FTIR after solvent evaporation and photoactivation. For biofilm assays, *S. mutans* was cultured on adhesive discs for 24 hours. Planktonic growth was measured via OD600, biofilm viability using luminescence, and biomass through crystal violet staining.

Results

Dentin discs incubated with 2% CHX or 10% QAM for 72 hours exhibited a significant increase in shear storage modulus ($p = 0.006$), indicating improved mechanical stability. Hydroxyproline assay results showed the highest collagen degradation in the H₂O group (35.4 $\mu\text{g/mL}$), with significantly lower degradation in CHX (20.8 $\mu\text{g/mL}$) and QAM (21.8 $\mu\text{g/mL}$) groups ($p = 0.005$), suggesting effective collagen preservation. Ethanol/water treatment led to intermediate degradation (29.7 $\mu\text{g/mL}$). The degree of conversion was highest in the 10% QAM group. CHX and QAM significantly reduced bacterial growth (OD600), biofilm biomass, and viability, with QAM being the most effective ($p < 0.001$ for all). Stereomicroscopy confirmed reduced biofilm formation in CHX and QAM groups compared to the control. These findings highlight the antimicrobial and enzymatic inhibition properties of QAM-modified adhesives, demonstrating their potential for improving the longevity of dental restorations by reducing bacterial colonization and collagen degradation.

Conclusions

Both 2% CHX and 10% QAM enhanced dentin stability and inhibited collagen degradation. QAM showed superior antimicrobial effects and improved polymerization, suggesting its potential to strengthen dentin and prevent biofilm formation in adhesive restorations.

QAM Inhibition of MMP Activity and Biofilm Formation in Composite Restorations

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Co-Authors: Steven Lewis, Fernanda Sandes De Lucena, Carmem Pfeifer

Introduction

Recurrent caries are a major cause of restoration failure and weakening of the tooth structure. Quaternary ammonium methacrylates (QAM) inhibit matrix metalloproteinase (MMP) activity by binding to the enzyme's active site, preventing collagen degradation. QAM also disrupts bacterial membrane, reducing cariogenic biofilm formation. Previous studies have focused on QAM effects in isolated systems, not in a physiologically relevant oral cavity model. This study aims to assess the combined effects of QAM on MMP inhibition and biofilm formation in a novel restorative interface model.

Methods

Fifteen extracted molars were prepared by removing the occlusal enamel to expose the dentin, then restored with one of three adhesives: Negative control (no inhibitor), Chlorhexidine (2% CHX), QAM (10% Q16). Samples were incubated with *Streptococcus mutans* for 5 days in a 5% CO₂, 37 degrees Celsius incubator. Pre- and post- incubation samples were molded to create epoxy resin replicas, sputter-coated with palladium, and imaged with scanning electron microscopy (SEM). Perimeter gap length (PGL) and occlusal gap width (OGW) were measured using ImageJ software. Samples were stained with crystal violet and propidium iodide, then cross-sectioned for confocal microscope.

Results

For PGL, the control group showed no significant change between initial and final measurements ($p=0.699$), while both 2% CHX and 10% QAM groups had significant increases in PGL ($p<0.001$ and $p=0.039$, respectively). No significant differences in PGL were found between groups. For OGW, the control and 2% CHX groups showed significant increases from final to initial measurements ($p<0.001$ for both), with QAM also showing significant increase ($p<0.001$). No significant differences in final OGW were observed between groups ($p=0.148$).

Conclusions

This pilot suggests that QAM may reduce gap formation at the restoration interface, similar to CHX, potentially by inhibiting MMP activity. Future studies will explore QAM's effects with mechanical challenges in a bioreactor model.

A Novel Organ-On-a-chip Model of Multiple Myeloma Interactions With Bone

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Introduction

Multiple myeloma (MM), the most common hematological malignancy, is characterized by proliferation of neoplastic plasma cells leading to osteolytic bone resorption in ~50% of patients. These bone lesions, often presenting as “moth-eaten” defects in the calvaria and mandible, result from disrupted bone homeostasis—typically maintained by balanced osteoclast and osteoblast activity, with osteocytes playing a critical regulatory role. In MM, osteocyte death and dysregulation, including reduced OPG and elevated SOST and FGF23, are reported to contribute to focal bone lesions. While 2D co-culture and transwell models suggest a Notch signaling mechanism in osteolytic lesions, they fail to replicate the complex bone microenvironment. We aimed to recreate the cellular and extracellular dynamics of MM-associated osteolytic lesions using a microfluidic bone-on-a-chip system.

Methods

Multiple myeloma (MM), the most common hematological malignancy, is characterized by proliferation of neoplastic plasma cells leading to osteolytic bone resorption in ~50% of patients. These bone lesions, often presenting as “moth-eaten” defects in the calvaria and mandible, result from disrupted bone homeostasis—typically maintained by balanced osteoclast and osteoblast activity, with osteocytes playing a critical regulatory role. In MM, osteocyte death and dysregulation, including reduced OPG and elevated SOST and FGF23, are reported to contribute to focal bone lesions. While 2D co-culture and transwell models suggest a Notch signaling mechanism in osteolytic lesions, they fail to replicate the complex bone microenvironment. We aimed to recreate the cellular and extracellular dynamics of MM-associated osteolytic lesions using a microfluidic bone-on-a-chip system.

Results

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High-Fidelity Bone-on-a-Chip Platform Recapitulates Bone Physiology and Oral Cancer Invasion

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Introduction

Bone homeostasis is maintained through a highly controlled and self-regulated interaction of osteoclasts, osteoblasts, and osteocytes. To date, it has been virtually impossible to recapitulate these interactions in vitro with precision without using exogenous growth factors. Reproducing the spatial arrangement and microenvironmental cues that govern this interplay, osteoclastogenesis, and bone resorption in-vitro remains challenging. Developing a platform that recapitulates these events in vitro would be a breakthrough in understanding bone pathology events, such as oral cancer invasion. We hypothesize that a biomimetic microenvironment replicating the native bone nanostructure and biological function of osteocyte cells three-dimensionally embedded in dense minerals, may enhance osteoclastogenesis and sustain osteoclast function without any exogenous growth factor, simulating bone physiology and oral squamous cell carcinoma invasion on-a-chip.

Methods

A bone-on-a-chip model was engineered using a biomimetic approach to achieve encapsulation of osteoblast cells in heavily calcified collagen (2.5 mg/mL) matrices, employing a 3-day protocol for collagen nanoscale intrafibrillar mineralization. Macrophages (THP-1 derived) and osteoblasts were layered on this matrix, creating a system with osteocytes, osteoblasts, osteoclasts, and macrophages as found in native bone. Osteoblast differentiation into osteocytes was assessed via immunofluorescence, and paracrine production for osteoclastogenesis (Luminex) and gene expression profiles of macrophages undergoing osteoclastogenesis (nCounter Nanostring) were compared to nonmineralized matrices supplemented with RANKL/MCSF (control). Osteoclast function was validated in both groups in response to therapeutics denosumab and alendronate. The bone invasion was evaluated by adding oral squamous cancer cells (UCSF-OT1109 cell line 3.103 into the lateral channel staining cancer cells with cytokeratin antibodies and quantifying them in the channel and matrix (invasion) after 24 or 48h.

Results

For the first time, we introduce a system where the mineralized matrix promotes osteocyte differentiation without growth factors, marked by increased expression of sclerostin and podoplanin ($p < 0.05$). Paracrine signaling from differentiated osteocytes alone resulted in more multinucleated, TRAP-positive osteoclasts ($p < 0.01$) than controls with RANKL/MCS-F. Mineralized samples stimulated RANKL and reduced OPG expression, while RANKL/MCS-F induced inflammatory cytokines like IL-1 β . Gene analysis showed the biomimetic matrix regulated osteoclastogenesis via cell/matrix interactions, while RANKL/MCS-F upregulated inflammatory pathways, and the chip model replicated patient response to denosumab and

Sousa (cont'd)

alendronate. Furthermore, more cytokeratin-positive cells (oral cancer invasion) were present in the groups containing osteoclasts compared to the non-osteoclast groups, showing that our model could illustrate bone events in physiology and pathology that were not able with previous models in vitro.

Conclusions

Our bone-on-a-chip model is the first to mimic bone structure and function with high fidelity and to support both osteocyte differentiation and osteoclastogenesis without external supplements. The model also mimics clinically relevant events in cancer invasion, bringing new avenues for drug testing and cancer therapy.

The salivary virome during childhood dental caries

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Introduction

The primary goal of this study was to use bioinformatic tools to characterize the salivary DNA virome in the context of severe caries versus healthy dentition through alpha and beta diversity metric analysis, co-occurrence analysis of phage, predicted host bacteria, and disease status, and identification of novel oral phage.

Methods

2,485 viral metagenome-assembled genomes (vMAGs) were identified, binned, and quantified from the metagenomic assemblies using ViWrap. fastANI (implemented with anvio) was used to dereplicate genetically similar vMAGs into 1,865 unique species-level vOTUs. The metagenomic assemblies were queried for all 3,858 unique species-level vOTUs of DNA viruses with a human host on NCBI Virus. Novel oral phages were identified using skani, querying the vOTUs against 16,185,359 viral genomes across 6 reference databases. QIIME 2 was used to perform alpha and beta diversity analyses, incorporating caries-versus-health associativity metadata generated by Songbird. Co-occurrence analyses were done using MMvec.

Results

The identified vMAGs were mostly phage; among the NCBI DNA viruses with a human host, all but Human betaherpesvirus 7 were at very low abundance in the saliva. The oral virome of the children with caries exhibited significantly different beta diversity compared to the oral virome of the children with healthy dentition. Co-occurrence analysis indicated that phage typically co-occurred with both their predicted hosts and with bacteria that were themselves associated with the same disease status.

Conclusions

This study illustrates the concept that there is still a wealth of unknown viral diversity within the oral microbiome and represents an important step towards the identification and study of phage therapy candidates which treat or prevent caries pathogenesis. Continued improvements in long-read sequencing technology concurrent with a continued reduction in sequencing costs will enable larger-scale future metagenomics studies producing more MAGs with greater contiguity.

Temporomandibular Joint Loads and Mandibular Length in Children

Valerie Truong
DMD Student, OHSU School of Dentistry

Mentor: Laura Iwasaki, Jeff Nickel

Co-Authors: L. R. Iwasaki, H. Liu, J. C. Nickel

Introduction

Temporomandibular joint (TMJ):
Cartilages are mechanosensitive

Loads are important to mandibular growth.

This pilot study aimed to determine if age and TMJ load during molar biting were related to mandibular length in children.

Methods

OHSU Institutional Review Board approved protocols

Inclusion criteria:

Age 10 – 14 years

Skeletal Class II relationship

Permanent incisors & 1st molars

Pre-orthodontic records included cone-beam computed tomography (CBCT)

Exclusion criteria:

Musculoskeletal disease

Craniofacial anomaly

Trauma to TMJ

Subjects gave informed consent

CBCT image and software (Dolphin, InVivo) used to measure:

Mandibular length (Co-Gn, mm; Fig. 1)

Craniomandibular geometry (Fig. 2)

Computer-assisted numerical models¹ used with each subject's geometry and objective of minimization of:

TMJ loads to predict effective sagittal eminence shape²

Muscle effort to predict TMJ loads during static biting³ on mandibular right 1st molar at a range of bite-force (BF) angles (Fig. 2)

Regression analysis tested relationship of

Mandible length (normalized to longest for each sex)

Age (years)

TMJ load (% applied BF)

Results

Sample: 21 children (Table 1)

Normalized mandibular length had a non-linear relationship ($R^2 = 0.40$) with age and average TMJ load (Fig. 3).

Conclusions

Age and TMJ load explained 40% of mandibular length variability in this group of children.

Acquired Pellicle Alpha Amylase Abundance in Hydrophilic/Hydrophobic Dental Materials

Jade Wong

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(Biomaterials and Biomedical Sciences)

Introduction

The salivary acquired pellicle (AP) is a thin film of proteins and proteoglycans from saliva. The AP functions as a protective layer on the tooth against acid challenges and acts as a mediator of the tooth's interaction with oral microbiome and bacteria adhesion. One of the most abundant proteins that make up the AP is salivary alpha amylase. It has a significant role as a modulator of the AP, facilitating the colonization of dental biofilm. The characteristics of a dental monomer, such as the pH, surface charge, and hydrophilicity/hydrophobicity directly influence on how the AP proteins are going to adhere to the surface. Therefore, this study aims to evaluate if a hydrophobic (BSEMA) and a hydrophilic (PEGDMA) monomer would have different abundance profile of alpha amylase within the acquired pellicle.

Methods

BSEMA (hydrophobic) or PEGDMA (hydrophilic) were mixed to 0.1wt% DMPA and inserted into a 10 mm x 0.8 mm (diameter x thickness) mold. The disks were photocured (700 mWcm², 1 min/side), stored for 24 h, and the degree of conversion (DC) was measured (near-IR). Disks were sanded to 0.4-0.6µm surface roughness (Ra). The saliva was collected from fifteen donors and the bacteria was removed with centrifugation and filter sterilized with 0.22 µm filter. The protein concentration was quantified with a DC Protein Assay (BioRad) with a bovine serum albumin standard. There were three treatments utilized: alpha amylase (50 ng/ml), saliva (5 ng/ml), alpha amylase + saliva (50 ng/ml + 5 ng/ml). The disks were incubated for 2h in treatment to form the AP (n=3). The initial and the final incubation treatments from the wells were saved to quantify the amount of human salivary alpha amylase that formed the AP with a colorimetric sandwich ELISA (Novus Biologicals NBP2-68204).

Results

Still in progress.

Conclusions

If the results demonstrate that the polymeric material characteristics and saliva proteins influence the quantity of alpha amylase to form the acquired pellicle, then it is possible to engineer dental materials to modulate/shift the ecology of the biofilm that interact with the AP.

The T7SS mediate the Interaction between *Streptococcus parasanguinis* and pathogenic cohabitant *Aggregatibacter actinomycetemcomitans*

Jinci Yan

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Mentor: Hui Wu

Co-Author: Hui Wu

Introduction

A balanced bacterial biofilm community is crucial for maintaining host health. Disruption of this balance can lead to bacterial dysbiosis and subsequent pathogenesis. While numerous mono-species infection models have been established and studied, providing valuable insights into the mechanisms of bacterial pathogenesis, the complex and dynamic interactions within multispecies communities remain poorly understood. In a previous study, our lab used a dual-species biofilm model consisting of the periodontal pathogen *Aggregatibacter actinomycetemcomitans* and the commensal *Streptococcus parasanguinis* to investigate bacterial interactions. Our findings demonstrated that *A. actinomycetemcomitans* interacts with *S. parasanguinis* both in vitro and in vivo. In this study, we aimed to explore the role of the T7 secretion system (T7SS) in mediating this interaction.

Methods

We first established an in vitro dual-species biofilm model to investigate the interaction between *A. actinomycetemcomitans* and *S. parasanguinis*. Synergistic effects were observed when the two species were co-cultured. Further comparative transcriptome analysis revealed significant changes in the expression of T7SS-associated genes between the co-culture and mono-species conditions. Main genes knockout mutant of *S. parasanguinis* was created to evaluate the involvement of the T7SS in the interaction between the two species.

Results

In our previous study, we observed that co-culturing *A. actinomycetemcomitans* and *S. parasanguinis* significantly enhanced biofilm formation. Interestingly, the viability of *A. actinomycetemcomitans* was notably reduced by *S. parasanguinis* in the co-culture. Conversely, the number of *S. parasanguinis* recovered was substantially higher in the dual-species biofilm. In the present study, we found that T7SS-associated genes exhibited significant changes between co-culture and mono-species conditions based on comparative transcriptome analysis. We then created different knockout mutant of the primary T7SS genes in *S. parasanguinis* and observed a surprising result: the mutant lost its ability to form a biofilm with *A. actinomycetemcomitans*. Furthermore, in the dual-species biofilm, the viability of *A. actinomycetemcomitans* was significantly increased in the presence of the *S. parasanguinis* mutant compared to the wild-type strain. In contrast, the number of *S. parasanguinis* mutants was drastically reduced in the dual-species biofilm compared to the wild-type.

Conclusions

In summary, our study identifies the T7 secretion system as a key mediator of the interaction between *A. actinomycetemcomitans* and *S. parasanguinis*, influencing the relative abundance of each organism within the dual-species biofilm. This study reveals a previously unrecognized mechanism through which an oral commensal bacterium responds to and compete with an opportunistic pathogen to enhance its own biofilm formation and overall fitness via the T7 secretion system.

Performance Comparison of AI Chatbots and Prosthodontic Residents on the National Prosthodontic Resident Examination (NPRE)

Christopher Yoon
DMD Student, OHSU School of Dentistry

Mentor: Despina Bompolaki, Hongseok An

Co-Authors: Dr. Despina Bompolaki, Dr. Hongseok An, Dr. Esha Mukherjee, Kelsey Lee

Introduction

Artificial intelligence (AI) and large language models (LLMs) are increasingly used in medical and dental education. AI chatbots such as ChatGPT, Gemini, Claude 3, and Llama demonstrate advanced natural language processing capabilities and have been tested on standardized exams in medicine and dentistry. However, their ability to accurately perform on specialty-specific assessments remains unclear. This study aimed to compare the performance of multiple AI chatbots against prosthodontic residents on the National Prosthodontic Resident Examination (NPRE) to assess their potential as educational tools in prosthodontics.

Methods

This study analyzed the performance of seven AI-based chatbots—ChatGPT 3.5, 4, 4.o, Gemini, Claude 3, CoPilot, and Llama—on the NPRE, comparing their scores to the average performance of prosthodontic residents from 2011 to 2023. The exam consists of 200 multiple-choice questions covering complex prosthodontic principles, occlusion, biomaterials, digital technology, and craniofacial anatomy. Each chatbot was provided the exam questions in a standardized format, ensuring consistency in evaluation. The AI responses were recorded and scored based on the official answer key. Resident performance data were obtained from the American College of Prosthodontists (ACP). Statistical analysis was conducted using repeated measures analysis of variance (ANOVA), with Bonferroni post-hoc tests for pairwise comparisons. Descriptive statistics, including mean scores, standard deviations, and score distributions, were analyzed. Chatbot performance variability and consistency were evaluated by comparing the standard deviations of each AI model.

Results

Significant differences were observed between AI chatbots and resident scores ($p < 0.001$). ChatGPT 3.5 achieved the highest average score (102.31 ± 14.28), surpassing all other chatbots and residents. Claude 3 and Llama demonstrated comparable performance to residents, whereas CoPilot scored significantly lower. ChatGPT 4 and 4.o exhibited high variability in their results, indicating inconsistency in responses. The overlapping subgroups in the pairwise comparisons suggest that while certain AI models perform well, ranking them definitively is challenging due to performance fluctuations.

Conclusions

AI chatbots can perform at or above the level of prosthodontic residents on the NPRE, highlighting their potential as educational tools. ChatGPT 3.5 outperformed all other models, while multimodal AI versions exhibited inconsistent results. The findings underscore the importance of task-specific optimization in AI models and caution against relying solely on AI for academic assessments. Future research should focus on refining AI capabilities for specialized disciplines and evaluating their reliability across different test formats.

Mechanism Underlying the Synergistic Interaction Between Veillonella and Prevotella

Zhengzhong Zou

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Co-Authors: Hui Wu

Introduction

Veillonella species are prominent anaerobes in the oral microbiome, frequently found in both caries lesions and healthy oral environments. While their coaggregation with Streptococcus and ability to utilize lactate instead of carbohydrates as an energy source are well documented, their broader metabolic interactions remain poorly understood. Here, we investigate the interactions between two abundant genera in the oral microbiome, Veillonella and Prevotella, to elucidate their potential synergistic relationship.

Methods

Biofilm formation was assessed using crystal violet staining. Gene expression differences between co-cultures and mono-cultures were analyzed via RNA sequencing (RNA-seq).

Results

Our findings revealed that biofilm formation was significantly enhanced in Veillonella-Prevotella co-cultures compared to their respective mono-cultures. Furthermore, colony-forming units (CFUs) of both genera were significantly increased in co-culture conditions, suggesting mutual growth promotion.

RNA-seq analysis of Veillonella-Prevotella co-cultures compared to Veillonella mono-cultures revealed upregulation of genes associated with bacterial microcompartments, the methylmalonyl-CoA pathway, and purine metabolism, while genes involved in thiamin pyrophosphate (TPP) biosynthesis, iron-sulfur (Fe-S) cluster assembly, and oxidative stress responses were downregulated. In Prevotella, co-culture conditions led to downregulation of genes related to pyruvate metabolism, threonine/serine export, and oxidative stress responses, while genes associated with energy metabolism and the Type 9 Secretion System (T9SS) were upregulated.

Conclusions

This study demonstrates that Veillonella and Prevotella, two dominant genera in the oral microbiome, enhance each other's growth and biofilm formation. Their metabolic interplay and high abundance suggest that their interactions play a crucial role in oral biofilm ecology, potentially influencing both health and disease states.

Research Day

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Session 2

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Bruce Havens	38	FACULTY	Evaluation of Cleft Volume as a Predictor of Alveolar Bone Graft Success
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David Anderson	6	STAFF	Networks Investigating social protein networks using Streptococcus mutans as a model system
Despina Bompolaki	32	FACULTY	Practice-Based Research at the SoD: Obstructive Sleep Apnea (OSA) Risk Assessment in Patients Seen at the SOD Clinics
Dillon Moya	18	POSTDOC	Machine Learning Modeling of Variables Affecting Longitudinal TMJ Structure Change
Elena Secaira, Ben Bratland	60	DMD/ CASECat	Effect of fluoride consumption on IQ in adolescents
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Fernanda Sandes De Lucena	24	POSTDOC	Selective glucosyltransferase inhibition: repurposing FDA-approved drugs to disrupt S. mutans biofilms
Finn Peck	58	DMD/ Research	Exploring in vitro Biofilm Formation and Interactions Among F. nucleatum, F. periodonticum, and P. micra
Graydon Gamache and Daniel Kim	52	DMD/ CASECat	Effects of Non-Surgical Periodontal Therapy on Blood Pressure in Hypertensive Patients
Henrico Strazzi Sahyon	22	POSTDOC	The Effect of Grain Particle Size on Surface, Mechanical, and Optical Properties of Recycled Zirconia
Hua Qin	8	STAFF	Development of a versatile toolbox for genetic manipulation of Prevotella melaninogenica
Hua Zhang	26	PhD STUDENT	Exploring LCP Protein Functions in Streptococcus mutans and Discovering Small Molecule Inhibitors
Huixin Wu	20	POSTDOC	Carcinogenic Potential of Oral Bacteria P. gingivalis, V. parvula, and P. melaninogenica in Oral Cancer
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Jeff Nickel	34	FACULTY	A Pilot Study of Temporomandibular Joint Alloplastic Implant Contact Mechanics
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Molly Burnside	4	STAFF	Shining Light on Oral Biofilm Fluorescence in situ Hybridization (FISH)
Molly McCoy	74	DMD/ Research	Enhancing Intraoral Scanning Accuracy for Mandibular Full-Arch Implant-Supported Prostheses Using Resin Markers: an in vitro study
Mona Sivaneri	28	RESIDENT	Dysbiotic oral microbial community selected by mouse immune system
Narita Narkhede	72	DMD/ CASECat	Guided Bone Regeneration (GBR for Treating Peri-implantitis Defects: Hope or Hype?
Nicole O'Dierno	46	DMD/ Research	Parvimonas micra: Identification and characterization of pathogenic P. micra from clinical dental abscesses
Peter Nguyen	50	DMD/ CASECat	Reduction of Silver Diamine Fluoride Staining In Carious Teeth Using Potassium Iodide To Form A Silver-Iodide Precipitate
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Ryan Knapp	80	DMD/ Research	Nocturnal Variation in Autonomic Nervous System Activity
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Tuesday, March 4

Robertson Life Science Building, Portland, OR



Investigating social protein networks using *Streptococcus mutans* as a model system

David Anderson

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OHSU School of Dentistry

Co-Authors: Justin Merritt

Introduction

Numerous protein interaction studies conducted in our laboratory have revealed a consistent repeating theme – proteins with no intuitive functional connection commonly associate with each other in the host cell. We believe that this phenomenon is a basic tenant governing all living cells in that many proteins have a core ancestral function (ex. catalysis), but also ancillary rolls via associating with proteins encoding disparate functions. Social protein networks would represent an energetically efficient way to harmonize the metabolic needs of a cell in a highly tunable fashion. Our initial test case will focus on the RNA degradosome from *S. mutans*.

Methods

We will combine several scientific disciplines to investigate RNA degradosome structure and composition, spanning: genetics, biochemistry, and structural biology. A crucial requirement to characterize these protein complexes is to generate empirical data on the interaction interfaces. Our goal is to develop a robust and economical in-house pipeline to process large-scale samples for crosslinking-mass spectrometry and cryo-electron microscopy. To this end, we are developing tandem and triple immunopurification strategies using recombinantly purified single-chain variable fragment (scFV) antibodies targeting workhorse epitopes (FLAG, HA, c-Myc) engineered into the genome of *S. mutans*. Our target proteins are also modified with a unique site-specific protease site prior to the epitope. This allows for specific, efficient, and importantly low volume elution of complexes from immobilized resins. Our near-term goal is to perform triple sequential pulldowns for J1 (HA, 3C) and J2 (FLAG, TEV) heterodimers, then finally a third protein (c-Myc, enterokinase) to generate a full picture of the interaction network of the RNA degradosome under various environmental conditions. We will build a graph network diagram of all the degradosome interacting proteins to elucidate how the complex constituents change as a response to environmental conditions. The third round of purification will allow us to parse our protein complex constituent exclusivity and inclusivity which may be important drivers governing the transcript-targeting profile.

Results

We have recently completed work regarding the production of our own recombinant antibodies from *Escherichia coli*. These are co-expressed with BirA ligase within the organism, which will target a c-terminal AviTag sequence appended to the antibody for site-specific biotinylation. A dual-cleavable system was tested in which a sequential pulldown of J2 RNase was performed targeting a 3x-FLAG tag. These complexes were release via TEV protease cleavage. A subsequent pulldown of J1 RNase was performed on this elution via a 3x-HA tag, followed by 3C protease elution. Mass spectrometry results on this purified pool of protein revealed nearly all the candidate interactors from past experiments, however, this protocol produced one of the lowest amounts of background we have seen.

Conclusions

We are developing a methodology for large-scale, low-background purification of native protein complexes using *S. mutans* as a model system. Initial experiments suggest we are on the cusp of successfully performing triple sequential pulldowns of native proteins, which we hypothesize will be necessary to achieve sufficient homogeneity for cryo-EM structural investigations.

The effect of *Beta vulgaris* (beetroot) on an in vitro oral microbiome of electronic cigarette users

Matthew Barbisan

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Co-Authors: Jonathon L. Baker, Jonah Tang, Daniela V. Staton (American Heritage School
Palm Beach, Delray Beach, FL, USA), Justin Nussbaum (Department of Biology, Lakeland
Community College, Kirtland, OH, USA)

Introduction

Although electronic cigarettes are often posited as a healthier substitute for conventional cigarette smoking, their use has been linked to a myriad of health issues, including—of particular interest in this study—negatively impacting the oral microbiome. Nitrate supplementation, suggested as a prebiotic, may provide a method to counteract these negative impacts. *Beta vulgaris*, commonly known as beetroot, is a nitrate-rich root vegetable that could show promise as a dietary method of nitrate supplementation. This pilot study aimed to investigate if beetroot extract supplementation on saliva-derived microcosms from e-cigarette users promotes the recovery of bacteria associated with good oral health.

Methods

10 mL of saliva was self-collected from each subject (7 e-cigarette users and 7 nonusers). 100 μ L of each saliva sample was cultured at 37 °C in liquid media for 5 hours under two conditions: 30 mL of liquid BHI media with or without treatment of 30 mg/mL of beetroot juice extract. The DNA from each of these microcosms (and the initial saliva) was extracted and sequenced. Sequencing of the 16S rRNA gene was performed on a MinION nanopore sequencer to collect data on taxonomic abundance at the species level for each sample. QIIME2, a bioinformatics and data science program, was used to perform downstream analyses on the collected data to investigate significant differences in alpha diversity (Shannon, Simpson, Chao1, and Faith's PD metrics) and beta diversity (Bray-Curtis, Jaccard, Weighted UNIFRAC, and Unweighted UNIFRAC metrics) between groups via a pairwise Kruskal-Wallis test and a pairwise PERMANOVA, respectively. Because of the natural subject-to-subject variability in the oral microbiome between subjects, repeated measure analysis via compositional tensor factorization (CTF) was also used to help mitigate these differences. In addition, differential abundance methods were used to investigate differences between groups for individual species: ANCOM-BC and Songbird were used in tandem to confirm results and increase confidence on significant differences between species abundance in groups.

Results

Alpha diversity and beta diversity of the starting saliva samples were significantly higher ($p < 0.05$) than that of all 4 microcosms under all metrics tested. In addition, the Shannon and Simpson metrics showed that, in the absence of beetroot, alpha diversity of the microcosms of the users was significantly lower than the microcosms of the nonusers and that the microcosms of the users and nonusers significantly decreased with beetroot treatment. With respect to beta diversity, beetroot treatment on the microcosms had a modest impact on these communities. CTF analysis led to more separation between users and nonusers though still not significant. Both differential abundance methods showed a higher abundance of *Neisseria* spp. and depletion of *Prevotella* spp. when comparing the saliva of nonusers to users. Furthermore, between microcosm groups, Songbird showed an increase abundance in *Rothia* spp. and *Haemophilis* spp. with beetroot treatment, though not significant according to ANCOM-BC.

Conclusions

In this pilot study, analyses showed modest differences in alpha and beta diversities between microcosm groups, with or without beetroot treatment, and suggested a slight recovery of bacteria associated with good oral health with treatment of beetroot juice extract. This was likely due to the reduced biodiversity of the microcosms by virtue of a selective culturing environment non-representative of the diverse oral cavity microenvironments in addition to a small sample size. Despite this, this study adds to contemporary research paving the way for more in-depth studies examining the role of nitrate-rich supplements as prebiotics to promote oral health.

Practice-Based Research at the SoD: Obstructive Sleep Apnea (OSA) Risk Assessment in Patients Seen at the SOD Clinics

Despina Bompolaki

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Co-Authors: Jack Ferracane, Robert Kravitz

Introduction

Obstructive sleep apnea (OSA) is a prevalent yet underdiagnosed condition with significant health implications. This study aims to assess the prevalence of moderate to severe OSA risk in patients seen at the OHSU School of Dentistry (SoD) clinics using the STOP-Bang questionnaire. Additionally, it evaluates patient follow-up behaviors regarding diagnosis and treatment. By integrating OSA risk assessment into routine dental visits, this study seeks to enhance patient awareness and facilitate early intervention. Findings will provide insights into the feasibility of dental clinics as screening sites and establish a framework for student-led research in dental education and patient care.

Methods

This study was conducted at the OHSU School of Dentistry (SoD) predoctoral and General Practice Residency (GPR) clinics. Adult patients (≥ 18 years) undergoing comprehensive or periodic exams were screened for obstructive sleep apnea (OSA) risk using the validated STOP-Bang questionnaire, that was recently embedded in the axiUm electronic health record. Patients scoring ≥ 3 were classified as moderate-to-high risk and provided educational materials encouraging physician evaluation.

Eligible patients were then identified through a retrospective search of completed STOP-Bang screenings and corresponding CDT code (D9957). Patients with a prior OSA diagnosis were excluded. Data were de-identified and analyzed using SPSS (IBM Corp.). Prevalence of OSA risk was determined based on STOP-Bang scores.

Results

A total of 387 patients were identified as having a sleep apnea screening form or code completed during the study period. Of these, 25 forms were incomplete, leaving 362 patients for analysis. Among them, 56.4% ($n=204$) were classified as low risk for obstructive sleep apnea (OSA), 31.2% ($n=113$) as moderate risk, and 12.4% ($n=45$) as high risk. As a next step for this study, follow-up calls will be conducted at 3 and 6 months by IRB-trained study team members. Verbal consent will be obtained, and structured questionnaires will evaluate whether patients sought further evaluation, received a diagnosis, and initiated treatment.

Conclusions

This study demonstrates the feasibility of integrating sleep apnea risk screening into routine dental visits using the STOP-Bang questionnaire and highlights the potential for dental providers to play a proactive role in early identification. A significant proportion (43.6%) of patients were classified as moderate to high risk for OSA, reinforcing the need for interdisciplinary collaboration in patient care. Future studies should explore ways to enhance patient adherence to physician referrals.

Additionally, the study establishes a framework for practice-based research within the dental school, providing valuable opportunities for students to engage in clinical research and improve patient outcomes.

Shining Light on Oral Biofilm Fluorescence in situ Hybridization (FISH)

Molly Burnside

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OHSU School of Dentistry

Co-Authors: Molly Burnside, Jonah Tang, Jonathan L. Baker, Justin Merritt, Jens Kreth

Introduction

Fluorescence in situ hybridization (FISH) has been crucial in understanding the biogeography of the oral microbiome, uncovering complex species interactions and providing a picture of their spatial arrangements. However, the use of FISH on undefined samples in the oral biofilm is in question due to the dependency on published sequences for probe design. With oral microbiome sequencing studies increasing and more availability of 16S rRNA sequences for individual species, we must assess how accurately previously defined species-specific FISH probes can discriminate among species. Here, we show that biogeographical associations in in situ oral biofilms, particularly for *Streptococcus* and *Corynebacterium*, may need to be revisited to accurately reflect the latest metagenomic information.

Methods

Bacterial strains were grown in BHI media aerobically or anaerobically for *Actinomyces* species. After sufficient growth, species were fixed overnight in 50% ethanol at 4 °C. Samples were permeabilized with solution (0.5M Tris-HCl pH 7.2, dH₂O, 0.5M EDTA pH 8, 35 mg Lysozyme) and incubated for 15 minutes at 37 °C. Samples then went through a series of dehydration with 50%, 80%, and 100% ethanol for 3 minutes each. 1 µL of 100ng fluorescent probe was added to 50 µL hybridization buffer (5M NaCl, 1M Tris/HCl pH 7.2, 25% Formamide, dH₂O, 0.01% SDS) and was incubated at 46°C for at least 2 hours. Formamide concentrations of 15% and 35% were also used to test the effect on hybridization. Following hybridization, samples were washed in buffer (5M NaCl, 1M Tris/HCl pH 7.2, 0.5M EDTA, 0.01% SDS) for 15 minutes at

48 °C. TE buffer was then added for storage at 4 °C. Samples were analyzed microscopically using a fluorescent microscope. To test the fluorescence intensity of each replicate, the imaging program, ImageJ, was used. Images were converted to grayscale, and the mean gray value of 10 distinct areas were collected by circling the fluorescent cells and using the measure tool. To control the fluorescent variation, the background area was also measured and subtracted from the fluorescent measurements. To determine fluorescence intensity percentage, the following equation was used:

$$\frac{((\text{Mean gray value (MGV) of fluorescence} - \text{MGV background}))}{(\text{MGV fluorescence})} \times 100$$

Results

Probe Ssan1 was designed previously to hybridize with *S. sanguinis* ATCC10556 and distinguish it from other *Streptococcus* species. However, Ssan1 hybridized with all species tested besides *S. pyogenes*, which is supported by low in silico hybridization. Probe Ssan3 was designed to specify to *S. sanguinis* SK36 based on the 23S rRNA gene sequence to introduce an alternative to 16S rRNA. Ssan3 hybridized with other *S. sanguinis* strains as well as *S. gordonii*. However, no hybridization was observed with *C. matruchotii*. Probe Cor633 was designed to hybridize with the *Corynebacterium* genus and was tested with *Corynebacterium* species as well as *Actinomyces* species. Cor633 hybridized with all species tested, with lower fluorescence observed with *Actinomyces*.

Conclusions

FISH is a powerful technique that has provided significant insights into the arrangement of microbial species in the oral cavity, yielding information crucial to the current understanding of microbial ecology. These studies are invaluable, as they can guide the development of novel approaches to understanding interspecies interactions. However, they come with an important caveat: promiscuity of widely used FISH probes. While 16S rRNA is a convenient target, it may not always provide the precision required. To enhance the reliability of FISH, probe design may need to incorporate other conserved genes for hybridization. As the saying goes, a picture is worth a thousand words, but it is the responsibility of scientists to select the most accurate tools to capture that picture. This commitment to precision and rigor underpins scientific accuracy, which is the foundation of meaningful discovery and progress.

Optic Dental Biopsy

Jonathan Cha
DMD Student, OHSU School of Dentistry

Mentor: Ginny Hsu

Co-Authors: Jonathan Cha, Siyu Chen, Saulo L. Sousa Melo, Ginny Ching-Yun Hsu

Introduction

The inability to detect the loss of cortical bone and vascularization in periodontal tissue represents a significant problem in evidence-based dentistry. Dental and periodontal tissues are delicate, have minimal regenerative properties, and rely on a simple blood supply. Therefore, early detection and timely adjustment of treatment plans, facilitated by continuous monitoring of dental and periodontal conditions, are essential for achieving successful treatment outcomes. A prototype swept-source optical coherence tomography (SS-OCT) was utilized for detecting and characterizing periodontal lesions, including gingival vascularization and alveolar bone morphology, to ultimately address current limitations in periodontal diagnostics.

Methods

A prototype SS-OCT system operating at a 1310 nm spectral range was employed to image dental and periodontal tissues. The patient was positioned in front of the OCT with a cheek retractor in place and a camera to align the area of interest. This system provided a transverse resolution of ~25 μm and axial resolution of ~9 μm . Imaging covered a $5 \times 5 \text{ mm}^2$ area with 500 B-scans, each containing 500 A-scans, completed within 3 seconds. Gingival vascularization was analyzed using angiograms generated through a split-spectrum amplitude-decorrelation algorithm. Alveolar bone morphology was visualized based on the backscattered signal and light scattering properties.

Results

SS-OCT successfully identified dental and periodontal anatomy such as the dentin-enamel junction (DEJ), enamel spindles, and enamel hypoplasia, with resolution comparable to histological imaging. Intricate vascularization networks including microvessels and arterioles were visualized with high clarity in healthy and recessed gingival tissues. SS-OCT also enabled the detection of alveolar bone fenestration, distinguishing sites with bone defects from sites with intact periodontal structures. These findings demonstrate the capability of SS-OCT to provide high-resolution, non-invasive imaging of both soft and hard tissues in periodontal and dental applications.

Conclusions

SS-OCT offers a novel, high-resolution imaging approach to non-invasive detection and characterization of periodontal and dental lesions. By enabling real-time visualization of vascularization, enamel defects, and bone integrity, SS-OCT establishes a potentially groundbreaking advancement in periodontal diagnostics, treatment planning, and monitoring. With its potential for widespread clinical adoption, SS-OCT could bridge the gap between dental research and routine patient care, marking a significant advancement in evidence-based precision dentistry. By enabling early disease detection, enhancing diagnostic accuracy, minimizing invasiveness, and optimizing treatment strategies, SS-OCT paves the way for a more effective and patient-centered approach to dental care.

Synthesis and characterization of cationic zinc-substituted hydroxyapatite nanoparticles as a potential anti-inflammatory therapeutic for periodontal disease

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Introduction

Periodontal disease (PD) is an infectious-inflammatory condition caused by an imbalance between the commensal microbiome and the host response, affecting two-thirds of individuals aged 65 years or older. Recent studies suggest that circulating cell-free DNA (cfDNA) plays a critical role in chronic inflammation. Consequently, therapeutic approaches utilizing cationic nanoparticles (NPs) to capture and bind negatively charged cfDNA have been proposed. While evidence supports cfDNA's involvement in PD, no clinically translatable therapies currently exist. Thus, this study aims to design and characterize cationic NPs and hydrogels to block downstream pro-inflammatory pathways associated with PD pathogenesis.

Methods

Cationic zinc-substituted hydroxyapatite nanoparticles (ZnHA NPs) were synthesized by mixing 1.0 M calcium nitrate tetrahydrate with 0.05 M zinc chloride in an ultrasound-assisted wet chemical process. The ZnHA NPs were divided into four groups and coated with three generations of polyamidoamine dendrimers (PAMAM-G3, PAMAM-G4, PAMAM-G5). Coating efficiency was evaluated using thermogravimetric analysis (TGA) and Fourier transform infrared (FTIR) spectroscopy, while morphology was observed via transmission electron microscopy (TEM) (n=3). Amino group concentration was measured by a ninhydrin assay (n=3). DNA binding efficiency was assessed by PicoGreen using calf, lambda, and saliva DNA (OHSU IRB #STUDY00027881) (n=5). Biocompatibility was evaluated through MTT assay in normal human dermal fibroblasts (n=6). For hydrogel formation, PAMAM@ZnHA NPs were reacted with 4-arm polyethylene glycol-aldehyde 10K at a 1:20 wt% ratio in a Schiff base reaction. Rheological properties of the hydrogel were evaluated using a rotational rheometer with frequency scanning from 1.0 to 100 rad/s under controlled strain of 0.5% (n=3). Anti-inflammatory properties were assessed in HEK-Blue™ hTLR9 cells using the QuantiBlue colorimetric assay to study NF-κB-dependent responses upon TLR9 stimulation (n=6). Statistical analysis was performed using ANOVA and Tukey's test (p<0.05).

Results

The successful synthesis and coating of PAMAM@ZnHA NPs was confirmed by FTIR, which exhibited absorption bands at 1550-1650 cm⁻¹. TGA results revealed an increase in mass compared to control ZnHA NPs, indicating the loss of organic compounds from the dendrimers. The coating did not alter the nanorod-like morphology of ZnHA NPs, as shown by TEM images. Amino group concentrations ranged from 3.3 to 5.6 mM, with the lowest values for PAMAM-G3@ZnHA and the highest for PAMAM-G5@ZnHA. DNA binding efficiency exceeded 90% for both calf and lambda DNA, with no significant differences between concentrations. For saliva DNA, binding efficiency was slightly reduced, though the nanoparticles remained resistant to corona protein formation. MTT assay results indicated cell metabolic activity ranging from 80% to 95%, suggesting excellent biocompatibility. Hydrogel formation was successful for PAMAM-G3 and G4-coated NPs, with storage and loss moduli ranging from 15 Pa to 300 Pa,

demonstrating injectable viscoelastic properties. TLR9 activation was completely inhibited in hydrogel-treated samples.

Conclusions

Our data indicates the newly developed PAMAM@ZnHA nanoparticles and hydrogels represent a promising therapeutic platform for targeting and binding cfDNA in order to reduce downstream pro-inflammatory processes associated with PD pathogenesis.

How Are Orthodontists Navigating the New Oregon Health Plan for Orthodontic Coverage?

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Introduction

In January 2023, Oregon Health Authority (OHA) expanded coverage for the Oregon Health Plan (OHP) to include severe handicapping malocclusion for beneficiaries under 21 years old

With the anticipated increase of eligible children and adolescents, it is important to understand the challenges affecting Oregon orthodontists to participate in OHP.

Methods

Clinical protocols approved by the OHSU Institutional Review Board (STUDY00025819)

Study Design: Mixed (qualitative and quantitative) methods study

Study Participants: Orthodontists recruited from active members of the Oregon State Society of Orthodontists (OSSO)

Data Collection: Qualtrics survey and semi-structured individual interviews

Data Analysis: Survey responses evaluated using Fisher's exact tests, chi-square tests, and logistic regression analyses with odds ratios. Individual interviews explored factors influencing OHP participation as well as recommendations from orthodontists to OHA and fellow peers.

Results

There were 41 participants (out of 131 active OSSO members) and 10 interviews were completed.

Most survey participants were male (69%), white (76%), solo practitioners (46%), >15+ years of practice (49%), and >45 y/o (71%). Many practitioners were not currently accepting OHP (66%) and perceived a fair reimbursement rate to be >\$4499 (74%) to provide orthodontics care.

Another factor was that practitioners believed it was important to limiting OHP case numbers to <30 annually (68%)

For factors affecting OHP participation, low reimbursement ($p=0.012$) and cancelled appointments ($p=0.043$) were significant with urban, corporate, university settings having higher OHP participation than solo and group private practice. Many themes were identified with a mixture of barriers and facilitators found at the systems and individual practice level.

Conclusions

There is a need to address changes in OHP policy and implementation in order to encourage utilization of the program and increase access to care for eligible OHP beneficiaries.

Evaluation of Cleft Volume as a Predictor of Alveolar Bone Graft Success

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Introduction

Orofacial clefts are one of the most common birth defects, and repair of the physical deficits typically includes an alveolar bone graft (ABG) to repair the alveolar cleft. Prior to ABG the maxillary segments separated by the cleft are typically expanded to a more normal width and orientation, frequently increasing the size of the cleft. While increased cleft size is commonly thought to be related to insufficient graft results, previous studies have been contradictory. The purpose of this study is to test the null hypothesis that alveolar cleft volume does not affect ABG success.

Methods

Case records were retrospectively selected for this pilot study according to protocols approved by Institutional Review Boards from three sites (OHSU, UCSF Fresno, and Valley Children's Hospital). Inclusion criteria were: 7-14 years-of-age at time bone grafting, unilateral cleft of the maxillary alveolus, pre-and ≥ 3 -month post-ABG surgery cone-beam computed tomography images (CBCTs). Exclusion criteria were: diagnosed underlying diseases or syndromes, ABG previously failed or used to support implants or prostheses, bilateral clefts, and orthodontic expansion prior to ABG. CBCTs were analyzed using software (Amira 2022.1) to create 3D masks of alveolar cleft sites before and after ABG and to calculate original and residual cleft volumes (mm³). Bony fill of the cleft site (%) was calculated by: (initial-residual cleft volumes/initial cleft volume) $\times 100$ and tested for correlation with case-specific factors. Statistical analysis for binary factors was performed using Student's t-tests, and linear regression was utilized for continuous factors. Statistical significance was defined as $p < 0.05$.

Results

Records from 13 cases (6 females, 7 males) met inclusion and did not meet exclusion criteria. Inter- and intra-rater reliabilities for calculating cleft volumes were excellent, with the intraclass correlation coefficient (ICC)=0.96 and 0.93, respectively. Mean bony fill was $47.6 \pm 27.6\%$ overall and significantly larger ($p < 0.01$) for females ($67.5 \pm 24.9\%$) compared to males ($30.6 \pm 16.4\%$) and significantly larger ($p = 0.01$) for right-sided clefts ($74.9 \pm 28.5\%$) compared to left-sided clefts (35.5 ± 17.3). Initial cleft volume versus bony fill (%) was inversely correlated ($R^2 = 0.21$, $p = 0.11$) overall, and the relationship was accentuated in cases without tooth structure exposed in the cleft ($R^2 = 0.37$, $p = 0.12$). Power analysis using these pilot data indicated a minimum sample size of $N = 40$ should achieve 80% statistical power with $\alpha = 0.05$ to detect the observed results. Extractions performed at the time of ABG, presence of exposed teeth in the cleft, and age at time of ABG were not found to influence bony fill significantly.

Conclusions

Increased bony fill of alveolar clefts after ABG was related to smaller initial cleft volume, being female, and clefts on the right side. Increased bony fill was not related to patient age at time of ABG, extractions performed during surgery, and the presence of exposed teeth in the cleft.

Havens(cont'd)

Increasing our sample size to N=40 will allow a more definitive examination of the relationship between alveolar cleft volume and ABG success.

Immediate Implant Placement in an Institutional Setting: Evaluating Surgical Complications and Implant Survival Outcomes

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Introduction

Dental implants are increasingly popular for treating partial edentulism. Traditionally, implants are placed after a few months of healing post-extraction. Immediate implant placement shortens treatment time, allows early return to function, and eliminates the need for a second surgery. Studies show similar survival rates for immediate and conventional implants. However, surgical complications may vary based on patient factors, operator experience, and use of surgical guide. This study aimed to assess implant survival and post-surgical complications in a dental institution and evaluated association with parameters like patient age, sex, tobacco use, medical history, implant site, provider experience, grafting, and surgical guide use.

Methods

A cross-sectional cohort study utilizing a retrospective chart review analyzed 305 patient records of recent immediate implant recipients at the OHSU School of Dentistry. Collected data included patient demographics (age, sex, tobacco use history, and medical history) and clinical factors (implant site, provider experience, grafting, and surgical guide use). Fisher's exact test assessed the statistical significance of individual variables, while univariate and multivariate logistic regression analyses explored associations between implant survival, surgical complications, and these factors.

Results

The overall survival rate for immediate implant placement was 95.4%. A statistically significant association was found between implant survival and the use of a surgical guide ($P = 0.0358$). Additionally, implant loss was significantly associated with patients presenting with asthma ($P = 0.0213$). Surgical complications occurred in 12.5% of cases, with a statistically significant association observed in patients with hypertension ($P = 0.0498$).

Conclusions

In this study, immediate implant placement demonstrated a high survival rate (95.4%), comparable to the reported survival rates of delayed implant placement in the literature (>95%). Implant survival was independent of patient age, gender, history of smoking, provider experience, and site of placement. The findings of this study support immediate implant placement as a reliable treatment option with predictable outcomes, particularly when a surgical guide is used.

Nocturnal Variation in Autonomic Nervous System Activity

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Introduction

Objective: To test inter-night reliability of home recordings of sympathetic and parasympathetic nervous system activity.

Methods

In accordance with institutional review board oversight and STROBE guidelines, adult subjects gave informed consent, and were trained in research protocols to record nocturnal heart rate. Subjects were asked to produce ≥ 2 night-time recordings of ≥ 6 hours in duration. Commercial software (MindWare Technologies LTD.) was used to quantify nocturnal ultradian cycling of parasympathetic and sympathetic activities. In 5 minute windows throughout each recording, the heart rate variability time domain measure of pNN50 (%) quantified parasympathetic activity. The frequency domain ratio of LF/HF characterized sympathetic activity relative to parasympathetic activity. Peaks and valleys of nocturnal ultradian cycling were identified by fitting a higher order polynomial to pNN50 and LF/HF data sets. For each recording, two peaks and two valleys were used for quantifying amplitude and duration of pNN50 and LF/HF activities and were used to quantify power densities (amplitude/time). Inter-night reliability of parasympathetic and ratio of sympathetic/parasympathetic power densities were determined based on ICC values. Interpretation of reliability were based on Cicchetti (1994): <0.4 = poor; $0.40-0.59$ = fair; $0.60-0.74$ = good; $0.75-1.00$ = excellent.

Results

Of 32 individuals screened and enrolled, 18 females and 11 males completed all study protocols. Subjects produced 87 night-time ECG recordings of average duration 7.7 ± 1.0 hours. Inter-night reliability of HRV measures was fair to good. Parasympathetic power densities, as measured by pNN50 had a mean ICC value of 0.71 (0.52 – 0.85). The ratio sympathetic/parasympathetic power densities had a mean ICC value of 0.66 (0.40 – 0.85).

Conclusions

Given that inter-night reliability of HRV data was only fair to good, it would be prudent for future investigations to collect multi-night data in order to have an ecological sample that reflects the intra-subject variability in nocturnal autonomic nervous system activity.

Selective glucosyltransferase inhibition: repurposing FDA-approved drugs to disrupt *S. mutans* biofilms

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Introduction

Streptococcus mutans (Sm) utilizes glucosyltransferases (Gtfs) to synthesize exopolysaccharides (EPS), enhancing its biofilm formation and cariogenic potential. Selectively inhibiting Gtfs without disrupting oral commensals presents a novel anticaries strategy. This study aimed to identify FDA-approved drugs that target Gtfs, validating their efficacy through microbiological and molecular assays.

Methods

Fifty-seven FDA-approved drugs were screened for Gtf inhibition via in silico docking and tested for their effects on Sm biofilms. Planktonic growth (OD600), biofilm biomass (crystal violet assay), and viability (luciferase assay) were assessed. EPS production and bacterial distribution were analyzed by confocal microscopy, with additional quantification of insoluble exopolysaccharides, biofilm strength, and colony-forming units. Three promising candidates that reduced Sm biomass without affecting viability were further tested in dual-species (Sm + *Streptococcus sanguinis* [Ss]) and triple-species (Sm + Ss + *Veillonella parvula* [Vp]) biofilms at 6.25–50 μ M. Biofilm biomass, planktonic growth, and viability were evaluated using a renilla reporter assay. Statistical analyses were performed using one-way ANOVA/Tukey tests ($\alpha = 0.05$).

Results

All tested drugs inhibited Sm biofilm formation in a dose-dependent manner ($p < 0.05$). Drug #3.8 reduced biofilm biomass without affecting Sm viability ($p = 0.507$) and was selected for further testing. In single-species biofilms, drugs #3.8 and #6.4 significantly disrupted Sm biofilms ($p < 0.001$), while drug #2 completely eradicated Sm biofilms. In dual-species biofilms, drug #2 selectively suppressed Sm without impacting Ss. However, in triple-species biofilms, Vp mitigated drug effects, reinforcing Sm biofilm resilience.

Conclusions

Repurposing FDA-approved drugs for selective Gtf inhibition offers a promising strategy for disrupting cariogenic Sm biofilms while preserving beneficial oral microbiota, advancing targeted caries prevention approaches.

Assessing Manual Dexterity in Novice Practitioners Using Typodonts and a Haptic Dental Simulator for Crown Preparation

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Introduction

The preclinical dental curriculum is designed to develop students' fine manual dexterity, essential for safe patient care, traditionally using typodonts in phantom heads. A new generation of simulation has emerged with the development of virtual reality (VR) and haptic dental trainers. The integration of both technologies allows users to see a 3D virtual environment while feeling the procedure through force feedback. The aims of this study were to evaluate whether additional training using a haptic dental simulator (SimtoCare Dente simulator- DS) would enhance crown preparation and self-evaluation performance. Additionally, it aimed to gather student feedback on their experience with the DS.

Methods

Forty-three second year dental students (DS2) participated in this study and were randomly assigned to either a test group (n=23) or a control group (n=20). Prior to the study, the students learned the fundamentals of crown preparation but had no previous experience with posterior all-ceramic crown preparations. All students began the experiment with a didactic lecture on how to prepare a posterior tooth for an all-ceramic crown, followed by a pre-test in which they performed an all-ceramic crown preparation on tooth #30 in a typodont in a phantom head. They then, self-evaluated their preparations. The test group practiced the same crown preparation three separate times using the DS while the control group had no additional practice. Afterward, all students completed a post-test and self-evaluated their final preparations. Two calibrated faculty members graded the preparations for both groups using a standardized rubric. Students in the test group also completed a Likert scale survey to share their perceptions on the DS. Statistical analysis included a paired t-test to compare faculty-assessed performance between pre-test and post-test scores within each group. A two-way ANOVA was used to evaluate student self-assessment accuracy compared to faculty evaluations across both groups.

Results

There was no statistically significant difference in student scores before and after practice on the DS. Likewise, there was no statistically significant difference in scores between the pretest and the post-test for the control group. Self-assessment analysis showed that both students and faculty exhibited similar scoring trends in the pre-test and post-test for both groups. Despite the lack of significant score improvement, 75% of students in the test group agreed or strongly agreed that practicing with the DS enhanced their hand-skills for the procedure. Additionally, 70% of the students in the test group stated that the practice sessions helped them with visualization, and 80% reported that practice sessions helped them increase their speed.

Conclusions

The result of this study shows that practicing crown preparations on a DS did not statistically improve students' post-test performance or self-assessment accuracy. However, the majority of the students (70% and above) who practiced on the DS felt that the experience was valuable, reporting that it enhanced their hand skills, visualization, and speed in performing the procedure.

Enhancing Intraoral Scanning Accuracy for Mandibular Full-Arch Implant-Supported Prostheses Using Resin Markers: an in vitro study

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Introduction

Digital workflows have advanced prosthodontics, but intraoral scanning (IOS) for complete-arch implant prostheses remains challenging. The lack of anatomical landmarks in edentulous arches causes registration errors, worsened in the mandibular arch. Strategies like modified scan bodies and photogrammetry aim to improve accuracy but add complexity. This study evaluates intraoral markers made from flowable composite resin to enhance IOS accuracy in edentulous mandibular scanning. The null hypothesis states that these markers will not affect scan precision or trueness.

Methods

A mandibular study model with four implants and simulated unattached mucosa was fabricated. A rubber dam was adapted around the study model to mimic movable mucosa during intraoral scanning. The study model was scanned with a laboratory scanner to create a reference scan (REF). Two intraoral scanners (Trios4, Primescan) were used for 15 consecutive scans each, following a standardized scanning protocol. Additional scans were taken after placing flowable composite resin markers on the ridge crests to evaluate their effect on scan accuracy, creating 4 different groups (Trios4-TR, Primescan-PS, Trios4 with resin markers-TRmark, Primescan with resin markers-PSmark). Indirect digitization (IND) involved making a conventional PVS impression with splinted impression copings, fabricating stone models, and scanning them with a laboratory scanner. All scans were analyzed using image analysis software, with soft tissue areas removed to assess only implant positions. Accuracy was evaluated through trueness (comparison with REF) and precision (repeatability of scans within each group). Root-mean-square (RMS) values were used for comparisons. Statistical analysis was performed using the Kruskal-Wallis test with pairwise comparisons ($\alpha=.05$).

Results

The Kruskal-Wallis test indicated that the scanning method significantly affected trueness ($P<.001$). Pairwise comparisons showed that the IND group had the highest trueness, followed by TR, PS, and PSmark groups, with no significant differences among TR, PS, and PSmark. The TR group had the lowest trueness. For precision, statistical analysis also showed a significant effect ($P<.001$). Pairwise comparisons revealed that PSmark, TRmark, and IND had the highest precision, with no significant differences among them. The PS group had lower precision than these three but higher than the TR group, which had the lowest precision.

Conclusions

Based on the findings of this study, indirect digitization remains the gold standard for full-arch implant scanning. Primescan showed higher accuracy than Trios4 when scanned without resin markers, but scanner type did not have a significant influence on accuracy when resin markers were used. Resin markers significantly improved both the trueness and precision of Trios4. In contrast, Primescan's trueness remained unchanged with markers, though its precision improved. These findings emphasize the importance of scanner selection and potential enhancements in accuracy through simple modifications like resin markers.

Machine Learning Modeling of Variables Affecting Longitudinal TMJ Structure Change

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Introduction

Objective: To test if machine learning modeling can identify anatomical, biomechanical, and psychosocial domain variables associated with longitudinal changes in temporomandibular joint (TMJ) osseous structures.

Methods

Ninety-seven subjects (82 females, average age 41.7 ± 10.3 years; 15 males, average age 37.6 ± 12.6 years) consented to participate according to institutional review board oversight. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) Axis I and II protocols were followed. Subjects' TMJs were imaged using cone-beam computed tomography (CBCT) at time of enrollment (T1) and on average >7 years later (T2). T1 to T2 changes were quantified by two calibrated radiologists, where right and left TMJs of each subject were categorized using DC/TMD Axis I criteria as "same-better" or "worse". Five T1 anatomical and 3 biomechanical measures that were derived from T1 CBCT images, and two T1 DC/TMD psychosocial measures were used in machine learning models (Support Vector Machine (SVM), Gradient Boosting Machine (GBM); Classification Tree (CT)) to rank order variables that segregated TMJs based on diagnostic category ("same-better" or "worse"). Machine learning model results were adjudicated based on accuracy, sensitivity, and specificity.

Results

A total of 192 TMJs were analyzed for changes in osseous structures, where 132 TMJs were classified as "same-better", and 50 TMJs were "worse". The GBM model had training classification accuracy of 1.00 (0.97-1.00). However, test classification accuracy was only 0.70 (0.63-0.76), with sensitivity and specificity of 0.76 and 0.54, respectively. The GBM model's top ranked variables were in the domains of i) condyle geometry (loading area, major axis length, aspect ratio, major axis to midsagittal plane angle); ii) TMJ biomechanics (anteroposterior position of the dentition, occlusal plane angle, mandibular ramus height), and iii) behavior (7-Question Behavior Checklist).

Conclusions

Machine learning modeling can be used as a tool to identify domain variables that are associated with TMJ ontology.

Exploring Novel Protein-Protein Interactions of Diadenylate Cyclase (DAC) in *Streptococcus mutans*

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Introduction

Diadenylate cyclase (DAC) is a critical enzyme that catalyzes the synthesis of the secondary messenger c-di-AMP from ATP or ADP molecules. This signaling molecule, found in Gram-positive and Gram-negative bacteria as well as certain Archaea, regulates essential cellular processes. While interactions between DAC and known regulators like GlmM and CdaR have been established, our study aims to identify novel protein-protein interactions involving DAC to further elucidate its regulatory mechanisms and diverse cellular roles.

Methods

Using *Streptococcus mutans* as a model, we employed co-immunoprecipitation coupled with mass spectrometry to identify potential DAC-interacting proteins. A FLAG-tagged DAC was purified from crosslinked and non-crosslinked cell lysates, followed by mass spectrometry analysis. To validate interactions in vivo, we utilized an intermolecular split luciferase assay, where luciferase fragments attached to interacting proteins produced luminescence upon interaction. Additionally, we assessed phenotypic changes in DAC-deficient and SMU_723-deficient strains, including cell growth, biofilm formation, acid production, acid tolerance, oxidative stress response, sugar metabolism, and in vivo colonization.

Results

Our proteomic screen expanded the list of potential DAC-interacting proteins beyond GlmM and CdaR. Selected interactions were confirmed in vivo using the split luciferase assay. Notably, SMU_723 emerged as a key interactor, with its deficiency mirroring phenotypic changes observed in the DAC-deficient strain, such as prolonged lag phase, irregular cell morphology, reduced acid production and tolerance, impaired sorbitol metabolism, and decreased colonization in a fly model. These shared phenotypes suggest a functional link between DAC and SMU_723.

Conclusions

This study confirms known interactions between DAC and GlmM/CdaR while identifying novel protein partners. The split luciferase assay validated several interactions in a cellular context. Furthermore, phenotypic analyses of DAC and SMU_723-deficient strains indicate that their interaction may play a significant role in shared pathways, including cell growth, stress responses, and colonization. These findings provide new insights into the regulatory network of DAC and its broader cellular functions.

A Pilot Study of Temporomandibular Joint Alloplastic Implants Contact Mechanics

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Introduction

Modeling of TMJ alloplastic mechanics requires ecological valid loading conditions. This pilot study used validated numerical modelling and ecological bite forces to estimate contact mechanics of temporomandibular joint (TMJ) alloplastic implants.

Methods

In accordance with university IRB oversight, deidentified CBCT of individuals who had alloplastic implants of the left, right, or both TMJ were used to construct three-dimensional anatomical geometry files of the positions of the mandibular condyles, teeth, and positions and orientations of the medial and lateral pterygoid, masseter, temporalis, and anterior digastric muscles. A validated computer-assisted numerical model with the objectives of minimization of muscle effort (MME) was used with subject-specific geometry files to predict TMJ loads for ecologically valid static bite-force of 10 Newtons (N) on central incisors, right canine, and right first molar. Average and peak normal stresses were calculated. ANOVA was used to test for significant differences in normal stress due to i) type of implant (unilateral, bilateral), ii) biting position, and iii) side (ipsilateral, bilateral).

Results

Seven unilateral and eight bilateral TMJ implant replacement subjects were analyzed. Both implant conditions produced higher mean normal stress (MPa) during incisor biting (2.49 ± 1.11) that was significantly higher (all $p < 0.001$) than for conditions of bite forces on canine and first molar teeth. Unilateral implants had overall mean normal stress of $2.1 (\pm 1.2)$ MPa which was significantly higher ($p = 0.038$) than bilateral implants (1.6 ± 0.62 MPa). Maximum peak normal stresses of >8.0 MPa occurred in subjects with unilateral TMJ implants.

Conclusions

Normal stresses were higher in subjects with unilateral TMJ implants and were of similar magnitude to normal stresses in prosthetic knee implants. Higher stresses may promote osteoarthritis in the normal TMJ of individuals with unilateral TMJ implants.

Parvimonas micra: Identification and characterization of pathogenic P. micra from clinical dental abscesses

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Introduction

P. micra is an obligate anaerobe and an inflammophilic bacterium that has been associated with periodontal disease, dental abscesses and colorectal cancers. Its persistence to survive and thrive in dental abscesses, where there is a strong host immune response demonstrates a unique interaction between the pathogen and host. The aim of this study was to isolate the bacterium and determine characteristics that could explain the ability of *P. micra* to manipulate the immune system.

Methods

P. micra was isolated from clinical dental abscess samples using PMM, a selective and differential media. The 16s rRNA genes from the isolates were amplified via PCR and sequenced to confirm their identity against HOMO BLAST and NCBI databases. A transformation assay using an ERM resistance cassette was ran for the most active isolate (A28) and transformation rate was calculated. A collagen cleavage assay using a serum derived from *P. micra* infected human neutrophils, was ran using SDS PAGE and silver stain to determine the bacterial response to inflammatory granules. It was also compared to serum derived from MRSA infected neutrophils and ionomycin treated neutrophils. Results of the collagen cleavage assay were analyzed using densitometry.

Results

Three isolates of *P. micra* were identified after 16s rRNA sequencing (A80.1, A82.1, A82.2) with 100% identity. Other potential pathogenic bacterial species were identified from the dental abscesses: *Cryptobacterium curtum* and *Capnocytophaga gingivalis* with 99.9-100% identity. The transformation rate of A28 isolate of *P. micra* was 3×10^{-4} (ERM/Total CFUs). For the collagen cleavage assay, there was a 64% decrease in signal with *P. micra* infected neutrophils compared to a 27% decrease in signal when infected with MRSA and an 82% decrease with ionomycin treatment.

Conclusions

Multiple pathogenic bacterial species were isolated from the clinical dental abscesses (e.g. *P. micra*) including some that have yet to be thoroughly studied, thus their characteristics are relatively unknown (e.g. *C. curtum*). This study highlights a potential mechanism for how *P. micra* manipulates the inflammatory immune response to provide a potential food source (cleaved collagen protein) and also outcompete other species by utilizing the inflammatory environment. Future paths of study could investigate additional isolates of *P. micra* to confirm the mechanism of manipulating the immune system and also investigation of other lesser-studied bacterial species for their roles in dental abscesses.

Exploring in vitro Biofilm Formation and Interactions Among *F. nucleatum*, *F. periodonticum*, and *P. micra*

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Co-Authors: Zhengzhong Zou Ph.D., Hui Wu Ph.D.

Introduction

Periodontitis is a multifactorial disease that stems from diet, genetics, hygiene, and the subgingival microbiome. In a healthy person, there are beneficial bacteria in the gingival sulcus. However, over enrichment of certain bacteria and subgingival biofilm formation has been correlated with periodontitis. *Parvimonas micra* (Pm), *Fusobacterium nucleatum* (Fn), and *Fusobacterium periodonticum* (Fp) are three species with levels found to be increased in patients with periodontitis. To broaden our understanding of symbiotic relationships in subgingival biofilm formation, we co-cultured strains of Pm, Fn, and Fp and assessed their biofilms and aggregating patterns.

Methods

Seven Fn, three Pm, and one Fp strains were grown overnight in sBHI broth inside an anaerobic incubator at 37 °C. The OD 600 was read and the growths were diluted to an OD 600 of 0.7. The standardized solutions were then diluted by a factor of one-thousand and co-cultured with a different species in a 96-well plate. This was put back into the anaerobic incubator to grow for seventy-two hours at 37 °C. The OD 600 was read to measure growth. The remaining media was poured out and the wells were washed three times with water. 30% acetic acid was added and shaken for ten minutes at 500 rpm. Crystal violet was added and shaken for thirty seconds at 1000 rpm. The OD 562 was read.

Select co-cultures that yielded increased biofilm were grown overnight in sBHI broth inside an anaerobic incubator at 37 °C in an 18 plate well. Phase contrast micrographs were taken at 100X.

Co-cultures were grown for seventy-two hours in the same conditions listed above. Proteins were pelleted, purified, and diluted to the same concentration using a coomassie protein assay. The standardized protein samples were then loaded on a SDS-page gel. Electrophoresis was performed and a photo was taken of the gel.

Results

Wide variability of biofilm formation was observed at the strain level with OD 562 levels ranging from 0.2 to 9.2. Pm str. A_28 consistently produced more biofilm than the other Pm strains. The addition of Pm regardless of the Fn strain always increased the amount of biofilm compared to the Fn monoculture.

The micrographs showed co-aggregation where the ratio was 50:50. In other co-culture micrographs, only one of the two strains were visible.

The SDS-page gel had six bands that were present in the various monocultures but not the co-cultures.

Conclusions

Our results showed that Fn, regardless of the strain, always produces more biofilm when cultured with Pm, suggesting a strong dependency of Fn for Pm in vitro.

Peck (cont'd)

The co-aggregation or domination displayed in the micrographs suggest that there might have been contamination or one species did not grow. We suggest that future studies include qPCR to quantify the bacteria present.

The protein bands present on the monocultures but not the corresponding co-cultures suggest that there may be certain protein expression pathways inhibited from quorum sensing. Mass spectrometry is recommended to identify the proteins and the mechanisms responsible for the symbiosis.

Development of a versatile toolbox for genetic manipulation of *Prevotella melaninogenica*

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Introduction

The *Prevotella* genus is comprised of Gram-negative obligate anaerobes and is second-most abundant genus in the human oral microbiome. Although numerous clinical studies have linked various *Prevotella* species to both oral and systemic diseases as well as multiple cancers, our mechanistic understanding of *Prevotella* pathobiology is rudimentary, largely due to potent genetic intractability throughout the entirety of the *Prevotella* genus. To address this issue, we established an efficient genetic toolbox to manipulate clinical isolates of *Prevotella melaninogenica* that will support future genetic studies of its host-pathogen interactions.

Methods

Prevotella melaninogenica strains were isolated from clinical odontogenic abscess specimens on MCDC blood agar plates containing kanamycin and vancomycin. 16S rRNA genes sequencing was performed to verify the strains. PCR-assembled constructs were transformed into *P. melaninogenica* using natural competence.

Results

In this study, we isolated *Prevotella melaninogenica* strains directly from odontogenic abscess clinical specimens and identified multiple strains exhibiting natural competence (exogenous DNA uptake). By exploiting this ability, we were able to obtain transformation efficiencies up to 2.65×10^{-6} using mutagenesis constructs assembled via cloning-independent methodologies. Two negative selection systems functioning in *P. melaninogenica* were also established based upon induced sensitivity to the 2-deoxy-galactose (2-DG) or sucrose. In addition, we successfully employed a codon-optimized version of the Green Renilla luciferase-encoding gene *renG* as a highly sensitive reporter gene in *P. melaninogenica*.

Conclusions

This study yields the first tractable genetic system in the *Prevotella* genus, which will provide new opportunities to systematically investigate *Prevotella* genetics, addressing a significant fundamental knowledge gap in the field.

Enhancing biocompatibility with Functionalized Melamine-Reinforced Self-Healing Microcapsules for Dental Restorative Biomaterials

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Introduction

Fifty percent of resin-based dental restorations fail within ten years, primarily due to recontamination of dental tissues and fractures in the restorative materials. Typically, restorations that fracture catastrophically initially develop microcracks caused by masticatory forces and thermal variations, which then propagate and merge. A proposed strategy to address this challenge involves equipping restorative materials with the ability to autonomously repair microcracks as they form. This is achieved by incorporating microcapsules containing a healing agent into the polymeric network. When microcracks form, the microcapsules rupture, releasing the healing agent, which polymerizes in situ, thereby repairing the damaged area and preventing further microcrack propagation. The most used systems are based on poly(urea-formaldehyde) (PUF) microcapsules, and although the results are promising, the potential release of formaldehyde from these systems is a significant concern due to its cytotoxicity and potential adverse effects on oral tissues. In other fields, modification of PUF networks by melamine incorporation and chemical functionalization have emerged as promising strategy to chemically bind and potentially reduce residual formaldehyde emission. Therefore, this study aims to design, synthesize, and characterize a toolkit of alternative microcapsules by integrating melamine and functionalizing agents based on methacrylate and thiol into the outer shell polymeric network, while also evaluating their potential to minimize formaldehyde release and enhance biocompatibility.

Methods

PUF and PUMF (poly(urea-melamine-formaldehyde)) microcapsules were prepared using a double-emulsion method with mechanical stirring at 400 rpm, following procedures described in the literature. Melamine was incorporated at a concentration of 0.349 M (0.22 g) and stirred with all the shell precursors. The healing agent consisted of triethylene glycol dimethacrylate (TEGDMA) tagged with 2-methyl-6-(4-dimethylaminostyryl)-4H-pyran dye (DCM) to facilitate microcapsule characterization. For the microcapsules functionalization, the methacrylate silane 3-(trimethoxysilyl) propyl methacrylate (TMSPM) and the thiol-based 3-mercaptopropyl trimethoxysilane (MPTMS), either individually or in combination with a tetraethyl orthosilicate (TEOS) pre-coating, were tested. In total, eight experimental groups were tested (PUF and PUMF modified with TMSPM, MPTMS, TEOS + TMSPM, or TEOS + MPTMS) and two control groups: pristine PUF and PUMF. After filtration and drying, the microcapsules were characterized morphologically using optical microscopy and scanning electron microscopy (SEM) (n=3). Size measurements (μm) and distribution (histogram) were assessed by measuring the microcapsules' diameter using ImageJ (n=50), and encapsulation efficiency (%) was analyzed using the acetone extraction method (n=3). Chemical surface modification was analyzed by energy-dispersive X-ray spectroscopy (EDS) and thermogravimetric analysis (TGA) (n=3). Formaldehyde release was assessed by the Purpald colorimetric assay at time points of 0h, 24h, 72h, 1 week, and 2 weeks (n=3). The cell viability of dental pulp stem cells (DPSC) and

human dermal fibroblasts (HDF) incubated with the microcapsules was evaluated using the Alamar Blue assay at 1, 3, 5, and 7 days. The collected data was statistically analyzed using a two-way ANOVA, followed by Tukey's post-hoc test ($p < 0.05$).

Results

In general, PUMF microcapsules presented greater healing agent (core) retention than PUF when evaluated under optical microscopy. These findings are associated with the enhanced mechanical and thermal stability of the microcapsule shells, attributed to the incorporation of melamine into the poly(urea-formaldehyde) polymeric network. The incorporation of melamine also resulted in microcapsule shells with increased surface roughness, which might be beneficial to enhance the mechanical interlocking of the microcapsules within the organic matrix of dental composites. Regarding size distribution, all tested groups exhibited high polydispersity where PUMF microcapsules were significantly smaller than the PUF microcapsules (110 μm and 185 μm , respectively). Regarding the healing agent encapsulation efficiency, it ranged from 92 to 98%. The success of the functionalization procedures was confirmed by the EDS and TGA analyses. For the EDS analysis, all functionalized groups exhibited silicon percentages ranging from 1.99% to 14.83%, in contrast to the 0% observed in the pristine PUF and PUMF controls. The different surface treatments were also reflected in the TGA results, which showed a curve shift to lower temperatures for the TMSPM-functionalized PUF and PUMF microcapsules, as well as higher T30 values for the MPTMS-containing groups (440.2°C and 336.3°C for the PUF and PUMF groups, respectively). These changes in the thermograms may be attributed to the lower thermal stability of TMSPM compared to MPTMS, due to the faster degradation of the methacrylate group compared to the thiol group. In terms of formaldehyde emission, TMSPM- and MPTMS-treated PUF microcapsules showed significantly lower values (5.0 and 6.5 μM , respectively) compared to pristine PUF microcapsules (9.0 μM). For PUMF microcapsules, surface functionalization also prevented peaks in formaldehyde emission as shown at 24h and 72h timepoints. This effect may be attributed to the ability of thiol and methacrylate silanes to establish stable chemical bonds with formaldehyde, thereby reducing its residual release. Interestingly, the PUMF microcapsules presented overall increased formaldehyde emission in comparison to the PUF, which may be related to mechanical retention of residual formaldehyde on an increased rough surface of melamine-modified shells. However, it did not lead to differences in cell metabolism observed in DPSC or fibroblast cells. As anticipated, DPSC cells were more sensitive to incubation with the microcapsules than fibroblasts, as evidenced by their overall lower cell metabolism. This increased sensitivity may be related to the higher metabolic activity and differentiation potential of these cells, making them more responsive to environmental changes. The groups with the highest DPSC metabolism percentages were PUF-MPTMS, pristine PUMF, and PUMF-TEOS+MPTMS (85.15 \pm 7.59%, 84.64 \pm 7.00%, and 93.99 \pm 6.42%, respectively). This outcome was expected, as thiol-functionalized surfaces are more conducive to cell attachment and growth compared to surfaces treated with methacrylate silane agents. It is important to emphasize that for the biocompatibility assessment, the microcapsules were dispersed directly into the cell growth media, a condition significantly harsher than clinical scenarios. In clinical applications, the microcapsules are embedded within dental resin matrices, which enhance their physical stability and reduce the likelihood of leaching of the healing agent or formaldehyde.

Conclusions

In summary, functionalization of self-healing microcapsules produces greater biocompatibility and melamine reinforcement enhances mechanical and thermal stability. These combined outcomes may culminate in the development of a self-healing resin composite which offers significant biosafety and potential extension of the clinical lifespan of dental restorations, thereby enhancing overall oral health care.

Dysbiotic oral microbial community selected by mouse immune system

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Introduction

The oral microbiome refers to the microorganisms in the human oral cavity. The oral cavity has over 700 species of bacteria. The bacteria in the mouth are responsible for the two most common bacterial diseases: dental caries and periodontal disease. *Streptococcus mutans* cause dental caries and can compromise pulpal tissue in an acute and chronic inflammatory process. *Porphyromonas gingivalis* (*P. gingivalis*) is one of the main pathogens in periodontal disease. The inflammation caused by *P. gingivalis* in the oral cavity can lead to several things such as disorder of the intestinal microbial community structure, destruction of the intestinal barrier, induction of endotoxemia, and systemic inflammatory response. Oral dysbiosis such as in the case of periodontal disease can be associated with a few systemic diseases such as chronic inflammatory diseases or degenerative diseases (Ex. Cancer, obesity, diabetes, atherosclerosis, and Alzheimer's disease). Certain oral bacteria have even been found in the tumor microenvironment, but it is not yet known if they promote or are attracted to these sites of inflammation.

Methods

How these bacteria can escape the oral cavity and travel to distant sites while simultaneously escaping the immune system is not clear. Daily activities such as eating or brushing our teeth release bacteria into the bloodstream. In healthy individuals, these bacteria are quickly dispatched by the innate immune system, despite being allowed to colonize the oral cavity. In contrast, an oral abscess may form when oral bacteria invade the pulp of a tooth secondary to caries, and the immune system cannot immediately dispatch all of the bacteria. The subset of the total oral community that survives in the abscess includes those which are associated with inflammatory diseases.

Results

Our goal is to identify a defined dysbiotic community that is sufficient to form persistent abscesses. Several of the bacterial species that are known to be associated with systemic disease can form transient abscesses but cannot effectively evade the immune system when isolated from the broader community. A defined dysbiotic community is a tool that can be used to better understand the interactions between the microbiota and the immune system.

Conclusions

1. Abscess model in the mouse has a similar selection criteria to human abscesses. They select for oral microbes that may be associated with chronic disease.
2. We can identify a set of these microbes either by differential plating or qPCR.
3. These microbes can evade the normal immune response and affect the immune response.

The Effect of Grain Particle Size on Surface, Mechanical, and Optical Properties of Recycled Zirconia

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Introduction

This study aimed to investigate how the grain particle size of recycled 3Y-TZP powder influences its surface, mechanical, and optical characteristics in comparison to commercial 3Y-TZP.

Methods

Zirconia powder was obtained from the milling waste of 3Y-TZP pucks (Ceramill Zi, Amann Girrbach). The recycled powder was sieved to achieve two granulometric ranges: 50 μm and 1 μm . A total of 129 discs (12 mm in diameter and 1 mm thick) were produced and categorized into three groups: a control group (commercial 3Y-TZP), recycled 3Y-TZP with 50 μm particles, and recycled 3Y-TZP with 1 μm particles. The discs were manufactured using uniaxial pressing and subsequently sintered at 1550 °C for 2 hours ($n=43/\text{group}$). Theoretical density ($n=10$) and scanning electron microscopy (SEM) ($n=2$) were employed for surface analysis. Optical properties, including translucency parameter and contrast ratio ($n=10$), were assessed. Mechanical characterization was performed using Vickers hardness and fracture toughness tests ($n=5$), while biaxial flexural strength was evaluated to determine characteristic strength and Weibull modulus ($n=16$), following ISO 6872:2016 standards. Statistical analysis included one-way ANOVA and Tukey's test ($P<0.05$) for density, optical, hardness, and fracture toughness data. Weibull statistics were used to assess characteristic strength and Weibull modulus, and SEM micrographs provided fractographic insights.

Results

The 1 μm particle group exhibited the highest density ($99.5 \pm 0.21 \%$), surpassing both the control ($98.70 \pm 0.49 \%$) and the 50 μm group ($99.10 \pm 0.09 \%$). No significant differences were found among groups regarding translucency ($P>0.05$). However, contrast ratios were higher for the 1 μm (0.86 ± 0.02) and 50 μm (0.84 ± 0.02) groups compared to the control (0.75 ± 0.02). Hardness values were greater in the control ($12.6 \pm 0.19 \text{ MPa}$) and 1 μm ($12.6 \pm 0.06 \text{ MPa}$) groups than in the 50 μm group ($12.4 \pm 0.04 \text{ MPa}$). Fracture toughness was highest in the 1 μm group ($4.31 \pm 0.02 \text{ MPa}\cdot\text{m}^{1/2}$), followed by the 50 μm group ($3.95 \pm 0.09 \text{ MPa}\cdot\text{m}^{1/2}$) and the control ($3.75 \pm 0.17 \text{ MPa}\cdot\text{m}^{1/2}$). The control ($1099.69 [1041.88 - 1160.71]$) and 1 μm ($1069.38 [1027.68 - 1112.77]$) groups had higher characteristic strength than the 50 μm group ($835.97 [787.23 - 887.72]$). However, Weibull modulus values did not significantly differ between groups ($P>0.05$). SEM imaging revealed enhanced particle compaction, and fractographic analysis indicated that fractures originated from defects on the tensile side and propagated toward the compression side of the discs.

Conclusions

The recycled 3Y-TZP powder with 1 μm particles demonstrated superior microstructure, mechanical strength, and optical properties compared to the 50 μm group. Additionally, its performance was comparable to that of commercial zirconia, suggesting that 1 μm recycled 3Y-TZP is a viable material for sustainable applications in restorative dentistry.

Masticatory Muscle Activity and Mandibular Ramus Length

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Introduction

The objective was to test for a relationship between the variables of diurnal and nocturnal masticatory muscle activities (duty factors, DF, %) and age (yrs.), and mandibular ramus length.

Methods

According to OHSU IRB oversight, skeletal Class II children, between the ages of 10-14-year-old, assented to participate. Each subject had a cone beam computed tomographic (CBCT) image which was used to measure mandibular ramus length (Condylion-Gonion). Subjects participated in laboratory protocols to quantify masticatory muscle activity per N of biting force. Subjects were trained how to use a portable EMG recorder. Subjects were asked to produce 3 awake and 3 night-time recordings of at least 6 hours in length. The recorder collected data of masticatory muscle activity (Duty Factor, % of time the muscle was active per recording period). Muscle duty factors were calculated for loading between 1 and 5 N, and for activations of ≤ 5 s. ANOVA was used to test for variable effects on mandibular ramus length. Ramus length data was normalized within females and males, and regression analysis was used to test for correlation between normalized ramus height (Co-Go), muscle duty factors (%), and age (years).

Results

Thirteen females (12.1 ± 1.1 yrs.) and twelve males (13.5 ± 1.3 yrs.) completed project protocols. Mandibular ramus lengths ranged from 41.5 to 62.4 mm, with significantly different ($p < 0.001$) average lengths in females and males of $48.6 (\pm 3.0)$ and $53.7 (\pm 4.7)$ mm, respectively. Subjects produced 72 awake and 69 night recordings of average lengths of 6.4 hrs. and 8.6 hrs, respectively. There was significantly more temporalis muscle activity during awake period recordings ($p < 0.01$). Regression analysis showed a correlation between awake muscle duty factor, age, and normalized ramus length ($R^2 = 0.41$).

Conclusions

There is a relationship between masticatory muscle activity, age, and growth of the mandibular ramus.

Effects of Sugar Substitutes on Streptococcus mutans Biomass Formation and Growth Dynamics

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Acknowledgement: Dr. Elizabeth Palmer

Introduction

Dental caries, a prevalent global health issue, are promoted by Streptococcus mutans & fermentable carbohydrates. While some sugar substitutes have been evaluated there is limited data on allulose and erythritol. In this study, the impact of allulose and erythritol on caries virulence traits were determined.

Methods

Fresh Streptococcus mutans UA159 subcultures prepared in both Todd Hewitt Broth (THB) & brain heart infusion (BHI) were grown to mid-log phase ($OD_{600} \approx 0.5$). For biomass, 1:1,000 subcultures were added to media supplemented with 1% sugar (Sucrose, fructose) or sugar substitute (Erythritol, xylitol, allulose) and grown for 16 hours in 5% CO₂. Biomass was assessed by crystal violet assay OD_{562} using a microplate reader. Growth curves were grown in 5% CO₂ with OD_{470} measured every 30 minutes for 16 hours Students T-test was used with significance set at $p < 0.05$

Results

Biomass Formation:

Compared to sucrose, all tested sugar alcohols significantly reduced biofilm formation. Allulose ($p = 9.20 \times 10^{-31}$), erythritol ($p = 8.83 \times 10^{-31}$), and xylitol ($p = 1.06 \times 10^{-30}$) all exhibited highly significant reductions ($p < 0.05$), confirming their inhibitory effects.

Bacterial Growth Curve:

For bacterial growth, polyols generally supported less proliferation than sucrose and fructose. Allulose ($p = 0.221$) and erythritol ($p = 0.570$) showed no significant difference from sucrose, while xylitol ($p = 0.055$) trended toward reduced growth, though marginally significant. Fructose ($p = 0.333$) exhibited similar growth to sucrose, reinforcing its role in promoting bacterial proliferation.

Conclusions

Sugar alcohols significantly reduced biomass formation versus sucrose ($p < 10^{-30}$), with allulose being most effective. Xylitol exhibited the strongest inhibitory effect on bacterial growth ($p = 0.055$), while fructose mirrored sucrose ($p = 0.333$). Although OD_{470} readings indicated comparable planktonic growth, subsequent growth curve analysis revealed a potential growth defect, suggesting the need for further investigation.

Use of Dental Adhesives to Improve Bond Strength of Titanium Implants to Bone

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Introduction

Common complications in biomedical device implantation are recurrent infection and loss of retention at the body-device interface. This study focused on improving the retention between bone and titanium implants by applying commercial dental adhesives to the bone prior to cementation with PMMA based bone cements.

Methods

Commercial dental adhesives Scotchbond Multi-Purpose – SBMP, Adper Single Bond Plus - ASB, Scotchbond Universal Plus – SBU (all from 3M) and Clearfil SE Bond – CFSE (Kuraray) were applied to cortical bone sourced from porcine jaws. Commercial PMMA-based bone cements (Jet Acrylic, Lang Dental or Arthroplasty Bone Cement R, BC, Biomet) were used to cement a 6 mm diameter Ti implant. Groups without prior adhesive application were used as controls. Shear bond strength was assessed after storage in MilliQ water for 24 h and post 1-week mechanical cycling. The bonded interface was imaged with confocal and scanning electron microscopy and the chemical composition was mapped using infra-red spectroscopy (ATR). Cytotoxicity against osteoblasts and fibroblasts were assessed with MTT assay. Quantitative data was analyzed with two-way ANOVA/Tukey's test ($\alpha=5\%$).

Results

The use of dental adhesives led to 10 to 20-fold increase in bond strength, with the self-etch system (CFSE) showing the greatest improvement after storage in water or after cycling in the bioreactor. Though not statistically different, ASB had the lower values. Mechanical cycling led to a slight decrease in bond strength for all materials but remained 10 to 15-fold greater than the control. Adhesive layer thickness was greater for SBMP and CFSE. ATR mapping suggested stronger interactions with the mineral content for CFSE, as expected. None of the adhesives showed cytotoxicity up to 0.6 $\mu\text{g/mL}$, much higher than the expected leachate concentration for these materials. In general, the FDA-approved adhesives utilized here were able to reinforce the adhesive interface between bone and the titanium implant, similar to what is observed in dentin-bonded restorations in the oral cavity.

Conclusions

This study demonstrates that applying dental adhesives prior to the PMMA bone cement materials can help increase retention at the body-device interface

Carcinogenic Potential of Oral Bacteria *P. gingivalis*, *V. parvula*, and *P. melaninogenica* in Oral Cancer

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Introduction

Oral cancer is the sixth most common cancer worldwide, affecting an estimated 58,000 individuals in the United States. Among oral cancers, oral squamous cell carcinoma (OSCC) is the most prevalent and aggressive form. Emerging evidence suggests that the oral microbiome, including specific bacterial species, plays a role in OSCC occurrence and progression. Pathogens such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis* have been shown to promote tumor progression in mice. However, given that the oral cavity harbors over 700 bacterial species, the precise relationship between specific microorganisms and OSCC remains unclear. This study investigates the potential cancer-promoting roles of select oral bacterial species, individually and in combination, using OSCC cell lines and a syngeneic mouse model.

Methods

For in vitro experiments, *P. gingivalis*, *Veillonella parvula*, and *Prevotella melaninogenica* were cultured separately and added at a multiplicity of infection (MOI) of 1 or 10 to human OSCC SCC-1 and murine OSCC MOC1 cell lines. Cell proliferation was assessed using the MTT assay, and the expression of STAT3, AKT, and apoptosis-related proteins was analyzed via western blotting. In the syngeneic mouse model, MOC1 cells were pre-incubated with individual bacterial strains or combinations before injection into the mouse tongue. After four weeks, tumor tissues were harvested for immunohistochemical (IHC) analysis of macrophage (F4/80) and T-cell (CD8a, CD38) markers, as well as the expression of p53 and Ki-67.

Results

Infection with *P. gingivalis*, *V. parvula*, and *P. melaninogenica*, either individually or in combination, promoted SCC-1 and MOC1 cell proliferation. The expression of STAT3, AKT, and Yap/Taz increased in cells co-incubated with *P. gingivalis* and *V. parvula*, as well as with the combination of all three bacterial strains. In vivo, tumors were significantly larger in mice injected with MOC1 cells co-incubated with *P. gingivalis* and *V. parvula* (P.g + V.p group) compared to single-strain and control groups. These tumors exhibited increased infiltration of F4/80+ macrophages and CD8a+ T cells. Although p53 and Ki-67 expression slightly increased, the differences were not statistically significant. Interestingly, mice in the P.g + V.p + P.m group exhibited reduced tumor size, accompanied by decreased F4/80+ and CD8a+ cell presence.

Conclusions

The combination of *P. gingivalis* and *V. parvula* enhances OSCC cell proliferation, activates the PI3K/AKT pathway, and upregulates oncogenic STAT3 expression. In vivo, this bacterial combination promotes OSCC tumor growth and increases immune cell infiltration into the tumor microenvironment. The reduced tumor size and immune cell presence in the P.g + V.p + P.m group suggest complex bacterial interactions in OSCC progression. Further analysis of macrophage polarization (M1 vs. M2) will provide deeper insights into the role of bacterial species in OSCC development.

Exploring LCP Protein Functions in *Streptococcus mutans* and Discovering Small Molecule Inhibitors

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Introduction

Proteins of the LytR-CpsA-Psr (LCP) family are widely distributed in Gram-positive bacteria, where they participate in cell envelope biogenesis. Biofilm regulatory protein A (BrpA), an LCP paralogue in *Streptococcus mutans*, plays a pivotal role in biofilm formation, although its function is not fully elucidated. This study seeks to explore BrpA's biological and structural mechanisms.

Methods

The *brpA* gene in *S. mutans* was disrupted by insertional mutagenesis, and the resulting mutant was examined for biofilm formation using crystal violet staining. Recombinant BrpA (rBrpA) was expressed in *Escherichia coli* and purified before being introduced into the mutant to test for functional complementation. Biolayer interferometry was performed to assess BrpA's interaction with the N-terminal catalytic domain of GtfC, and the crystal structure of BrpA was determined at 1.8 Å resolution, pinpointing two positively charged surface residues essential for Gtf binding. In silico screening was used to identify candidate small-molecule inhibitors, which were subsequently synthesized. Binding between BrpA and these compounds was measured by microscale thermophoresis, while biofilm assays evaluated the inhibitory effects on BrpA function.

Results

Disruption of *brpA* significantly diminished extracellular polysaccharide (glucan) production in *S. mutans*, leading to compromised biofilm formation. Complementation with rBrpA fully restored these phenotypes, confirming the protein's role in glucan synthesis. Microscopy revealed that BrpA colocalized with glucans in the biofilms. Structural analysis identified two positively charged residues on BrpA's surface required for binding to GtfC. The synthesized small-molecule inhibitors effectively bound BrpA and blocked its activity, as evidenced by reduced biofilm formation.

Conclusions

These findings demonstrate that BrpA is essential for biofilm formation by interacting with the GtfC catalytic domain to facilitate glucan production. The identified small-molecule inhibitors specifically bind BrpA and inhibit its function, providing a promising candidate for controlling biofilm-related oral diseases.



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