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**Front Cover Image:**

*Farahnaz Fahimipour, Research Assistant, Department of Oral and Craniofacial Health Sciences.*

Artistic illustration of **junctons between normal human gingival epithelial cells** (hGEPs) in different magnifications.
Dear Colleagues,

We would like welcome everyone to the 35th Annual Research Day in the School of Dentistry. This year we have more than 90 poster and oral presentations representing the research accomplishments of our students, faculty, staff, research fellows, and visiting scholars. Many of these presentations are the products of interdepartmental, campus-wide, national and international collaborations.

The School of Dentistry’s research mission places an overarching emphasis on the promotion of oral health and function. Discoveries and new knowledge generated in the basic, translational and clinical sciences, as well as in the areas of health services, health policy and health education, are represented in the abstracts presented for you today.

The day’s events include a keynote address by Dr. Mary L. Marazita, Ph.D. professor and director of Director, Center for Craniofacial and Dental Genetics at the University of Pittsburgh. She has dedicated her career to identifying genes for complex craniofacial and dental disorders in humans. We hope you will attend Dr. Marazita’s talk, “Genomic and Phenomic Studies of Orofacial Cleft Birth Defects.” There are also two workshops and eleven lunch and learn sessions, during which you can discuss the latest updates in emerging technologies in dentistry, digital dentistry, gold foil to restore conservative lesions, oral microbiome, and HPV associated lesions to name a few.

Please join us and enjoy the day,

Eric T. Everett, PhD
Professor and Interim Associate Dean for Research

Scott S. De Rossi, DMD, MBA
Dean and Professor
Dear Colleagues,

It is our great pleasure to welcome you to the 2019 Research Day of the UNC-CH School of Dentistry. It is a day when we engage students and trainees of all levels, faculty, staff, visiting scholars, alumni and exhibitors with one another and foster stimulating discussions among and between clinicians and scientists.

The Student Research Group (SRG) and the North Carolina Section of the American Association for Dental Research (NC-AADR) are thrilled to see this historic event continue to grow. This year, there are 30 dental students presenting their research work. Additionally, there are presentations from 22 Master’s and 11 PhD students, 8 Post-doctoral fellows or associates, 2 visiting scholars, 5 staff members and 5 faculty members—90 total abstracts. We would like to particularly welcome two student guest presenters, selected by their institutions, the University of Michigan and the University of Pittsburg, as representatives to the 35th UNC-CH SOD Research Day. We look forward to building upon this scientific exchange and collaborative momentum in the future!

We hope that you enjoy your day as you interact with the presenters and colleagues, and participate in activities including the keynote address, lunch & learn sessions and workshops. This year, we have a record 11 Lunch and Learn sessions, covering a wide range of themes and interests, hosted by our expert faculty!

Please, take time to visit our exhibitors who have provided substantial support to this scientific event.

We look forward to another successful scientific event!

Sincerely,

Danielle Burgess    Dani Fox     Kimon Divaris
Third-year Dental Student  Third-year Dental Student  Associate Professor
Co-President    Co-President    President
Student Research Group  Student Research Group  NC-AADR
35th UNC-CH SOD Research Day Keynote Presentation
Kirkland auditorium, Koury Oral Health Sciences Building, UNC School of Dentistry
noon-1:00PM, Wednesday February 6, 2019

Mary Marazita, Professor, University of Pittsburgh

Genomic and Phenomic Studies of Orofacial Cleft Birth Defects

Nonsyndromic orofacial cleft birth defects (OFCs) are the most common craniofacial anomalies in humans, affecting approximately 1 in 700 newborns, and are one of the most common structural birth defects worldwide. On average, a child with an OFC initially faces feeding difficulties, undergoes 6 surgeries, spends 30 days in hospital, receives 5 years of orthodontic treatment, and participates in ongoing speech therapy, leading to an estimated total lifetime treatment cost $200,000 or more. Interest in the etiology of OFCs goes back centuries, as does formal scientific investigation. Most OFCs occur as isolated malformations and are considered “non-syndromic (NS)”. There is now general consensus that NS OFCs represent etiologically complex birth defects that result from genetic variants, environmental exposures and their interactions. In this talk, Dr. Marazita will summarize the results of the large consortium that she is leading, investigating the genomics and phenomics of OFCs worldwide, including recent results from whole-genome sequencing.

Dr. Mary Marazita is Director of the Center for Craniofacial and Dental Genetics, and Professor and Vice Chair of the Department of Oral Biology in the School of Dental Medicine at the University of Pittsburgh. Her research areas include human genetics of complex traits, primarily facial birth defects, and oral disease. She has more than 300 peer-reviewed publications, and substantial grant support totaling about $6 million annually. Her studies have led to collaborations with colleagues across the globe.
## Schedule of events

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity and Location</th>
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<tr>
<td>7:00 am – 8:00 am</td>
<td><strong>Poster and Vendors Set-up</strong>&lt;br&gt;Atrium and Main Street, Koury Oral Health Sciences Building</td>
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<tr>
<td>8:00 am</td>
<td><strong>Dean’s Welcome Breakfast</strong></td>
</tr>
<tr>
<td>8:00 am - 4:30 pm</td>
<td><strong>Exhibition open</strong></td>
</tr>
<tr>
<td>8:30 am – 8:40 am</td>
<td><strong>Dean’s Welcome and Opening Remarks</strong>&lt;br&gt;<strong>Dean Scott De Rossi, DMD, MBA</strong>&lt;br&gt;Atrium, Koury Oral Health Sciences Building</td>
</tr>
<tr>
<td>8:45 am – 10:15 am</td>
<td><strong>Oral Presentation Sessions I-V</strong>&lt;br&gt;Room assignment details in the program&lt;br&gt;<strong>General attendance</strong></td>
</tr>
<tr>
<td>10:15 am – 11:45 am</td>
<td><strong>Poster Presentations</strong>&lt;br&gt;Atrium and Main Street, Koury Oral Health Sciences Building</td>
</tr>
<tr>
<td>12:00 pm – 1:00 pm</td>
<td><strong>KEYNOTE PRESENTATION</strong>&lt;br&gt;<strong>Mary Marazita, PhD. University of Pittsburgh</strong>&lt;br&gt;Kirkland Auditorium, Koury Oral Health Sciences&lt;br&gt;<strong>General attendance</strong></td>
</tr>
<tr>
<td>1:15 pm - 2:15 pm</td>
<td><strong>Lunch and Learn Sessions</strong>&lt;br&gt;Room assignment details in the program&lt;br&gt;<em>By registration only</em></td>
</tr>
<tr>
<td>2:15 pm – 3:15 pm</td>
<td><strong>Technology in Dentistry Showcase</strong>&lt;br&gt;with Drs. Ibrahim Duqum, Ryan Cook, Wendy Clark, Dewey Chapa&lt;br&gt;<strong>Session 1. Koury G508</strong>&lt;br&gt;<em>By registration only</em></td>
</tr>
<tr>
<td>3:30 pm – 4:30 pm</td>
<td><strong>Technology in Dentistry Showcase</strong>&lt;br&gt;with Drs. Ibrahim Duqum, Ryan Cook, Wendy Clark, Dewey Chapa&lt;br&gt;<strong>Session 1. Koury G508</strong>&lt;br&gt;<em>By registration only</em></td>
</tr>
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</table>

**SAVE THE DATE!**

Friday, February 8th 5:00 pm – 7:00 pm

**What:** **Awards Ceremony** (snacks and beverages provided) – Sponsored by the **Dental Foundation of North Carolina**

**Where:** **TRUDeli** (114 Henderson St, Chapel Hill)
# Lunch and Learn Sessions
(1:15 pm – 2:15 pm; registration required)

<table>
<thead>
<tr>
<th>Room</th>
<th>Presenter</th>
<th>Session Title</th>
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<tbody>
<tr>
<td>Brauer 475</td>
<td><strong>Dr. Taiseer Sulaiman</strong>, Assistant Professor, Restorative Sciences</td>
<td>Composite resins</td>
</tr>
<tr>
<td>Brauer 353</td>
<td><strong>Dr. Julie Marchesan</strong>, Assistant Professor, Periodontology</td>
<td>The scientific evidence base for flossing</td>
</tr>
<tr>
<td>Koury 3615</td>
<td><strong>Dr. Apoena Ribeiro</strong>, Associate Professor, Diagnostic Sciences</td>
<td>The oral microbiome in dental caries and its impact on oral health and care</td>
</tr>
<tr>
<td>OD 1080 (Breeland)</td>
<td><strong>Dr. Don Tyndall</strong>, Professor and Director of Radiology, Diagnostic Sciences</td>
<td>Emerging technologies in radiographic imaging for dentistry.</td>
</tr>
<tr>
<td>Brauer 259</td>
<td><strong>Dr. Siggi Saemundsson</strong>, Professor and Graduate Program Director, Pediatric Dentistry</td>
<td>Hypnosis and dentistry—where do they connect?</td>
</tr>
<tr>
<td>Koury G502</td>
<td><strong>Dr. Ibrahim Duqum</strong>, Associate Professor</td>
<td>Digital Dentures: Technology and Materials Update.</td>
</tr>
<tr>
<td>Koury G508</td>
<td><strong>Dr. Wendy Clark</strong>, Assistant Professor, Restorative Sciences</td>
<td>Digital approach to implant restorations</td>
</tr>
<tr>
<td>Koury 5615</td>
<td><strong>Dr. Ricardo Padilla</strong>, Diagnostic Sciences</td>
<td>Oral mucosa lesions associated with HPV</td>
</tr>
<tr>
<td>Koury 5401</td>
<td><strong>Prof. Beth Kornegay</strong>, Dental Ecology</td>
<td>Embracing change: how to implement strategies at the UNC-CH School of Dentistry</td>
</tr>
<tr>
<td>Koury 4615</td>
<td><strong>Dr. Valerie Murrah</strong>, Professor, Chair Diagnostic Sciences</td>
<td>Implementing Strategies for Dealing with Infectious Pathogens in Every Day Practice</td>
</tr>
<tr>
<td>Brauer 266</td>
<td><strong>Dr. Robert Bridgeman</strong>, Private Practice, NC</td>
<td>Gold foil as direct restorative material in conservative lesions.</td>
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Workshops (registration required)

<table>
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<tr>
<th>Time</th>
<th>Room</th>
<th>Presenters</th>
<th>Session Title</th>
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</thead>
<tbody>
<tr>
<td>2:15 pm – 3:15 pm</td>
<td>Koury G508</td>
<td>Drs. Ibrahim Duqum, Ryan Cook, Wendy Clark and Dewey Chapa</td>
<td>Technology in Dentistry Showcase (session 1)</td>
</tr>
<tr>
<td>2:15 pm – 3:15 pm</td>
<td>Koury Sim Lab 1st Floor</td>
<td>Drs. Robert Bridgeman* and Rick Walter</td>
<td>Gold foil hands-on workshop (session 1)</td>
</tr>
<tr>
<td>3:30 pm – 4:30 pm</td>
<td>Koury G508</td>
<td>Drs. Ibrahim Duqum, Ryan Cook, Wendy Clark and Dewey Chapa</td>
<td>Technology in Dentistry Showcase (session 2)</td>
</tr>
<tr>
<td>3:30 pm – 4:30 pm</td>
<td>Koury Sim Lab 1st Floor</td>
<td>Drs. Robert Bridgeman* and Rick Walter</td>
<td>Gold foil hands-on workshop (session 2)</td>
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*Robert Bridgeman, DDS

Dr. Robert H. Bridgeman majored in biology at Marshall University and Appalachian State University from 1998-2002, and is a 2006 graduate of the UNC School of Dentistry. After obtaining his dental degree, he moved home to Boone, NC and associated with his father, Craig Bridgeman, where they both continue to practice. From 2012-2014, Dr. Bridgeman also provided pediatric dental services for the Appalachian Regional District Health Department.

Dr. Bridgeman is dedicated to excellence in operative dentistry in his private practice, through his membership in the Academy of Operative, his service to the American Academy of Gold Foil Operators as the sitting Vice President, and as the Secretary Treasurer for the Hollenbach-Medina Operative Dentistry Seminar (Club #71 of the Academy of Richard V. Tucker Study Clubs). Dr. Bridgeman is also an alumnus of the Pankey Institution (2009), and is a current member of the NCDS and the ADA.
### Oral Presentations Sessions I—II (8:45 am – 10:15 am)

Turner award finalists are denoted with an asterisk *

<table>
<thead>
<tr>
<th>Oral Session 1</th>
<th>Room: Old Dental 0150</th>
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<tr>
<td>abstract #</td>
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<td>75</td>
<td>8:45-9:00</td>
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<td>81</td>
<td>9:00-9:15</td>
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<td>80</td>
<td>9:15-9:30</td>
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<td>51</td>
<td>9:30-9:45</td>
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<td>55</td>
<td>9:45-10:00</td>
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<tr>
<th>Oral Session 2</th>
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<td>8:45-9:00</td>
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<td>36</td>
<td>9:00-9:15</td>
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<td>35</td>
<td>9:15-9:30</td>
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<td>32</td>
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<td>14</td>
<td>9:45-10:00</td>
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<td>10:00-10:15</td>
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## Oral Presentations Sessions III—IV (8:45 am – 10:00 am)

Turner award finalists are denoted with an asterisk *

### Oral Session 3

<table>
<thead>
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<th>Department</th>
<th>Title</th>
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<tr>
<td>84</td>
<td>8:45-9:00</td>
<td>Awab Abdulmajeed</td>
<td>Restorative Sciences</td>
<td>Effect of Fatiguing and Preheating on the Mechanical Properties of Bulk-fill versus Conventional Composite Resin</td>
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<tr>
<td>83</td>
<td>9:00-9:15</td>
<td>Elizabeth Griffis</td>
<td>Restorative Sciences</td>
<td>Cuspal Coverage Indications with CAD/CAM Lithium Disilicate Restorations</td>
</tr>
<tr>
<td>86</td>
<td>9:15-9:30</td>
<td>Elisa Arnarsdottir</td>
<td>Endodontics</td>
<td>Periapical Microsurgery: Assessment of Different Light Emitting Diode (LED) Transilluminator Types for Dentinal Defects Detection</td>
</tr>
<tr>
<td>15</td>
<td>9:30-9:45</td>
<td>Michael Mittelstead</td>
<td>Endodontics</td>
<td>Influence of Clinician Background on Endodontic Surgery vs. Retreatment Recommendations</td>
</tr>
<tr>
<td>85</td>
<td>9:45-10:00</td>
<td>Nicholas Pettit</td>
<td>Endodontics</td>
<td>Assessment of Light Emitting Diode (LED) Transilluminators in Detection of Coronal Cracks and Fractures</td>
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### Oral Session 4

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<tr>
<th>abstract #</th>
<th>Time</th>
<th>Presenter</th>
<th>Periodontology</th>
<th>Title</th>
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<tr>
<td>40</td>
<td>8:45-9:00</td>
<td>Kevin Byrd*</td>
<td>Oral &amp; Craniofacial Health Sciences</td>
<td>Lrig1 Marks a Quiescent Stem Cell Population in the Oral Cavity</td>
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<td>43</td>
<td>9:00-9:15</td>
<td>Si On Lim*</td>
<td>Diagnostic Sciences</td>
<td>Differential Expression of PD-L1 in Proliferative Verrucous Leukoplakia Lesions</td>
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<td>26</td>
<td>9:15-9:30</td>
<td>Marta Musskopf</td>
<td>Periodontology</td>
<td>Mini-pig Implant Intra-oral Model: Peri-implant Bone Characteristics</td>
</tr>
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<td>28</td>
<td>9:45-10:00</td>
<td>Eugenia Monaghan</td>
<td>Periodontology</td>
<td>Volumetric Analysis of Hard Tissue Healing after Tooth Extraction and Socket Grafting</td>
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**Oral Presentations Session V (8:45 am – 9:45 am)**

Turner award finalists are denoted with an asterisk *

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<th>abstract #</th>
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<th>Presenter</th>
<th>Department</th>
<th>Title</th>
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<tr>
<td>24</td>
<td>8:45-9:00</td>
<td><strong>Stephen Tuin</strong></td>
<td>Oral &amp; Craniofacial Health Sciences</td>
<td>Nitric Oxide Release Enhances Osteogenic Effects of Diethylenetriamine and Spermine</td>
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<td>47</td>
<td>9:00-9:15</td>
<td><strong>Adele Musicant</strong>*</td>
<td>Genetics &amp; Molecular Biology Curriculum</td>
<td>Dual EGFR and HDAC Inhibition Exploits a Unique Therapeutic Vulnerability in Mucoepidermoid Carcinoma</td>
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<td>63</td>
<td>9:15-9:30</td>
<td><strong>Karin Schey</strong>*</td>
<td>Periodontology</td>
<td>Clinical Changes and Oral Microbiome Shifts in HIV+ Patients Following Periodontal and Restorative Therapy</td>
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<td>59</td>
<td>9:30-9:45</td>
<td><strong>William Seaman</strong>*</td>
<td>Lineberger Comprehensive Cancer Center</td>
<td>Oral Pathogens Induce EBV+ B-cell/Epithelial Cell Adherence Resulting in Virus Transfer to Naïve Oral Keratinocytes</td>
</tr>
<tr>
<td>abstract #</td>
<td>Presenter</td>
<td>Department/Program</td>
<td>Title</td>
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<tr>
<td>1</td>
<td>Valerie Acquesta</td>
<td>Periodontology</td>
<td>Addressing Access to Care Through Teledentistry</td>
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<td>Leiana Edwards</td>
<td>Office of Teledentistry</td>
<td>Teledentistry Knowledge and Attitudes Before and After a Statewide Summit</td>
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<td>Ricardo Padilla</td>
<td>Diagnostic Sciences</td>
<td>Interprofessional Collaboration to Integrate Oral Health into Medical Education</td>
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<td>Brittany Klein</td>
<td>DDS curriculum</td>
<td>Audiology and Oral Health Professionals: An Interprofessional Collaboration</td>
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<td>5</td>
<td>Debin Warren*</td>
<td>DH Education</td>
<td>Interprofessional collaboration among students on oral health for cancer patients</td>
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<td>6</td>
<td>Beth Kornegay</td>
<td>Periodontology</td>
<td>Structured Focus Group Data Inform Curriculum Innovation in Dental Education</td>
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<td>7</td>
<td>Sarah Liebkemann*</td>
<td>DH Program</td>
<td>Perceptions and Attitudes of Dental Hygiene Students Toward Interactive Virtual Games (IVGs)</td>
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<td>Monica Kim*</td>
<td>DDS curriculum</td>
<td>Changes in Dental Students’ Knowledge and Perceptions of Constructive Feedback Associated with Educational Intervention</td>
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<td>9</td>
<td>Sarah Lowman</td>
<td>DDS curriculum</td>
<td>Dental Student Perspectives on Digital Dentures: A Pilot Study</td>
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<td>Ryan Gross</td>
<td>DDS curriculum</td>
<td>Peer-Tutoring Digital Resources Are Used and Accepted in Dental Education</td>
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<td>Megan Inclan*</td>
<td>Pediatric Dentistry</td>
<td>Pre-Doctoral Special Health Care Needs Education: Lost in a Crowded Curriculum</td>
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<td>Miguel Simancas-Pallares</td>
<td>Oral and Craniofacial Health Sciences</td>
<td>Dental Students’ Perceived Stress is Associated with Psychological Distress and Burnout—their Self-Efficacy is Protective</td>
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<td>13</td>
<td>Alexandra Yarborough</td>
<td>Restorative Sciences</td>
<td>A Removable Prosthodontic Laboratory Survey</td>
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<td>Nathan Yip*</td>
<td>DDS curriculum</td>
<td>Association of Apical Periodontitis and Diabetes in a Large Hospital Network</td>
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<td>Damian Slaczka</td>
<td>DDS curriculum</td>
<td>The Impact of Study Design on the Association of Endodontic Disease and Cardiovascular Disease</td>
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<td>Kamaira Philips</td>
<td>Oral and Craniofacial Health Sciences</td>
<td>Periodontal Disease Classification Affects Associations with Systemic Conditions</td>
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<td>Yizu Jiao</td>
<td>DDS curriculum</td>
<td>GWAS for Interleukin-1β Levels in Gingival Crevicular Fluid Identifies IL37 Variants in Periodontal Inflammation</td>
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<td>Grant Egnatz</td>
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<td>Inflammasome Priming in Experimental Periodontitis</td>
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<td>21</td>
<td>Sabrina Collias</td>
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<td>Modulation of Experimental Periodontitis through IFI16</td>
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<td>Mustafa Girnary</td>
<td>Periodontology</td>
<td>Sex Impact of Caspase-1 Mediation of Periodontal Bone Destruction</td>
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<td>23</td>
<td>Meng Deng*</td>
<td>Oral and Craniofacial Biomedicine PhD Program</td>
<td>TRAF3IP3 Negatively Regulates Type I Interferon Signaling by Suppressing TBK1</td>
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<td>Sing Wai Wong*</td>
<td>Oral and Craniofacial Biomedicine PhD Program</td>
<td>Pre-clinical Model of Paget’s Disease Reveals Interferon-beta as a Therapeutic Target</td>
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<td>Colin LaPrade</td>
<td>DDS curriculum</td>
<td>Bitewing Dosimetry of 3D Intraoral Tomosynthesis Dental X-ray Imaging System</td>
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<td>Beatrice Tejera</td>
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<td>3D Maxilla and Defect Quantification Using Automatic Segmentation for Patients with Unilateral Cleft Lip and Palate</td>
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<td>31</td>
<td>Ryan Kearney</td>
<td>Orthodontics</td>
<td>Alveolar Bone Grafting Success in Clefts – A 5 Year Study</td>
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<td>33</td>
<td>Hannah Bodnar*</td>
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Abstracts

[1] Addressing Access to Care Through Teledentistry

Liebkemann S¹, Dunham C¹, Gallo G², Dua S³, Acquesta V⁴, Okereke K⁵, Harbison A⁴, Hernandez A⁴, Matthews NS⁵

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Objectives: Access to dental care is burdensome for many underserved and rural North Carolinians due to the maldistribution of practitioners, which has generated an untreated dental disease crisis. To address this problem, the University of North Carolina School of Dentistry (UNC SoD) hosted the first Teledentistry Summit in the state with the intention of exploring the challenges and solutions of implementing this dental care delivery model, and establishing the North Carolina Teledentistry Task Force. This project highlights the major events that took place at the summit and suggests a mechanism by which the task force can work to educate, establish, and implement teledentistry models to eliminate the disparities in oral health care between rural and urban communities.

Methods: Faculty and class representatives from the dental surgery/hygiene programs of the UNC SoD and over 70 stakeholders from across the country assembled to develop a shared understanding of teledentistry, discuss perceptions and attitudes, and identify the benefits and challenges of introducing teledentistry in North Carolina. Keynote speakers presented aspects of currently established uses of telehealth, including telemedicine and the Virtual Dental Home model utilized in California. Participants were then divided into six groups: finance/reimbursement, community settings, private practice, practice act and policies, IT, and education. Each group explored the advantages and barriers to establishing a statewide teledentistry program and presented their findings to all participants of the summit.

Results: The individual breakout groups presented common themes of teledentistry and made recommendations on how to increase access to dental care, value of care, and improve oral health through teledentistry.

Conclusions: The summit established the North Carolina Teledentistry Task Force and the Teledentistry Student Group, the first of its kind in the USA, to educate our state and promote the implementation of teledentistry models. Funded by: Blue Cross Blue Shield of North Carolina Foundation, The Duke Endowment, The North Carolina Dental Society, and Delta Dental
[2] Teledentistry Knowledge and Attitudes Before and After a Statewide Summit

Weintraub JA1, Edwards L2, Brame J3, Lampiris L4, White BA1,5, Adatorwovor R6, Matthews NS7

1Department of Dental Ecology, School of Dentistry, University of North Carolina at Chapel Hill, 2Office of Teledentistry, School of Dentistry, University of North Carolina at Chapel Hill, 3Department of Periodontics, School of Dentistry, University of North Carolina at Chapel Hill, 4Department of Dental Ecology, School of Dentistry, University of North Carolina at Chapel Hill, 5Department of Health Policy and Management, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, 6Department of Biostatistics, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, 7Department of Oral and Maxillofacial Surgery, School of Dentistry, University of North Carolina at Chapel Hill

Objectives: The first statewide North Carolina (NC) teledentistry (TD) summit was convened by the University of North Carolina at Chapel Hill School of Dentistry. The 2018 all-day summit had multiple goals including increasing participants’ understanding of TD, reimbursement policies, IT options, benefits, challenges and solutions. Aimed to compare participants’ pre- and post-summit knowledge and attitudes toward TD. Methods: Summit attendees included invited stakeholders and experts in related policy, education, advocacy, legislation, business and technology and selected UNC students. The pre- and post-surveys were administered using Qualtrics® to 67 individuals. Descriptive statistics and McNemar’s matched pair test were performed comparing proportions for pre-post matched pairs. Results: The response rates were pre-summit 86.6% (n= 58) and post-summit 70.1% (n= 47); matched pre-post survey pairs (n=42). Pre-summit respondents reported their primary role in administration (48%), teaching and mentoring (21%), patient care (12%) or were students (19%). Excluding the 10 students, 53% of participants were dentists or hygienists. Knowledge items were assessed from 1 (no knowledge) to 7 (very knowledgeable). Overall TD knowledge (scored 5-7) and knowledge about NC telemedicine policies for Medicaid reimbursement increased from 38.1% to 92.9% and 16.7% to 69.1%, respectively. For both questions, there was a significant pre-post increase in proportions with knowledge, both p <.0001. From 1 (not at all) to 5 (a great deal) the extent TD should be developed in NC scored 4-5 increased from 78.6% to 95.2%, p=0.03; the extent hygienists should have a role in TD services scored 4-5, increased from 83.3% to 88.1%., p=0.53. The most frequently mentioned challenge was “state practice acts requiring direct supervision of dental hygienists, limiting their teledentistry use in community settings” which increased pre- to post-survey from 33.3% to 59.5%, p = 0.02. Conclusions: Stakeholder knowledge was high and attitudes favorable for moving forward with TD in NC. Funding sources: BlueCross BlueShield of North Carolina Foundation, The Duke Endowment, Delta Dental and the North Carolina Dental Society

[3] Interprofessional Collaboration to Integrate Oral Health into Medical Education

Padilla R1, Quinonez R2, Gilliland K3, Kowlowitz V3, Koonce T3, Gilchrist MJ3, Lampiris L2, Ciarrocca K2

1Department of Diagnostic Sciences, School of Dentistry, University of North Carolina at Chapel Hill, 2School of Dentistry, UNC-CH, 3School of Medicine, University of North Carolina at Chapel Hill

Objectives: 1- To incorporate oral health knowledge and examination skills into the history and physical exam in medical education, 2- To evaluate medical school faculty’s and students’ perceptions of the effectiveness of the experience and attitudes toward integrating oral health into primary care. Methods: Analysis of the medical school’s curriculum identified an appropriate place for this intervention. The video “Oral Health For The Primary Care Provider” was developed for the Smiles For Life Program and utilized to demonstrate the examination. Students and faculty viewed the video prior to the instructional session. Dental faculty and students were calibrated to teach the examination. They rotated among student groups to demonstrate and supervise students’ hands-on practice. Medical students and their faculty evaluated the effectiveness of the video, demonstration/practice session and their attitudes towards primary care physicians including oral health as part of the history and physical examination, provide oral health counseling, and refer patients to a dentist. Results:
During two years, 301 medical students and 43 faculty completed surveys. On a 4-point scale, 99% of the students rated the practice session “Effective” or “Very Effective”. Of the medical school small group faculty tutors, 100% rated it “Helpful” or “Very Helpful”, to have School of Dentistry faculty teach the session. Most students and faculty rated that including oral health in aspects of primary care was important (88.8% - 99.3%). In 2018, 95.5% of faculty also rated including oral health history, screening, counseling and referral) “Important” or “Very Important”. **Conclusions:** The Schools of Dentistry and Medicine successfully collaborated in identifying, designing, and delivering opportunities to integrate oral health education into the medical school curriculum utilizing an interprofessional education format. Medical faculty and students evaluated the hands-on interprofessional education experience positively and rated the integration of oral health into primary care as important.

**[4] Audiology and Oral Health Professionals: An Interprofessional Collaboration**

Klein B1, Brame J2, Kowlowitz V3, McKenna NM4, Weintraub JA2, Ciarrocca K2

1Class of 2020, School of Dentistry, University of North Carolina at Chapel Hill, 2Department of Dental Ecology, School of Dentistry University of North Carolina at Chapel Hill, 3Academic Affairs, School of Dentistry, University of North Carolina at Chapel Hill, 4Division of Audiology and Hearing Sciences, School of Medicine, University of North Carolina at Chapel Hill

**Objectives:** An interactive learning experience for dental, dental hygiene (DH), and audiology students was created to engage students in learning about, from, and with each other. Goals for dental and DH students included learning about the audiology profession, hearing assessments, hearing health, and patient communication for those with hearing loss. Audiology students were instructed on head and neck exams and referral to oral health care providers. The purpose of this study was to evaluate the educational experience. **Methods:** Audiology students received didactic and hands-on instruction by dental and DH students and faculty on performing head and neck exams and appropriate referrals. Audiology students presented to senior DH and second-year dental students and offered supervised hearing screenings by audiology students. Dental and DH students were asked to complete post-experience surveys. Student opinions and perceived knowledge levels were assessed using descriptive statistics. **Results:** Fifty-seven students completed the survey; 39 dental students (46% response rate), and 12 DH students (response rate 33%); six did not report their program. Thirty-three participated in the hearing assessments; 14 reporting this as their first hearing assessment. Students were asked their level of knowledge of the audiology profession, hearing assessments, noise-induced hearing loss, and patient communication before and after the experience. For each, students’ rating improved from majority of respondents reporting they were not knowledgeable at all or slightly knowledgeable prior to the experience to majority of respondents reporting they were moderately or very knowledgeable following the experience. Eighty-six percent agreed this should be added to curriculum and prepared them to communicate with patients who have hearing loss; 92% agreed the experience increased their understanding of the importance of collaborating with other health professionals. **Conclusions:** This experience increased students’ knowledge of the audiology profession and understanding of hearing loss, while also increasing their appreciation of interprofessional education.

**[5] Interprofessional collaboration among students on oral health for cancer patients**

Warren DL1, Ciarrocca K2, Williams J3, Brame JL1

1Dental Hygiene Education Program, School of Dentistry, University of North Carolina at Chapel Hill, 2Department of Dental Ecology, School of Dentistry, University of North Carolina at Chapel Hill

**Objectives:** Educate nursing and dental hygiene (DH) students on oral considerations during cancer treatment; evaluate changes in knowledge, confidence, and willingness to provide oral screening, counseling, and referrals for patients undergoing cancer therapy. **Methods:** Pilot study utilizing mixed-methods design including all first-year
DH and accelerated nursing (ABSN) students at the University of North Carolina at Chapel Hill (UNC). Data was collected using baseline and post-intervention surveys and debriefing session. Students received a presentation regarding oral considerations and provision of screening, counseling, and referral for cancer patients. A control group (16 ABSN, 8 DH) was asked to complete an immediate post-survey; others were organized into small, mixed groups and evaluated an unfolding case study and post-survey completion. A debriefing session followed. Tests with Row Mean Score Differ statistics and Chi-Square tests were used. **Results:** 93 matched surveys returned (61 ABSN, 31 DH). Baseline surveys revealed 82% (n=76) indicated no knowledge to complete oral health screenings, 68% (n=63) counseling, or 65% (n=60) referral; post-survey results denoted positive changes to knowledge in screening (72%, n=67), counseling (81%, n=75) and referral (89%, n=83). Baseline confidence assessment revealed 22% (n=20) had confidence to complete oral screening, 25% (n=23) for counseling, and 47% (n=44) for referral. Post-survey results showed confidence increases to screen (75%, n=70), counsel (83%, n=77), and refer (91%, n=82). Baseline and post-intervention levels of willingness to screen, counsel, and refer were high. Results revealed 96% (n=89) had willingness to collaborate with other healthcare professions and 99% (n=92) agreed shared learning would help them become a more effective team member. **Conclusions:** Educating students in an interprofessional collaborative learning environment can increase their knowledge, confidence, and willingness to provide screenings, counseling, and referrals for patients undergoing cancer therapies. Responses indicate a willingness and desire to collaborate with other disciplines to enhance patient care.

[6] Structured Focus Group Data Inform Curriculum Innovation in Dental Education

Kornegay B,1 Jackson T,2 La Garry A,3 Quinonez RB,4 Teal R,5 Carda-Autin J,5 Reside J,1 Swift E,4 King J,6 Ciarrocca K,7 Kowlowitz V,4 Zomorodi M,8 Greene J9

1Department of Periodontology, School of Dentistry, University of North Carolina at Chapel Hill, 2Department of Orthodontics, School of Dentistry, University of North Carolina at Chapel Hill, 3School of Education, University of North Carolina at Chapel Hill, 4Office of Academic Affairs, School of Dentistry, University of North Carolina at Chapel Hill, 5CHAI Core, University of North Carolina at Chapel Hill, 6Department of Restorative Sciences, School of Dentistry, University of North Carolina at Chapel Hill, 7Department of Dental Ecology, School of Dentistry, University of North Carolina at Chapel Hill, 8Office of the Provost, University of North Carolina at Chapel Hill

**Objectives:** The need to innovate predoctoral dental education is well established, but few data-driven methods for implementation of curricular change have been published. The University of North Carolina at Chapel Hill School of Dentistry (SOD) incorporated a qualitative research approach to gain faculty insight on curriculum needs, strategies, and barriers. This approach was part of Phase one of four innovation phases for curriculum redesign. **Methods:** Eighty faculty participated in eight separate focus groups during a curriculum summit. Focus groups lasted 60 minutes and were conducted and digitally recorded by four professional facilitators in breakout rooms in a campus facility. Focus group guides were developed to elicit perceptions about (a) what the SOD graduate should know in 2040; (b) what SOD does well and needs improvement in the context of the proposed curriculum framework of Advocate-Clinician-Thinker (ACT); and (c) barriers to faculty engagement in curriculum change. A summary report was generated from a qualitative thematic analysis. **Results:** Focus group findings concluded that: (a) faculty placed high value both on clinical technical skills and broader healthcare system skills to produce a competent dental graduate; (b) more standardized advocate related leadership experiences for all students would be beneficial, but not at the expense of clinical training; (c) greater focus on technical skill experiences and more evidence based training should be developed; (d) opportunities to break down silos between departments should be increased, and (e) teaching should be valued more, through tenure and promotion, curriculum centralization that promotes collaboration, and with support to address workload and improve growth opportunities. **Conclusion:** Qualitative data are powerful in providing insight into critical issues affecting curriculum innovation. This process can help guide structural changes that promote faculty engagement and curriculum success.
[7] Perceptions and Attitudes of Dental Hygiene Students Toward Interactive Virtual Games (IVGs)

Liebkemann SL¹, Kornegay B¹, Brame J¹

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Objectives: Interactive Virtual Games (IVGs) are active learning tools that promote critical thinking, problem solving, and/or content memorization through timed, interactive simulations and activities. The purpose of this study was to determine the attitudes and perceptions of dental hygiene students toward learning with an IVG.

Methods: An anonymous Qualtrics link was emailed to 70 first and second year dental hygiene (DH) students in the undergraduate baccalaureate program at the University of North Carolina at Chapel Hill School of Dentistry. The link contained a short animated video describing the concept of an IVG, followed by a 28 question survey related to demographic identification, technology usage, preferred learning styles, and IVG integration. Univariate analysis yielded descriptive data about the participants' opinions.

Results: Fifty students submitted responses to the survey (response rate 71%). Ninety percent (N=45) reported having played some type of educational video game in the past. Ninety-two percent (N=46) indicated that they were interested using IVGs to supplement the information presented in class. Students reported interest in using IVGs to improve their critical thinking ability (98%, N=49), memorization ability (96%, N=48), and tactile skills (96%, N=48). Students stated that they believed IVGs were versatile and would improve their preparedness for clinic.

Conclusions: The positive attitudes and perceptions of DH students toward the notion of IVGs indicate that further research involving the design, implementation, and effectiveness of an IVG is appropriate.

[8] Changes in Dental Students’ Knowledge and Perceptions of Constructive Feedback Associated with Educational Intervention

Kim M¹, Ciarrocca K², Brame J³, Divaris K⁴

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Objectives: In current modalities of feedback assessment in the professional classroom, data gathering is typically done post-course instruction. This project examines student attitudes and perceptions of receiving and providing constructive feedback changes to include greater competence after being exposed to a professional workshop lead by a professional educator.

Methods: A survey assessing second-year dental students’ perceived preparedness, knowledge and utility of constructive feedback, both as recipient and provider in a professional setting, was administered via Qualtrics before and after an interventional calibration workshop. The survey included open-ended and Likert-type questions. We used Atlas.ti software and interpretative and descriptive coding to analyze students’ qualitative responses and identify prominent themes including the transition from unconscious incompetence to conscious incompetence. To determine changes in quantitative measures between pre- and post-workshop we employed unpaired t-tests with a p<0.05 criterion using Stata 15.1. Results: Sixty students completed the pre-intervention and 44 completed the post-intervention surveys—response rates of 73% and 54%, respectively. We found statistically significant increases (p<0.05) in students’ preparedness, comfort and perceived relevance of constructive feedback after the interventional calibration workshop when using closed-ended questions and discovered an increase in the frequency of certitude phrases in the post-interventional survey.

Conclusion: Preliminary results indicate using both closed and open-ended variable surveys support that an
Interventional workshop has a positive effect for students to assess not only their current comfort level of communicating constructive feedback, but also improving their current standing in a professional environment. Students’ levels of unconscious incompetence and conscious incompetence in receiving and providing feedback increased after professional development and can be applied to increased performance in their dental educational experience. Statistically significant values corroborate that a calibration training and even continuous education in this arena would not only boost student competence levels but facilitate better communication among faculty and patients. **Supported by:** ADEA Academic Dental Careers Fellowship Program and UNC-CH Odum Institute

**[9] Dental Student Perspectives on Digital Dentures: A Pilot Study**

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**Objectives:** To assess the knowledge, experience, skill, and interest in digital dentures among third- and fourth-year dental students at a dental school in the southeastern United States. **Methods:** Perspectives on digital and conventional dentures were assessed using an anonymous survey that was distributed to third- and fourth-year dental students (n=156) at the UNC School of Dentistry. Additionally, a group of senior dental students participating in a digital denture pilot program was given the survey (n=11). Students participating in the pilot program were excluded from the broader student cohort. A five-point sliding scale was used to measure responses. **Results:** A total of 35 surveys were completed by third-year students (51.5%; 57.1% men; mean age [SD] 26.2 [2.1] years) and 33 surveys were completed by fourth-year students who were not participants in the digital denture pilot program (48.5%; 57.8% women; mean age [SD] 27.1 [3.0] years). Among the pilot program cohort, 10 surveys were completed (90.9%; 50.0% women; mean [SD] 26.3 [1.7] years). Senior students rated their mean perceived ability to fabricate complete dentures using conventional techniques higher than third-year students. However, neither class felt highly certain with their skill set. Students who participated in the digital denture pilot program reported the highest perceived ability to fabricate complete dentures using conventional techniques. Additionally, they were moderately certain that they could fabricate complete dentures using digital techniques, while those who did not participate in the pilot program rated their abilities with digital techniques as low. Students in all groups indicated a strong desire to incorporate digital denture fabrication techniques into their future clinical practice. **Conclusion:** In response to student interest in digital denture technologies and a need to prepare graduates for increasingly digital practice environments, an opportunity exists to incorporate digital approaches into dental training to enhance student skills in removable prosthodontics and augment curricular offerings.

**[10] Peer-Tutoring Digital Resources Are Used and Accepted in Dental Education**

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**Objectives:** The project aims were to 1) describe the user base of an existing open-source web-based peer-tutoring video set centered on dental education topics and 2) describe current dental students’ knowledge and use of digital peer-tutoring resources. **Methods:** Eighty-nine peer-tutoring videos on dental education topics were produced by a UNC dental student using freely available software and uploaded to YouTube. With a cross-sectional observational study, voluntary anonymous surveys were disseminated to two populations. One survey asked
YouTube viewers about their usage of and satisfaction with the dental peer-tutoring resource. The second survey asked current dental students about use, perception, and satisfaction with peer-tutoring videos and other digital learning resources. Both surveys recorded Net Promoter Score (NPS) for the video set, a validated customer satisfaction tool that measures willingness of users to recommend products or services to others. Dental students were also asked about use of different digital learning resources and the perceived value of these resources. Descriptive statistics were calculated for both surveys. **Results:** Participants included members of the UNC DDS classes of 2019 (37.96%), 2020 (21.9%), 2021 (25.55%), and 2022 (14.6%) (N=137). 26.79% of subjects reported using videos like those available on YouTube to supplement educational resources provided by faculty. NPS demonstrated positive perception of the peer tutoring videos with a score of 54.46. Participants also included YouTube viewers of the peer tutoring video set (N=52). This cohort was comprised of 58.82% dental students or residents, 21.57% dental professionals, 5.88% pre-dental students, 3.92% dental hygiene students, 3.92% dental school faculty, 1.96% dental patients, and 3.92% other. NPS demonstrated positive perception of the peer tutoring videos with a score of 78.26. **Conclusions:** These results demonstrate that dental students use digital peer-tutoring resources, primarily YouTube videos, and find them to be helpful and acceptable learning tools. **Supported by:** UNC DDS Short-Term Research Fellowship sponsored by the School of Dentistry, Dental Alumni, and the Dental Foundation of North Carolina


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**Objectives:** The purpose of this study was to gain an in-depth understanding of the ramifications of the changed Commission of Dental Accreditation standards for pre-doctoral special healthcare needs (SHCN) education. **Methods:** A mixed methods, descriptive study paired one-on-one interviews with administrators at dental schools across the country with surveys of current and recent dental school graduates. Semi-structured phone interviews were conducted with faculty members in various leadership positions in dental education to assess the current state of SHCN in pre-doctoral curricula. The interviews were transcribed verbatim and analyzed using a qualitative descriptive framework. Surveys were sent to recent graduates and fourth year dental students. Survey participants were asked questions regarding their educational background, personal experiences with patients who have SHCN, and their attitudes concerning treatment of patients with SHCN. Descriptive statistics and bivariate analysis were employed. **Results:** Eleven faculty members were interviewed from nine institutions. All participants acknowledged that the bulk of their education in treating patients with SHCN occurred during their post-doctoral training. Participants identified inconsistent SHCN curricular experiences, which was attributed to time constraints in an increasingly crowded curriculum. Survey data from a single institutions’ alumni reveal 55% providers treat five or fewer patients with SHCN per month. Though most alumni work in a practice environment capable of handling this population, 50% endorse financial barriers to seeing more patients with SHCN. The majority of students surveyed plan to refer patients with SHCN to specialists. **Conclusions:** Many practicing dentists treat five or fewer SHCN patients a month and graduating dental students will likely refer these patients to a specialist. The vast majority of providers who treat SHCN patients gained this experience during residency training. Potential barriers to treating SHCN patients include a lack of exposure to SHCN individuals as a pre-doctoral student and financial barriers.
Dental Students’ Perceived Stress is Associated with Psychological Distress and Burnout—their Self-Efficacy is Protective

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Objectives: Dental students experience considerable levels of stress during their training. “Some stress” is inherent in dental education, but prolonged or excessive levels can lead to clinically-manifested psychological distress and eventually burnout, especially in susceptible individuals. In this study we sought to a) test the hypothesis of stress being associated with distress and burnout among dental students and b) determine whether students’ self-efficacy is protective against distress and burnout.

Methods: We used data from a large-scale survey of dental students’ wellness (n=5,520; 69% females; mean age=21 yrs.) conducted in 17 Colombian dental schools. Information on stress was obtained via a 17-item version of the Dental Environment Stress questionnaire, psychological distress using the 10-item version of the Symptom Checklist Revised, burnout using the student version of the Maslach Burnout Inventory, and general self-efficacy using the 10-item General Self-Efficacy Scale. Using structural equation modeling (SEM) we developed measurement models for each construct and examined how stress affects distress and burnout. We used Mplus v.8.1 to estimate standardized coefficients (B) and corresponding standard errors (se) and applied a conventional p<0.05 criterion for hypothesis testing.

Results: The structural model demonstrated acceptable fit: RMSEA=0.038, CFI=0.90, TLI=0.90, and SRMR=0.06. We confirmed the existence of significant direct effects of stress on distress (B=0.93, se=0.03), distress on burnout (B=0.36, se=0.02), stress on burnout (B=0.66, se=0.04), self-efficacy on distress (B=-0.43, se=0.04) and self-efficacy on burnout (B=-0.18, se=0.04); and indirect effects of stress on burnout (B=0.35, se=0.01). Importantly, we found that all these associations were moderated by the protective influence of students’ self-efficacy (p<0.05).

Conclusions: Acknowledging the limitations of the study’s cross-sectional design, our results highlight key pathways underlying dental students’ mental health and psychological morbidity development. Future research should elucidate the temporality of these associations and identify specific approaches for the alleviation of stress and the promotion of self-efficacy in dental education.

A Removable Prosthodontic Laboratory Survey

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Objectives: To assess the current trends for 1. The techniques that dentists are using for removable prosthetics and 2. The quality of communication to their dental laboratory technician. Methods: A ten-question anonymous
response survey was developed and emailed with a cover letter to National Association of Dental Laboratories (NADL). The NADL sent the survey to its list-serve of dental laboratories across the United States. The received responses were handled in Qualtrics software to maintain respondent confidentiality in accordance with IRB protocol. Results were tallied and converted to percentages in order to correspond with similarly published data. **Results:** 52 survey responses were received from dental laboratory technicians. Only 3.6% of the responding technicians felt work authorizations for removable prosthetics were complete enough to provide their best service. Additional findings include that removable partial dentures (RPDs) are typically designed by the technician at the dentists’ request and that most cases are mounted on a plain-line (hinge articulator). Responses also indicated that while most of the technicians felt their RPD impressions were properly extended, the rest seats were typically underprepared. **Conclusions:** Similar to previously published data, dentists tend to trend toward clinical procedures that minimize patient chair time. While the surveyed technicians appear generally satisfied with the quality of work they received, there was a consistent message that communication was not always adequate to fabricate their best work.

[14] Understanding Technology Adoption by Orthodontists: A Qualitative and Quantitative Study

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**Objectives:** Orthodontics is evolving due to advances in 3D imaging, additive fabrication, digital scanning and treatment planning. With digital tools, orthodontic treatment may become more predictable, efficient and effective, while reducing side effects. These technologies are impacting patient care, but knowledge of their adoption patterns and influence is incomplete. We aim to identify adoption decision makers, information sources, perspectives, incentives and barriers. **Methods:** Twenty-four privately practicing orthodontists were interviewed in a semi-structured format following a topic guide. Interview transcripts were analyzed to identify factors in technology adoption and its perceived influence on practice. Thematic patterns were established through iterative systematic analysis, and qualitative validity was ensured by researcher triangulation. After identifying key factors in adoption using interviews, we developed and conducted the first national survey of AAO members on their technology adoption habits; data were assessed using descriptive and bivariate analyses. **Results:** Interviews (n=24) and survey responses (n=270) revealed orthodontists make purchasing decisions independently of staff, after consulting other dentists and company representatives. Meetings, residency training and continuing education courses are influential information sources, while research literature is not. Early and middle adopters are integrating digital imaging, planning and fabrication technologies into practice and view enhanced ease of use, capabilities, performance and procedural efficiency as primary incentives to adoption. Improving outcomes and patient comfort are not cited as primary incentives, whereas all interviewees view cost as the largest barrier. Orthodontists positively perceive the influence of technology on their practices but are concerned that further innovation and direct-to-consumer products will cause loss of market share. **Conclusions:** Orthodontists anticipate broad adoption of intraoral scanning, digital treatment planning, and CAD/ CAM appliances as future standards of care, although there is concern about self-treatment trends and direct-to-consumer products. Understanding the technology adoption process can guide innovation to improve treatment and ease the transition into a digital workflow.
Influence of Clinician Background on Endodontic Surgery vs. Retreatment Recommendations

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Objectives: Endodontists face a uniquely challenging task when planning treatment of infected teeth with prior endodontic treatment. Treatment options endodontists must consider typically include nonsurgical retreatment, apical microsurgery, and extraction. The literature suggests that retreatment and surgical treatment have advantages over one another depending on the etiology of failure and clinical situation. Clinicians may be biased in their treatment planning of these cases based on factors in their training and personal background. By recognizing important clinical factors and potential personal biases, endodontists will be better equipped to make the best treatment recommendation for their patients. The objective of this study is to evaluate for potential factors in a clinician’s background which may bias them toward retreatment or apical surgery.

Methods: A survey was sent out to practicing members of the American Association of Endodontists which recorded information about their background as well as their initial treatment decisions for a given set of previously treated teeth.

Results: Initial results exhibited significant variability in background, practice style and treatment decisions for the teeth provided. Bivariate and multivariate analysis was performed to evaluate the relationship between the treatment planning responses, explanatory variables and covariates.

Conclusions: Factors in a clinician’s background may play important roles in treatment planning tendencies, aside from known prognostic data in the literature. Understanding of potential biases may enable clinicians to more thoroughly evaluate their recommendations in order to make the best recommendations for their patients.

Association of Apical Periodontitis and Diabetes in a Large Hospital Network

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Objectives: Previous studies have shown an association between endodontic infections and type 2 diabetes (T2DM); however, these studies are underpowered, often failing to control for confounding variables. To determine if there is an independent association between apical periodontitis and T2DM in a large data set available from a hospital network database.

Methods: Carolina Data Warehouse for Health (CDW-H) is a central data repository containing clinical, research, and administrative data sourced from UNC Health Care, including both Epic and Legacy hospital systems. An initial search of this database yielded a total of 5,995,011 patients, of whom 7,749 were diagnosed with apical periodontitis (ICD-10 codes K04.4-K04.8) between October 1st, 2015 and September 30th, 2018. These codes include the following diagnoses, acute apical periodontitis of pulpal origin, chronic apical periodontitis, periapical abscess with or without sinus, or radicular cyst. Patient demographics, T2DM, HbA1c%, periodontal disease, oral cellulitis, hypertension, atherosclerosis, renal disease, smoking status, BMI, metformin use, statin use and hospital inpatient data were collected from their most recent visit. A control group of 7,749 patients were sampled from the remaining pool of patients in CDW-H for those without apical periodontitis and matched exactly to the age, race/ethnicity, and sex of each patient in the initial group. Multivariable logistic regression was used to estimate the association between T2DM and apical periodontitis after controlling for the effects of the aforementioned confounders.

Results: T2DM was associated with significantly
greater odds of apical periodontitis (OR=2.0, 95% CI=1.7, 2.4). The use of metformin (OR=0.7, 95% CI=0.6, 0.9) or statins (OR=0.7, 95% CI=0.6, 0.8) was associated with lower odds of apical periodontitis. **Conclusions:** Those with T2DM may have greater odds of having apical periodontitis even after controlling for demographic factors and potential confounding factors. Metformin and statin use may be associated with lower odds of apical periodontitis.

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**[17] The Impact of Study Design on the Association of Endodontic Disease and Cardiovascular Disease**

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**Objectives:** Studies have shown an association between endodontic disease and systemic disease. The strength of the association varies and may depend on the level of evidence of the study. The purpose of this study was to systematically review the available evidence to determine if the study design influenced the strength of the association of endodontic disease and cardiovascular disease. **Methods:** Cohort, cross-sectional, case-control and case control with composite measure studies were included in this review. The primary research question was defined following the PICO format. Search strategies were developed for MEDLINE, Embase, Cochrane library and Scopus. Additionally, the bibliography of relevant articles and textbook chapters were manually searched. Studies identified were independently reviewed by two reviewers, and the full texts of the articles were reviewed, and the data analyzed. **Results:** Among the 1724 articles identified, 31 comprised the final selection. Six were cohort studies, four case control, eight case-control with composite measures, and 13 cross-sectional studies. There was a high degree of heterogeneity among the studies selected. There was consistency in the general trend for association between the two diseases of interest. The available data does not allow to assess the effect of endodontic treatment on cardiovascular disease. **Conclusions:** The association of endodontic disease and systemic disease was consistent across the evidence hierarchy. There may be a more important role for study design that should be investigated in future studies with more control of confounding variables.

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**[18] Periodontal Disease Classification Affects Associations with Systemic Conditions**

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**Objectives:** We tested how periodontal disease classification definitions created using supervised and unsupervised learning systems identify associations with systemic conditions. We compared three periodontal disease classification models: 1) Centers for Disease Control and Prevention & American Academy of Periodontology (CDC/AAP) and the 2) World Workshop Stages and Grades System (WW17), representing supervised learning systems, and the 3) Periodontal Profile Class System (PPC), representing an unsupervised learning system. Supervised learning is a system that uses expert-drawn ‘lines in the sand’ to delineate disease categories. Unsupervised learning is a data-driven method to determine disease categories (reading ‘patterns in the sand’), which produces homogenous groups of people based on a variety of clinical measures. **Methods:** Atherosclerosis Risk in Communities study data were used to compare all three models of periodontal disease
classification. PPC categories were converted to WW17 Stages and are referred to as PPC-Stages. SAS PROC Logistic was used to create adjusted odds ratios for the following systemic conditions: 1) Intima-media arterial wall thickness (IMT > 1mm), 2) Calcified Arterial Plaque, 3) Diabetes Mellitus, 4) Hypertension, 5) High-Density Lipoprotein cholesterol (HDL < 40), and 6) Obesity (BMI > 30). Results: Fully adjusted models for IMT, Calcified Arterial Plaque and Diabetes showed no statistically significant odds ratios for periodontal disease classification models based on supervised learning systems. However, there were significant odds ratios for the classification model based on unsupervised learning systems. Fully adjusted models for Hypertension, HDL and Obesity had significant odds ratios for models created with both supervised and unsupervised learning. Conclusion: PPC-stages appear to be more sensitive in identifying relationships between periodontal disease and systemic conditions. Thus, periodontal definitions created with unsupervised methods allow us to explore in more depth how new disease phenotypes are related to systemic conditions. This understanding may help clinicians make informed treatment decisions.

[19] GWAS for Interleukin-1β Levels in Gingival Crevicular Fluid Identifies IL37 Variants in Periodontal Inflammation

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Objectives: Periodontal disease is a common, dysbiotic inflammatory condition. IL1β has been established as a robust Gingival Crevicular Fluid (GCF) biomarker for a hyperinflammatory phenotype, as well as severe clinical inflammation, bone loss and periodontal disease progression. IL-1β expression has been reported to be strongly genetically controlled. IL-37 is a key suppressor of innate immunity and a master regulator of inflammation at mucosal surface in humans. However, there is no evidence for the genetic control of IL-1β expression by IL-37 in periodontal disease. Methods: IL-37, the major locus associated with high GCF IL-1β levels, were identified by GWAS. IL-37 expression and localization in human gingival tissue were assessed by qPCR and immunohistochemistry. IL-37 variants leading to the expression of IL-1β and more severe periodontal disease were examined by human PBMCs and transgenic mice. Results: Here we report a GWAS for “high” gingival crevicular fluid IL-1β expression among 4910 European-American adults and identify association signals in the IL37 locus. We demonstrate that elevated IL-37 levels are expressed in multiple cells of diseased periodontal tissues. rs3811046 at IL-37 locus (p=3.3x10−22) is associated with severe chronic periodontitis (OR=1.50; 95% CI=1.12–2.00). The minor allele at rs3811046 is associated with increased expression of IL-1β in periodontal tissue due to impaired production of the IL-37 protein. A second signal in the IL37 locus (rs2708943, MAF=0.09, p=4.2x10−7) is associated with severely attenuated IL37 mRNA expression and protein synthesis. Conclusions: Our findings demonstrate two IL37 variants with functional roles in decreased expression of IL-37, leading to up-regulation of IL-1β, constituting a hyper-inflammatory trait associated with periodontitis. Overall, these findings demonstrate the strong association between IL37 SNP and GCF IL-1 β levels. Supported by: NIDCR R01DE023836 (S.O.). T90DE021986 and F32 DE026688 (Y.Z.J.)
[20] Inflammasome Priming in Experimental Periodontitis

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Objectives: Inflammasomes are multimolecular complexes formed after recognition of microbial components for the activation of caspase-1, which leads to the activation and secretion of IL-1β and IL-18. Inflammasome activation occurs in two-steps. The first step is priming, which upregulates inflammasome components, while the second step induces the formation of inflammasomes. The impact of inflammasomes in periodontal disease and its potential for therapeutics is still unclear. The goal of this study was to evaluate inflammasome priming in experimental periodontitis.

Methods: Experimental periodontitis was induced in wild-type (WT) mice aged 8-10 weeks. Suture-silk ligatures were inserted between the 1st and 2nd maxillary molars for 10 days to induce disease. Gingival tissues were collected for mRNA expression analysis by qRT-PCR, and maxillae were analyzed for bone loss by µCT analysis. Statistical significance was determined by student’s t-test. Results: The amount of bone loss for mice subjected to the ligature model is significantly greater compared to controls [0.35 mm (SD= 0.018 mm) vs.0.23 mm (SD= 0.014 mm), p≤ 0.01]. The measurements of the mRNA expressions of critical inflammasome components are significantly higher in the gingival tissue of mice treated with the ligature model. Gingival tissue showed significant increases in expression of Aim2 (2.8-fold), Ifi204 (2.4-fold), Nlrp3 (7.7-fold), and Il1b (~20-fold) as compared to tissue from healthy controls (p≤0.05, p≤0.05, p≤0.01, p≤0.05 respectively). Conclusions: Our data show that inflammasome priming occurs in the ligature-induced periodontitis model. Future studies will determine whether therapies targeting inflammasome components will alter the progression of periodontal disease. Funded by: grant K01DE027087 by the National Institute of Dental and Craniofacial Research (National Institutes of Health) awarded to J.T.M, and through grant KL2TR002490 awarded to K.V.S.

[21] Modulation of Experimental Periodontitis through IFI16

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Objectives: Interferon, gamma-inducible protein 16 (IFI16) and absent in melanoma 2 (AIM2) SNPs are associated with higher levels of periodontal microorganisms and increased extent of periodontal disease. The purpose of this functional study was to evaluate the effect of IFI16 as a modulator of periodontitis and characterize the cellular source of the phenotype. Methods: Periodontitis was induced in wild-type (WT) and Ifi204−/− mice (murine homologue for IFI16) via the ligature model for 10-days. Alveolar bone loss was measured by µCT analysis. In order to verify that monocyte lineage cells could be identified in this model, we made chimeric mice. WT mice were depleted of hematopoietic cells (CD45.1) by irradiation followed by bone marrow transfer (BMT) (CD45.2). The reverse chimera was also created. The gender, age and weight were matched. Animal’s weight was monitored daily for 14 days. After, ligature-induced periodontitis was done. Flow cytometric analysis of the bone marrow was done using CD45 as a hematopoietic marker to determine success of the BMT. Human gingival tissues collected from individuals with periodontitis were analyzed by immunofluorescence for CD14 and IFI16. Results: Ifi204−/− mice
showed a significantly higher mean bone loss compared to WT mice [0.42mm(SD=0.092mm) vs. 0.34mm(SD=0.083mm), p<0.05]. In the BMT experiments, mice showed initial expected body weight loss that was recovered after 14 days. Thirty days after the BMT, 95.3% of the stained and live cells from recipient mice presented the same CD45 as the donor mice. IFI16 was observed in multiple cells of the human gingival tissues. In the gingival inflammatory infiltrate, IFI16 expression co-localized with CD14+ monocytes/macrophages. **Conclusions:** Our data show that Ifi204 modulates experimental periodontitis. Macrophages are a potential cell source of IFI16. The chimeric mouse model will allow studying the role of macrophages in experimental periodontitis. Future studies are necessary to better understand IFI16 in the context of periodontitis.

**[22] Sex Impact of Caspase-1 Mediation of Periodontal Bone Destruction**

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**Objectives:** Activation of inflammasomes activates caspase-1; activation of caspase-1 in-turn induces cleavage, activation, and secretion of the pro-inflammatory cytokines IL-1β and IL-18, both of which are recognized as important mediators of inflammatory diseases. The goal of this study was to determine whether inhibition of caspase-1 results in a decreased periodontal inflammatory response in vitro, and in experimental periodontitis. **Methods:** THP-1 cells pre-treated with vehicle (DMSO) or 10 µg/mL caspase-1 inhibitor (VX-765) for 1 hour were stimulated with periodontal pathogens (Pg A7436, Pg 33277, Fn, and Aa) for 6, 12, and 24 hours. This was repeated with 3-hour LPS priming prior to stimulation with pathogens and pathway inflammasomes (Nigericin, Poly(dA:dT)). Cell supernatants were evaluated for IL-1β and IL-18 levels by ELISA. One day prior to ligature placement, C57BL/6 WT female and male mice (8-10 weeks) were orally administered vehicle (DMSO), 10 mg/kg, or 100 mg/kg VX-765 twice daily. After 10 days, maxillae were collected for µCT bone-loss analysis. **Results:** Caspase-1 inhibition significantly decreased IL-1β and IL-18 levels upon periodontal pathogen stimulation as compared with vehicle (p<0.05). This was also demonstrated when cells were primed with LPS and stimulated with inflammasomes (p<0.01). In experimental periodontitis, baseline µCT measurements indicated that linear ABC-CEJ distance was different between female (0.18mm) and male (0.24mm) age-matched mice. However, a significant decrease in bone-loss was observed only for males given 100 mg/kg of VX-765 (0.10mm; SE=0.02mm) when compared to vehicle (0.20mm; SE=0.03mm). **Conclusions:** Our data show that caspase-1 inhibition inhibits activation of inflammasome by periodontal pathogens in vitro and prevents ~50% periodontal bone-loss in male mice with experimental periodontitis. Further experiments will be performed to understand the influence of sex on caspase-1 bone-loss inhibition, and whether inhibition of caspase-1 activation is a promising therapeutic target.
[23] TRAF3IP3 Negatively Regulates Type I Interferon Signaling by Suppressing TBK1

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Objectives: Type I interferon (IFN-I) signaling is important for antiviral and autoimmune response, and it is subjected to tight control, however molecular mechanisms to tune down these pathways is incompletely understood. A golgi-associated factor, TRAF3-interacting protein 3 (TRAF3IP3), has been found to be crucial for thymocyte development and T regulatory cell functions. However, its role in myeloid cells has not been explored.

Methods: Upon sensing cytosolic viral RNA, retinoic acid-induced gene I (RIG-I) and melanoma-differentiation-associated gene 5 (MDA5) interact with mitochondrial antiviral signaling protein (MAVS) and activate TANK binding kinase 1 (TBK1) to induce IFNs. To determine the role of TRAF3IP3 in IFN-I pathway, IFNB and ISRE luciferase reporter assays were performed in 293T with overexpressed TRAR3IP3. THP-1 cells with TRAF3IP3 deficiency generated by CRISPR/Cas or bone marrow derived macrophage (BMDM) with Traf3ip3 deficiency were challenged with cytosolic poly(I:C), 5’ ppp-dsRNA, or vesicular stomatitis virus (VSV) infection. Scramble THP-1 or BMDM from wildtype (WT) mice were used as controls. For in vivo, WT or Traf3ip3 deficient mice were infected with VSV via i.p. infection. The mice were monitored for two weeks. Western blot, RT-PCR, ELISA, Co-IP, confocal microscopy, and plaque assay were performed determined the role of TRAF3IP3 in viral RNA triggered IFN-I signaling pathway.

Results: We find that the overexpression of TRAF3IP3 suppressed cytosolic poly(I:C), 5’ ppp-dsRNA, and vesicular stomatitis virus (VSV) triggered IFN production, whereas deficiency of Traf3ip3 potentiated viral RNA triggered IFN production. In support of the in vitro data, Traf3ip3-deficient mice were infected with VSV and found to exhibit enhanced susceptibility to VSV challenge. Mechanistically, TRAF3IP3 interacted with TBK1 and targeted TBK1 for ubiquitination and degradation.

Conclusions: These results uncovered a previously unrecognized role of TRAF3IP3 in the regulation of RNA induced IFN pathway.


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Objectives: Nitric oxide (NO) is a positive modulator of osteogenesis shown to increase bone formation. Local controlled release may result in enhanced bone healing. Objective: Evaluate the effects of NO releasing small molecules diethylenetriamine (DETA) and spermine on osteogenesis in an MC3T3-based in vitro model. Methods: MC3T3-E1 Subclone-4 cells were cultured in media containing 1 or 50 μM DETA or spermine (NO releasing (+NO)) and polymer only controls (-NO)) and tested for cytotoxicity (4 and 24-hours, n=6), proliferation (days 3-9, n=6), calcium accretion (alizarin-red (n=2) and calcium quantification (n=4), days 21 and 28), collagen organization by picrosirius red (PSR, days 14 and 21, n=3), and PCR for osteogenic markers RUNX2, COL1α1, BSP, OSX, and OCN. Statistical significance: One-way ANOVA Bonferroni post-hoc analysis. Results: DETA and spermine (+ NO) were not toxic at 1 μM for 4 and 24-hour timepoints. Spermine was toxic at 50 μM and this dose was discontinued for spermine. DETA (+ NO) was not toxic at 50 μM. DETA +NO led to a small decrease in proliferation at 50 μM, no
change in proliferation was detected at 1 μM for DETA and spermine (+ NO). Alizarin staining and calcium quantification demonstrated spermine itself led to increased calcium, with a further increase with +NO release. DETA itself did not increase calcium but did with +NO release. PSR data agreed and showed an increase in red signal (more mature collagen) for spermine, with a further increase with +NO, and an increase in red for DETA (+NO only). PCR data showed increases in COL1α1, OSX, and OCN related to DETA and spermine themselves, and not addition of NO release. **Conclusion:** We have demonstrated that DETA and spermine alone affect osteogenesis, with further increases when engineered to release NO. These results are promising for development of treatments for bone restoration using NO releasing small molecules. **Funding Source:** UNC-CH

[25] **Pre-clinical Model of Paget’s Disease Reveals Interferon-beta as a Therapeutic Target**

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**Objectives:** Paget’s disease of the bone (PDB) is the second most common bone disease and is characterized by focal areas of increased osteoclastogenesis and bone turnover in older adults. Recent studies have revealed that Optineurin (*OPTN*) is genetically linked to PDB, but less than 10% of *Optn* mutant knock-in mice developed PDB. In this study, we aim to generate a highly penetrant PDB mouse model using an *Optn* knockout (KO) strategy that will allow us to uncover the molecular mechanisms by which *Optn* regulates osteoclastogenesis. **Methods:** We generated *Optn* global deficient (*Optn*−/−) mice. Axial bones collected from 3, 8, 12, 16 and 22 months old *Optn*−/− and WT mice were phenotyped. **In vitro** osteoclastogenesis and osteogenesis were performed. To dissect the molecular mechanisms of how *Optn* loss contributes to PDB, we profiled the gene expressions of *Optn*−/− and WT osteoclasts using Affymetrix GeneChip arrays. **In vitro** rescue experiments were performed with recombinant Interferon-beta. **Results:** 100% aged *Optn*−/− mice exhibited polyostotic localized osteolytic and mixed lesions in their axial bones. While loss of *Optn* does not affect **in vitro** osteogenesis, differentiation of primary osteoclasts **ex vivo** revealed that *Optn* deficiency results in an increased osteoclastogenesis. Microarray reveals that *Optn*−/− osteoclasts displayed a significantly decreased type I interferon (IFN) signature. q-PCR or western blot showed that *Optn*−/− osteoclasts produced significantly lower levels of Interferon-beta, a negative regulator of osteoclastogenesis, and displayed defective IFNa/bR signaling. Furthermore, *Optn*−/− osteoclasts were resistant to cell death. Exogenous Interferon-beta treatment completely reduced the hyper-differentiation and cell survival advantage observed in *Optn*−/− osteoclasts. **Conclusions:** We established a penetrant PDB preclinical model that recapitulated key features observed in PDB patients, and our results indicate that interferon-beta is a potential therapeutic target of PDB. **Funding:** NIH/NIDCR R01DE022816; R90DE022527; NIH Intramural Research Program 1ZIAES10328601; NIH/NEI K08EY021520

[26] **Mini-pig Implant Intra-oral Model: Peri-implant Bone Characteristics**

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Objectives: The mini-pig has become the animal model of choice for pre-clinical studies investigating new implant technologies. Aim to describe the peri-implant bone characteristics of the mini-pig implant intra-oral model, according to intra-oral distribution and healing period. Methods: Twenty-four Yucatan mini-pigs (20–24 months old) received four dental implants (NobelActive™ ø3.5 x 10.0mm CC NP) connected to multi-unit abutments (NP 2.5 mm) and healing caps (Ti ø5.0 × 4.1 mm). Animals were euthanized at 3, 6, and 13 weeks after implant placement. MicroCT and histological analyses were performed. Generalized estimating equations, accounting for clustering of observations within animals, were used to estimate parameters according to implant site, implant position in the arch, and healing period. The significance level was set at 5%. Results: For the microCT analysis, marginal bone loss was significantly higher on I2 and I3 than I1; no significant differences were observed between buccal and lingual sites. Bone volume density and trabecular thickness significantly increased from 3 to 6 weeks, leveling off afterwards. Trabecular spacing was significantly lower at 13 weeks compared to 3 weeks. For the histological analysis, no statistically significant differences were observed for osseointegration according to site, implant position, or healing time. Bone density within the implant threads and immediately outside the implant threads was significantly higher at posterior than anterior sites and a significant increase in density over time was observed. The first bone-implant contact was significantly smaller at I2 and I3 than I1. Conclusions: The mini-pig implant intra-oral model appears adequate to investigate the effect of new implant technologies on the peri-implant bone. Judgment should be exercised when considering intra-oral distribution of the implants and healing periods.


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Objectives: Long-term functional and aesthetic success of dental implants depends on the integration of the surrounding soft tissue. Recently, the minipig intra-oral model has become the preferred animal model to investigate new implant technologies; however, the model is not well described and very few studies have evaluated the peri-implant soft tissue characteristics. Aimed to describe the peri-implant soft tissues characteristics of the minipig implant intra-oral model according to intra-oral sites and healing period. Methods: Twelve Yucatan minipigs (16–19 months old) received three dental implants (NobelActive™ TiUnite™ ø3.5 x 10.0mm CC NP) in each jaw quadrant. Anodized or machined titanium abutments and titanium healing caps were connected to the implants for transmucosal healing. Animals were euthanized at 6 and 13 weeks. Generalized estimating equations accounting for clustering of observations were used to estimate soft tissue parameters according to site, implant position, and healing period. Results: Greater soft tissue volume was observed at buccal than lingual sites. Soft tissue attachment to the abutment, including epithelium and connective tissue, was frequently observed. In the multivariable analysis, mucosal height and epithelium length were significantly higher on buccal than lingual sites (p<0.05), and on posterior than anterior implants (p<0.05). Healing period did not significantly affect these parameters. Inflammation scores at the peri-implant mucosa margin increased significantly over time, and it was significant higher on buccal than lingual sites (p<0.05); no significant differences were observed between anterior/posterior sites. Inflammation scores at the platform level were significantly higher at buccal than lingual sites, no other factors significantly affect the inflammation scores. Conclusions: The minipig implant intra-oral model appears appropriate to investigate the effect of new implant technologies on the soft tissues. Intra-oral distribution of the implants and healing time should be considered when designing studies.
[28] Volumetric Analysis of Hard Tissue Healing after Tooth Extraction and Socket Grafting

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Objectives: The main objective of this study was to evaluate clinical and radiographic bone volume changes following socket graft with a 3D-collagen matrix, compared to socket graft with the use of a collagen dressing.

Methods: This randomized controlled clinical trial recruited 24 subjects into 2 groups: 1) Extraction treated with xenograft bone substitute (BioOss Collagen®) + collagen dressing (HeliPlug®), 2) Extraction treated with xenograft bone substitute (BioOss Collagen®) + 3D-collagen matrix (Mucograft Seal®). Subjects received a Cone beam computed tomography (CBCT) prior to extraction for the baseline hard-tissue volume measurement, and at 6-month post-extraction CBCT for volumetric measurement. Hard tissue analysis was performed to compare linear ridge remodeling and buccal volume changes. CBCT images were segmented and reconstructed (Invesalius®) prior to being analyzed by non-contact reverse engineering software (Geomagic - Control®). Results: Results derived from the linear ridge remodeling analysis demonstrated that the use of a 3-D collagen matrix lost an average of 2.17mm, 1.85mm, and 1.63mm at 1mm, 3mm, and 5mm below the buccal bone crest, respectively. The use of a collagen dressing showed a reduction average of 1.75mm, 1.25mm, and 0.85mm at 1mm, 3mm, and 5mm below the buccal bone crest, respectively. The volumetric analysis demonstrated that the use of a 3-D collagen matrix lost an average of 72.65mm³ compared to 60.93mm³ found in the collagen dressing group. There were no statistically significant differences between the groups. Conclusions: This human investigation provides early evidence of the volumetric bone changes after tooth extraction and socket grafting. The results from this study demonstrated no significant difference between the use of 3-D collagen matrix and collagen dressing regarding the amount of linear and volumetric remodeling of buccal bone. Funding source: Geistlich, Inc.; NIH/NIDCR K23-DE025093

[29] Bitewing Dosimetry of 3D Intraoral Tomosynthesis Dental X-ray Imaging System

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Objectives: There is substantial evidence for a cumulative dose related response to ionizing radiation in the form of cancer developing years after initial exposure. Therefore, this study focused on effective dose, a quantity with direct correlations to biologic risk from dental x-ray exposures. The purpose of this study was to measure doses and calculate effective doses (E) resulting from exposure parameters that are used for intraoral tomosynthesis and conventional imaging for adult posterior bitewing exams of the dentition. Additionally, this study sought to evaluate the effect of sensor attenuation on patient dose. Methods: To meet these aims, a human tissue equivalent adult phantom and optically stimulated luminescent (Landauer, Inc., Glenwood, IL) dosimeters were used to measure dose produced during simulated posterior bitewing examinations (n=4). Optically stimulated luminescent dosimeters were used to measure x-ray dose at 24 head/neck tissue sites of interest. Dosimetry was acquired using a tissue equivalent phantom simulating the anatomy of an average adult male (Atom Max Model 711 HN, CIRS Inc., Norfolk, VA). Exposures parameters used were 70 kV/7mA (0.7mAs & 0.12mAs) for s-IOT and conventional (KaVo FOCUS®, Charlotte, NC), respectively. ANOVA and Tukey HSD statistics on dose were utilized to demonstrate significant data relationships. Results: Sensor-present doses were significantly lower than sensor-absent for all modalities(p=0.0001). Significant differences in E were found for all modality combinations with the exception of s-IOT sensor-present vs. conventional rectangular sensor-absent(p=0.0482).
Effective Dose by Modality (µSv)

<table>
<thead>
<tr>
<th>Modality</th>
<th>Dose (µSv)</th>
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<tbody>
<tr>
<td>Rectangular Conventional (with) Sensor</td>
<td>1.1</td>
</tr>
<tr>
<td>Rectangular Conventional (without) Sensor</td>
<td>4.6</td>
</tr>
<tr>
<td>S-IOT (with) Sensor</td>
<td>5.9</td>
</tr>
<tr>
<td>S-IOT (without) Sensor</td>
<td>11.9</td>
</tr>
<tr>
<td>Circular Conventional (with) Sensor</td>
<td>8.2</td>
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<td>Circular Conventional (without) Sensor</td>
<td>15.7</td>
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**Conclusions:** Unadjusted s-IOT dose was 26% less than conventional-circular and 61% greater than conventional-rectangular, for sensor-absent exposures. Unadjusted, sensor-present s-IOT dose was 28% less than conventional-circular and 81% greater than conventional-rectangular. Despite a four-fold increase in mAs for s-IOT imaging compared to conventional, $E$ from s-IOT imaging was at least 26% less than the current most commonly implemented bitewing technique, conventional-circular, while providing substantially greater diagnostic yield in the form of 3-D information.

[30] 3D Maxilla and Defect Quantification Using Automatic Segmentation for Patients with Unilateral Cleft Lip and Palate

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**Objectives:** Accurate quantification of the complex 3D cleft defect structure is key for optimal treatment planning and patient outcomes. For a condition which can seriously affect quality of life, formulating a universal system for accurate quantification of each unique defect would prove incredibly beneficial. The aims of this study were to automatically segment cone-beam computed tomography (CBCT) images of non-syndromic unilateral cleft lip and palate, and characterize maxilla and defect 3D structural parameters underlining maxilla and defect relationship.

**Methods:** CBCT images were acquired from 60 patients presenting unilateral cleft lip and palate. ITK-SNAP software was used for 3D imaging analysis. Advanced machine learning techniques were applied for sophisticated automatic segmentation of the maxilla and defect. To fully characterize the defect, the distribution probability was mapped from superimposed 3D models, paired t tests were performed for statistical analysis, and a multiple linear regression was completed. **Results:** The maxilla and defect were successfully auto-segmented from surrounding bony structures in a time-effective manner and with morphologically similar features to those obtained via manual segmentation. The defect side demonstrated a significant decrease in maxillary length, anterior width, and volume with mean measurements of $34.31±2.56mm$, $17.83±2.06mm$, and $21.26±3.33×10^3mm^3$, and an increased maxillary anterior height with a mean of $25.91±4.12mm$, respectively, as compared to the non-defect side. Defect superimposition displayed a concentrated distribution near the alveolar bone region and anterior maxillary structures appeared to contribute to defect variability. **Conclusions:** Successful and accurate 3D defect models were obtained, with structural parameters defined and quantified, to achieve an enhanced understanding of non-syndromic unilateral cleft lip and palate with potential for widespread future clinical applications.
[31] Alveolar Bone Grafting Success in Clefts – A 5 Year Study

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Objectives: The primary aim of this study was to compare radiographic success of alveolar bone grafting in the cleft population from 1.1.2012 to 12.31.2016 at the University of North Carolina at Chapel Hill. The secondary aim was to investigate factors contributing to the radiographic success of alveolar bone grafting in cleft patients.

Methods: The sample is comprised of 126 patients (59 male, 67 female) who received alveolar bone grafting to repair a novel cleft at the UNC-CH Craniofacial center. The patients age range at the time of graft was 6-56 years, with a mean of 11 years and 3 months. A total of 169 cleft sites were assessed and were comprised of 53 left unilateral, 29 right unilateral, and 44 bilateral clefts. Information regarding the presence of lateral incisors, supernumerary teeth, years of surgeon experience, canine height, root development, and canine angulation was gathered. Alveolar bone grafting success was determined based off the Kindelan index by two assessors who were blinded from the patient demographics, surgical procedures, and surgical experience.

Results: According to pre-graft data, there was a statistical significance (p<0.05) involving graft success based upon the presence of a permanent lateral incisor in the cleft site. Additionally, the pre-graft data displayed a statistically significant (p<0.05) correlation regarding canine height and success, with the success group demonstrating a canine location that is 12% higher than the failure group relative to the occlusal table.

Conclusion: This retrospective study provides valuable insight into the factors that can predict success in cleft patients receiving alveolar bone grafting. The results demonstrate that the presence of a permanent lateral incisor and a higher location of a permanent canine in the cleft site can provide higher success rates in alveolar bone grafting on novel cleft sites. Supported by: UNC School of Dentistry DDS Short-Term Research Fellowship

[32] Association between Dental Characteristics and Sleep Disordered Breathing in Patients with a Dentofacial Disharmony

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Objectives: The purpose of this study was to investigate the association between dental characteristics and sleep disordered breathing (SDB) in patients with a dentofacial disharmony (DFD).

Methods: 138 subjects between the ages of 12 and 60 who have a skeletal or dentofacial disharmony severe enough to warrant a referral for orthognathic surgery workup were invited to participate. The subject’s weight, height, and neck circumference were recorded. Intraoral measurements included dental classification, overjet and overbite in millimeters. Subjects completed two self-administered validated questionnaires: Pittsburg Sleep Quality Index (PSQI) and the Functional Outcomes of Sleep Questionnaire (FOSQ) to determine their perception of their sleep quality and effects of sleep on daily activities. Total scores and clinical screening criteria were calculated for each questionnaire. Bivariate and regression models were fitted to assess the relationship between age and the dental clinical factors and the indicators of sleep quality and daytime sleepiness.

Results: Of the 138 subjects, 59% were female and the average age was 18.5 (SD = 6.9). 9% of the subjects were Class I, 30% Class II, and 61% Class III. The average overjet was 1.2mm (SD = 5.7) and overbite was 1.3 (SD = 3.7). 63% self-reported having poor sleep quality and 38% reported poor daily functioning. There were no statistically significant relationships between age, overjet, or skeletal class and the clinical indicators of good sleep quality or good daily functioning. (P>0.10) Good sleep quality and daily functioning were related to overbite. The odds of having good sleep quality was 1.11 times (95% CO: 1.01;1.22)
and 1.12 times (95%: 1.02; 1.24) greater for good daily functioning with one-point increase in overbite.

**Conclusions:** Patients with a dentofacial disharmony severe enough to warrant an orthognathic surgery consultation should be screened for SDB as an important consideration in planning surgery.

[33] Sleep Quality in Patients with Dentofacial Disharmonies Requiring Orthognathic Surgical Intervention

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**Objectives:** To determine the prevalence of poor-quality sleepers and those with poor daily functioning in a population of patients diagnosed with dentofacial disharmonies before and after orthognathic surgery. **Methods:** One hundred thirty-nine patients, diagnosed with dentofacial disharmonies severe enough to warrant orthognathic surgery were enrolled in this study (IRB # 14-1132). Subjects completed the Pittsburgh Sleep Quality Index (PSQI) and the Functional Outcomes of Sleep Questionnaire (FOSQ). Total FOSQ and PSQI scores as well as percentages based on published clinical cut points were calculated. Descriptive statistics and confidence intervals were calculated for total scores and percentages of those with poor sleep quality and poor daily functioning.

**Results:** Fifty-nine percent of the subjects were female, and the average age was 18.5 (SD = 6.9%). The median total score for the PSQI was 5 (95% CI: 5, 6) and for the FOSQ, the median total score was 18.57 (95% CI: 18.28, 18.83). Based on the published cut point of >5 for the PSQI, 88 of the subjects (63%; 95% CI: 0.55, 0.71) reported poor sleep quality (1,2,3). For the published cut point for the FOSQ (<18) indicating greater effect of sleepiness on daily life, 52 subjects (36%; 95%CI: 0.28, 0.45) reported diminished daily functioning. **Conclusions:** A substantial proportion of patients planned to undergo surgical correction of dentofacial deformities reported poor sleep quality prior to surgery. Clinicians should carefully consider the impact of the planned surgical movements on the airway especially for those patients who report sleep disordered breathing.

[34] Orthognathic Speech Pathology: Evaluating the Effect of Class III Jaw Discrepancies on Disordered Speech

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**Objectives:** Patients with severe jaw disproportions seek orthodontic care and orthognathic jaw surgery to address issues with function, esthetics and speech. Studies show that speech concerns surpass impaired chewing function as a motivator for jaw surgery. Pathologic speech impedes communication, profoundly impacting quality of life, but existing data regarding the relationship between jaw discrepancies and speech are qualitative assessments of small sample sizes. We hypothesized that deviations from normal central frequencies of consonant sounds correlate with severity of anterior-posterior and jaw disproportions. **Methods:** To provide quantitative data to test our hypothesis, 36 patients with dentofacial deformity and 15 matched reference individuals were recruited to this study. All subjects were examined by an orthodontist for dental measurements, then qualitatively assessed by a speech pathologist and audio recorded while speaking a series of words. **Results:** Patients pronounced words that required them to make sounds against oral structures such as the teeth and palate, which are likely to be impacted by skeletal disproportions that manifest as underbite or open bite. The recordings were quantitatively analyzed to measure sound frequency distortions. **Conclusions:** Spectral moment analysis found a shift in /tʃ/ and /t/ central frequencies relative to controls; perceptual analysis indicated 81% of Class III subjects produced abnormal dentalized sounds compared to 8% of controls. These findings provide critical insight into the complex interplay
between craniofacial and vocal structures and lay foundational knowledge for exploring how treatment of dentofacial deformities may impact speech-sound disorders.

[35] Effects of Malocclusion Factors on Extraction Thresholds in Orthodontics

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Background: We hypothesize that there exists a threshold (cut-off) of overjet, overbite, crowding to empirically determine whether or not to extract. Objectives: (1) To define cut-offs for the predisposed factors that influence the extraction decision. (2) To create two prediction models to aid in this decision. Methods: Using the data of 2,814 patients, sensitivity and specificity analysis was used to determine extraction cut-offs for each factor. Two prediction models, dichotomous tree and logistic regression, were built to draw a decision rule by incorporating these cut-offs. Results: The cut-offs are 4.5mm (overjet), 3.5mm (overbite), 6.5mm (upper crowding), and 5.5mm (lower crowding). For the decision tree, a composite score <1.5 has a low probability of extraction (16%), while a score >2.5 has a high probability (80%). For the logistic regression formula, the extraction cut-off was 36%. Conclusion: These predictive models are a useful tool to be used in extraction decisions. Supported by: NIH/NIDCR R01DE022816, Southern Association of Orthodontics Research Grant 2018

[36] Effect of Bracket Type and Wire Dimension on Orthodontic Alignment: A Randomized Clinical Trial

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Objectives: We hypothesize that bracket type and wire dimension can influence the rate of orthodontic tooth alignment. Methods: To test this hypothesis, 49 patients were enrolled in a randomized, prospective clinical trial to analyze the effect of bracket type and wire dimension on orthodontic leveling and aligning. Participants were allocated into 1 of 4 groups: twin brackets with either .014” or .016” CuNiTi wire, or self-ligating (SL) brackets with either .014” or .016” CuNiTi wire. Intraoral scans taken at baseline and at 6 and 12 weeks after bonding were analyzed. Little Index measurements and superimpositions were completed to determine changes in alignment as well as translation and rotation of the teeth in 3D. Results: Partial analysis of the incisal rotation data (10 subjects with twin brackets and 5 with SL brackets) suggest that .014” wire and twin brackets yielded greater mean derotation (6.0+/−1.7 and 5.4+/−2.3 deg, respectively) than .016” wire and SL (3.8+/−1.9 and 3.7+/−2.7 deg, respectively). Conclusions: While .014” CuNiTi wire and twin brackets yielded greater mean derotation than .016” wire and SL brackets, the difference was not statistically significant. Complete data analysis will increase the study power and include Little Index measurements to analyze efficiency of alignment. Supported by: Southern Association of Orthodontics Research Grant
[37] Accelerated Aligner Treatment with Vibrations: Molecular Mechanisms of Tooth Movement

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Objectives: Previous clinical trials suggest orthodontic patients assigned to an accelerated aligner schedule and patients using vibration devices will experience accelerated tooth movement. However, very few studies exist describing the bone metabolism induced by these treatment modalities. Bone remodeling is controlled by a balance between resorption and formation activities, which are regulated via TNFSF11 binding and other cytokines. Gingival crevicular fluid (GCF) analysis is a noninvasive, practical sampling procedure to observe molecular mediators. The purpose of this study is to investigate the relationship between the levels of IL1B, IL6, TNFA, and TNFSF11 expression in accelerated Invisalign® in conjunction with AcceleDent® treatment compared to standard Invisalign treatment time. We hypothesize that accelerated Invisalign® plus AcceleDent® application can synergistically accelerate tooth movement without causing an adverse bone remodeling response. Methods: Eight patients treated in the UNC Graduate Orthodontic Clinic were recruited (UNC-IRBH 216-0167). Inclusion/exclusion criteria were applied. Three groups were randomized into one 2-week and two 4-day aligner wear schedules with one 4-day group receiving AcceleDent® treatment. Non-invasive sampling of GCF was done at baseline, 4-days and 2-weeks. GCF samples were processed using the Multiplex Luminex Platform. IL1B, IL6, TNFA, and TNFSF11 concentrations in GCF are expressed as log values. Results are expressed as the General Linear Model correlation and p-value. Results: This preliminary data shows no significant difference in levels of cytokines or TNFS11 at compression or tension sites for tooth movement between accelerated and accelerated coupled with AcceleDent® treatment groups compared to controls at baseline, 4-days or 2-weeks. Conclusions: The biological effects of accelerated Invisalign® and AcceleDent® application have not been determined via molecular biomarkers due to the current small sample size. Supported by: AADR Student Research Fellowship (SHL).

[38] Salivary Protein Biomarkers associated with Orthodontic Tooth Movement

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Objectives: Orthodontic tooth movement can have an effect through changes in salivary proteins. Thus, saliva can theoretically provide a non-invasive means to measure effectiveness and physiological effects of orthodontic tooth movement. We applied a systematic review to analyze current evidence on salivary biomarkers associated with orthodontic tooth movement identified by mass spectrometry proteomics and other protein detection techniques. Methods: Literature search was done using PubMed, EMBASE, and Web of Science databases, through the 15th of November 2018, using the following keyword combinations: “saliva”, “protein”, and “orthodontics”; “saliva”, “tooth movement”, and “proteins”; “saliva”, “proteomics” and “orthodontics”; “saliva”, “proteomics”, and “tooth movement”; “orthodontics” and “salivary biomarkers”; “tooth movement” and “salivary biomarkers”; and “tooth” and “movement” and “saliva” and "biomarkers”. Review articles, opinions, case reports, letters to the editors, news, technique articles or non-human studies were excluded. Only studies using human saliva with orthodontic treatment and saliva sample analysis were included and then selected for full-text review. Results: Out of 482 articles screened, 7 studies were selected. Sample size ranged from 3-72 subjects. Unstimulated whole saliva
sample collection protocol was used. Most studies used either mass spectrometry proteomics or ELISA. Twenty biomarkers were identified as associated with orthodontic tooth movement. **Conclusions:** Salivary proteins in the near future may be used to develop a non-invasive tool for measuring effectiveness of orthodontic treatment and orthognathic treatment as well as adverse orthodontic treatment consequences, such as root resorption.

[39] Development of primary craniofacial muscle-derived cell cultures for applications in craniofacial tissue engineering

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**Objectives:** Reconstruction of soft tissue defects secondary to orofacial cancer resection remains a challenge. Craniofacial soft tissue engineering exists as a promising therapy. Identification and modulation of the ideal cell population is key. This study determined the optimal culture methods for rat primary craniofacial muscle-derived cells (MDC) for utilization in tissue engineering applications. **Methods:** Rat cell cultures were established using three techniques: 1) explant culture; 2) 0.1% collagenase digestion; and 3) digestion followed by cell pre-plating. Population doubling times (PDTs) were calculated. qRT-PCR determined gene expression levels of Pax7, a satellite cell marker, and the myogenic regulatory factors MyoD and MyoG. Explant MDCs were assessed for myogenicity over a 14-day period. Thereafter, explant MDCs were cultured with either IGF-1, a positive regulator of muscle growth, or a GDF-8 blocking peptide, an inhibitor of muscle growth. **Results:** PDTs were similar between culture techniques. Pax7 expression was present at passage 0 and absent by passage 6 for all cultures. Digest and pre-plate methods demonstrated 5- and 14-fold upregulation, respectively, compared to explant cultures. During the 14-day explant MDC culture period, MyoD expression peaked at day 7 and MyoG appeared at day 10. Expression of both genes decreased by day 14. Explant cultures treated with exogenous IGF-1 or GDF-8 blocking peptide expressed MyoD earlier than the control. **Conclusions:** The digest and pre-plate techniques were superior for supporting rat primary craniofacial MDCs. Explant MDCs still had potential to differentiate into muscle fibers. The addition of IGF-1 or GDF-8 blocking peptide resulted in earlier commitment to a myogenic lineage for this cell population. **Supported by:** UNC Oral and Craniofacial Health Sciences

[40] Lrig1 Marks a Quiescent Stem Cell Population in the Oral Cavity

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**Objective:** The oral cavity is lined by a diverse array of oral mucosal epithelia (OE) that are uniquely adapted for feeding, speech, and protection from the environment. In the gastrointestinal tract, it is believed there are at least two stem cell (SC) pools: 1) those active during maintenance and 2) a reserve population, which infrequently divide and are uninvolved in homeostasis. However, whether oral epithelial stem cells (OESCs) contain subpopulations and how they are regulated remains unknown. **Methods:** We adopted genetic label retention approaches to identify label-retaining cells (LRCs) using two distinct promoters (Krt14 and Krt5) to drive expression of a doxycycline-
regulable histone-GFP, which is diluted as cells divide during a variable chase period. We complemented these assays with 1) *in vivo* immunofluorescence-based proliferation assays 2) FACS/qPCR, 3) lineage tracing (*K5-; K14-creER*), 5) genetic knockout (KO) mice, 4) diet modification, and 6) mechanical wounding assays. **Results:** We found that, of three diverse OE regions, palatal epithelium is unique in displaying marked proliferative heterogeneity by short- and long-term lineage tracing. Palatal epithelium contains a previously uncharacterized, infrequently-dividing OESC population that resides within a specialized niche, the junctional zone. These OESCs display a tendency to self-renew by symmetric divisions, respond to masticatory stresses, and promote wound healing. We isolated these reserve OESCs by FACS and performed RNAseq on GFP HI and GFPlo populations. GFPhi OESCs expressed higher Lrig1, a pan-Egfr/ErbB negative regulator. Lrig1 HI OESCs are enriched in the junctional zone and are inversely correlated to Lrig3/Egfr expression. Furthermore, Lrig1 is enriched in this population and is essential for maintaining quiescence as assessed by Lrig1 KO. **Conclusion:** Collectively, these data suggest that the hard palate harbors OESCs reminiscent of reserve stem cells and that it is an exception to the accepted single progenitor model for stratified epithelia. **Supported by:** 1K08DE026537-01 (KMB), Center for Gastrointestinal Biology & Disease (supported by P30 KD034987; SEW), Kimmel Scholar Award SKF-15-165 (SEW), P50 CA095103 (RJC), R35 CA197570 (RJC), R35 DE026602 (ODK).

**[41] Hav 3cd Protease Can Cleave Sdme and Shift Gsdme-Induced Pyroptosis to Apoptosis**

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**Objectives:** To explore the mechanism of HAV proteases cleaving GSDME. Pyroptosis is characterized as gasdermin-mediated programmed necrotic cell death. The N terminal of gasdermin (GSDM) can generate pores on the cell membrane, which results in the cell swelling, burst, release of cellular contents and triggers inflammation. Currently, we found that GSDME, an understudied member of GSDM, can be cleaved by HAV proteases 3CD and 3C, mutants of which lose the ability to cleave GSDME. Further, we found the cleavage residue of HAV 3CD protease on GSDME, mutant of which would escape the cleavage of HAV protease. More excitingly, we observed that HAV 3CD protease can prevent GSDME being cleaved by caspase-3 and reverse pyroptosis into apoptosis.

**Methods:** Western blot, TNF treatment, LDH essay. **Results:** 1. GSDME is cleaved in HAV infected liver cells. 2. HAV 3CD and 3C protease can cleave GSDME, but not other members in the family. 3. HAV 3CD mutant cannot cleave GSDME. 4. HAV 3CD protease can prevent GSDME being cleaved by caspase-3 and reverse pyroptosis into apoptosis. **Conclusions:** HAV 3CD protease can cleave GSDME and shift pyroptosis into apoptosis.

**[42] Determining Sox10’s Potential to Induce Potency in Adult Epithelial Salivary Gland Duct Cells**

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**Objectives:** Every year, over half a million head and neck cancer patients are treated with radiotherapy, which results in the severe dry mouth syndrome, xerostomia, due to co-radiation of healthy salivary glands. Poor quality of life in such patients is a result of conditions associated with xerostomia, such as hyposalivation, dental caries, fungal infections, decaying teeth, and masticatory dysfunctions. Current therapies to rescue hyposalivation only provide temporary relief, thus new therapies for permanent tissue repair are needed. Our lab has extensive expertise in salivary glands’ stem cell research, and was the first to demonstrate the clinical potential of KIT+ (c-Kit, CD117) stem/progenitor cells in rescuing hyposalivation. Our current work shows that transcription factor SOX10 plays a major role in the proliferation and differentiation potential of fetal KIT+ cells. Interestingly, forced overexpression of Sox10 also increased the differentiation potential of an adult mouse epithelial duct cell line *in vitro*. However, it is
still unclear whether Sox10, similar to the fetal stage, is capable of inducing self-renewal and potency in normal adult duct cells. Based on our previous data, we hypothesize that Sox10 can induce potency in adult duct cells. **Methods:**
To test this hypothesis, we will utilize well-established *in vitro* self-renewal assays to score cell proliferation and stemness in adult ductal epithelial cells. A cell derived from the adult epithelial duct cells, named SIMS, will be utilized to test the hypotheses. These experiments will be repeated in primary epithelial salivary cells. **Results:** In short-term self-renewal assay, Sox10 overexpression in SIMS cells results in a larger spheroid formation. Our current efforts are invested in determining self-renewal over multiple passages. **Conclusions:** Completion of these aims will substantially improve our understanding of Sox10’s function as a master regulator and its capacity to alter stemness in epithelial duct cells. Future objectives could be targeted at applying Sox10 overexpression in clinical settings to rescue radiation-induced hyposalivation. **Supported by:** F30, R01DE027034

### [43] Differential Expression of PD-L1 in Proliferative Verrucous Leukoplakia Lesions

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**Objectives:** Upregulation of program cell death ligand 1 (PD-L1) in some cancers allows immune surveillance evasion and proliferation of the cancer cells. Expression of PD-L1 is currently utilized as a biomarker for treatment with anti-program death 1 (PD-1) drugs in certain cancers. Thus far, investigation of PD-L1 expression in precancerous conditions has been minimal. We will explore the expression of PD-L1 in precancerous lesions from patients with proliferative verrucous leukoplakia (PVL), a precancerous condition with high malignant transformation rate. **Methods:** The archived residual formalin-fixed, paraffin embedded tissue of oral mucosal biopsies diagnosed as hyperkeratosis, dysplasia, verrucous hyperplasia, and carcinoma in-situ from patients with PVL were retrieved from the University of North Carolina at Chapel Hill School of Dentistry Oral and Maxillofacial Pathology Service. The specimens were divided into low-risk lesions (hyperkeratosis, low-grade dysplasia, verrucous hyperplasia) and high-risk lesions (high-grade dysplasia and carcinoma-in-situ) and patient-matched. Amalgam tattoo biopsy specimens were selected as control. Immunohistochemistry was performed using PD-L1 antibody. The expression of PD-L1 was scored using the tumor proportion method. **Results:** Eight patients were selected for the low-risk and high-risk groups. Seven out of eight patients were female. The average ages at the time of biopsy were 57, 55, and 59 years for the control, low-risk, and high-risk groups, respectively. The most common sites for PVL lesions were gingiva and tongue. All control specimens showed < 1% PD-L1 expression. Of twelve low-risk specimens, one specimen showed > 1% expression (8.3%). Of eighteen high-risk specimens, fifteen specimens showed ≥ 1% PD-L1 expression (83%). **Conclusion:** A significant number of high-risk lesions demonstrated ≥ 1% PD-L1 expression, which is the threshold utilized in anti-PD-1 drug studies for head and neck squamous cell carcinoma. Our results suggest that anti-PD-1 therapy may be beneficial for patients with PVL who have developed high-risk lesions. **Funding sources:** NC TraCS $2K Grants and MS Research Support Grant Award

### [44] Abnormal Molecular Phenotypes of Collagen in Canine Mammary Carcinoma

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**Objectives:** In spite of recent advances in diagnosis and treatment, breast cancer remains a global cause of morbidity and premature death for both human and veterinary patients. Due to molecular and histologic similarities, canines provide an excellent model of human breast cancer since mammary tumors are the most prevalent neoplasms in female dogs and ~50% of all cases are malignant. Despite an increasing awareness of the critical role of fibrillar collagens in breast cancer biology and therapeutics in women and dogs, tumor-permissive collagen features are still not well defined. To characterize the molecular and morphological phenotypes of type I collagen in canine mammary carcinoma. **Methods:** Tissues were obtained from canine mammary glands (8 containing tumors and 3 non-tumor controls), reduced with NaB3H4, hydrolyzed and subjected to amino acid and collagen cross-link analyses. Aliquots were also subjected to mass spectrometric analysis to determine lysine post-translational modifications at specific molecular sites of collagen. Tissues were also histologically examined by H&E and picrosirius red staining. **Results:** In tumor samples, the total number of cross-links enriched in the stable hydroxylysine-aldehyde derived cross-links were significantly increased compared to controls (p<0.001). The mass spectrometric analyses revealed that, in tumors, all lysine residues in the type I collagen telopeptides were specifically over-hydroxylated (1.4-2.8-fold increase) in comparison to controls. Histological analysis showed that the collagen fibers appeared to be thick and straight in tumor samples. **Conclusions:** These results demonstrated, for the first time, that all telopeptidyl lysine residues in mammary tumor type I collagen are “over” hydroxylated resulting in an increase in the stable collagen cross-links. The resulting biophysical and biomechanical consequences of these stable collagen cross-links are predicted to promote efficient tumor cell metastasis. **Supported by:** Canine Health Foundation (CHF2489), Lineberger Tier III Development Award and University Cancer Research Funds.

[45] Transcriptomic Signatures of Rare Breast Cancer Histological Types

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**Objectives:** The WHO classifies breast epithelial tumors into 21 histological types. Amongst these, the most common type of breast cancer histology is invasive ductal carcinoma – not otherwise specified (IDC) which accounts for 50-80% of all breast cancers. IDC exhibits heterogeneity in terms of clinical characteristics, treatment and prognosis, which are heavily determined by its gene expression derived ‘intrinsic’ molecular subtype. The other histology types then together make up for the remaining 20-25% of breast cancers and are known as ‘special’ histopathological types of breast cancer. These special histologies also exhibit intrinsic subtypes but they have specific molecular profiles extending beyond intrinsic subtypes. In this work, we study the transcriptomic profiles of special histological types in the cancer genome atlas (TCGA) breast cancer database. **Methods:** Molecular profiles were retrieved (http://cancergenome.nih.gov/): RNAseq gene expression (Illumina HiSeq RNASeqV2 Level 3.1.9.0). DESEQ2 R package was used for fold change analysis. Gene set enrichment analysis was conducted using GenePattern. Unsupervised and supervised hierarchical clustering was done using Cluster 3.0 and exported using Java Tree View. **Results:** We identify histology discriminatory genes independent of intrinsic subtype, perform a supervised clustering analysis and study inter-relationships between them. We demonstrate that this comparison helps highlight a relevant subtype of the ‘papillary’ histology. Moreover, we apply this list in the context of the mucinous histology and build a predictive model that can predict ‘mucinous’ histology in cancers of other organ systems. Finally, utilizing single cell RNAseq derived gene signatures we identify a cell of origin for the special histological types. **Conclusions:** Overall, through this work, we put forth a framework for identifying significant biological pathways in understanding cancer histogenesis.
CRISPR/Cas9 Genetic Screen Identifies Targets that Enhance Efficacy of FTIs for Treating HRAS-Dependent HNSCC

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Objectives: Activating mutations in the HRAS oncogene are present in 5-11% of head and neck squamous cell carcinomas (HNSCC). My studies will determine if HRAS is a driver of HNSCC growth and whether pharmacologic inhibitors of HRAS membrane association (farnesyl transferase inhibitors or FTIs) have therapeutic value in HNSCC. I will also identify novel treatment targets that will enhance the efficacy of the clinical candidate FTI, tipifarnib, in HRAS mutant HNSCC.

Methods: We genetically depleted HRAS from HRAS-mutant HNSCC cell lines by shRNA and treated HNSCC cells with inhibitors of farnesyltransferase (FTIs: tipifarnib and lonafarnib). We monitored target inhibition by aqueous/detergent fractionation and immunoblotting; quantitated cell viability by Alamar blue assay; and assessed cell cycle distribution and apoptosis by flow cytometry. To identify novel genes that would enhance sensitivity to FTIs, we applied a CRISPR/Cas9 loss of function screen to silence the expression of 2500 druggable genes in combination with tipifarnib.

Results: Genetic depletion of mutant HRAS inhibited the growth of HRAS-mutant HNSCC cell lines in vitro and robustly induced apoptosis. We found that FTIs blocked HRAS farnesylation at nanomolar range and inhibited the growth of HRAS-mutant HNSCC in a target-dependent manner. Using a CRISPR/Cas9 loss of function screen we identified genes that enhanced the sensitivity of HRAS mutant cells to tipifarnib. KEGG pathway enrichment analysis showed that these genes regulated MAPK/PI3K signaling pathways, chemokine signaling, autophagy and chromatin structure. Co-treatment with pharmacological inhibitors of these targets caused increased growth inhibition and apoptosis compared to single agent alone.

Conclusions: We established that genetic suppression of mutant HRAS blocks HNSCC growth, validating HRAS as a potential therapeutic target. HRAS-mutant HNSCC cell lines are HRAS-dependent and sensitive to FTI. Further, the combination of FTI with small molecule inhibitors of targets identified in our CRISPR screen will improve treatment outcomes. Supported by: Royster Society of Fellows fellowship (SJ), NIH P01CA203657 (ADC and CJD)

Dual EGFR and HDAC Inhibition Exploits a Unique Therapeutic Vulnerability in Mucoepidermoid Carcinoma

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Objective: Mucoepidermoid carcinoma (MEC) is the most common salivary gland cancer and is frequently treated by surgical resection with or without adjuvant radiation therapy. However, treatment options are limited for patients who develop recurrent and/or metastatic disease. Thus, we sought to identify novel targeted therapies for MEC.

Methods: We performed a high-throughput drug screen in MEC cells using a focused library of receptor tyrosine kinase and epigenetic inhibitors to assess effects on MEC viability. Candidate drug leads were validated in vitro in four MEC cell lines. We assessed the effects of these leads on cell proliferation, apoptosis, cell cycle distribution, 2D colony formation and 3D sphere formation. qPCR and Western blotting were used to probe changes in the expression

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levels of gene/protein targets known to contribute to MEC tumorigenesis. **Results:** Based on the results of our drug screen, we identified a promising dual-action EGFR/HDAC inhibitor (CUDC-101) and compared its efficacy to that of widely-used single-agent EGFR (Erlotinib) and HDAC (SAHA/Vorinostat) inhibitors. CUDC-101 is ~10-fold more potent than SAHA and ~2-fold more potent than Erlotinib at blunting MEC cell proliferation. Specifically, CUDC-101 treatment induces a rapid and robust down-regulation of key MEC driver genes (CREB, MYC, and CRTC1/MAML2) accompanied by increased cellular apoptosis. While SAHA also induces apoptosis, ten-fold more drug is required to achieve this effect. Notably, Erlotinib treatment causes cells to enter a quiescent state where they remain viable and can rapidly resume proliferation upon Erlotinib withdrawal. **Conclusions:** While individual single-agent EGFR or HDAC inhibitors are somewhat effective at blunting MEC cell growth and inducing apoptosis, these approaches suffer from critical setbacks including acquired resistance to long-term therapy and significant off-target cytotoxicity. Given these critical setbacks, dual EGFR/HDAC targeting using low doses of CUDC-101 is an attractive therapeutic option for MEC. **Funding Sources:** UNC Cancer Research Funds (ALA), Ruth L. Kirschstein National Research Service Award (NRSA) F31 Fellowship (AMM).

[48] Investigating Aberrant Non-Canonical Activation of the CREB Co-Activator CRTC2 in Head and Neck Cancer

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**Objectives:** Head and neck cancers are the sixth most commonly occurring cancer worldwide and often display aberrant signaling that promotes cell growth and proliferation. Previous studies by the Amelio Lab discovered dysregulated cAMP/CREB signaling in HNSCC cells lines and identified the CREB coactivator CRTC2 as the predominant isoform expressed in oral keratinocytes as well as HNSCC cells. Mitogen-Activated Kinase Kinase 1 (MEKK1) can induce non-canonical CRTC phosphorylation and nuclear translocation where it binds CREB and initiates transcription of downstream target genes. This study investigated 1) CRTC2 localization/activation, and 2) the role of MEKK1 in CRTC2 activity in HNSCCs. **Methods:** Luciferase assays were used to measure endogenous CRTC2 activity in the HNSCC cell lines UMSCC74A (HPV-negative) and UMSCC47 (HPV-positive). Cells were treated with vehicle or compounds that activate cAMP signaling and CRTC nuclear translocation. Immunofluorescence assays were used to examine localization (cytosolic vs. nuclear) of CRTC2 following treatment. Since the MEKK1 pathway is often activated in HNSCCs, we also performed loss-of-function luciferase assays by stably expressing MEKK1 shRNAs in UMSCC74A and UMSCC47 HNSCC cells. Transduced cells were selected with puromycin and MEKK1 knockdown validated using qPCR. **Results:** CRTC2s are regulated by subcellular localization and upon further characterization, we discovered that CRTC2 displays enhanced nuclear localization in non-stimulated HNSCC cell lines compared to oral keratinocytes. Notably, stimulating cAMP signaling only had modest effects on induced transcription, however knockdown of MEKK1 resulted in a 40% reduction in CRTC2/CREB transcription activity in HPV-positive cells, but not HPV-negative cells. **Conclusions:** These data suggest that MEKK1 differentially mediates CRTC2 activation based on HPV status and that MEKK1 pathway inhibitors may be effective for treating HPV-positive oral cancers. Our future goals are to 1) examine the effects of MEKK1 pharmacologic inhibition on CRTC2 localization, and 2) characterize the impact of CRTC2 knockdown on proliferation and cell migration in HNSCC cell lines. **Supported by:** NIH/NIDCR T90-DE021986 and NIH/NCI T32-CA211056 to MB Carper and Lineberger Tier III Development Award and University Cancer Research Funds to AL Amelio.
Novel Roles of E3 ubiquitin Ligase RNF168 in Genome Maintenance and Sustaining Squamous Cell Carcinomas

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Objectives: Mechanisms by which cancer cells acquire genome instability and resist therapy are poorly understood. The broad long-term goal of this project is to define mechanisms by which Squamous Cell Carcinomas (SCC) arise, evolve and resist therapy which will help identify new molecular vulnerabilities. RNF168, which encodes an E3 Ubiquitin ligase and DNA repair protein, is amplified in 22.1% of SCC but less significantly in other cancers. Our objective is to define the role of RNF168 in genome maintenance and SCC biology. RNF168 is known to affect the choice of error-prone vs error free DNA Double Strand Break (DSB) repair. Our preliminary data suggest that RNF168 also has novel roles in promoting error-prone resolution of stalled DNA replication forks. We seek to test the hypothesis that RNF168 promotes error-prone genome maintenance thereby aiding cancer sustenance. Methods: We are using gene editing, silencing and overexpression to manipulate RNF168 levels and determine how RNF168 impacts DNA repair pathway choice, proliferation, viability and chemoresistance of cultured cancer cell lines. Results: RNF168 depletion using siRNA reduces viability of cancer cell lines indicating a new role for RNF168 in sustaining cell viability. RNF168 co-localizes with the DNA replication factor PCNA during S-phase indicating a new role of RNF168 in DNA synthesis. Using mutational analyses, we identified a PCNA-Interacting Peptide (PIP) domain in RNF168 that is necessary for mediating PCNA association which when deleted prevents RNF168 from localizing to DNA replication factories. Conclusions: RNF168 is important for cancer cell survival and has a new role in DNA synthesis unrelated to its known roles in DSB repair. Experiments are underway to characterize the biological activities of PCNA-interaction-defective RNF168. These will determine the extent to which canonical DSB repair functions and new PCNA-mediated activities are involved in sustaining cancer cells. Funding source: NIH R01-ES09558 (CV)

Non-thermal plasma specifically kills oral squamous cell carcinoma cells in a catalytic Fe(II)-dependent manner


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Objectives: Oral cancer accounts for ~2% of all cancers worldwide, and therapeutic intervention is closely associated with quality of life. Here, we evaluated the effects of non-thermal plasma (NTP) on oral squamous cell carcinoma (OSCC) cells with special reference to catalytic Fe(II). Methods: We used seven OSCC cell lines and two fibroblast cell lines derived from skin and lung. Cells were treated with NTP for 30~120 s at 25 °C. The effects of NTP on cells were evaluated by cell viability, apoptosis, reactive oxygen species, cell migration. In addition, the effects of ferric ammonium citrate and desferrioxamine on NTP application were investigated. Results: NTP
exerted a specific effect on OSCC cells compared to fibroblasts. Furthermore, the effect was dependent on the amounts of catalytic Fe(II) present, especially in lysosomes. Following NTP application, lipid peroxidation occurred, and peroxides and mitochondrial superoxide were generated. Positive TUNEL and annexin V staining indicated the potential involvement of NTP-induced apoptosis. NTP exposure significantly suppressed the migratory, invasive and colony-forming abilities of OSCC cells. **Conclusions:** The oral cavity is easily accessed and monitored; therefore, NTP can be directly applied to the oral cavity to target OSCC without damaging fibroblasts. In conclusion, NTP treatment is a potential therapeutic option for oral cancer.

[51] **Efficacy of Propranolol Compared to Placebo for Treatment of TMD Pain: SOPPRANO Randomized Controlled Trial**


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**Objectives:** Preliminary studies suggest that propranolol, a non-selective beta-adrenergic receptor antagonist used for migraine prevention, may alleviate pain from temporomandibular disorder (TMD). The objective of this study was to evaluate the efficacy of propranolol compared to placebo in reducing facial pain for people with TMD. **Methods:** SOPPRANO, a multicenter, randomized, double-blind, placebo-controlled, parallel group, phase IIb trial, enrolled subjects aged 18-65 years with TMD myalgia (with/without arthralgia). Subjects were randomized 1:1 to either extended release propranolol hydrochloride (60 mg, b.i.d.) or placebo during a 10-week treatment period. The primary endpoint was change in an index of facial pain, computed as pain intensity (0-100 scale) multiplied by pain duration (0-100% of waking day) from Daily Symptom Diaries (DSDs). Efficacy was analyzed as absolute change in pain index from baseline to week 9 of treatment and as relative change (absolute change divided by baseline) dichotomized as ≥30% or ≥50% (i.e., “responder analysis”). Generalized linear mixed models tested for treatment-group differences adjusting for study site, sex, race, and pain index at baseline. **Results:** Of 299 subjects screened, 200 fulfilled selection criteria and were randomized; 199 completed at least one post-randomization DSD and were included in an intention-to-treat analysis; 167 of them provided data through week 9. At week 9, model-adjusted absolute reductions in pain index did not differ significantly between propranolol and placebo (P=0.40). However, relative reductions in pain index of ≥30% were significantly more likely (odds ratio=2.0, P=0.028) for propranolol (69.6% responders, 95%CL=58.0%, 79.2%) than placebo (52.8% responders, 95%CL=41.3%, 64.0%), and associated number-needed-to-treat (NNT) was 5.9. Relative reductions of ≥50% were also more likely in propranolol than placebo (P=0.034, NNT=5.9). Adverse event rates were similar between treatment arms. **Conclusion:** Despite having a non-significant effect on absolute change in facial pain, propranolol was efficacious in achieving ≥30% and ≥50% relative reductions after 9 weeks of treatment among TMD subjects. **Supported by:** NIH/NIDCR U01DE024169

[52] **A Randomized Clinical Trial of Buffered 1% vs Unbuffered 2% Lidocaine in Children**


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Objective: The administration of local anesthetics can be painful, and the risk of adverse events limits the maximum local anesthetic amount that can be safely administered to young children. Buffered local anesthetics are promising alternatives to conventional, unbuffered anesthetic formulations; however, there is little evidence about their use in children. We sought to determine the anesthetic and physiologic differences associated with use of buffered 1% and unbuffered 2% lidocaine (both with 1:100,000 epinephrine) in children. Methods: In this randomized, double-blinded, crossover study, 24 children ages 10-12 received 2 IAN blocks, at least one week apart, randomized to alternating sequences of 2 drug formulations: formula A—3cc buffered 1% lidocaine (i.e., including 0.3cc of 8.4% sodium bicarbonate) or formula B—3cc unbuffered 2% lidocaine. Outcome measures included, among others, peak blood lidocaine levels (15min. post-injection), timing of clinical signs onset (i.e., “lip sign”), response to pain on injection and duration of anesthesia. Initial analyses relied upon paired t-tests and a p<0.05 statistical significance criterion. Results: We found significantly lower peak blood lidocaine levels associated with the buffered versus unbuffered formulation in the first 16 randomized participants: 484 ng/mL vs 1156 ng/mL, respectively—a 53% (95 confidence interval=43%-64%; p<0.05) weight-adjusted relative decrease. We found no important differences in pain upon injection, onset and duration of anesthesia. Conclusion: These preliminary results suggest equal effectiveness of a buffered local anesthetic formulation compared to a double-concentration unbuffered one, while resulting to lower peak blood lidocaine levels—an important gain for the prevention of anesthetic toxicity. Supported by: Sunstar/AAPD post-graduate research fellowship; MS Research Support Grant

[53] A Multi-modal Analgesic Protocol Reduced Opioid Use/Misuse after 3rd Molar Surgery

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Objective: This IRB-approved prospective study was designed to assess the number of opioid doses available to patients from filled prescriptions (Rx) and the opioid doses taken and left-over after adoption of a multimodal analgesic protocol to manage pain post-surgery in 2018. Methods: The inclusion criteria were American Society of Anesthesiologists risk classification I, II, age 18-35 years, and at least two lower 3rd molars removed. The exclusion criteria were patients being treated for opioid addiction/abuse. All enrolled, consented subject-patients were treated with the multi-modal analgesic protocol. In addition, subject-patients were given two Rx for 4 doses of Hydrocodone 5mg/Acetaminophen 325mg each; one Rx dated to be filled day of surgery, one Rx dated to be filled any subsequent day, with both Rx filled at patient’s discretion. Data were derived from an encounter form completed at surgery by patient, a 14-day diary by patient which tracked opioid drugs taken, and the Rx filled as recorded in the NC RxSentry Prescription Drug Monitoring Program. The primary outcome variable was the number of opioid doses filled by Rx for each subject-patient. The primary predictor variable was the multimodal analgesic protocol. Descriptive statistics were used for reporting outcomes. Results: Data from 50 subjects were analyzed; 32 (64%) were females. Median age was 22 years (IQR 19y, 26y). Twenty-nine (58%) subject-patients filled no Rx, 9 filled one Rx (18%), 12 filled two Rx (24%). Within the group that filled one Rx, there were a total of 23 left-over doses not consumed out of 36 doses (64%). Within the group that filled two Rx, there were a total of 36 left-over doses not consumed out of 96 doses (38%). Conclusions: Outcomes suggest that implementation of a multi-modal analgesic protocol with 3rd molar surgery may be effective in reducing use and misuse of opioid drugs. Supported by: OMS departmental funds
[54] Relating Levels of Omega-3/Omega-6 Polyunsaturated Fatty Acids to Chronic Pain as Measured by LC-ESI-MS/MS

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Objectives: Human studies show that omega-3 polyunsaturated fatty acids (PUFAs) reduce pain through anti-inflammatory and anti-nociceptive mechanisms, whereas omega-6 PUFAs induce pain. This study aimed to assess the utility of liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) for quantifying PUFAs in erythrocytes, and to determine PUFA concentrations in a small sample set. Methods: Study participants (n=50 total) included patients with painful temporomandibular disorder and one to four other chronic pain disorders (case, n=25) and control participants with none of the chronic pain conditions (non-case, n=25). Participants provided general dietary habits. PUFAs were extracted from erythrocytes and extract solutions were spiked with isotopically-labeled PUFA internal standards to correct for instrumental variation during analysis. Reversed-phase LC was utilized to separate PUFAs based on polarity prior to ionization. Negative polarity electrospray ionization was used to generate gas-phase ions from solution-phase molecules, allowing for MS detection. MS/MS was used to dissociate precursor ions corresponding to the individual PUFAs and measure the resulting product ions with greater selectivity and sensitivity than single stage MS. PUFAs concentrations were calculated using calibration curves generated from known concentrations of PUFA standards. Results: Five PUFAs (omega-3: eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid; omega-6: arachidonic acid, 12(S)-HETE) were detected and quantified in all 50 samples. The remaining 5 PUFAs were at quantities below the method detection limit in some samples. Average concentration ratios in case/non-case samples were 1.34 and 1.68 for arachidonic acid and 12(S)-HETE, respectively; 0.99, 1.47 and 1.63 for eicosapentaenoic, docosahexaenoic and docosapentaenoic acids, respectively. Conclusions: Omega-6 PUFAs concentrations were higher in case samples, matching their proposed relation to chronic pain. Omega-3 PUFAs were hypothesized to be higher in non-case samples, but the dataset did not show this trend. Participant’s dietary information will be incorporated in forthcoming data analysis to improve our understanding of the dataset. Supported by: NIH/NIDCR U01-DE017018; NC TraCS CTSA UL1TR001111

[55] Painful TMJ osteoarthritis is not associated with a higher presence of comorbid bodily pain

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Objectives: The study objective is to investigate the presence of Bodily Pain Conditions (BPC) in patients with Temporomandibular Joint Arthralgia (TMJA) with and without TMJ Osteoarthritis (TMJOA). We hypothesized that painful TMJOA is not associated with a higher presence of BPC. Methods: This is a retrospective cross-sectional study involving consecutive patients who sought treatment at the UNC Orofacial Pain Clinic between 01/01/2010 and 12/31/2014 with a diagnosis of Temporomandibular Disorders (TMD) according to the RDC/TMD criteria (Dworkin et al, 1992). TMJA was determined based on self-reported pain in one or both TMJs. Cases fulfilled the Ahmad classification for TMJOA (Ahmad et al, 2009) in addition to TMJA (TMJA+OA), while controls had TMJA with normal condyles (TMJA-OA). Data was collected from the imaging reports of the Cone-Beam Computerized Tomography (CBCT) of bilateral TMJs performed using the CareStream 9300. Self-reported BPC (such as fibromyalgia and low back pain) was obtained via a clinical interview. Statistical analysis was performed using chi square test and logistic regression. Results: Twenty-eight cases (TMJA+OA), with a total of 45 osteoarthritic
condyles, and 25 controls (TMJA-OA), with a total of 50 normal condyles, were included in the study. The mean ages of the cases and controls differed significantly (47 and 36 years, respectively, p=0.03). Nineteen (67%) cases and 15 (60%) controls were females. The presence of at least one BPC was reported by 19 (68%) cases and 18 (72%) control subjects (p=0.74), while the presence of two or more BPC was reported by 9 (32.1%) cases and 8 (32%) control subjects (p=0.99). Conclusions: In this cohort of patients with TMJA, the presence of comorbid pain conditions was high and independent of the presence of TMJOA.

[56] The presence of TMDH is associated with greater psychiatric comorbidity in patients with chronic TMD

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Objectives: The study aim is to investigate the association between Headache Attributed to Temporomandibular Disorders (TMDH) and the presence of psychopathology in patients with chronic Temporomandibular Disorders (TMD). We hypothesize that TMD patients with TMDH have a greater number of psychiatric disorders compared to TMD patients without TMDH. Methods: This is a retrospective cross-sectional study of consecutive patients who sought treatment at the UNC Orofacial Pain Clinic between 01/01/2013 and 12/31/2014 with a diagnosis of chronic TMD according to the RDC/TMD criteria (Dworkin et al, 1992). Cases fulfilled the ICHD-3 criteria for TMDH (IHS, 2018) in addition to TMD (TMD+TMDH), while controls had TMD only (TMD-TMDH). Data on the presence and the number of self-reported psychiatric disorders (such as anxiety, depression, and PTSD) were collected via a clinical interview. Chi-Square and F-test were used for data analysis. Statistical significance was set at 0.05 and power at 0.80. Results: One hundred and nineteen patients, including 34 cases (TMD+TMDH) and 85 controls (TMD-TMDH), satisfied the inclusion criteria. Females accounted for 68% of the cases and 72% of the controls (p=0.80). The mean age of cases and controls were similar (43.2 and 44.1 years respectively; p=0.75). The number of psychological conditions ranged from 0 to 3 in both groups, with the TMD+TMDH group reporting a statistically significantly greater mean number of psychiatric disorders (mean= 0.94; sd=0.89), compared to the TMD-TMDH group (mean= 0.56; sd=0.89) (p=0.045; OR=1.51 [CI=1.001-2.28]). Statistically significantly more cases reported at least 2 psychiatric disorders compared to controls (32% of cases and 13% of controls; p=0.036; OR=2.64 [CI=1.04-6.71]). Conclusion: In patients suffering from chronic TMD, the presence of TMDH was associated with greater psychiatric comorbidity. Supported by: National Institute of General Medical Sciences of the National Institutes of Health T32GM086330 (DV).

[57] Lifestyle Factors that Effect Self-Perceived Health in HIV+ Individuals Receiving Comprehensive Dental Care

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Objective: Inadequate dental care can significantly compromise the health and well-being of those with human immunodeficiency virus (HIV). This longitudinal study investigated the impact of comprehensive dental intervention on quality of life using a validated short form 8 (SF-8) in patients with HIV. Mental composite scores (MCS) and physical composite scores (PCS) ranged from 0 (poor) to 100 (excellent). Methods: 196 participants were provided 2 years of comprehensive dental care at 6-month intervals. Per-protocol visits involved dental
prophylaxis/debridement, oral hygiene instructions, and interview, which included the SF-8, factors affecting oral health, HIV antiretroviral therapy (ART), and demographics. **Results:** The majority of subjects were male (75.0%) and African-American (59.8%) with a median age of 43.4 years. At baseline, although 80.6% of participants report taking ART, only 46.2% of these participants were virally suppressed (< 50 copies/mL) at the most recent laboratory visit. Although viral load was not significantly associated with SF-8 scores in the longitudinal data, interestingly, patient-reported ART was strongly associated with mental self-perceived wellness (p=0.0002, Wald chi-square). This association did not depend on oral care provision or date of diagnosis. Compared to patients who were either unemployed or disabled, those patients who were employed scored, on average, 3.6 points higher (p=0.0002, Wald chi-square) on the MCS and 2.4 points higher (p=0.004, Wald chi-square) on the PCS. Tobacco use was associated with lower MCS (p=0.001, Wald chi-square) and PCS (p=0.002, Wald chi-square). **Conclusion:** The results from these secondary analyses suggest that lifestyle can greatly influence self-perceived health. It is already well-established that tobacco users are at higher risk of periodontal disease, and those who are employed are more likely to have dental insurance. Thus, it is important that dental medicine focus on risk factors associated with poor quality of life, as these same risk factors are associated with oral disease.

**[58] Herpesviral Detection in HIV+ Subjects is Related to Pathogenic Bacterial Microbiomes**

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**Objectives:** Epstein-Barr virus (EBV) is a gammaherpesvirus important to oral and gastric carcinogenesis and to periodontal disease. CMV is a beta herpesvirus that has also been detected in periodontal disease. Determining herpesviral interactions in the context of periodontal disease is critical to understanding periodontal pathogenesis. **Methods:** Salivary EBV, CMV viral loads were determined for 27 HIV+ subjects at baseline, 12 months, and 24 months post-dental intervention by qPCR. Salivary microbial content was analyzed by sequencing the 16S rDNA gene V1-V3 hypervariable region using MiSeq (Illumina, CA) and reads were evaluated using SILVA Taxonomy. **Results:** Detection of EBV and CMV were associated with pathogenic microbiomes and significant clinical periodontal disease. While elevated EBV VL was detected in the majority of subjects, only a single subject consistently demonstrated elevated CMV VL at baseline. This CMV+ subject had the highest EBV copy number at 22,500 copies/ul and had severe periodontal disease. Dental intervention reduced mean oral EBV VL across most subjects (by 1-2 logs), reduced CMV to undetectable in the CMV positive subject, and shifted the microbial profile to a more commensal based bacterial profile. **Conclusions:** We have begun to decipher mechanisms of oral bacteria driven viral pathogenesis. EBV VL was related to pathogenic bacterial loads in vivo. CMV however was only detected with extraordinarily high EBV VL and severe periodontal disease.

**[59] Oral Pathogens Induce EBV+ B-cell/Epithelial Cell Adherence Resulting in Virus Transfer to Naïve Oral Keratinocytes**

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Objectives: Epstein-Barr virus (EBV) is a gammaherpesvirus important in human oral and gastric carcinogenesis including AIDS-associated malignancies. Latent EBV resides in circulating B-cells. Viral reactivation leads to productive infection and virus transfer to epithelial cells resulting in viral spread. Determining viral reactivation mechanisms is essential to combat EBV-associated disease. EBV viral load (VL) correlates with periodontitis severity, suggesting bacterial pathogens contribute to viral reactivation. This study identified mechanisms underlying bacteria-mediated EBV reactivation and B-cell/oral epithelial cell virus transfer. Methods: EBV+ B-cells were treated with bacterial spent media (BSM) obtained from cultures of oral pathogens, F. nucleatum and P. gingivalis. Following BSM treatment of EBV+ B-cells, epigenetic marks (histone modifications) were analyzed by immunoblot. BSM-induced EBV reactivation was assessed by detection of lytic viral proteins. Pathways important in BSM-induced EBV reactivation were determined pharmacologically. B-cell/epithelial cell interactions were determined by co-culturing BSM-treated EBV+ B-cells with naïve normal oral keratinocytes (NOK). Viral transfer was detected by visualizing virus-encoded GFP in NOK cells. Results: Pathogen BSM increased global expression of activating epigenetic modifications (total H3Ac, H3K9Ac, H3K27Ac) in EBV+ B-cells. Viral lytic proteins and viral DNA replication was detected in cells treated with pathogen BSM. Commensal BSM (S. sanguinis) did not induce epigenetic modifications, virus reactivation/DNA replication or B-cell/epithelial cell adherence in co-culture. Strong adherence between pathogen BSM-treated EBV+ B-cells and naïve NOKs was detected. Following removal of B-cells, GFP+ NOKs were detected suggesting viral transfer occurred. Conclusions: EBV lytic reactivation was induced in latently-infected EBV+ B-cells treated with oral pathogen BSM. This highlights the potential for circulating EBV+ B-cells to adhere to oral epithelium at sites of active periodontitis. Virus transfer to oral epithelial cells can result in productive de novo infection and increase VL in the oral cavity. Potential increased latently-infected cell populations can contribute to EBV-associated pathologies and malignancies.

[60] Developing tools to understand how Oral Pathogenic Bacteria modulate HPV E6/E7 transcription

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Objectives: Human papillomaviruses (HPV) is a major world health concern and infection of high-risk HPV can lead to the development of a variety of cancers. Epidemiologic data that supports a relationship between oral periodontal infections and the development of oral cancer. Previous studies from our group demonstrate that bacterial spent media from periodontal pathogens can increase transcription of HPV associated oncogenes. This study sought to develop a stable cell line containing the luciferase gene under the transcriptional control of the HPV long control region (which serves as promoter for HPV associated oncogenes E6 and E7), in order to study specific bacterial products involved promoter activation. Methods: The HPV LCR, was PCR-amplified and cloned into the reporter plasmid, pGL2-Basic to produce pHPVLCRpLuc. The HPV promoter/luciferase cassette was cloned into the lentiviral vector, pLKO.1. A promoterless luciferase was constructed as a negative control. Results: Treatment of HPV+ cells treated with Fn or Pg BSM resulted in expression HPVE6/E7 genes as determined by RT qPCR. No increase in viral transcripts was seen in commensal-treated cells. PCR products were obtained using primers specific for the HPV LCR promoter. Plasmids were generated and restriction digest analysis confirmed the presence of the viral promoter/luciferase cassette in the lentiviral vector. Conclusions: BSM-induced HPV E6/E7 expression suggested that bacterial driven transcription occurred at the HPV LCR. Stable cell lines containing viral promoter/luciferase cassettes will be useful in elucidating mechanisms of oral pathogenic bacteria driven HPV pathogenesis.
[61] How Oral Pathogenic Bacteria Stimulate Epstein-Barr Virus (EBV) Reactivation

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Objectives: Epstein-Barr virus (EBV) is associated with human oral and gastric cancers. Lytic reactivation from latency contributes to EBV spread. Reactivation is dependent on the initial expression of two immediate-early genes, BRLF1 and BZLF1. Increased oral EBV load correlates with periodontitis severity suggesting bacterial pathogens contribute to reactivation. This study sought to develop a stable cell line containing the luciferase gene under the transcriptional control of the promoters for the EBV immediately-early genes, BRLF1 and BZLF1, to study bacterial products involved in lytic reactivation. Methods: The latently-infected EBV+ human gastric carcinoma cell line, AGS-EBV, was treated with bacterial spent media (BSM) from anaerobic cultures of F. nucleatum (Fn) or P. gingivalis (Pg). The oral commensal bacteria, S. sanguinis (Ss), was used as a negative control. Lytic reactivation was determined by immunoblot to detect immediate-early viral lytic proteins. The promoters of the EBV immediate-early genes, were PCR-amplified and cloned into the reporter plasmid, pGL2-Basic to produce pEBVRpLuc and pEBVZpLuc, respectively. EBV promoter/luciferase cassettes were cloned into the lentiviral vector, pLKO.1. A promoterless luciferase was constructed as a negative control. Results: Treatment of latently-infected EBV cells treated with Fn or Pg BSM resulted in expression of the immediate-early viral lytic proteins. The promoters of the EBV immediate-early genes, were PCR-amplified and cloned into the reporter plasmid, pGL2-Basic to produce pEBVRpLuc and pEBVZpLuc, respectively. EBV promoter/luciferase cassettes were cloned into the lentiviral vector, pLKO.1. A promoterless luciferase was constructed as a negative control. Conclusions: BSM-induced EBV reactivation suggested that transcription occurred at the viral immediate-early lytic genes. Stable cell lines containing viral promoter/luciferase cassettes will be useful in elucidating mechanisms of viral reactivation by oral pathogenic bacteria. This is an important connection to show that improving oral hygiene can limit EBV reactivation and improve systemic health. Funding: NIDCR R56 DE023940-01

[62] Pathogenic Oral Microbes are Associated with Increased Epstein–Barr Virus Levels in Vivo and Modulate Viral Chromatin via Brd4 to Increase Viral and Host Gene Expression

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Objectives: Epstein-Barr virus (EBV) is a gammaherpesvirus important to oral and gastric carcinogenesis and to periodontal disease. Determining viral reactivation mechanisms is essential to combat EBV-associated disease. This study identified mechanisms underlying bacteria-mediated EBV reactivation. Methods: Oral commensal (Lactobacillus/Strep Sanguis [Ss]) and pathogen (F. nucleatum [Fn]/P. gingivalis [Pg]) bacterial loads were determined for 8 HIV+ subjects at baseline and 12months post-dental intervention by qPCR. Latently infected gastric cancer cells (AGS-EBV) were treated with spent media (BSM) from cultures of Fn, Pg or Ss to assess EBV reactivation. RT-qPCR and immunoblot analysis determined gene expression. A role for bromodomain proteins in BSM-induced reactivation was determined using the BRD4 inhibitor JQ1. Cancer related transcripts (UPAR) were assessed by RT-qPCR. The FAIRE assay was performed to measure BSM-mediated nucleosome ejection from viral promoters. Results: Dental intervention reduced mean oral EBV VL and Pg/Fn while increasing Ss/Lacto bacterial
loads. In vitro, BSM treatment increased EBV lytic mRNA up to 3.5 fold, EBV BRLF1 protein levels, and cancer-
related transcripts uPAR, by 3 fold. Transcript levels and BRLF1 protein levels diminished in the presence of JQ1.
FAIRE analysis indicated Fn, Pg-mediated nucleosome ejection at the EBVBRLF1 promoter, demonstrating 50%, 60%
nucleosome occupancy compared to WC media. Conclusions: We have begun to decipher mechanisms of oral
bacteria driven EBV pathogenesis. EBV VL was related to pathogenic bacterial loads in vivo. In vitro, the pathogens
promoted nucleosome ejection at viral promoters that likely preceded Brd4 driven Fn- and Pg-mediated EBV
reactivation. This process drives enhanced transcription of both viral and host cancer genes.

[63] Clinical Changes and Oral Microbiome Shifts in HIV+ Patients Following Periodontal and Restorative Therapy

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Objectives: The microbiome may exert significant effects in the context of immunosuppression. The objective of
this study was to compare clinical outcomes and microbiome changes following comprehensive dental care in HIV+
patients. Methods: Thirty HIV+ patients received periodontal treatment, oral hygiene instructions, caries control,
and extraction of hopeless teeth. Systemic (viral load, CD4 counts, ART regimen) and oral clinical parameters
(presence of caries, gingival and plaque indices were measured at baseline(BL), 12-months(12M), and 24-
months(24M) after therapy. Periodontal disease severity was determined using the biofilm-gingival interface (BGI)
index. Saliva and throatwash samples were collected at the clinical visits. Their microbial content was analyzed by
sequencing the 16S rDNA gene V1-V3 hypervariable region using MiSeq (Illumina, CA) and reads were evaluated
using SILVA Taxonomy. We determined the microbiological profiles of patients and whether differences in OTU
abundance in paired samples (BL and 12M or 24M) were associated with changes in clinical correlates. Results:
Extent of periodontal disease, measured by number of sites with attachment loss ≥4mm with BOP, was inversely
correlated with alpha diversity (p=0.016758). Overall, the relative abundance of taxa typically associated with
periodontal disease and inflammation were increased in patients with probing depths ≥4mm with BOP, including
Tanerella (p=0.020984) and Treponema (p=0.027488). Microbiological differences between BL and 12M were
related to increases in OTU abundance in Campylobacter (p=0.008177), Haemophilus (p=0.010398), Oriabacterium
(p=0.03396), and Veillonella (p=0.034006). Microbiological differences between BL and 24M were related to
increases in OTU abundance in Veillonella (p=0.001414) and Campylobacter (p=0.0069006) and decreases in
abundance of Gemella (p=0.012314), Solobacterium (p=0.035119), Afipia (p=0.039213), Parvimonas (p=0.043312),
Granulicatella (p=0.044623), Bergeyella (p=0.044807), Peptostreptococcus (p=0.044923), and Peptococcus
(p=0.046161). Conclusions: Periodontal treatment in HIV+ patients improved clinical outcomes, reduced levels of
pathogenic microbiota and facilitated the establishment of a more diverse community conducive to clinical
improvement. Supported by: HRSA H97HA07519 (JWC), NIH/NIDCR: 1R56DE023940-01(JWC) NIH/NIDCR: R01-
DE024767 (FT)
[64] 16S rRNA Sequencing Toolkit Comparison and Implementation to Analyze the Oral Microbiome in Fanconi Anemia

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Objectives: To compare two 16S ribosomal RNA sequencing pipelines’ ability to describe mock microbial communities’ composition at the species (or de-novo sub-species) level, and then apply these tools to a 16S rRNA sequence dataset from Fanconi Anemia (FA) patients to determine whether strain abundances are associated with disease phenotype. Methods: Five mock microbial communities were prepared using different proportions of 42 microbial species catalogued in the Human Oral Microbiome Database. Illumina 1.8 Sanger sequencing was then performed in triplicate for 16S rRNA V1-V2 and V3-V4 regions. We then run sequences through Oligotyping and DADA2, two 16S rRNA toolkits used to classify microbes at a high taxonomic resolution, and optimize the pipelines for our data. The pipelines are then applied to 16S rRNA sequencing data taken from salivary samples of 62 FA patients, calculating abundances of all reads clustered in selected genera. We then build logistic/linear regression models to test the association between phenotype and specific oligotypes, incorporating patient demographical covariates. Results: Oligotyping and DADA2 pipelines’ ability to detect spiked species in mock microbial communities reach a threshold of approximately 0.73 for all sequencing protocols, yet Oligotyping, which can be optimized through manual checkpoints during processing, achieves greater specificity than DADA2. When applied to FA data, Oligotyping was able to identify 233 oligotypes clustered in genera of interest. Regression analysis indicates eight oligotypes are associated with significant differences in patient diagnosis, gingival bleeding index, and platelet counts in multiple models accounting for covariates. Whether these oligotypes represent species/strains will be further tested against bacterial reference genomes. Conclusions: We successfully demonstrated different pipelines’ ability to characterize microbial communities at species-level resolution, yet Oligotyping, optimized through user decision-making, has greater sensitivity and specificity than DADA2. Pipeline analysis of FA data indicates specific strain abundances are significantly associated with certain FA phenotypes.

[65] Genome-Wide Association Study of Dental Caries in Diverse Caucasian Populations

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Objectives: Dental caries is a common chronic disease influenced by a combination of genetic and environmental factors. In this study we conducted a genome-wide association study (GWAS) to identify associated genetic loci affecting susceptibility to caries in multiple Caucasian populations. Methods: The study subjects were Caucasians ascertained through the Pittsburgh Orofacial Cleft study (POFC, N=1970) and through the Center for Oral Health Research in Appalachia (COHRA, N=1755). We analyzed caries separately in the primary (ages: 2-12 yrs. N=672 from OFC, 925 from COHRA) and permanent (ages: 18-60 yrs. N=1298 from OFC, 830 from COHRA) dentitions. For each participant, dental caries was assessed by counts of decayed and filled teeth (dft/DFT) and genetic variants
were genotyped or imputed across the entire genome. More than 8 million genetic markers were tested in each study. In POFC, the covariates were age, gender, presence/absence of any type of orofacial cleft, and recruitment site. In COHRA, the covariates were age, gender, and recruitment site. GWAS in each sample was carried out using linear mixed models to test genetic association while simultaneously accounting for relatedness and population structure. **Results:** In the OFC, we identified genome-wide significant genetic locus (p<5E-08) near MAPK9 (primary dft). There was suggestive evidence of association (p<5E-06) near NFX1 (permanent DFT), and PIK3CD (primary dft). NFX1 and PIK3CD are involved in immune and inflammatory response, and MAPK9 in cellular processes. Those genes also showed evidence of association in COHRA. Interestingly, there were also suggestive findings for permanent DFT in both OFC and COHRA near taste receptor genes (TAS2R38, TAS2R3, TAS2R4, TASR25). Meta-analysis combining results from OFC and COHRA will be presented. **Conclusions:** This study identified several genetic loci associated with dental caries risk in multiple Caucasian populations. These results may lead to better understanding of cariogenesis, and could improve dental caries predictions, prevention, and/or treatment in the future. **Funding:** R01-DE014899, R01-DE016148, U01-DE018903, X01-HG00784

[66] **ZOE 2.0: A Community-Based Early Childhood Oral Health Study**

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**Objectives:** Improving children’s oral health is a long-standing area of priority and sustained efforts by many stakeholders. Despite these efforts, early childhood caries (ECC) persists as a clinical and dental public health problem with multilevel consequences. We describe the activities and early findings of the ZOE 2.0 study – a large scale, community-based, genetic epidemiologic study of early childhood oral health in North Carolina (NC). **Methods:** Between August 15, 2016 and January 11, 2019, 7,882 children ages 3-5 attending Head Start centers across NC were enrolled in ZOE 2.0. Children’s parents or guardians provided written informed consent and completed a questionnaire. Clinical examinations were done in participating centers by trained and calibrated examiners using ICDAS criteria. Several domains of clinical information were collected including anthropometry, extra-oral characteristics, dental occlusion, surface-level dental caries diagnoses, tooth-level hypoplastic defects of the enamel, evidence of dental trauma history, and Frankl behavior score at the exam. Saliva and supragingival dental plaque samples were collected to enable downstream genomics, microbiomics and metabolomics studies. **Results:** There were 5,981 clinical encounters with study participants through January 11, 2019 and >99% of them resulted to usable clinical data and a saliva sample for DNA extraction. Enrolled children attended 241 different HS centers across NC, had mean age of 53 months and were equally split between boys and girls. Forty-seven percent self-reported as African-American, 22% white, 13% multi-racial and 20% were of Hispanic ethnicity. Fifty-four percent of examined children had ECC (defined at the ICDAS>2 threshold) but the distribution of caries experience and unrestored disease varied across the state. **Conclusions:** We anticipate that this long-term research program will illuminate foundational domains for the advancement of precision oral health and care. Ultimately, this new knowledge can help catalyze the development of effective preventive and therapeutic modalities via actions at the policy, community, family, and person level. **Supported by:** NIH/NIDCR U01DE025046.
[67] Childhood Adiposity in a Community-Based Oral Health Study

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Objectives: Childhood obesity is an important public health problem with significant comorbidities—one-third of US children and adolescents are overweight or obese. In this study, we examined the prevalence and correlates of adiposity [as measured by the body mass index (BMI)] among a large, community-based sample of preschool-age children. Methods: The analytical sample comprised 5,593 preschool-age children (mean age=53 months; range=36-72 months) enrolled in NC Head Start (HS) programs/centers and participating in the ZOE 2.0 study. Height and weight were measured in participating HS centers using portable equipment. BMI percentiles and Z-scores for age and sex were used to define categories of underweight (<5th percentile), normal weight (5th-<85th percentile), overweight (85th-<95th percentile) and obesity (≥95th percentile). Self-administered questionnaires were given to caregivers to collect sociodemographic characteristics, caregivers' self-reported oral health status (OHS; excellent/very good/good/fair/poor), caregiver-reported child OHS, and other health-related behaviors. Surface-level dental caries experience was recorded using ICDAS criteria by trained and calibrated examiners. Analyses included descriptive and bivariate methods based on X² and non-parametric trend tests. Results: Thirteen percent of participating children were overweight and 9% were obese, while 68% were of normal weight and 10% were underweight. Girls were more likely to be overweight or obese (25%) compared to boys (21%; p<0.0005). Children of Spanish-speaking families were more likely to be overweight or obese (31%) versus 22% in non-Spanish-speaking families (p<0.0005). Parents’ education was inversely associated with their children’s BMI. We did not find any important associations between behavioral or dietary variables, or self-reported measures of oral health and BMI or BMI category. Conclusions: In this community-based sample of preschool-age children we found that more than one out of five children were overweight or obese. Although the specific mechanisms underlying this link need further study, postulated explanations include poor access to affordable, healthy food for low-income families. Supported by: NIH/NIDCR U01-DE025046

[68] Child Nutrition Patterns Associated with Primary Dentition Dental Caries

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Objectives: The classic definition of early childhood caries (ECC) does not consider patterns of dental caries lesion presentation (e.g., smooth surface versus interproximal). Understanding these patterns and associated nutritional influences is key for the development of effective, individualized caries prevention and management strategies. We aimed to identify nutrition patterns associated with different childhood dental caries presentations among patients of a private pediatric dental practice. Methods: We have enrolled and examined 38 healthy (ASA I or II) children (out of a target of 120) under the age of 12 between July 2018 and January 2019. All children underwent a comprehensive dental caries examination by a board-certified pediatric dentist after their parents/caregivers had completed a 20-item nutrition questionnaire. Three clinical groups were defined according to children’s age and
dental caries patterns: group A: 0-3-year-olds, with maxillary anterior caries lesions with or without posterior lesions; group B: 4-12-year-olds, with posterior proximal caries lesions; group C: patients with no caries experience, age-, gender-, and payment method-matched to groups A and B. We used a latent class analysis approach to identify dietary patterns and examined their association with dental caries patterns using bivariate association testing methods and a p<0.05 statistical significance criterion. Results: We identified two clusters (i.e., latent classes) of nutrition patterns: “cluster 1” (68% of participants) correlated with frequent soda/sweet tea and fruit juice consumption, and “cluster 2” (32%) with fruit snacks, milk and water consumption. Clinical group memberships and diet patterns were significantly associated (Fisher’s exact, p=0.02): all children in group B were in “cluster 1” diet (i.e., more liquid-based), whereas groups A and C were almost evenly split between the two diet clusters. Conclusions: We identified nutritional patterns that appear to be associated with distinct clinical presentations of childhood caries. Upon validation and replication, these findings highlight possible targets for individualized preventive interventions for ECC.

[69] Clinical Subtypes of Early Childhood Caries in Preschool-Age Children: the ZOE 2.0 study

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Objectives: Early childhood caries (ECC) manifests with a variety of clinical presentations, but this heterogeneity is neither appreciated nor operationalized by its current taxonomy. The objective of this investigation was to identify distinct clinical subtypes of dental caries’ presentation in preschool-age children. Methods: We used ICDAS-based surface-level dental caries experience information from a large sample of preschool-age children (n=5,596), participants in a community-based oral health study in North Carolina. We used latent class analysis (LCA) with Mplus v.8.1 software to identify subtypes (i.e., latent classes) of dental caries lesion distributions—the optimal number of classes was determined using both model-fit (e.g., Bayesian information criterion) and clinical relevance (e.g., interpretability) criteria—overall, and within strata of restored and untreated ECC. To facilitate class interpretation, we used UV mapping via Blender software to illustrate tooth surface-level caries probabilities onto three-dimensional models. Results: We identified 6 distinct and clinically-relevant subtypes of ECC in the entire sample, with class membership probabilities ranging between 60.0% and 3.8%. Stratified analyses identified 6 and 3 classes for the restored and non-restored groups, respectively. The unrestored ECC subtypes segregated according to recognizable patterns of caries lesion distribution, e.g., “class 1” affected both molars and maxillary incisors, “class 2” maxillary anteriors, “class 3” affected molars. Conclusions: Upon further validation and replication, these ECC subtypes can be used to determine optimal dental caries management approaches based on possibly distinct risk factor profiles. Overall, this empirically-derived classification can help identify more effective interventions, aligned with the paradigm of precision dentistry. Supported by: NIH/NIDCR U01DE025046

[70] Examiner Reliability in Assessing Caries Activity Using ICDAS and QLF-D

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Objectives: Caries activity assessment is critical for proper diagnosis and treatment planning. This study aims to investigate the reliability of examiners assessing caries activity using the International Caries Detection and Assessment System (ICDAS) visual criteria and quantitative light-induced fluorescence digital (QLF-D; Inspektor Research Systems, The Netherlands) technology. Methods: Extracted permanent human teeth with sound surfaces and non-cavitated carious lesions (ICDAS 1-3) on smooth (n=60) and root (n=60) surfaces were selected. Three calibrated examiners evaluated the teeth using ICDAS as sound, active, or inactive, repeating the exam one week later. The same examiners used the Plaque Patch function of QA2 image analysis software (Inspektor Research Systems) on QLF-D images of the teeth. QA2 White Spot Analysis was used to determine lesion presence. Results: Inter-examiner reliability for ICDAS for caries lesion detection was Fleiss kappa 0.83 for smooth and 0.84 for root surfaces, while intra-examiner results ranged from 0.69-1.00 for smooth surfaces and 0.97 for root surfaces. When assessing activity using ICDAS, inter-examiner agreement was 0.64 for smooth and 0.59 for root surfaces, while intra-examiner ranged from 0.70-0.78 for smooth and 0.69-0.75 for root surfaces. Inter-examiner reliability with QLF-D ∆Rmax values using ICC was 0.80 for smooth and 0.60 for root surfaces. Compared against each other, the agreement between White Spot 95Q Analysis and ICDAS demonstrated a Rank Biserial Correlation of 0.66 for smooth and 0.81 for root surfaces. Conclusions: ICDAS criteria demonstrated “almost perfect” agreement when simply identifying lesion presence. However, intra- and inter-examiner reliability decreased when using ICDAS for activity assessment. QLF-D ∆Rmax presents an off-purpose possibility to increase reliability of activity assessment. White Spot Analysis demonstrated near perfect agreement in confirming the presence of root lesions. Studies with clinical validations are warranted to support these uses, and these results emphasize the need for a reliable means of activity assessment.

[71] Nucleic Acid Yield of Saliva and Plaque Samples in a Large-Scale Epidemiologic Study in Children

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Objectives: Saliva and supragingival plaque offer convenient and non-invasive media for the collection of human DNA and oral microbiome samples that can inform the development and application of precision oral health care. However, there is little information on the fidelity of oral biospecimen collection among very young children. Our objective was to determine the yields of these approaches as implemented in a community-based genetic epidemiologic study of early childhood oral health. Methods: We have conducted comprehensive oral/dental clinical examinations including saliva and supragingival plaque collections in > 6,000 3-5-year-old children participating in a community-based early childhood oral health study in NC. Saliva samples were obtained using the Oragene-DNA Genotek OG-575 kit. DNA was extracted with a Perkin-Elmer Magnetic bead-based MSMI robot, quantified with PicoGreen® (PG) or a human DNA-specific concentration estimation RNAseP assay and quality-assessed using A260/A280 ratios. Plaque was collected with sterile toothpicks, embedded in RNA later and frozen in -80°C until analysis. We have used both manual (MO Bio Power-Biofilm RNA kit) and high-throughput (Norgen Biotek Total RNA Purification kit) plaque NA extraction protocols—quantitation is based on PG. Results: The median human DNA yield of the first 4,163 saliva samples was 7.7μg and 99.9% had yields of >200ng—the minimum required for a contemporary high-density genotyping array. In terms of purity, 97% had A260/A280 ratios between 1.7-2.1 and 76% had 1.8-2.0. Human-specific yields were on average 34% lower than those obtained with PG. The first 333 plaque samples’ NA yield was mean=1.4μg (SD=1.3) and median=1.1μg (range=0.09-8.0). Human DNA obtained from saliva and NA obtained from plaque have been subsequently
successfully used for pilot human genotyping, as well as metagenomics, metatranscriptomics and metabolomics analyses. **Conclusion:** Oral biospecimen collection for precision oral health investigations and applications, involving human DNA and aspects of the oral microbiome, are feasible among preschool-age children and yield high-quality information. **Supported by:** NIH/NIDCR Grant #U01-DE025046

[72] **Measurement of Domestic Drinking Water Source Fluoride Levels in a Community-Based Dental Public Health Study**

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**Objectives:** Fluoride is the most widespread and efficacious agent for dental caries prevention. Community water fluoridation (CWF) is an ideal means for oral health promotion at the population level. Nevertheless, direct measurement of household fluoride exposure via CWF is rare in studies of early childhood caries (ECC). We describe the methods and early outcomes of domestic water source fluoride concentration measurement in the context of an ongoing community-based study of early childhood oral health in North Carolina. **Methods:** After their clinical examination at participating Head Start centers, all study participants are asked to provide a domestic water sample and mail it back to the investigators’ team—they are provided with a water sample vial, a pre-addressed and postage pre-paid mailer, as well as visual and written instructions in English and Spanish. All samples are forwarded to the NC State Laboratory of Public Health, where Fluoride is measured with the EPA 300.0 (i.e., ion chromatography) method. For descriptive purposes, water sample fluoride concentrations are categorized as ‘non-detectable’: <0.20ppm F-, ‘sub-optimal’: 0.20-0.64ppm, ‘optimal or above’: ≥0.65ppm, or ‘unsatisfactory’: whenever the measurement could not be performed for any reason. We explored the association between fluoride levels and clinically-determined dental caries experience via logistic regression and marginal effects estimation using a p<0.05 criterion. **Results:** Of 5,638 children with clinical encounters in ZOE 2.0 as of December 7, 2018, 1,300 (23%) samples had been returned. Sixty-six (5%) were unsatisfactory. Among those with measured F- concentration, 500 (41%) were non-detectable, 379 (31%) were sub-optimal and 355 (29%) were optimal or above. Detectable F- levels were associated with a statistically non-significant lower prevalence of ECC (defined at the ICDAS>2 level): 49.5% versus 54% (p=0.1). **Conclusion:** Measuring fluoride in public health studies is practically challenging but adds value to comprehensive investigations of oral health, given its demonstrable positive oral health effects. **Supported by:** NIH/NIDCR U01-DE025046

[73] **Examination of Trends in Pediatric Dental Treatment**

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**Objectives:** Determine the impact of radiographs on treatment decision in the primary dentition. **Methods:** Participants were presented a series of 10 clinical cases with varying degrees of severity (ICDAS 2-6), with and without radiographs via Qualtrics survey and provided a bank of non-invasive and/or invasive treatment options. Non-invasive treatment options ranged from no-treatment to fluoride and dental sealants and invasive treatment options ranged from filling to crown and extraction. Clinically, 1 lesion was ICDAS 2, 1 ICDAS 3, 4 ICDAS 4, 3 ICDAS 5, and 1 ICDAS 6. Radiographically, 4 of 10 lesions penetrated the outer dentinal 1/3, 1 the middle 1/3, 5 the inner
1/3. **Results:** The survey was sent to 7934 (dental school faculty, AAPD members, and private practice dentists). Response rate was 12.9% (N=1026) with 838 qualified responses. Participant’s age ranged from 25 to 82 (6.9% were 25-29; 34.7% 30-39; 20.9% 40-49 years; 15.9% 50-59; and 21.6% 60+). In 66.67% of cases there was agreement before and after radiograph presentation. Three cases were noticeably more likely to be treated restoratively after radiograph presentation, 5.3% (outer dentinal 1/3), 12.9% (inner dentinal 1/3), and 15.4% (outer dentinal 1/3) respectively. One outer dentinal 1/3 lesion was treated non-restoratively noticeably more with radiographs, 14.7%. Overall, 90% of cases were more likely to be treated restoratively, regardless of radiographs. Deep lesions (middle to inner third of dentin) were predominantly treated restoratively. Shallow lesions were treated predominantly restoratively, but with more variation. Regardless of radiographs, restorative options were significantly more likely to be selected for ICDAS 3+. **Conclusions:** The presence of radiographs did not impact the treatment decision making for this group of participants and this group of cases. This may be explained by the clinical severity of the lesions included in this study.

[74] Geographic and Community Descriptions where Silver Diamine Fluoride is Used in North Carolina

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**Objective:** Early childhood caries (ECC) imparts significant burden on the public health system that transcends individual, family, and community levels. The time-honored strategy for managing ECC involves surgical care, often in a hospital setting while the child is under general anesthesia (GA), an extraordinarily expensive venue for a disease that is largely preventable. Silver diamine fluoride (SDF) has been introduced to manage ECC, mitigate its effects, and reduce reliance on GA for dental treatment in young children. Our objectives were to: a) describe the socio-contextual and community health characteristics where dentists use SDF and b) determine the effects of SDF use on GA use. **Methods:** This cross-sectional, time-series study used North Carolina Medicaid dental claims from January 1, 2016 to June 30, 2018. Provider data were extracted from the claims including practice address, number of children treated with SDF, and number of children treated with GA. Utilization rates were normalized using census data. North Carolina County Health Ranking data provided county level indicators for a number of socio-contextual and community health factors. Descriptive statistics and spatial analysis maps, including bivariate choropleths, were reported to compare the adoption of SDF against GA use. **Results:** SDF use spread outward from areas of good social and health indicators to areas of worse indicators. The number of areas with high/moderate SDF utilization and no GA utilization increased, while the number of areas with no SDF utilization and high/moderate GA utilization decreased. Among providers, the SDF utilization rate increased (7.42/1000 to 15.31/1000) and the GA utilization rate decreased (97.33/1000 to 53.65/1000). **Conclusion:** Early adopters of SDF were in resource rich areas. However, over this limited time period, it appears to be reaching communities with more limited resources. It remains too early to determine the public health impacts of SDF treatment on early childhood caries.

[75] Use of Select Non-Surgical Caries Management Techniques in Pediatric Patients Among North Carolina Dentists

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Objectives: Recent advances in the science and practice of dentistry have resulted in the expansion of available dental caries treatment options and increased emphasis on its non-surgical management. However, the adoption of existing and new non-surgical caries management techniques by the dental community is arguably slow and varied. To understand this phenomenon, we undertook this investigation seeking to identify patterns, correlates and determinants of using non-surgical caries management techniques amongst dentists who primarily treat children in NC. Methods: In the first part of this mixed-methods (qualitative and quantitative) study, between August and December 2018, we conducted 16 in-depth interviews with pediatric and general dentists who primarily treat children. The sample comprised a purposefully diverse population according to specialization, years, location and type (i.e., public health or private) of practice. We used a semi-structured interview guide that was pilot tested and iteratively revised throughout the study. The guide included probing questions on participants’ experiences, influences and decision-making regarding the use of non-surgical caries management techniques. Interviews were digitally recorded, transcribed verbatim and thematically analyzed using MAXQDA software. Reporting was based on emerging and recurring themes and insightful quotes. Results: Making the best possible clinical decisions for pediatric patients while balancing parental acceptance have so far emerged as major motivators for caries management technique choice. Staining associated with SDF placement on anterior teeth and perceived pressure by referring providers were reported as barriers for more widespread use of SDF. Respondents’ practice was reportedly influenced by guidelines and research evidence, although more experienced dentists reported relying more on personal clinical experiences compared to less experienced ones. Conclusion: These early findings provide valuable insights into practitioners’ influences, motivations and clinical decision-making; emerging themes and barriers can be further investigated in quantitative studies and serve as possible targets in the context of implementation and quality improvement programs. Supported by: MS Research Support Grant, School of Dentistry, University of North Carolina at Chapel Hill

[76] The Effects of Silver Diamine Fluoride on Future Care of Early Childhood Caries

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Objectives: Silver diamine fluoride (SDF) arrests the progression of dental disease and promotes remineralization of demineralized tissues. Its use is now advocated for in caries management programs. Our objective was to compare dental treatment and expenditure differences between children treated with SDF and children treated with traditional restorative/surgical care. Methods: In this retrospective matched cohort study, we collected demographic, medical history, and treatment procedure information from administrative claims and patient records at a single private practice in Raleigh, North Carolina. Our primary outcome was the number and type of procedure and its associated expenditures performed during 2017. We compared groups according to child demographic information, special health care needs (SHCN) status, and child behavior. Analyses relied upon descriptive statistics (means and standard deviations for continuous variables, counts and frequencies for categorical variables) and bivariate methods (student’s t-tests for continuous variables, Pearson chi-square tests for categorical variables). Results: One hundred four children received SDF treatment, and 250 received traditional restorative/surgical treatment. Children treated with SDF children were younger, had poorer behavior, and had more SHCN. There was no difference in the number of restorative procedures between groups (95% Confidence Interval [CI] for Difference: -0.54, 1.27). However, children treated with traditional treatment had significantly less endodontic and surgical procedures than the SDF treated group (95%CI: -0.95, -0.10) and were less likely to receive restorative/surgical treatment under general anesthesia. There was no significant difference in overall treatment expenditures between the two groups. Conclusion: SDF led to no significant changes in restorative treatment or overall treatment expenditures in this single private practice in urban North Carolina after one year of follow-up. Further research in larger settings and for longer follow-up periods is needed to provide better context about the
public health and financial impacts of SDF treatment.

[77] Dental Fear in Pediatric Patients Treated with Silver Diamine Fluoride: A Preliminary Report

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Objective: The purpose of this study was to evaluate the effects of SDF treatment on dental fear. Specifically, we compared dental fear before and after various caries management strategies while accounting for demographic information. The final results of this study should provide novel insight into the effects of different caries management strategies on dental fear. Methods: This cross-sectional report was conducted at a large multi-center private practice in Charlotte, North Carolina. Data collection began in September 2018, and the first three months are reported here. Child and family demographic information (such as age in years, gender, race, parent education level, family income, and dental insurance status) as well as dental treatment needs was collected. We used the Modified Corah’s Dental Anxiety Scale (MDAS) to measure dental fear in both the adult caregiver and the child patient. All study data were recorded using REDCap. We compared pre-treatment responses across three major categories: (1) in-office treatment with SDF, (2) conventional, in-office restorative/surgical treatment, and (3) restorative treatment using hospital-based general anesthesia. Descriptive statistics (means, standard deviations, and frequencies) and bivariate methods (student t-tests and Pearson chi-squared tests, as indicated) comprised the analysis. Results: The three treatment groups were similar across all child and family demographic domains except for age, where children treated with SDF were significantly younger than the other groups. Children treated with general anesthesia had the most treatment needs. There was no significant difference in MDAS scores among the three treatment groups for both adult caregivers and children. Overall, anxiety was highest for having a “tooth drilled” and receiving an injection. Conclusions: This pre-treatment assessment shows no demographic or dental fear differences aside from age among the three treatment groups. However, the study remains ongoing and conclusions will be reassessed when post-treatment surveys are added to the data base.

[78] Oral Health-Related Quality of Life in Children Treated with Silver Diamine Fluoride: A Preliminary Report

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Objectives: Non-surgical strategies for managing early childhood caries (ECC), such as silver diamine fluoride (SDF), have recently gained traction in pediatric dentistry as part of caries management programs. The impact of SDF on oral health-related quality of life (OHRQoL) remains largely unexplored. This study evaluates the impact that SDF has on OHRQoL for children affected by ECC. Methods: Caregivers of children less than 6 years of age at a multi-center private practice in Charlotte, North Carolina were enrolled for this cross-sectional report. Data presented represents pre-treatment data collected between September and December 2018. Caregivers completed the 13-item Early Childhood Oral Health Impact Scale (ECOHIS) and a demographic survey. Child behavior and dental treatment plans were recorded, and all data were compared across four groups: (1) in-office treatment with SDF, (2) conventional, in-office restorative treatment, (3) restorative treatment using hospital-based general anesthesia, and (4) control group of children requiring no treatment. Analysis was confined to descriptive statistics (means, standard deviations, and frequencies) and bivariate methods (student t-tests and Pearson chi-squared tests, as appropriate). Results: Aside from age, the four groups were similar according to all demographics (the SDF group
was the youngest). There was a significant difference among treatment groups on the ECOHIS items of oral/dental pain (P = 0.045) and difficulty drinking hot or cold beverages (P = 0.016), where children treated with general anesthesia most affected. All other items demonstrated no significant difference among groups. The hospital general anesthesia group experienced the most extensive dental needs and the SDF group showed the least. **Conclusion:** These pre-treatment data show similarities among the four study groups with respect to demographics and OHRQoL. However, the study is ongoing, so we cannot fully compare the degree to which various caries management strategies succeed in improving OHRQoL among pediatric dental patients and their families.

[79] Implementing a Prenatal Oral Health Program: Infrastructure Evaluation and Participant Perceptions

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**Objectives:** The Prenatal Oral Health Program (pOHP) was developed to promote interprofessional collaboration between dental, dental hygiene, and medical students to provide pregnant women with increased access to comprehensive healthcare. This study evaluates program infrastructure via an analysis of various implementation models employed, as well as participant perceptions. **Methods:** This mixed methods study combines qualitative data from a focus group (FG) discussion with quantitative data from a retrospective analysis of pre/post survey data. The FG data weighs the advantages/disadvantages of different clinical structures (integrated with main student clinic vs. stand-alone), program timing (3rd vs. 4th year), management systems (no patient care coordinator (PCC) vs. PCC), and learning styles (traditional vs. interprofessional experiences (IPE)). FG participants included students who had previously participated in pOHP, as well as staff and faculty who were involved in its development (N=7). Surveys evaluated participating dental students’ perceptions prior to and following pOHP training in 2017-2018 (N=81; matched N=76; response rate=90.47%). **Results:** FG and survey data demonstrate that an integrated clinic is preferred over a stand-alone model, though students who have seen a pOHP patient (vs. no patient) are more likely to support a stand-alone model. FG and survey data both demonstrate support for pOHP as a program for 3rd year dental students, though students who have seen a pOHP patient believe it would be effective as a 2nd year program. FG and survey data both emphasize the importance of a PCC and IPE. **Conclusion:** Innovating new clinical models require a period of evolution to determine the most preferred and sustainable infrastructure. This evaluation compares the advantages and disadvantages of different clinical models and demonstrates that student perceptions are influenced by whether or not the student saw a pOHP patient in clinic. The findings will inform the program’s future implementation and ensure maximal outreach and effectiveness.

[80] Developing a Business Model to Implement Childhood Oral Care into General Dentistry Practices

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**Objectives:** Pediatric dental clinics are specifically equipped for and focused on the care of young children. General practitioners should also be able to provide most oral health well-care in early childhood; however, the financial viability of implementing an infant oral health program in general practice has not been demonstrated. To address this knowledge gap, we sought to systematically develop a business model to aid in the decision-making of implementing a childhood oral health program in general dental practice, in a financially viable or profitable manner. **Methods:** We followed a systematic information gathering and expert consensus-building approach to
construct a business model, which can serve as a template for general dentists to input their individual practice and financial parameters. We have used expert opinion (i.e., consensus by an experts’ panel) and evidence from the literature to build and inform the model. Subsequently, we will seek to further validate these parameter values via a survey among general dentists in the Northeast region of the US. Finally, we will seek input from a focus group of general dentists practicing in NC, aiming to reach consensus on these input parameters. **Results:** The experts’ panel identified 10 parameters (e.g., average patient retention rate, patient pool insurance profile, current net annual income, etc.) that would be most influential on the proposed business model. Some mean values obtained from the literature for these input parameters include: annual patient retention rate=80%; actual recall interval=11 months; net annual income=$197,190; 8.8% of patients with Medicaid insurance. Using these input values, it appears that implementation of childhood oral care into a general dentistry office can be financially beneficial. **Conclusions:** Upon further validation and replication, the model and practice parameters offered here may be used to inform decisions regarding the financially viable implementation of early childhood oral health care programs in general dentistry practices.

[81] Promoting Early Childhood Oral Health in Clinical Practice: Development of a Smartphone Application

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**Objectives:** The age-1 dental visit is supported by national guidelines and is an essential component of early childhood oral health promotion. Barriers prevent the widespread adoption of the age-1 visit including dentists’ insufficient training. New educational technologies support the development of effective teaching and learning tools—as these are progressively integrated in dental curricula, they offer opportunities for developing educational programs to enhance learning related to early childhood oral health. In this project we aimed to 1) develop an instructional smartphone application (app) based on the Baby Oral Health Program (bOHP), 2) determine its acceptability by learners of different levels, and 3) measure its educational effectiveness in the context of early childhood oral health knowledge. **Methods:** We used established processes to plan, design and develop the prototype. A similar systematic process utilizing engineering standards was followed to assure product quality and we iteratively revised the product according to learners’ feedback. This was provided by a convenience sample of 30 dental students, dental residents and private practitioners. Additional evaluation will employ a pre- and post-survey design to determine the app’s impact on users’ content knowledge, decision-making, comfort, and stage of readiness to care for young children. **Results:** With learner testing completed, the prototype is being lengthened for pilot testing. The prototype will then be the foundation for the bOHP clinical simulator app. Results thus far indicate dental students’ preference for technology in their education, with most study participants providing positive feedback for the prototype’s usability, user-friendliness and intuitiveness. Importantly, students appreciate the immediate feedback and logical progression of the prototype app and indicate that a smartphone application would add value to their education. **Conclusion:** Upon further evaluation, refinement and testing, we anticipate that the smartphone app will offer a user-friendly and effective means of early childhood oral health education for learners of all levels. **Supported by:** Hillsdale Fund, Greensboro NC, and bOHP Fund, School of Dentistry, University of North Carolina at Chapel Hill
[82] Accuracy and Precision of 3-Dimensional Printed Dental Models Produced by Different Rapid Prototyping Technologies

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Objective: To evaluate the accuracy of dental models manufactured by 3 different rapid prototyping processes: stereolithography apparatus (SLA), Liquid Crystal Display with Unidirectional Peel (LCD-SLA/UDP), and digital light processing (DLP). The secondary objective was to test the accuracy of the models printed at different Z-plane resolution. Materials and Methods: Twelve 3-D digital surface models (6 maxillary and 6 mandibular) from the UNC Orthodontic database were randomly selected. Digital scans were obtained via TRIOS scanner (3Shape) and exported as surface model stereolithography (STL) files. The digital models were printed using 3 rapid prototyping (RP) techniques: 1) SLA (Form2), 2) LCD-SLA/UDP (UniZ), and 3) DLP (Moonray). Each model was printed at 50 and 100 microns resolution in the Z plane. The printed models were rescanned with the TRIOS scanner to create digital STL models and superimposed to the original scan using a Procrustes best fit algorithm with VAM (Canfield Scientific). Absolute and root mean square errors were calculated along the entire surface of the models to determine accuracy. T-Test and ANOVA statistical analysis were performed. Results: The absolute mean error for models printed at 100 microns were 0.08mm for the Form2 (95% CI -0.01-0.13mm), 0.14mm for the MoonRay (95% CI -0.01-0.33mm), and 0.17mm for the UniZ (95% CI -0.01-0.41mm). The accuracy of the Form2 models were statistically significant compared to the MoonRay and UniZ (P= 0.02). There were no differences in the accuracy of the models when printed at 50 versus 100 microns (P=0.82). Conclusion: Models produced from SLA print mechanism were more accurate than DLP and LCD-SLA/UDP. Changing the print resolution from 50 to 100 microns did not affect the overall accuracy of the models. Supported by: Southern Association of Orthodontists Award

[83] Cuspal Coverage Indications with CAD/CAM Lithium Disilicate Restorations

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Objectives: 1) To examine the in vitro fracture resistance of premolars with varying preserved cusp thicknesses restored with CAD/CAM lithium disilicate onlays. 2) To determine if functional and nonfunctional cusps have different cuspal coverage indications. Methods: Human maxillary premolars (N=48) were randomly assigned to six groups. Samples were prepared for IPS e.max CAD onlays using the CEREC Bluecam CAD/CAM system. Onlays for groups 1-2 replaced the nonfunctional cusp and all but 3mm(G1) or 2mm(G2) buccal-lingual width of the functional cusp. Onlays for groups 3-4 replaced the functional cusp and all but 3mm(G3) or 2mm(G4) buccal-lingual width of the nonfunctional cusp. Onlays for group 5 covered both cusps with 2mm thick complete cuspal coverage. Group 6 samples were identical to group 1 with added retentive mesial and distal boxes. The samples were exposed to simultaneous thermocycling (10,000 cycles, 5-55°C, 30s/cycle) and mechanical loading (1.2million cycles, F=70N/cycle, 1.4Hz) using a stainless steel stop (diameter 4mm) contacting the functional cusp then moving laterally to the central fossa. The samples were examined visually for onlay debonding or fracture, cusp fracture or fracture combination to determine mode of failure. Results: The failure rates were as follows: 75% (G1), 0.0% (G2), 12.5% (G3), 0.0% (G4), 0.0% (G5) and 0.0% (G6). The cumulative failure rate for the functional cusp groups (G1+G2) was 37.5%. The cumulative failure rate for the nonfunctional groups (G2+G3) was 6.3%. The difference in percent failure between the functional cusp groups and nonfunctional cusp groups was statistically significant (p=.04;
95%CI:2.11-55.66). No cusp or restoration fractures were observed; all failures were due to debonding of the restoration. **Conclusions:** Within the limitations of this study, added retentive features or margin location in relation to occlusion, not remaining cusp thickness, influences the success of CAD/CAM generated lithium disilicate onlays. Thin preserved cusps were not prone to fracture.

[84] **Effect of Fatiguing and Preheating on the Mechanical Properties of Bulk-fill versus Conventional Composite Resin**

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**Objectives:** To evaluate the effect of fatiguing and preheating on the mechanical properties of a bulk-fill composite resin and its conventional counterpart. **Methods:** Hundred and eighty specimen of Filtek One Bulk Fill Restorative (FOBR, 3M ESPE) and Filtek Supreme Ultra (FSU, 3M ESPE) were prepared for each of the following tests: Fracture Toughness (ISO 23146), Diametral Tensile Strength (No. 27 of ANSI/ADA), Flexural Strength and Elastic Modulus (ISO standard 4049). Specimens in the preheated group were heated to 68º C for 10 minutes, and in the fatiguing group were cyclically loaded and thermocycled for 600000 cycles and then tested. Statistical analysis was performed using analysis of variance (ANOVA) and Tukey’s multiple comparison tests for pairwise comparisons. **Results:** Means and standard deviations can be found in tables 1-4. Preheating and fatiguing had a significant effect on the properties of both FSU and FOBR. Fracture toughness was increased for FOBR when specimens preheated and decreased when fatigued, FSU was not affected. Diametral tensile strength was decreased significantly after fatiguing for the FSU. FOBR had lower tensile strength for all groups when compared to FSU. Fatiguing significantly reduced flexural strength of both FSU and FOBR with latter significantly different than FSU. Preheating had no effect on the flexural strength of both. Preheating significantly decreased the elastic modulus of both composite resins equally. **Conclusion:** Conventional and bulk fill composite resins have minimal difference in their mechanical properties. Preheating does not yield negative effect on the mechanical properties. In-vitro fatiguing yields useful information by emulating the intraoral challenge and predicting the effect of that on properties of composite resins.

<table>
<thead>
<tr>
<th>Composite Resin</th>
<th>Baseline (Mean ± SD)</th>
<th>Preheated (Mean ± SD)</th>
<th>Fatigued (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtek Supreme Ultra</td>
<td>1.53 ± 0.21</td>
<td>1.57 ± 0.13</td>
<td>1.57 ± 0.11</td>
</tr>
<tr>
<td>Filtek One Bulk Fill Restorative</td>
<td>1.78 ± 0.13 #</td>
<td>1.94 ± 0.16 * #</td>
<td>1.66 ± 0.07 * #</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Composite Resin</th>
<th>Baseline (Mean ± SD)</th>
<th>Preheated (Mean ± SD)</th>
<th>Fatigued (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtek Supreme Ultra</td>
<td>62.05 ± 5.06 #</td>
<td>64.90 ± 7.74</td>
<td>51.54 ± 7.80*</td>
</tr>
<tr>
<td>Filtek One Bulk Fill Restorative</td>
<td>55.74 ± 3.34</td>
<td>59.69 ± 6.70</td>
<td>57.52 ± 5.08</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Composite Resin</th>
<th>Baseline (Mean ± SD)</th>
<th>Preheated (Mean ± SD)</th>
<th>Fatigued (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtek Supreme Ultra</td>
<td>150.74 ± 11.52</td>
<td>144.33 ± 7.00</td>
<td>137.78 ± 7.27 * #</td>
</tr>
<tr>
<td>Filtek One Bulk Fill Restorative</td>
<td>149.40 ± 13.66</td>
<td>161.42 ± 4.40 #</td>
<td>125.62 ± 16.28 *</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Composite Resin</th>
<th>Baseline (Mean ± SD)</th>
<th>Preheated (Mean ± SD)</th>
<th>Fatigued (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtek Supreme Ultra</td>
<td>15.17 ± 0.73</td>
<td>13.74 ± 1.35 *</td>
<td>14.95 ± 0.60 *</td>
</tr>
<tr>
<td>Filtek One Bulk Fill Restorative</td>
<td>16.46 ± 1.43</td>
<td>14.15 ± 2.03 *</td>
<td>15.02 ± 1.05 *</td>
</tr>
</tbody>
</table>

*indicates statistical difference (P<.05) when compared to baseline; # indicates statistical difference (P<.05) between conventional and bulk-fill composite resin.
Assessment of Light Emitting Diode (LED) Transilluminators in Detection of Coronal Cracks and Fractures

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Objectives: The detection and diagnosis of cracks and fractures in teeth has been a perpetual challenge in dentistry. Transillumination and magnification have been shown to be effective diagnostic aids. To date there has been no assessment made to verify the ideal beam width and lumens of transilluminators needed for this purpose. This study evaluated the ability of three LED-transilluminator devices with different diameter heads (A-0.97mm, B-1.58mm, C-4.56mm) at varying intensities (L-Low, H-High) to detect cracks in teeth through an ex-vivo model; the effect of magnification was also analyzed. Methods: Forty-four extracted posterior teeth were evaluated. With the exception of five negative controls, all samples were known to have cracks. The teeth were examined by two calibrated evaluators and a consensus agreement reached of tooth status. Assessment of each sample was randomized and analyzed with the overhead dental light through direct vision followed by different light sources through the microscope at 7.5x magnification. The samples were assessed through the microscope light source followed by the three masked LED-transilluminator devices at two distinct intensities. Results: The overall accurate diagnosis was reported in 83.8% of the samples: magnification only 72%, no magnification 74%, AL 78%, AH 86%, CL 86%, BL 89%, BH 89%, CH 89%. The odds of an accurate diagnosis differed among the transilluminators compared to the no magnification group; the odds of agreement for the AH, BH, and CH groups were 3.06, 2.49, and 3.27 times greater respectively. Conclusion: Within the limitations of this study, cracked teeth were more often accurately diagnosed with increased magnification applied with light intensity LED-transilluminators. Supported by: AAE Foundation for Endodontics

Periapical Microsurgery: Assessment of Different Light Emitting Diode (LED) Transilluminator Types for Dentinal Defects Detection

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Objective: LED-transilluminators in periapical microsurgery have been used to detect dentinal defects (DD), however little is known about their ideal size and lumens needed for this purpose. Finding the ideal LED-transilluminator can enhance the precision of this diagnostic test. This study evaluated the ability of four LED-transilluminators in the detection of DD through an ex-vivo TRUEJAW™ surgical model. Methods: Forty-four extracted and previously endodontically treated mandibular premolar teeth were evaluated. Teeth were randomly mounted in the TRUEJAW™ followed by a surgical flap and osteotomy to expose the apical third of the root. After 3 mm apical resection, the root-end surface was inspected through the microscope at 19.4X magnification by two calibrated evaluators. The assessment was made with the microscope light alone followed by 4 masked LED-transilluminators of different diameters and quantity of light output (lumens: lu): Small-Low (0.97mm, 1,126lu), Small-High (0.97mm, 4,476lu), Medium-Low (1.58mm, 9,237lu) and Medium-High (1.58mm, 29,184lu). A consensus agreement was reached for presence or absence of DD. After evaluation, the teeth were removed from the model and examined to establish the true status of the resected surface. This was accomplished with a direct 16X microscopic examination with a Large-High (4.76 mm, 1,2 million lu) LED-transilluminator and methylene-blue dye. The sensitivity and specificity values of each light source were calculated. Results: Ninety-seven percent of roots identified to have DD with transillumination agreed with the established true status. Sensitivity for the
microscope light, Small-Low, Small-High, Medium-Low and Medium-High transilluminators was 55.6%, 63.9%, 63.9%, 81% and 78.4% respectively. Specificity for the microscope light, Small-Low and Small-High transilluminators was 87.5% and for the Medium-Low and Medium-High transilluminators it was 100%.

**Conclusion:** Within the limitations of this ex-vivo surgical study, DD were more often detected with LED-Transilluminators with a larger diameter and higher lumens.

[87] Development of a standardized method to induce cracks in extracted human teeth

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**Objectives:** Cracked teeth are the third most common cause for tooth loss in industrialized countries. Current imaging modalities often have low resolution, which do not show cracks clearly. Our long-term goal is to create and validate a reliable, reproducible and automatic technique for detection and quantification of cracks. The goal of this current project was to create a standardized method to induce cracks in extracted human teeth. **Methods:** We used anonymized, extracted human premolars and molars. The teeth (n=10) were placed in resin trays and stabilized using dental wax to simulate the periodontal ligament. A customized compression insert for the Instron machine was used to fit into the central grooves of these teeth and evenly distribute forces onto the occlusal surfaces. A continuous force (≤400N) was exerted on the grooves using an Instron machine (INSTRON E3000 Electropuls). Ten sound teeth were used as controls. The teeth were then visually examined by two masked investigators. The presence/absence of cracks, their location, extent and orientation was recorded. Data were analyzed by chi square. **Results:** Our standardized method induces cracks in extracted teeth in a reliable manner (p=0.017). The cracks are mesio-distally oriented and propagate from the crown towards the root of the tooth, which mimics the common clinical presentations of cracked teeth. **Conclusions:** Completion of this project is the first step in developing a reliable and quantifiable method for crack detection. Future work will focus on developing and validating an automatic technique for crack detection in high resolution cone beam computed tomography scans. We believe that this work will ultimately lead to improved and early detection of cracks and thus prevent tooth loss. **Funding Sources:** DDS Short-term Research Fellowship - Grover C. Hunter Research Fund, UNC School of Dentistry and NIH/NIDCR R43 DE027574

[88] Prevention of Coronal Discoloration with Novel Topical Endodontic Antimicrobial Agents

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**Objectives:** Tetracyclines are a component of triple antibiotic paste and other antibiotic formulations commonly used in pulp regeneration procedures. However, tetracyclines cause tooth staining. A previous case report suggested that use of a bonding agent lining in the pulp chamber may reduce discoloration. Tigecycline is a newer tetracycline antibiotic that has been proposed for regenerative procedures, but whose discoloration potential relative to other endodontic disinfectants remains unclear. The aim of this study was to evaluate prevention of coronal discoloration from a novel combination of tigecycline and Emdogain® when used with a bonding agent. **Methods:** Forty extracted human teeth with single root canals were collected and instrumented. The access cavities were acid-etched/bonded followed by topical application of Emdogain + 10mg/mL tigecycline, Emdogain + 10mg/mL doxycycline, calcium hydroxide or no medication. All teeth were incubated for four weeks. To assess
discoloration effects, color measurements were obtained using a spectrophotometer in a light-controlled chamber at D65 artificial light setting. Baseline color measurements were obtained prior to the procedure. Following application of medicaments, color measurements were obtained for the incisal, middle and cervical thirds of the crown at weeks 1 and 4. Mean color change (ΔE) from baseline were evaluated using analysis of variance with repeated measures. Results: Teeth in treatment and control groups showed no significant change in color at the cervical, middle or incisal thirds (P>0.05) at weeks 1 and 4 from baseline. Conclusions: Use of a bonding agent in conjunction with antimicrobial thirds appears to minimize tooth discoloration with these tetracycline medicaments. Funding: Department of Endodontics

[89] Adhesion and Remineralization Potential of Proteoglycan-infused Dentin
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¹Pediatric Dentistry, Federal University of Santa Catarina, Florianopolis, SC, Brazil, ²DDS Class of 2022, School of Dentistry, University of North Carolina at Chapel Hill, ³Department of Restorative Sciences, University of North Carolina at Chapel Hill

Objectives: This study tested the effects of the addition of small leucine-rich proteoglycan (SLRP) proteins on phosphoric acid (PA)-treated dentin in microtensile bond strength (MTBS); and the role of such SLRPs in the remineralization potential of demineralized dentin collagen. Methods: Fifty human molars were used to obtain coronal dentin sections. SLRPs were either decorin (DE) or biglycan (BI) in their core or proteoglycan form (with glycosaminoglycans, GAGs). Groups were: 1. no treatment (no-tx), 2. DE core, 3. DE+GAGs, 4. BI core, 5.BI+GAGs. All samples were etched with PA for 15s, rinsed, and treated accordingly prior to application of Adper Single Bond Plus and a composite buildup. Twenty-four hours or 6 months after the procedure, samples were tested for MTBS. Dentin blocks were fully demineralized, infused with the SLRPs, and placed in remineralization solution or water for 2 weeks and evaluated by TEM. Results: MTBS test presented a mean of 53.2 ±15.6 MPa in the no-tx group with no statistically significant difference to DE core (48.4±13.7 MPa) and BI core (47.1±15.4 MPa). The GAGs groups DE+GAGs (27.0±13.7) and BI+GAGs (37.2±19.4) showed decreased MTBS compared to no-tx (p<0.001). All no-tx samples had adhesive failures. A low number (<10%) of cohesive failures in dentin was noticed for the other groups. At 6 months the SLRPs with GAGs showed statistically significant improvement on bonding (62.2 ±5.9 for DE core and 52.9±8.1 for BI core) (p<0.001). TEM images indicated a tighter collagen fibril pattern of samples treated with SLRPs+GAGs in saliva compared to water and no-tx. Conclusions: Core proteins seem not to affect dentin bonding but the presence of GAGs is detrimental to immediate bond strength. Aging of samples showed proteins with GAGs are able to improve bonding overtime and favor collagen fibril packing, which can help with the remineralization process and fibril stability.
North Carolina’s Limited Supervision Act: Utilization, Knowledge, and Opinions

Alalkami J1, Lampiris LN2, Sams LD3, Harmon JB1, Brame JL3
1Dental Hygiene Education Program, School of Dentistry, University of North Carolina at Chapel Hill, 2Department of Dental Ecology, University of North Carolina at Chapel Hill, 3Department of Periodontology, School of Dentistry, University of North Carolina at Chapel Hill

Objectives: The North Carolina Dental Hygiene Limited Supervision Statute (DHLS) was passed in 2007 addressing access to care needs by allowing dentists to employ dental hygienists (DH) to provide care without direct supervision in facilities identified by the Office of Rural Health and approved by the State Board of Dental Examiners (SBDE). Dentists are responsible for identifying and examining patients, planning care, and reporting and maintaining records. There are no existing outcomes assessment data to indicate effectiveness, utilization or awareness of DHLS. Study aims included evaluation of utilization, knowledge, and opinions of DH.

Methods: This study was exempt by the Institutional Review Board at the University of North Carolina at Chapel Hill. A mixed-methods study design was used. A publicly-available statistical report indicating utilization, types of services performed, and location of care was obtained from the NC SBDE. A 19-item survey was developed and distributed to assess knowledge and opinions of NC DH attending a continuing education (CE) course. Survey responses were analyzed using descriptive statistics and the chi-square test for proportions.

Results: Data showed an average of 11 dentists per year utilizing DHLS between 2008-2016. Services by DH varied and included prophylaxis, scaling and root planing, fluoride, and radiographs. No adverse occurrences were reported. Surveys were completed by 115 DH. Data revealed 80% (N=88) wanted to utilize DHLS, 96% (N=109) wanted to learn more, and 99% (N=113) agreed it is important for DH and dentists to learn about DHLS. A majority of respondents lacked knowledge regarding DHLS allowable procedures and requirements. Qualitative feedback showed trends with lack of knowledge, underutilization, and a need for DHLS.

Conclusion: DHLS is an underutilized service in NC; however, DH indicate a willingness to learn and utilize this statute more. Increasing knowledge and awareness may positively impact access to care in NC.
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