



# **BOSTON UNIVERSITY SCHOOL OF DENTAL MEDICINE**

**25th Annual**

# **SCIENCE DAY**

**March 16, 2006**

**SPONSORED BY:**

**BUSDM PREDOCTORAL RESEARCH PROGRAM**

**BUSDM STUDENT RESEARCH GROUP**

**BUSDM AMERICAN STUDENT DENTAL ASSOCIATION**

Image: Immunolocalization of  $\alpha$ -catenin (green) in infiltrating epithelial islands of squamous cell carcinoma of the lateral tongue border (10X). Tissue was counterstained with DAPI (blue) for nuclei. Courtesy of Drs. Noonan and Kukuruzinska



**The mission  
of the  
Predoctoral Research Program  
at  
Boston University  
School of Dental Medicine  
is  
to shape the future of dental medicine  
and dental education through  
research;  
to educate students from diverse  
backgrounds about the importance of  
research in dental medicine; and  
to mentor students to make informed  
decisions about research career  
opportunities.**

## **Contents**

Message from the Dean	2
Message from the Associate Dean for Research	2
Research Faculty Mentors	3
Predoctoral Research Committee Members	4
Science Day 2006 Judging Committee	5
Student Research Group (SRG) 2006 Officers	5
American Student Dental Association (ASDA)	5
Administration	5
Program	6
Keynote Speaker	6
Vendors	6
Science Day 2006 Winning Presentations	7
Science Day 2006 Presentations	8
Junior Investigator Awards	10
Abstracts	11

## Message from the Dean

Since our inaugural Science Day back in 1981, much has changed in the research landscape. Demystification of the human genome, unprecedented increases in the ability of technology to process large amounts of data quickly, and innovations in biomaterials and drug delivery methods mean we are on the verge of revolutionary discoveries.

BUSDM is part of that revolution. As a school of dental medicine with a strong research mission, we offer predoctoral and postdoctoral students alike access to some of the world's foremost researchers in their field. We are the only dental school with a Department of Molecular and Cell Biology, and are pioneers in health disparities research.

The abstracts in these pages highlight the work presented by our students, residents, and fellows during the school's March 2006 Science Day. Their achievements are testament to the strength of our students and faculty mentors.

Spencer N. Frankl, DDS, MSD  
Professor and Dean



## Message from the Associate Dean for Research

Nurturing a new generation of researchers is an integral part of the school's mission. This charge is amply displayed here in this booklet, which encapsulates our students' and residents' research presentations from BUSDM's 25th Annual Science Day in March 2006.

The research presented reflects the breadth of multidisciplinary approaches taken in our school and the strong commitment to translate these findings in day-to-day dental practice. Because biomedical research plays an increasingly important role in dental medicine, it is crucial that today's practitioners remain up-to-date on advancements that will affect their patients' health.

Twenty-five years ago Dean Frankl recognized the tremendous impact research would have not only in dental education but in the profession as a whole. Accordingly, he has strongly supported research at the school, putting resources behind the enterprise of discovery to improve health and ensure our students remain at the forefront of innovation. His dedication to instilling a culture of research at the school is evidenced on the pages that follow.

Salomon Amar, DDS, PhD  
Associate Dean for Research



## Research Faculty Mentors

### *General Dentistry*

- Judith Jones, DDS, MPH, professor and chair. Research Area: Health Outcomes Research, Oral-systemic Relationships
- Paula Friedman, DDS, MSD, MPH, professor. Research Area: Geriatric Dentistry

### *Health Policy and Health Services Research*

- Raul Garcia, DMD, MMS, professor and chair. Research Area: Epidemiology
- Michelle Henshaw, DDS, MPH, assistant professor. Research Area: Public Health
- Elizabeth Kaye, MPH, PhD, associate professor. Research Area: Public Health
- Ana Karina Mascarenhas, BDS, MPH, DrPH, associate professor. Research area: Oral Epidemiology and Health Services Focusing on Access Utilization and Quality of Care in Orthodontics

### *Molecular and Cell Biology*

- Carlos Hirschberg, PhD, professor and chair. Research Area: Biochemistry/Molecular Biology
- Claudia Abeijon, PhD, assistant professor. Research Area: Cell Biology/Microbiology
- Maria Kukuruzinska, PhD, professor. Research Area: Molecular and Cell Biology/Development
- Phillips Robbins, PhD, professor. Research Area: Molecular and Cell Biology
- Miklos Sahin-Toth, MD, PhD, associate professor. Research Area: Biochemistry
- John Samuelson, MD, PhD, professor. Research Area: Microbiology

### *Oral and Maxillofacial Surgery*

- David Cottrell, DMD, associate professor and chair. Research Area: Oral Medicine/Surgery

### *Orthodontics and Dentofacial Orthopedics*

- Donald Ferguson, DMD, MSD, professor and chair. Research Area: Bone
- Anthony Gianelly, DMD, PhD, MD, professor. Research Area: Bone

### *Orthopedic Surgery*

- Louis Gerstenfeld, PhD, associate professor. Research Area: Cell Biology/Bone
- George Barnes, PhD, assistant professor. Research Area: Cell Biology/Bone

### *Pediatric Dentistry*

- Christopher Hughes, DDS, PhD, associate professor and chair. Research Area: Oral Microbiology

#### *Periodontology and Oral Biology*

- Salomon Amar, DDS, PhD, professor and associate dean for research. Research Area: Cell Biology
- Frank Oppenheim, DMD, PhD, professor and chair. Research Area: Biochemistry
- Dana Graves, DDS, DMSc, professor. Research Area: Cell Biology
- Robert Gyurko, DDS, PhD, assistant professor. Research Area: Periodontology/Immunology/Bone Physiology
- Eva Helmerhorst, MS, PhD, assistant professor. Research Area: Biochemistry
- Alpdogan Kantarci, DDS, PhD, assistant professor. Research Area: Periodontology/Immunology/Molecular Biology
- Cataldo Leone, DMD, DMSc, associate professor. Research Area: Biochemistry/Periodontology
- Philip Trackman, PhD, professor. Research Area: Cell Biology
- Thomas Van Dyke, DDS, PhD, professor. Research Area: Periodontology/Immunology

#### *Restorative Sciences/Biomaterials*

- Dan Nathanson, DMD, MSD, professor and chair. Research Area: Biomaterials
- Laisheng Chou, DMD, PhD, professor. Research Area: Cell Biology/Oral Medicine
- Russell Giordano, DMD, DMSc, associate professor. Research Area: Biomaterials
- Zhimon Jacobson, DMD, MSD, clinical professor. Research Area: Implantology

### **Predoctoral Research Committee Members**

- Maria Kukuruzinska, PhD, professor, director and chairperson of the Predoctoral Research Program
- Salomon Amar, DDS, PhD, professor and associate dean for research
- Donald Ferguson, DMD, MSD, professor and chair of the Department of Orthodontics & Dentofacial Orthopedics
- Paula Friedman, DDS, MSD, MPH, professor and associate dean for administration
- Deborah Fournier, PhD, associate professor and associate dean for institutional planning and evaluation
- Jeffrey Hutter, DMD, MEd, associate professor, chair and associate dean for academic affairs
- Raul Garcia, DMD, MMS, professor and chair of the Department of Health Policy and Health Services Research
- Russell Giordano, DMD, DMSc, associate professor and director of the Division of Biomaterials
- Anita Gohel, BDS, PhD, assistant professor in the Department of General Dentistry
- Kathy Held, MS, assistant professor and associate director of extramural programs

- Afaf Hourani, MS, MPH, manager of the Office of Science Information
- Judith Jones, DDS, MPH, professor and chair of the Department of General Dentistry
- Cataldo Leone, DMD, MD, associate professor and co-chair of the Predoctoral Research Program
- Mari Megias, MS, director of communications
- Elisa Sin DMD 07, president of the Student Research Group
- Thomas Van Dyke, DDS, PhD, professor and director of the Clinical Research Center

### **Science Day 2006 Judging Committee**

- John Bowley, DDS, MS
- Paula Friedman, DDS, MSD, MPH
- Anita Gohel, BDS, PhD
- Eva Helmerhorst, PhD
- Elizabeth Kaye, MPH, PhD
- Alpdogan Kantarci, DDS, PhD

### **Student Research Group (SRG) 2006 Officers**

- Elisa Sin DMD 07, president
- Nathaniel Caldon DMD 07, vice president
- Anush John DMD 07, secretary
- Sophia Lalani DMD 09, treasurer

### **American Student Dental Association (ASDA)**

- David Blackburn DMD, 06 advisory board
- Leon Yu DMD 07, first delegate
- Monika Srivastava DMD 07, second delegate
- Chrys Constantinou DMD 08, legislative liaison
- Kerry Anzenberger DMD 08, DMD I representative

### **Administration**

- Spencer Frankl, DDS, MSD, professor and dean of BUSDM
- Jeffrey Hutter, DMD, MEd, associate professor, chair and associate dean for academic affairs
- Richard Rabbett, associate director for administration
- Mari Megias, MS, director of communications
- Andrew Burke, manager of facilities

## Program

- Exhibitions: 9 AM-5 PM
- Poster Presentations: 10 AM-12 PM
- Keynote Speaker: 12 PM
- Oral Presentations: 2 -4:30 PM
- Awards Ceremony: Friday, March 17, at Science Night Gala Dinner

## Keynote Speaker

### Mark Klempner, MD “Sharpening the NEIDL at BUMC: Paths Forward for Emerging Infectious Diseases Research”

Dr. Klempner is the associate provost for research at BUMC, the Conrad Wesselhoeft Professor of Medicine at MED, and director of the National Emerging Infections Diseases Laboratory (NEIDL). As principal investigator, on the NEIDL grant, he has been instrumental in securing the funds for Boston University to construct this Biohazard Level 4 facility. Klempner received his MD from Cornell University Medical College, did his residency at Massachusetts General Hospital, and completed a fellowship at the National Institute of Allergy and Infectious Disease, National Institutes of Health. His research includes investigations into the basic molecular biology and pathogenic mechanisms of the Lyme disease spirochete, *Borrelia burgdorferi*, patient-based clinical research on prevention, diagnosis and treatment of Lyme disease, and novel molecular methods for detecting, identifying, and quantifying microorganisms.

## Vendors

- |  |                                    |
|--|------------------------------------|
| • Aspen Dental                           | • Lexi-Comp                        |
| • Brasseler USA                          | • Liaison International            |
| • Carl Zeiss Surgical Ltd                | • Massachusetts Dental Society     |
| • Delta Dental of Massachusetts          | • Nobel Biocare                    |
| • Dentsply North America                 | • Patterson Dental Supply          |
| • Designs for Vision                     | • The Procter & Gamble Company     |
| • EDIC                                   | • Southern Dental Industries       |
| • Garrison Dental Solutions              | • Sonicare/Philips Oral Healthcare |
| • General Scientific Corp/Surgitel       | • Sullivan Schein Dental           |
| • Gentle Communications/<br>GentleDental | • 3M ESPE                          |
| • Hu-Friedy                              | • Tom's of Maine                   |
| • Ivoclar Vivadent                       | • Ultradent Products               |
| • Laclede, Inc./Biotene                  | • United States Army               |
| • Langham Hotel Boston                   | • United States Navy               |
|  | • United States Airforce           |



## Science Day 2006 Winning Presentations

### Poster Presentations

#### *Predoctoral Students*

Khadija Rhourida, Thomas Van Dyke and Robert Gyurko: "Gender-dependent modulation of osteoclast formation by inducible nitric oxide synthase."

#### *Postdoctoral Students*

Hesham Al-Mashat, Suneel Kandru, Rongkun Liu, Yugal Behl, Tesfahun Desta and Dana Graves: "Diabetes impairs healing by enhancing caspase activity."

#### *Postdoctoral Fellows*

Camille C. Siqueira, Thomas Van Dyke and Robert Gyurko: "p47phox activation and alveolar bone loss in hyperglycemic ins2akita mice."

#### *ADA/Dentsply Award*

Tsung-Lin James Lee and Russell Giordano: "Spectrophotometer evaluation of the influence of multiple firing on color and opacity of dental porcelains."



Khadija  
Rhourida



Hesham  
Al-Mashat



Camille  
Siqueira



James Lee



John Turner



Ariel Bales-  
Kogan



Walter  
Siqueira

### Oral Presentations

#### *Predoctoral Students*

John Turner, Donald Ferguson, John-David Sebaoun, Alpdogan Kantarci and Robert Carvalho: "Anabolic modeling of trabecular bone following selective alveolar decortication."

#### *Postdoctoral Students*

Ariel Bales-Kogan and Ana Karina Mascarenhas: "Treatment quality between Boston University students and ABO-certified orthodontists."

#### *Postdoctoral Fellows*

Walter L. Siqueira, Marek Kloczewiak, Eva Helmerhorst, Martin Steffen and Frank Oppenheim: "Discovery of new enamel pellicle proteins by mass spectrometry."

## Science Day 2006 Presentations

### Poster Presentations

#### *Predoctoral Students*

Alok Ahlawat, Donald Ferguson, Omid Rajaei, Bill Wilcko and Tom Wilcko: "Influence of DI on orthodontic outcome following selective alveolar decortication."

Carolyn Dicus and Ana Karina Mascarenhas: "Development of a middle school oral health curriculum."

Chris Kelson, Donald Ferguson, Jean-David Sebaoun, Alpdogan Kantarci, Robert Carvalho and Thomas Van Dyke: "Anabolic modeling of the lamina dura following selective alveolar decortication."

Asal Kohandel-Shirazi, Kevin Oliveira, Donald Ferguson, Bill Wilcko and Tom Wilcko: "Orthodontic stability of advanced lower incisors following selective alveolar decortication."

Tsung-Lin James Lee and Russell Giordano: "Spectrophotometer evaluation of the influence of multiple firing on color and opacity of dental ceramics."

Khadija Rhourida, Thomas Van Dyke and Robert Gyurko: "Gender-dependent modulation of osteoclast formation by inducible nitric oxide synthase."

David Park, Martha Nunn, Takashi Kumagai, Judith Jones and Takanari Miyamoto: "Caries prediction vs actual incidents; multivariate risk analysis of dental caries in complaint adult population."

Elizabeth Walker, Donald Ferguson, Bill Wilcko and Tom Wilcko: "Orthodontic treatment and retention outcomes following selective alveolar decortication."

#### *Postdoctoral Students*

Hesham Al-Mashat, Suneel Kandru, Rongkun Liu, Yugal Behl, Tesfahun Desta and Dana Graves: "Diabetes impairs healing by enhancing caspase activity."

Satheesh Elangovan, Marek Kloczewiak, Eva Helmerhorst and Frank Oppenheim: "Comparison of hydroxyapatite synthesis under neutral and basic conditions."

Li Gao, Thomas Van Dyke and Robert Gyurko: "Accelerated bone loss after molar ligation compared to bacterial challenge."

Homan Hanasab, Eva Helmerhorst and Frank Oppenheim: "Oral candida carriage and salivary antifungal activity."

Rayyan Kayal, Dimitris Tsatsas, Megan Bauer, Elizabeth Krall, Elise Morgan, Louis Gerstenfeld and Dana Graves: "Diabetes may impair fracture healing by excess removal of cartilage."

Jean-David Sebaoun, Donald Ferguson, Alpdogan Kantarci, Robert Carvalho, Thomas Van Dyke and Harry Prasad: "Catabolic modeling of trabecular bone following selective alveolar decortication."

### *Postdoctoral Fellows*

Greg Pezza, Sharron Rich, Carolyn Wehler, Raul Garcia and Judith Jones:  
“Periodontal care improves short-term quality-of-life and satisfaction with diabetes care?”  
Camille C. Siqueira, Thomas Van Dyke and Robert Gyurko: “p47phox activation and alveolar bone loss in hyperglycemic ins2akita mice.”

### **Oral Presentations**

#### *Predoctoral Students*

Monica Dosanjh, Donald Ferguson, Omid Rajaei, Bill Wilcko, Tom Wilcko:  
“Orthodontic outcome changes during retention following selective alveolar decortication.”  
John Turner, Donald Ferguson, John-David Sebaoun, Alpdogan Kantarci and Robert Carvalho: “Anabolic modeling of trabecular bone following selective alveolar decortication.”

#### *Postdoctoral Students*

Emad Al-Badawi and Dan Nathanson: “In vitro testing parameters effect on adhesive shear bond strength.”  
Ariel Bales-Kogan and Ana Karina Mascarenhas: “Treatment quality between Boston University students and ABO-certified orthodontists.”  
Afaf Dahlan, Russell Giordano, Richard Pober and Donald Ferguson: “Evaluation of self-ligating bracket clip (SLB) breaking force.”  
Adnan Ishgi and Russell Giordano: “The effect of different surface treatments on flexural strength and microstructure of Y-TZP zirconia ceramics.”  
Taera Kim, Takanari Miyamoto and Thomas Dietrich: “The effect of root proximity on alveolar bone loss.”  
Xiuli Sun, Eva Helmerhorst and Frank Oppenheim: “Assessment of the kinetics of histatin 1 and Histatin 3 proteolysis in human saliva.”  
Caterina Venuleo, Eva Helmerhorst, Ana Fraga and Frank Oppenheim: “C. glabrata secretes a histatin 5 neutralizing component.”  
JuliaYang, Michelle Siqueira, Xianoren Tang, Salomon Amar and Dana Graves: “ST18 regulates TNF-alpha-induced apoptosis and inflammatory gene expression.”  
Michael Davey and Caroline Genco: “Defining the roles of Toll-like receptor (TLR)2 and TLR4 in P. gingivalis 67-kDa fimbria-mediated activation of IL-8 production by human aortic endothelial cells.”

#### *Postdoctoral Fellows*

Walter L. Siqueira, Marek Kloczewiak, Eva Helmerhorst, Martin Steffen and Frank Oppenheim: “Discovery of new enamel pellicle proteins by mass spectrometry.”

## **Junior Investigator Awards**

Dr. Hatice Hasturk, assistant professor, periodontology & oral biology

Dr. Alp Kantarci, assistant professor, periodontology & oral biology

Dr. Vikki Noonan, assistant professor, oral pathology

Dr. Greg Pezza, clinical instructor, general dentistry

Dr. Ramzi Sarkis, clinical instructor, restorative sciences/biomaterials

Dr. Xiaoren Tang, research associate, periodontology & oral biology

## Abstracts

### Influence of DI on Orthodontic Outcomes Following Selective Alveolar Decortication

Alok Ahlawat, Donald J. Ferguson, Bill Wilcko and Tom Wilcko, Private Practice, Erie, PA, USA and Boston University School of Dental Medicine, Department of Orthodontics

Discrepancy Index (DI) was released by the ABO as a measure of pre-treatment malocclusion severity. It is expected that orthodontic treatment outcome and retention success is related to severity of initial malocclusion. **Objectives:** To evaluate post treatment and retention outcomes relative to pre-treatment malocclusion severity in a population of patients treated with orthodontic therapy facilitated by selective alveolar decortication. **Methods:** DI scores were derived from pre-treatment study casts and radiographs for 77 patients and grouped into 4 groups according to total DI score or severity: 1=0-9, 2=10-19, 3=20-29, and 4=30+. Seventeen ABO Objective Grading System (OGS) criteria were used to score orthodontic outcomes at immediate post treatment or T1 (n=77), at least 1 year retention or T2 (n=56) and at least 2 years retention or T3 (n=23). **Results:** Non-parametric Kruskal Walls H-testing revealed statistical differences between DI severity group 3 and the following DI severity groups: groups 1 and 2 (7.6 vs 1.7 and 2.2, p=.001) for T1 occlusion relationship; group 1 (7.9 vs .04, p=.000) and group 2 (7.9 vs 2.3, p=.003) for T2 occlusal relationship; group 1 (6.1 vs 2.2, p=.02) for T2 overjet; and group 1 (31.7 vs 15.2, p=.03) for T2 OGS total score. **Conclusions:** In the sample studied, treatment of malocclusions with DI severity scores between 20 and 29 resulted in higher OGS scores for occlusion relationship at T1 and T2 and for overjet and OGS total scores at T2.

*Supported by the Department of Orthodontics, BUSDM*



## Development of a Middle School Oral Health Curriculum

Carolyn Dicus, Ana Karina Mascarenhas, Boston University School of Dental Medicine, Department of Health Policy and Health Services Research

**Introduction:** To date, the health curriculum presented by the Boston Area Health Education Center (BAHEC) includes no references to oral health. This situation proffers an ideal opportunity to collaborate with BAHEC with the goal of helping students and their teachers to grasp the importance of oral health as well as to expose minority students to dental careers early in their education. A search of the literature showed that current curricula targeting this age group are scarce.

**Conclusion:** Thus, a new curriculum was developed based on information culled from existing (but outdated) curricula, dental school lectures, and other sources. The curriculum consists of six modules:

- 1) Dental Anatomy
- 2) Dental Disease Process and Oral Hygiene
- 3) Nutrition and Oral Health
- 4) History of Tobacco, Smokeless Tobacco and Oral Cancer
- 5) Adolescent Issues in Oral Health
- 6) Dental Procedures and Dental Careers

Each incorporates hands-on activities and is designed to enable instruction with minimal preparation. Pre- and post-lesson tests to assess the effectiveness of the curriculum in meeting these goals were also developed.

*Supported by the Department of Health Policy and Health Services Research/Division of Dental Public Health, BUSDM*

## Anabolic Modeling of the Lamina Dura Following Selective Alveolar Decortication

Chris Kelson, Jean-David Sebaoun, Donald J. Ferguson, Alpdagon Kantarci, Robert Carvalho and Thomas E. Van Dyke, Boston University School of Dental Medicine, Department of Orthodontics

It has been clinically demonstrated that orthodontic treatment time is reduced 60% to 70% following selective alveolar decortication. Decortication injury is followed by an increase in tissue turnover. **Objective:** The aim of this study was to analyze bone apposition pattern following selective alveolar decortication. **Methods:** Six rats underwent unilateral selective alveolar decortication buccal and lingual to the upper left first molar with the right side serving as control; animals were divided into 2 groups. In group 1, vital bone stains were injected sub-peritoneal at 1 week intervals beginning 1 week post-op starting with calcein, then tetracycline, and lastly alizarin red. The same series of injections were made in group 2 starting at post-op week 4. Group 1 animals were killed at post-op week 4 and group 2 was killed at post-op week 7. Maxillary halves were harvested and processed for undecalcified fluorescent stain histology. Multiple systematic measurements (80 to 294 values per specimen) of bone apposition surrounding the 5 roots of the first molar were made using Olympus MicroSuite FIVE analysis software and expressed as total 3-week apposition width as well as apposition length as percent of overall root perimeter. **Results:** One-way ANOVA with Tukey post hoc testing demonstrated that apposition width was significantly greater ( $p=.000$ ) following surgery at 4 weeks post decortication (.051mm) compared with the 4 week control (.037mm), and the 7 week surgery (.037mm) and control (.032mm). No differences were observed in apposition length as a percentage of root perimeters. **Conclusion:** Apposition of the lamina dura (anabolic modeling) increased 46% at the 4-weeks stage following selective alveolar decortication in the rat.

*Supported by the Department of Orthodontics, BUSDM*

## Orthodontic Stability of Advanced Lower Incisors Following Selective Alveolar Decortication

Asal Kohandel-Shirazi, Donald J. Ferguson, Bill Wilcko and Tom Wilcko, Private Practice, Erie, PA, USA and Boston University School of Dental Medicine, Department of Orthodontics

Re-crowding of lower incisors following labial positioning is consistently cited in orthodontic retention literature. The tendency for incisors to return toward pre-treatment positions is due, in part, to periodontal tissue memory. Selective alveolar decortication plus alveolar grafting (AOO™) when combined with orthodontic treatment results in greater post treatment and retention stability. This finding is likely due to enhanced tissue turnover and loss of periodontal tissue memory.

**Objectives:** To assess the effects of labial advancement of lower incisors of at least 3mm on stability with and without AOO™. **Methods:** The orthodontic study cast records of 75 patients were examined at pre-tx (T1), post treatment (T2) and retention (T3) and grouped as follows: L1 advanced >3mm with AOO™ (n=8), L1 advanced <3mm with AOO™ (n=16), L1 advanced >3mm without AOO™ (n=25), and L1 advanced <3mm without AOO™ (n=26). Irregularity Index (NDX) and inter-canine distance (3-3) were measured at the 3 time intervals. **Results:** For the L1 advanced >3mm without AOO™ group, T3 NDX was greater ( $p<002$ ) than all other groups and magnitude of NDX change from T2 to T3 was greater ( $p<001$ ) than both groups with AOO™; both non-AOO™ groups relapsed T2 to T3. No differences were observed in 3-3 width treatment changes (T1-T2), but 3-3 width relapsed during retention more in the L1 advanced >3mm without AOO™ group than the L1 advanced <3mm without AOO™ group. **Conclusions:** Advancement of the lower incisor greater than 3mm did not result in significant re-crowding when selective alveolar decortication was combined with orthodontic treatment; relapse was absent.

*Supported by the Department of Orthodontics, BUSDM*

## Gender-Dependent Modulation of Osteoclast Formation by Inducible Nitric Oxide Synthase

Khadija Rhourida, Thomas Van Dyke and Robert Gyrko, Boston University  
School of Dental Medicine, Department of Periodontology and Oral Biology

**Objective:** To investigate the effect of gender on the interaction between inducible nitric oxide synthase (iNOS) and osteoclast formation. **Materials and Methods:** Bone mineral density (BMD) was measured with dual energy x-ray absorption on femurs of wild type and iNOS KO mice. Primary osteoclast cultures were induced with M-CSF and RANKL in isolated bone marrow cells. Periodontal osteoclasts were induced by two methods: oral inoculation with *Porphyromonas gingivalis* or by tying a 9-0 silk ligature on the upper 2nd molar. Osteoclasts were stained with TRAP in cultures and in histological sections. **Results:** BMD was significantly increased in female iNOS KO femurs ( $41.7 \pm 1.9$  mg/cm<sup>2</sup>) compared to that of female wild type mice ( $32.0 \pm 2.9$  mg/cm<sup>2</sup>,  $P < 0.01$ ). Male iNOS KO mice however had similar BMD ( $34.4 \pm 2.9$  mg/cm<sup>2</sup>) as wild type male mice ( $33.4 \pm 3.7$  mg/cm<sup>2</sup>). When primary osteoclast cultures were induced with M-CSF and RANKL, significantly fewer TRAP-positive multinuclear cells were found in female iNOS KO cultures ( $1089.3 \pm 140.6$  cells/mm<sup>2</sup>) than in female wild type cultures ( $2192.3 \pm 177.6$  mm<sup>2</sup>,  $P < 0.01$ ). Between male mice a much smaller difference exists when comparing iNOS KO cultures ( $2450 \pm 576$ /mm<sup>2</sup>) with wild type cultures ( $3243 \pm 403.1$ /mm<sup>2</sup>,  $P = 0.12$ ). When female mice were inoculated with *P. gingivalis* orally, iNOS KO mice developed fewer osteoclasts on the alveolar bone surface ( $3.73 \pm 0.85$  cells/mm) than wild type ( $5.19 \pm 0.32$  cells/mm,  $P < 0.05$ ). Placement of silk ligature in female mice on the maxillary 2nd molar similarly resulted in lower osteoclast induction in iNOS KO mice ( $7.69 \pm 0.52$  cells/mm), compared to wild type mice ( $11.96 \pm 1.73$  cells/mm  $P < 0.05$ ). **Conclusions:** iNOS enhances osteoclast formation in female mice both in vivo and in vitro, while its role in male osteoclast formation is less pronounced. This finding suggests that iNOS and its product, nitric oxide, may modulate the effects of estrogen or other sex hormones on bone metabolism.

*Supported by NIDCR/NIH grants DE14568 (R.G.) and RR00533 (T.E.V.D.)*

# Spectrophotometer Evaluation of the Influence of Multiple Firing on Color and Opacity of Dental Ceramics

Tsung-Lin James Lee and Russell Giordano, Boston University School of Dental Medicine, Department of Restorative Sciences/Biomaterials

**Goal:** Quantitative analysis of the color and opacity changes of dental porcelains and framework materials after multiple firing procedures. **Rationale:** All ceramic restorative materials may be fabricated using high strength framework materials milled from partially sintered blocks using CAD/CAM systems. Two of these materials are In-Ceram Alumina ( $\text{Al}_2\text{O}_3$ ) and Yttria partially stabilized Zirconia ( $\text{ZrO}_2$ ), "YZ cubes". In-Ceram is an interpenetrating phase material, consisting of a porous alumina network (about 80% dense) which is milled and then infiltrated with a glass in different shades to produce a 100% dense core. VITA has developed new material called YZ cubes, approximately 97 wt.% Zirconia and 3 wt.% Yttria. This is about 60% dense and requires oversized milling to compensate for the 20% shrinkage which occurs during a 6 hour firing cycle to produce fully dense framework. VM 13 is a refined particle size veneering porcelain also introduced by VITA with claims of improved color stability. Several factors can influence the opacity and color during the fabrication of the porcelain restorations, including the size of the grain, surface texture, firing temperature, number of firings. Many color stability experiments published to date use a colorimeter or visual grading of the samples. A colorimeter, however, does not generate a full spectrum and is susceptible to variations in the illumination lamps. Spectrophotometers allow for generation of a full detailed spectrum as well as compensate for variations in illumination and metameric effects. Most opacity tests were performed with direct transmission technique. A hiding power technique using a spectrophotometer may be performed to generate quantitative measurements of translucency. Experimental technique involves measuring reflectance of light through the specimen with a black and white background separately. Black; Yb: low reflectance and high absorption. White; Yw: high reflectance and low absorption. Contrast ratio ( $\text{CR} = \text{Yb}/\text{Yw}$ ) can be calculated to compare translucency. This experiment compared the translucency of In-Ceram Alumina, Group CA and YZ zirconia, Group CZ. In addition the color stability of these materials and the veneering porcelain, VM13, was measured for repeated firings to simulate procedures actually used in a dental laboratory. **Specific Aims:** In this experiment, In-Ceram Alumina, YZ Zirconia and 7 shades of VM 13 are tested. Each sample is fired 1, 3, 5, and 7 times to simulate actual fabrication procedures required to produce the final restoration.

1. A GretagMacbeth i5 Spectrophotometer is used to generate a spectrum within the visual light wavelength range (300-700nm) with single reflectance in order to measure the color change and translucency via the hiding power method.
2. The opacity change of specimens after multiple firing are measured and translucency via the hiding power method.
3. ANOVA and Turkey test are performed to compare and determine if significant changes occur during repeated firing.

*Supported by Vident and Vita Zahnfabrik.*



## Caries Prediction Vs. Actual Incidents; Multivariate Risk Analysis of Dental Caries in Complaint Adult Population

David J. Park, Martha.E. Nunn, Takashi Kumagai, Judith A Jones and Takanari Miyamoto, University School of Dentistry at Matsudo, Chiba, 271-8587, Japan and Boston University School of Dental Medicine, Department of Health Policy and Health Services Research and Department of General Dentistry

**Objectives:** Previously, the factors for determining the incidence of dental caries have been well investigated. Emerging evidence suggests that the incidence of dental caries can be predicted by certain diagnostic tests, such as microbiological tests for *Streptococcus mutans* (SM) and *Lactobacillus* (LB), saliva buffer capacity test (SBC), saliva quantity tests (SA), and diet habit analysis (DHA). However relatively few studies have demonstrated the association between the commonly taught caries risk factors and actual incidence of dental caries in adult subjects. The purpose of this study was to investigate the relationship of these diagnostic tests to incidence of dental caries over time. **Methods:** Data from 148 fully compliant patients with 3111 teeth who were treated and maintained for at least 15 years were collected from one private practice in Japan. Diagnostic Caries Tests that were conducted included *Streptococcus mutans* (SM) and *Lactobacillus* (LB), saliva buffer capacity (SBC), amount of saliva (SA), and diet habit analysis (DHA). (Orion, Sweden). Fluoride use, oral hygiene, individual tooth experience of dental caries (ITE), diabetic status (DA), and smoking status were also assessed. Multivariate survival analysis was utilized to determine the impact of these risk factors on the incidence of dental caries in this population of fully compliant patients.

**Results:** Positive DA, ITE, increased meals (DHA), high levels of SM and LB, smoking, lack of fluoride, poor oral hygiene tended to increase the incidence of dental caries although only diabetic status, individual tooth experience of dental caries, and increased meals achieved statistical significance ( $p < 0.05$ ). Increased SA and poor SBC did not affect incidence of dental caries. **Conclusions:** Positive DA, ITE, DHA, SM, LB, smoking, lack of fluoride and poor oral hygiene appear to be risk factors of dental caries. Only SM, LB, smoking, lack of fluoride and poor oral hygiene can be controlled with patient compliance. Further research should be conducted in a larger sample to evaluate the role of diagnostic testing in dental practice.

*Supported by the Department of General Dentistry and the Department of Health Policy and Health Services Research, BUSDM*

## Orthodontic Treatment and Retention Outcomes Following Selective Alveolar Decortication

Elizabeth D. Walker, Donald J. Ferguson, Bill Wilcko and Tom Wilcko, Private Practice, Erie, PA, USA and Boston University School of Dental Medicine, Department of Orthodontics

Success in orthodontically treating malocclusion is somewhat dependent upon the severity of the initial malocclusion. The American Board of Orthodontics developed the Discrepancy Index (DI) as a measure of pre-treatment malocclusion severity and the Objective Grading System (OGS) to assess orthodontic treatment outcomes.

**Objectives:** To compare non-extraction orthodontic treatment and retention outcomes with and without selective alveolar decortication plus grafting (A00tm) for pre-treatment malocclusion with DI scores greater than 10. **Methods:** Pre-treatment patient records were screened for DI scores greater than 10 and grouped for treatment with (n=26) and without (n=28) A00tm. Study casts and panoramic x-rays for non-extraction, straight-wire therapy were scored at post treatment (T1) and retention (T2) using the OGS for 8 criteria plus total OGS score. **Results:** For the A00tm group, total post treatment (T1) OGS score (21.7) was significantly lower ( $p<.001$ ) than the group without surgery (30.6), as were buccal-lingual inclinations (4.6 v 6.6,  $p=.04$ ), marginal ridge relationships (2.7 v 5.9,  $p<.001$ ) and interproximal contacts (.3 v 1.1,  $p=.03$ ). At retention (T2), the A00tm group had significantly lower scores for alignment (1.3 v 3.8,  $p<.001$ ) and marginal ridge relationships (1.4 v 4.9,  $p<.001$ ) as well for effect sizes. **Conclusions:** Orthodontics combined with selective alveolar decortication plus grafting (A00tm) to resolve malocclusions with pre-treatment Discrepancy Index scores greater than 10 produced better orthodontic and retention outcomes in non-extraction, straight wire therapy cases.

*Supported by the Department of Orthodontics, BUSDM*

## Diabetes Impairs Healing by Enhancing Caspase Activity

Hesham A. Al-Mashat, Suneel Kandru, Rongkun Liu, Yugal Behl, Tesfahun Desta and Dana T.Graves, Boston University School of Dental Medicine, Department of Periodontology and Oral Biology

**Background:** Diabetics suffer from many complications including enhanced tissue loss from periodontal disease and impaired healing. Several mechanisms have been proposed, many of which focused on the negative impact of diabetes on fibroblast proliferation and differentiation function. Other mechanisms which may include the loss of fibroblast through apoptosis have not been well studied. **Objective:** To investigate if type 2 diabetes enhances the rate of fibroblast apoptosis during healing and the mechanisms by which that might occur? **Methods:** We induced a bacterial wound by inoculating the scalp of control or db/db diabetic mice with the periodontal pathogen *P. gingivalis*. Mice were killed at 0, 5, 8 days after inoculation (n=6). RNA profiling and caspase activity were measured following inoculation of *P. gingivalis*. The functional significance of diabetes-induced apoptosis was studied by treating diabetic mice with a pancaspase inhibitor, Z-VAD-FMK. **Results:** The results indicate a significantly higher rate of fibroblast-specific apoptosis in the diabetic group measured by the TUNEL assay ( $P < 0.05$ ). Apoptosis was evident during the peak healing period and coincided with a significant diminished in collagen 1 and III expression and significantly reduced formation of new connective tissue matrix in diabetic mice. Diabetes caused a more than two fold induction of 71 genes that directly or indirectly regulate apoptosis and significantly enhanced caspase -8, -9 and -3 activity. Inhibiting apoptosis significantly improved several parameters of healing including fibroblast density, enhanced mRNA levels of collagen I and III and increased matrix formation. **Conclusion:** Therefore, studies presented here provide the first direct evidence that diabetes caused a global induction of pro-apoptotic genes during healing and increased apoptosis of fibroblasts which functionally contributes to impaired wound healing. The RNA level and the Functional studies presented in this study provide the evidence that caspase-3 activity was enhanced in the diabetic group during healing along with both caspase-8 and caspase-9, suggesting that both extrinsic and intrinsic apoptotic pathways are involved.

*Supported by NIDCR/NIH grants DE07559 and DE11254*

## Comparison of Hydroxyapatite Synthesis under neutral and basic conditions

Satheesh Elangovan, Marek Kloczewiak, Eva Helmerhorst and Frank Oppenheim, Boston University School of Dental Medicine, Department of Periodontology and Oral Biology

**Introduction:** Pure Hydroxyapatite (HA) has a numerous biomedical applications apart from having served as an enamel model for years. This study focused on methodologies of HA synthesis ranging from minimal to high content in carbonate since carbonate content is inversely related to its stability in acidic environments. **Objective:** The main goal of this work was to elucidate the effect of pH during synthesis on the final carbonate content of HA preparations. **Methods:** First, we used calcium nitrate and ammonium phosphate at pH 11.0 and second, we employed calcium chloride and sodium phosphate at pH 7.4, describing the principal features of 2 different methods to prepare HA. In addition, we carried out HA synthesis under room conditions or excluding air utilizing mineral oil to cover the reaction solutions. Contents of carbonate were determined by FTIR and Conway diffusion. **Results:** HA preparations made under neutral pH conditions were low in carbonate content whether mineral oil was used or not during synthesis. The carbonate content ranged between 0.2 to 0.5%. In comparison, HA preparations made at pH 11.0 showed drastic differences in carbonate content depending on the use of mineral oil to exclude air during the formation of HA. Oil coverage of reactions mixtures resulted in HA preparations of 0.2-0.5 % carbonate while synthesis under room conditions yielded mineral containing 1.5-1.9 % carbonate. **Conclusion:** Based on the methodology described, HA preparations with minimal carbonate content can be readily achieved at neutrality with calcium chloride and sodium phosphate without using air exclusion strategies such as mineral oil coverage, nitrogen gassing or applying vacuum conditions. The techniques described may be useful for the preparation of pure HA samples needed for a variety of biomedical applications in oral medicine.

*Supported by NIDCR/NIH Grants, DE 07652, DE 05672 and DE 14950*

## Accelerated bone loss after molar ligation compared to bacterial challenge

Li Gao, Thomas Van Dyke and Robert Gyurko, Boston University Goldman School of Dental Medicine, Department of Periodontology and Oral Biology

**Objectives:** To compare the extent and time course of alveolar bone loss and osteoclast activation in two murine models of periodontal disease: molar ligation and *Porphyromonas gingivalis* oral inoculation. **Methods:** A split-mouth design was applied to two groups of mice (C57BL/6, 6-8 weeks old, n = 12 in both groups), resulting in four treatment groups: 1. ligation of a 9-0 suture around the upper left 2<sup>nd</sup> molar, 2. untreated upper right 2<sup>nd</sup> molars as controls, 3. ligation of the upper left 2<sup>nd</sup> molar in combination with oral inoculation with 10<sup>9</sup> CFU *P. gingivalis*, 4. unligated upper right 2<sup>nd</sup> molar receiving *P. gingivalis* challenge only. Alveolar bone loss was measured as the CEJ-ABC (cemento-enamel junction and alveolar bone crest) distance. Osteoclasts were counted on histological sections following tartrate-resistant acid phosphatase (TRAP) staining and counts were normalized to alveolar bone surface. Data were analyzed with one-way ANOVA. **Results:** *P. gingivalis* oral challenge resulted in moderate increase in CEJ-ABC distance (alveolar bone loss) 6 weeks after treatment (0.12±0.01 mm vs. control 0.1±0.01 mm, *P*=0.16). In comparison, molar ligation induced marked alveolar bone loss after 3, 6, 9 and 12 weeks (0.16±0.04 mm, 0.16±0.02 mm, 0.18±0.03 mm, 0.17±0.02 mm vs. corresponding controls, *P* <0.05). Combined treatment with molar ligation and *P. gingivalis* did not further increase the CEJ-ABC distance. Evidence for osteoclast activation was found one day after molar ligation, and TRAP-positive cell numbers peaked at day 3 (12±4/mm vs. control 2±2/mm, *P* <0.01). **Conclusion:** Molar ligation is a rapid and effective way to induce periodontal bone loss in mice. Osteoclast activation occurs within 24 hours of ligature placement, and the extent of bone loss well exceeds that of the *P. gingivalis*-induced bone loss. The combination of *P. gingivalis* and ligature did not exhibit an additive effect.

Supported by NIDCR/NIH grants DE16191 and RR00533



## Oral Candida Carriage and Salivary Antifungal Activity

Homan Hanasab, Eva J. Helmerhorst and Frank G. Oppenheim, Boston University School of Dental Medicine, Department of Periodontology and Oral Biology

**Introduction:** When local or general predisposing factors are present, *Candida*, an oral opportunistic pathogen, may cause acute or chronic oral infections with clinical manifestation such as pseudomembraneus (oral thrush), atrophic (erythematous) or hyperplastic candidiasis or angular cheilitis. It is well known that healthy individuals differ with respect to the carrier status of *Candida albicans*. **Aims:** Our aims were 1. to study the variation in oral *Candida* levels among and within the same subject and among different subjects, and 2. to investigate the antifungal activity of parotid salivary secretion. **Methods:** Whole saliva was collected from thirteen subjects every 2 to 3 days for a 2 week time interval using masticatory stimulation. A 1 ml aliquot of whole saliva was centrifuged, the sediment was suspended in PBS and plated on Sabouraud dextrose agar containing chloramphenicol (20 mg/l), or on ChromAgar allowing a specific *C. albicans* count. After 48 hrs of incubation at 30 °C, colonies were counted. To determine the antifungal activity of parotid saliva, parotid secretions were collected under gustatory stimulation from two subjects. The fungistatic activity of the parotid secretions was evaluated in growth media assaying 15 medically important fungi including 6 strains of *C. albicans*. **Results:** Four of the 13 subjects harbored *C. albicans* in their saliva. The total number of *C. albicans* in these subjects showed fluctuation. However, non-carriers maintained their non-carrier status during the entire interval of examination. Parotid saliva exhibited a surprising trend of being fungistatic toward many fungal species while exhibiting remarkably less activity toward *C. albicans*. **Conclusion:** Despite variable salivary *Candida* counts within carriers, the carrier status appears to be constant. It is hypothesized that the observed lower parotid saliva activity toward *C. albicans* may explain why this fungus is the main oral fungal pathogen.

*Supported by NIDCR/NIH grants DE05672, DE07652 and DE14950*

## Diabetes May Impair Fracture Healing By Excess Removal of Cartilage

Rayyan Kayal, Dimitris Tsatsas, Megan Bauer, Elizabeth Krall, Elise Morgan, Lou Gerstenfeld and Dana Graves, Boston University School of Medicine, Department of Orthopedic Surgery and Boston University School of Dental Medicine, Department of Periodontology and Oral Biology

**Objectives:** Fracture healing involves the formation of cartilage which is then resorbed and replaced by bone. Diabetes negatively affects repair of many tissues including bone. To gain insight into how diabetes affects fracture healing studies were carried out focusing on the potential impact of diabetes on cartilage. The aim of this study is to identify how diabetes affects fracture healing. **Methods:** CD-1 mice were treated with low dose injections of streptozotocin which induces type I diabetes. After three weeks, fracture of the tibia was induced and the mice were sacrificed after 12, 16 and 22 day. Tibias were collected, embedded, sectioned and stained with several stains. Histological analysis of the center of fracture, 0.5 and 1.0 mm proximal and distal of the fracture center was done using Image Pro plus software. Another group of 16 day samples were examined under micro CT. RNA was isolated from a separate set of animals using Trizol for real time PCR. **Results:** On day 12 callus size and cartilage area were similar in normoglycemic and diabetic mice. However, on day 16, callus size was 60% larger and cartilage area was 88% larger in the normoglycemic group ( $p < 0.05$ ). New bone formation showed a 31% higher amount in the normoglycemic groups at day 16 ( $p < 0.05$ ). Osteoclast number was measured using the TRAP stain and normalized by the area of cartilage and bone. There were 78% more chondro/osteoclasts in the diabetic group at day 16 ( $p < 0.05$ ). Micro CT images showed 23% increase in callus volume and 30% increase in bone volume in the normoglycemic animals. Real time PCR shows an increased expression of several genes that promote osteoclastogenesis. **Conclusion:** In diabetes, cartilage is lost prematurely. This may lead to a reduced scaffold for new bone formation. This loss of cartilage may be due to increased chondro/osteoclast activity.

*Supported by NIH grant AR49920 01A1*

## Catabolic Modeling of Trabecular Bone Following Selective Alveolar Decortication

Jean-David Sebaoun, Donald J. Ferguson, Alpdagon Kantarci, Robert Carvalho, and Thomas Van Dyke, Boston University, Boston University School of Dental Medicine, Department of Orthodontics

Significant hard and soft tissue turnover increases are reported following bone injury but little is known about the effect of alveolar injury. Clinical orthodontics is 60 to 70% more rapid following selective labial-lingual alveolar decortication but the biological rationale for rapid tooth movement remains obscure. **Objective:** The objectives were to evaluate bone modeling in 1) trabecular bone, 2) PDL and 3) 1st versus 3rd molars as a function of time and proximity following decortication. **Methods:** Nine rats underwent selective buccal-lingual alveolar decortication adjacent to the left maxillary first molar in a split mouth design. The animals were sacrificed in groups of three at 3, 7, and 11 weeks, and maxillas were removed, stripped, and prepared for decalcified histology using TRAP or H&E stains. Bone modeling dynamics was histomorphometrically examined for osteoclast and/or precursor count (OC) within the geometric center defined by the 4 most distal 1st molar roots, the 2 mesial roots of the 3rd molar, and within the 1st molar PDL. **Results:** Kruskal-Wallis testing showed that trabecular bone OC at 3 weeks post decortication (56.3) was significantly greater ( $p < .02$ ) than control (26.3) and 7 week surgery (29.7). At 3 weeks post decortication, one-way ANOVA testing demonstrated bone surface volume (4.4mm<sup>2</sup>) was significantly less ( $p < .05$ ) than control (7.5mm<sup>2</sup>) and 7 week surgery (6.2mm<sup>2</sup>) and all other groups; PDL surface volume was greater (7.2mm<sup>2</sup>) than 3 week control (3.1mm<sup>2</sup>) and 7 week surgery (5.3mm<sup>2</sup>). Moreover, Regional Acceleratory Phenomena (RAP) was shown as PDL OC for 3-week surgery was greater than all other groups, and the 1st molar 3-week surgery group had significantly greater OC than the 3rd molar and all other groups. **Conclusion:** Selective alveolar decortication in the rat resulted in approximately a 50% increase in catabolic modeling of alveolar trabecular bone adjacent to the surgery and RAP was demonstrated.

*Supported by the Department of Orthodontics, BUSDM*

## Periodontal Care Improves Short-Term Quality-Of-Life And Satisfaction With Diabetes Care?

Gregory F. Pezza, Sharron E. Rich, Carolyn J. Wehler, Raul I. Garcia and Judith A. Jones, VA Center for Health Quality, Outcomes and Economic Research, Bedford and Boston University School of Dental Medicine

**Objectives:** We are conducting a clinical trial on the effects of periodontal therapy in veterans with poorly controlled diabetes. This paper describes the impact of periodontal therapy on patient's satisfaction with diabetes care and on self-reported oral-specific and general health. **Methods:** Veterans with poorly controlled diabetes were randomized into two groups: immediate periodontal therapy (n=82) or usual care (therapy delayed for four months, n=83); half of each group continued care for 12 months and the other half was returned to their usual care. Outcomes were change in self-reported health measures (score at time<sub>x</sub> - score at baseline) at four months and one year. Measures included the Diabetes Treatment Satisfaction Questionnaire (DTSQ: Bradley, 1994); the single-item self-report of oral health (OH1), the Geriatric Oral Health Assessment Index (GOHAI: Atchison and Dolan, 1990); and the single-item self-report of general health (GH1). We examined means for continuous variables, frequencies for categorical variables, and compared groups by t-tests and chi-square tests. **Results:** After four months 43% of the treated versus 13% of the usual care group had improvements in OH1 (chi-square=18.1, p<0.001). Other differences were marginally significant: in the early treatment group there was 1.5-point improvement in DTSQ versus none in the usual care group, t=-1.62, p=0.1. GOHAI mean improvement was 0.32 in the early treatment group, versus -0.38 in the usual treatment group, t=-1.6, p=0.1. GH1 improved 0.2 in the treated versus none in the usual care group, t=-1.4, p=0.15. No differences were evident at 12 months. **Conclusions:** Periodontal therapy in veterans with poorly-controlled diabetics has a short term positive effect on patient's satisfaction with diabetes care and on their oral-specific and general health-related quality-of-life.

*Supported by VA HSR&D QUERI DII-99.206, NIH K24 DE00419 and Boston University*

## p47phox Activation and Alveolar Bone Loss in Hyperglycemic Ins2Akita Mice

Camille Siqueira, Thomas Van Dyke and Robert Gyrko, Boston University School of Dental Medicine, Department of Periodontology and Oral Biology

**Objectives:** To investigate the effect of chronic in vivo hyperglycemia on the assembly of the superoxide-generating enzyme NADPH oxidase. In addition, the rate and severity of periodontitis in diabetic mice was investigated. **Methods:** A novel mouse model of insulin-dependent diabetes, the Ins2Akita mice, was used in these experiments. Neutrophils (polymorphonuclear leukocytes, PMNs) were harvested from the bone marrow of femurs and separated from mononuclear cells by Histopaque step-gradient centrifugation. PMNs were stimulated in vitro with 1  $\mu$ M fMLP for 10 minutes. Protein extracts were analyzed with Western blotting using antibodies to p47phox and gp91phox. Superoxide release was measured by superoxide dismutase-inhibitable reduction of cytochrome C. To induce alveolar bone loss, maxillary second molars of wild type and Ins2Akita mice were ligated using a 9-0 silk ligature for 3 weeks. Alveolar bone loss was measured morphometrically on defleshed and methylene blue-stained skulls. **Results:** Ins2Akita mice have markedly higher blood glucose levels ( $545 \pm 144$  mg/dL) compared to wild type mice ( $262 \pm 39$  mg/dL,  $P < 0.01$ ). p47phox was found exclusively in the cytoplasm of unstimulated wild type PMNs. However, in Ins2Akita PMNs p47phox was partially translocated to the cell membrane even in the unstimulated state. gp91phox was found exclusively in the cell membrane of PMNs of both genotypes. Correspondingly, increased superoxide release was found in unstimulated and in fMLP-stimulated Ins2Akita PMNs compared to wild type PMNs. When alveolar bone loss was induced by molar ligation, Ins2Akita mice lost more alveolar bone as measured by the cemento-enamel junction and alveolar bone crest (CEJ-ABC) distance ( $0.26 \pm 0.11$  mm) than wild type mice ( $0.18 \pm 0.04$  mm,  $P < 0.05$ ). **Conclusions:** Chronic hyperglycemia leads to premature activation of the superoxide-generating enzyme NADPH oxidase, which in turn may contribute to increased periodontal bone loss.

*Supported by NIDCR/NIH grant DE016933*



## Orthodontic Outcome Changes During Retention After Selective Alveolar Decortication

Monica Dosanjh, Donald Ferguson, Omid Rajaei, Bill Wilcko and Tom Wilcko, Boston University School of Dental Medicine, Department of Orthodontics

Surgical scarring of alveolar bone induces an increase in hard and soft tissue turnover, a process collectively known as Regional Acceleratory Phenomena. If the purpose of orthodontic retention is to hold the treatment result until the periodontium reorganizes (turns-over), then enhanced alveolar turnover should reduce relapse of treatment outcome. **Objectives:** To assess post orthodontic treatment changes during retention following selective alveolar decortication and augmentation grafting. **Methods:** The study cast and panoramic records of 51 selective alveolar decortication patients were scored using the ABO Objective Grading System (OGS) at immediate post treatment (T1), at least 1 year retention (T2) and at least 2 years retention (T3). **Results:** Wilcoxon signed-rank non-parametric testing revealed 5 of 16 study variables improved ( $p < .05$ ) from T1 to T2 ( $n=51$ ) as follows: Mn posterior alignment (1.5 v .5,  $p=.000$ ), Mx B-L inclinations (1.8 v 1.5,  $p=.04$ ), Mx marginal ridges (1.1 v .6,  $p=.02$ ), Mn marginal ridges (1.3 v .8,  $p=.04$ ) and Mn interproximal contact (.3 v .02,  $p=.01$ ). From T2 to T3 ( $n=24$ ), Mn B-L inclinations improved (3.7 v 2.6,  $p=.01$ ). **Conclusion:** Orthodontic treatment combined with selective alveolar decortication and grafting resulted in improved orthodontic treatment outcome during the retention period; relapse was absent.

*Supported by the Department of Orthodontics, BUSDM*

## Anabolic Modeling of Trabecular Bone Following Selective Alveolar Decortication

John Turner, Donald Ferguson, Jean-David Sebaoun, Alpdagon Kantarci, Robert Carvalho and Thomas Van Dyke, Boston University School of Dental Medicine, Department of Orthodontics

When orthodontic clinical treatment is combined with selective alveolar decortication, it has been demonstrated that clinical treatment time is 60 to 70% more rapid. However, the biological rationale for rapid tooth movement after alveolar decortication remains obscure. Corticotomy was performed by Bogoch, et al. on the rabbit tibia condyle, the rabbits were feed calcein ad libitum in the drinking water post-op, and 5X turnover of trabecular bone was demonstrated adjacent to the boney incision at post-op day 28. In contrast, little is known about the anabolic hard tissue response to alveolar bone injury. **Objectives:** The objective was to evaluate anabolic modeling of alveolar trabecular bone as a function of time and location following selective alveolar decortication. **Methods:** Five rats underwent selective buccal-lingual alveolar decortication adjacent to the left maxillary first molar; the contra lateral side acted as the control in the split mouth design. The animals were feed vital bone stain calcein ad libitum in the drinking water post-op and sacrificed at 3 weeks (n=3) or 7 weeks (n=2). Maxillas were removed, stripped, and prepared for non-decalified fluorescent stain histology. Pixel counts were made in the transverse sections of trabecular bone in the first molar and third molar areas that were analyzed independently using a 100 x 100 pixel square grid (10,000 pixels). **Results:** Percent of new bone apposition in the 1st molar area was significantly greater ( $p<.03$ ) in the post decortication group (5968 pixels) at 3 weeks compared to the 3 week 1st molar control (2446) and the 3 week 3rd molar control (2290) and surgery (1778). **Conclusion:** Anabolic modeling of alveolar trabecular bone adjacent to the decortication site increased by about 1.5 times at 3 weeks; this increase represented a 2.6 to 3.4 fold greater anabolic modeling activity as compared to the 3rd molar area.

*Supported by the Department of Orthodontics, BUSDM*

## In Vitro Testing Parameters Effect on Adhesive Shear Bond Strength

Emad Al-Badawi, Richard Pober, R. L'herault, and Dan Nathanson, Boston University School of Dental Medicine, Department of Restorative Sciences/Biomaterials

**Purpose:** To evaluate the effect of loading geometry and sample diameter on adhesive shear bond strength. **Methods:** The occlusal surfaces of 450 human molars were sectioned to the dentin surfaces and polished. Optibond Solo Plus (Kerr) and Optibond FL (Kerr) were used to bond composite resin (Z100, 3M) cylinders of 1, 2 & 4mm diameter (n=15/group). Bond strength was tested at 24 hours in shear mode using a wire loop, knife blade, flat blade, notched blade and flat blade on iris, at 0.5 mm/min (Instron). ANOVA and Tukey multiple comparison tests were used for analysis (P<.05). **Results:** The knife and wire loop methods produced significantly lower bond strengths. The 1mm diameter samples produced significantly higher bond strengths than 4mm samples. Bond strength differences between the flat, notched and flat-on-iris methods were not significant. Mean shear bond strengths (MPa) and standard deviations:

	Optibond Solo Plus			Optibond FL		
	1mm	2mm	4mm	1mm	2mm	4mm
Wire loop	31.57 (2.9)	26.77 (3.7)	20.06 (7.6)	28.11 (2.5)	21.77 (4.5)	16.67 (5.2)
Knife blade	25.05 (1.9)	15.91 (2.5)	10.62 (1.5)	20.09 (2.2)	13.99 (2.4)	10.81 (2.9)
Flat blade	38.39 (8.9)	32.20 (3.7)	30.34 (5.3)	35.91 (6.9)	31.96 (5.3)	29.99 (5.5)
Notched blade	41.54 (6.2)	35.95 (3.6)	33.91 (4.3)	39.79 (7.1)	34.36 (8.9)	32.47 (4.8)
Flat blade on Iris	39.13 (7.6)	35.63 (5.7)	31.65 (8.4)	42.70 (5.7)	33.81 (7.6)	30.13 (4.8)

**Conclusion:** Shear blade geometry and sample diameter had a significant effect on shear bond strength. In comparing bond strength results from various studies, the methodology, blade geometry and sample cross section should be considered.

*Supported by 3M-ESPE, GC America and Kerr*

## Treatment Quality Between Boston University Students and ABO-Certified Orthodontists

Ariel Bales-Kogan and Ana Karina Mascarenhas, Boston University School of Dental Medicine, Department of Health Policy and Health Services Research

**Objective:** To evaluate the quality of orthodontic care provided by graduate students at the Boston University Goldman School of Dental Medicine Orthodontics Department compared to patients treated by American Board of Orthodontics (ABO)-certified orthodontists, or Diplomates, in private practice. **Methods:** The Peer Assessment Rating (PAR) index and the ABO's Objective Grading System (OGS) were used as measures of quality of care. Treatment quality and outcome were evaluated by post-treatment PAR, percent PAR reduction, PAR pass proportion (cases achieving >70% PAR reduction), OGS point deductions, OGS pass proportion (cases receiving 30 point deductions or less), and treatment duration. A total sample of 479 cases were examined, 213 from the graduate clinic and 266 from 4 volunteer Diplomates. **Results:** The PAR index demonstrated no difference in quality of care between patients treated at the graduate clinic and those treated by Diplomates according to all calculated PAR values. The OGS indicated that quality of care at the graduate clinic was significantly better than that of the Diplomates ( $p=0.003$ ). Further, cases from the graduate clinic had a 2.6 times higher likelihood of meeting the OGS criteria for quality compared to cases completed by the Diplomates. There was no significant difference in treatment duration between the two groups ( $p<.0001$ ), after controlling for confounders (such as gender, age, extractions, and retention methods). Although overall average quality of care at the graduate clinic appeared favorable according to ABO guidelines, a large proportion of 33.3% of cases from the graduate clinic and 58.6% from Diplomates would likely fail the ABO final certification process. **Conclusions:** There is a significant difference in the quality of care rendered to patients between the graduate clinic and by ABO Diplomates when measured by the OGS, but not by the PAR or length of treatment.

*Supported by the Department of Health Policy and Health Services Research/Division of Dental Public Health, BUSDM*

## Evaluation of self-ligating bracket clip (SLB) breaking force

Afaf Dahlan, Russell Giordano, Richard Pober and Donald Ferguson, Boston University School of Dental Medicine, Department of Orthodontics and Restorative Sciences/Biomaterials

**Objectives:** 1) To evaluate the force at which the retaining clips/slides of self-ligating brackets break 2) to compare between the active self ligation system and the passive self ligation system in terms of clip/slide failure. **Materials and Methods:** The clip breaking force of twelve specimens of In-Ovation R was evaluated using two test designs; 1) Typodont test in which a metal rod with a bonded plastic tooth was attached to the cross head of the Instron machine, a 0.016 x 0.022 Bio Force Sentalloy wire was then self-ligated to the plastic tooth and to a modified lower jaw with bonded In-Ovation R in both. The wire was drawn by the moving rod in an upward direction until the retaining clip broke, 2) Non-Typodont design in which 12 In-Ovation brackets and 12 Damon SL II brackets bonded to a metal piece, a 0.016 x 0.022 Bio Force Sentalloy wire was held between two screws then attached to the Instron machine cross head, the wire was pulled in an upward direction until the clips broke. Amount of wire displacement and the force at which the clips broke were recorded. A Mann-Whitney statistical analysis was used to analyze the data. **Results:** There is a significant difference between the breaking force of In- Ovation R (mean = 0.069kN) and the breaking force of Damon 2 (mean = 0.023kN), with In-Ovation R showing higher values (exact significance=0). There is no significant difference (exact significance=0.755) between breaking force tested with Typodont design (mean = 0.066) and that with Non-Typodont design (mean= 0.069). **Conclusions:** In-Ovation R clip is significantly stronger than Damon 2 slide; No difference was found between the failure loads for clip breakage using a Typodont vs. Non-Typodont models.

*Supported by GAC and Ormco*

# The Effect of Different Surface Treatments on Flexural Strength and Microstructure of Y-TZP Zirconia Ceramics

Adnan Ishgi and Russell Giordano, Boston University School of Dental Medicine, Department of Restorative Sciences/Biomaterials

**Objectives:** The purpose of this study is to investigate the effect of grinding, polishing, sandblasting, and sintering on biaxial flexural strength and the microstructure of two different commercial types of zirconia ceramics. **Methods:** Two types of zirconia ceramics were used in this study, LAVA™ blocks from 3M ESPE and YZ blocks from VITA®. Disc-shaped specimens were fabricated and randomly divided into six groups (n=10); Sintered (Control), Ground, Polished, Sandblasted, Ground/Heated, and Simulated Veneer Firing (SVF). Biaxial flexural strength was tested using an Instron at a crosshead speed of 0.5 mm/min. The microstructure was examined using an SEM with EDS for elemental analysis. The weight% of the monoclinic phase was determined using X-Ray diffractometry. The statistical analysis was performed using ANOVA and Tukey multiple comparisons ( $p < 0.05$ ). **Results:** There was no significant difference between YZ and LAVA in the mean strength among all the groups. The “sandblasted” and the “polished” groups in both materials showed a higher mean strength than that of the “sintered” (control) group ( $p < 0.05$ ). “Ground and Heated” showed the lowest mean strength in both materials ( $p < 0.05$ ). There was no significant difference in the mean strength between the “Simulated veneer firing” and the “sintered” group. “Sandblasted” group showed the highest weight percent of monoclinic phase. In all groups that were subjected to a firing cycle the monoclinic phase was negligible. Biaxial flexural strength (MPa) YZ LAVA Sintered 812.39 842.07 Ground 810.58 808.54 Polished 979.24 1004.27 Sandblasted 1081.28 1133.22 Ground/Heated 652.21 632.07 SVF 772.99 840.49. **Conclusion:** Sandblasting and polishing increased the mean strength values of both materials tested. Contrary and unexpected, a simulated firing cycle after grinding led to significant degradation in strength values.

*Supported in part by 3M/ESPE, Vident, and Vita Zahnfabrik*

## The Effect of Root Proximity on Alveolar Bone Loss

Taera Kim, Takanari Miyamoto, and Thomas Dietrich, VA Boston Healthcare System, MA, USA and Boston University School of Dental Medicine

**Objective:** The purpose of the present longitudinal study was to evaluate the association between root proximity and alveolar bone loss. **Methods:** We used data of the VA Dental Longitudinal Study, a closed cohort study with 1231 predominantly white men enrolled in 1968 and triennial follow-up exams. Periapical radiographs from baseline and the last examination of lower incisors with  $\geq 10$  years of follow-up were selected. Root distance at CEJ level and alveolar bone levels were measured on digitized radiographs. The primary outcome was the average rate of bone loss over the time of follow-up. Secondary outcomes were the risk of losing at least 0.5mm or 1.0mm of bone over ten years. Site-specific linear and generalized linear models were fit to evaluate the association between root proximity and alveolar bone loss, adjusting for covariates. Empirical standard errors and general estimating equations were used to account for the correlation between sites within subjects. Fractional polynomial regression was used to evaluate the dose-response function. **Results:** The final sample consisted of 473 men (mean age 46, range 28-71 years) with a mean follow-up of 23 (range 10-35) years. The mean root distance was  $1.0 \pm 0.3$  mm and mean bone loss over 10 years was  $0.61 \pm 0.59$  mm. There was a significant and non-linear association between root proximity and bone loss ( $p < 0.005$ ). Compared to root distances  $> 0.8$  mm, sites with root distances 0.6-0.8mm and  $< 0.6$ mm were 13% (95% CI: 0%, 26%;  $p = 0.041$ ) and 28% (11%, 48%;  $p = 0.001$ ) more likely to loose  $\geq 0.5$ mm, and 10% (-16%, 40%;  $p = 0.54$ ) and 52% (9%, 213%;  $p = 0.013$ ) more likely to loose  $\geq 1.0$ mm of bone over ten years, respectively. **Conclusions:** The results of this study suggest that root distances smaller than 0.8mm are a risk factor for alveolar bone loss.

*Supported by U.S. Dept. of Veterans Affairs and NIDCR/NIH grant K24 DE00419*



## Assessment of the Kinetics of Histatin 1 and Histatin 3 Proteolysis in Human Saliva

Xiuli Sun, Eva Helmerhorst and Frank Oppenheim, Boston University Medical Center, Goldman School of Dental Medicine, Department of Periodontology and Oral Biology

**Introduction:** Histatins are human salivary antifungal proteins that are prone to proteolytic degradation upon their release into the oral cavity. Histatin 1 and histatin 3 are primary gene products of the *HIS1* and *HIS2* genes, respectively. The primary cleavage products and the disappearance of intact histatin proteins can be expected to have functional consequences. **Objectives:** To investigate the rate and pattern of degradation of histatin 1 and histatin 3 induced by whole saliva proteases. **Methods:** The proteins investigated were the native form of histatin 1, which is phosphorylated at serine 2, synthetic non-phosphorylated histatin 1 (dHis1), and native histatin 3 which is non-phosphorylated. Protein samples were incubated with aliquots of pooled human whole saliva supernatant for various time intervals (0 to 3 hours), and the resultant digests were analyzed by RP-HPLC. **Results:** Histatin 1 and histatin 3 differed from each other by both the degradation pattern and the degradation rate. Histatin 3 yielded more degradation products than histatin 1 under identical incubation conditions. The degradation rate of histatin 1 was approximately 50% of the degradation rate of histatin 3. The degradation rate of native histatin 1 was about 60% of that of dHis1, indicating that phosphorylation of serine 2 in native histatin 1 plays a protective role with respect to proteolysis in human saliva. **Conclusion:** Even minor sequence differences as present in histatin 1 and histatin 3, as well as the degree of phosphorylation shows an effect on both proteolytic kinetics and the degradation pattern.

*Supported by NIDCR/NIH grants DE05672, DE07652 and DE14950*

## **Candida glabrata Secretes a Histatin 5 Neutralizing Component**

Caterina Venuleo, Eva Helmerhorst, Ana Fraga and Frank Oppenheim, University of Coimbra, Center for Neurosciences and Cell Biology, Portugal and Boston University School of Dental Medicine, Department of Periodontology and Oral Biology

**Introduction:** *Candida glabrata* shows an unusual resistance toward the antifungal activity of a large number of cationic antifungal proteins differing in their mechanism of action, but sharing a net positive charge. *C. glabrata* is particularly resistant to histatin 5, a human salivary antifungal protein which exhibits strong fungicidal and fungistatic activities to many other classes of fungi. **Objective:** To evaluate cell-associated and supernatant-associated factors which may explain the resistance of *C. glabrata* to histatin 5. **Methods:** Anionic surface charges on *C. albicans* and *C. glabrata* strains were compared by determination of the adherence of cells to chromatographic anionic exchange resin. To assess whether *C. glabrata* supernatant would neutralize histatin 5 activity, growth inhibition assays with *C. albicans* cells were conducted in a) fresh diluted Sabouraud dextrose broth (control), b) *C. albicans* supernatant (control), c) *C. glabrata* supernatant, d) boiled *C. glabrata* supernatant. **Results:** Anionic surface charges of *C. albicans* and *C. glabrata* strains were very similar and this parameter therefore is not likely related to differences in histatin susceptibility between these two fungal species. A major finding however was that unboiled *C. glabrata* supernatant caused a seven to eight-fold increase in the  $IC_{50}$  values of histatin 5 toward *C. albicans* compared to a 1.5 fold increase observed with *C. albicans* supernatant. Notably, boiled *C. glabrata* supernatant did not increase the  $IC_{50}$  values. **Conclusion:** *C. glabrata* secretes a histatin 5-neutralizing, heat-sensitive component, which may be associated with the reduced sensitivity of this fungus toward histatin 5.

*Supported by NIDCR/NIH grants DE05672, DE07652 and DE014950*

## ST18 Regulates TNF- $\alpha$ -Induced Apoptosis and Inflammatory Gene Expression

Julia Yang, Michelle Siqueira, Xiuli Tang, Salomon Amar and Dana Graves, Boston University School of Dental Medicine, Department of Periodontology and Oral Biology

**Introduction:** TNF- $\alpha$  regulates inflammation and apoptosis by modulating the expression of other genes through activation of transcription factors such as NF $\kappa$ B AP-1, CREB. TNF- $\alpha$  is one of the prime signals that induces apoptosis in various types of cells. TNF- $\alpha$  is also a potent inflammatory mediator, which in turn activates a number of pro-inflammatory molecules through transcriptional regulation. A prototype of transcription factor regarding inflammation and apoptosis is NF $\kappa$ B. However it has anti-apoptotic effect and cannot account for the pro-apoptotic effect of TNF. Therefore other transcription factors are likely to be involved in this process. Our previous protein/DNA array results indicated that the transcription factor ST18 (suppression of tumorigenicity 18) protein level was elevated 4 folds upon stimulation of TNF- $\alpha$ . The purpose of this study is to investigate the role of ST18 in mediating TNF- $\alpha$  effects. **Methods:** Adult dermal fibroblasts were stimulated *in vitro* with 20ng/ml of the recombinant human TNF- $\alpha$  for 0, 1 and 3 hours. Nuclear proteins were extracted and EMSA (Electrophoretic Mobility Shift Assay) was performed to evaluate promoter binding interactions (protein/DNA probe). The cells were also transfected with siRNA targeting ST18 to evaluate the effect of silencing of ST18 on the TNF- $\alpha$ -induced fibroblasts apoptosis at 24hrs. After 6 hours of TNF- $\alpha$  stimulation, the RNA was extracted and the real-time PCR was performed on pro-inflammatory gene IL-6 and pro-apoptotic genes, TNF- $\alpha$ , Caspase-3 and Fas, to evaluate the effect of silencing of ST18 on the gene expression. In order to further investigate the role of ST18 play in relation to TNF- $\alpha$  stimulation on the rest of human genome, the microarray analysis was performed using GeneChip human genome U133 plus 2.0 arrays. **Results:** EMSA result showed that ST18 nuclear protein levels were elevated 1hr after TNF- $\alpha$  stimulation, indicating protein/DNA interaction. The level of activation returned to baseline after 3 hours. Apoptosis assay results showed that silencing ST18 resulted in 80% inhibition of TNF- $\alpha$ -induced apoptosis. Furthermore, TNF- $\alpha$ -stimulated mRNA levels of the pro-apoptotic genes, TNF- $\alpha$ , caspase-3 and Fas were reduced significantly by silencing of ST18 by 44%, 40% and 46% respectively ( $P < 0.05$ ). Regarding pro-inflammatory genes, IL-6 protein levels and mRNA levels were both reduced by silencing of ST18 upon TNF- $\alpha$  stimulation by 90% and 70% respectively. ( $P < 0.05$ ). The RNA profiling analysis further confirmed our results: with over 47,000 genes analyzed, 8855 genes were significantly ( $P < 0.05$ ) activated/inactivated by silencing of ST18, including 193 apoptosis-related genes and 201 inflammation-related genes. **Conclusion:** Taken together, these studies show that ST18 is elevated by TNF- $\alpha$ , both at the protein level and at mRNA level. Silencing of ST18 inhibits TNF- $\alpha$ -induced apoptosis, possibly via the inhibition of pro-apoptotic gene expression. Inhibition of ST18 also inhibits the pro-inflammatory gene expression.

*Supported by NIH grant DE07559*

## Defining the roles of Toll-like receptor (TLR) 2 and TLR4 in *P. gingivalis* 67-kDa fimbria-mediated activation of IL-8 production by human aortic endothelial cells

Michael Davey and Caroline A. Genco, Boston University School of Medicine, Department of Infectious Diseases and Department of Microbiology and Boston University School of Dental Medicine, Department of Periodontology and Oral Biology

Toll-like receptors (TLRs) have been demonstrated to mediate the host response to microbial infection and chronic inflammatory diseases such as atherosclerosis. Epidemiological data support the idea that infection with the periodontal pathogen, *Porphyromonas gingivalis*, may be a risk factor for acceleration of atherogenesis. Previously, we reported that invasive *P. gingivalis* and fimbria stimulate endothelial cell activation, a necessary initial event in the development of atherosclerosis.

**Methods:** As endothelial cells can present antigen via TLRs and play an important role in the development of atherosclerosis, we cultured human aortic endothelial cells (HAEC) with wild-type *P. gingivalis*, a fimbria-deficient mutant, and purified antigen to determine if fimbria-mediated activation of HAEC occurs via direct interaction with TLR2 and/or TLR4. We observed that although the 41-kDa (major) fimbria are required for efficient *P. gingivalis* invasion of HAEC, the 67-kDa (minor) fimbria appear to mediate tight adherence and invasion. **Results:** The purified native form of the 67-kDa fimbria induced pro-inflammatory IL-8 expression similar to invasive *P. gingivalis* while treatment of endothelial cells with cytochalasin D abolished the observed IL-8 response, suggesting that the 67-kDa fimbria elicit chemokine production in HAEC via an internalization process. Fluorescence-activated cell sorter (FACS) analysis of non-permeabilized, unchallenged HAEC demonstrated constitutive surface expression of TLR4 and little TLR2 while permeabilized, non-challenged HAEC demonstrated large pools of intracellular TLR2. Treatment of HAEC with functional blocking antibodies to TLR2 and TLR4, prior to stimulation with 67-kDa fimbria, resulted in anti-TLR4, but not anti-TLR2 blocking of the 67-kDa-mediated IL-8 response by HAEC. Stimulation of HAEC transfected with dominant negative (dn) TLR2 and TLR4 confirmed 67-kDa fimbria activation of TLR4-mediated IL-8 production, but also blocked TLR2-mediated IL-8 production. Indirect ELISA-like binding assays with purified TLR2-Fc fusion proteins demonstrated specific, saturable binding of 67-kDa fimbria with an estimated K<sub>d</sub> of ~31nM. This result was confirmed by a homologous competitive binding. Non-specific binding of 67-kDa fimbria to TLR4-Fc was also observed. **Conclusion:** Collectively, these results suggest that 67-kDa fimbria-mediated IL-8 production by HAEC appears to occur via a specific interaction with TLR2 and an indirect activation of TLR4.

Supported by NIH grant R01 HL 080387

## Discovery of New Enamel Pellicle Proteins by Mass Spectrometry

Walter Siqueira, Marek Kloczewiak, Eva Helmerhorst, Martin Steffen and Frank Oppenheim, Boston University School of Dental Medicine, Department of Periodontology and Oral Biology

**Introduction:** The acquired enamel pellicle (AEP) is well known to be a protein film with unique composition and properties. While its importance has been well recognized with respect to bacterial biofilm formation and mineralization of enamel very little information is available on its composition and structure. **Objective:** To use mass spectrometry to identify and characterize proteins and peptides of the human, in-vivo formed, AEP. **Methods:** AEP was collected using PVDF membrane saturated with sodium bicarbonate. Proteins were extracted with water, desalted, concentrated and then subjected to tryptic digestion in SDS acrylamide gel or in solution. The digests were applied to an ion trap mass spectrometer (ProteomeX LTQ, Thermo Finnigan, San Jose, CA) equipped with a nanospray reversed phase column. **Results:** Mass spectra were analyzed using the SEQUEST software program and m/z values were queried against human proteins in the RefSeq database. This resulted in the identification of at least 46 human proteins ranging in coverage between a low 0.3% to a high of 69.0 %. A total of 18 new AEP proteins were identified. In addition to the classical salivary pellicle precursor proteins a variety of non-exocrine proteins which derive from gingival fluid and epithelial cells were discovered. **Conclusion:** Mass spectrometry seems to be uniquely suited to characterize AEP protein/peptides available at ng levels. The data showed that well known acidic salivary proteins participate in pellicle formation. Unexpected is the finding that gingival fluid and epithelial cells make significant contributions to the AEP. This fact may have major functional consequences for the AEP which will have to be considered.

*Supported by NIDCR/NIH Grants DE05672, DE07652 and DE14950*