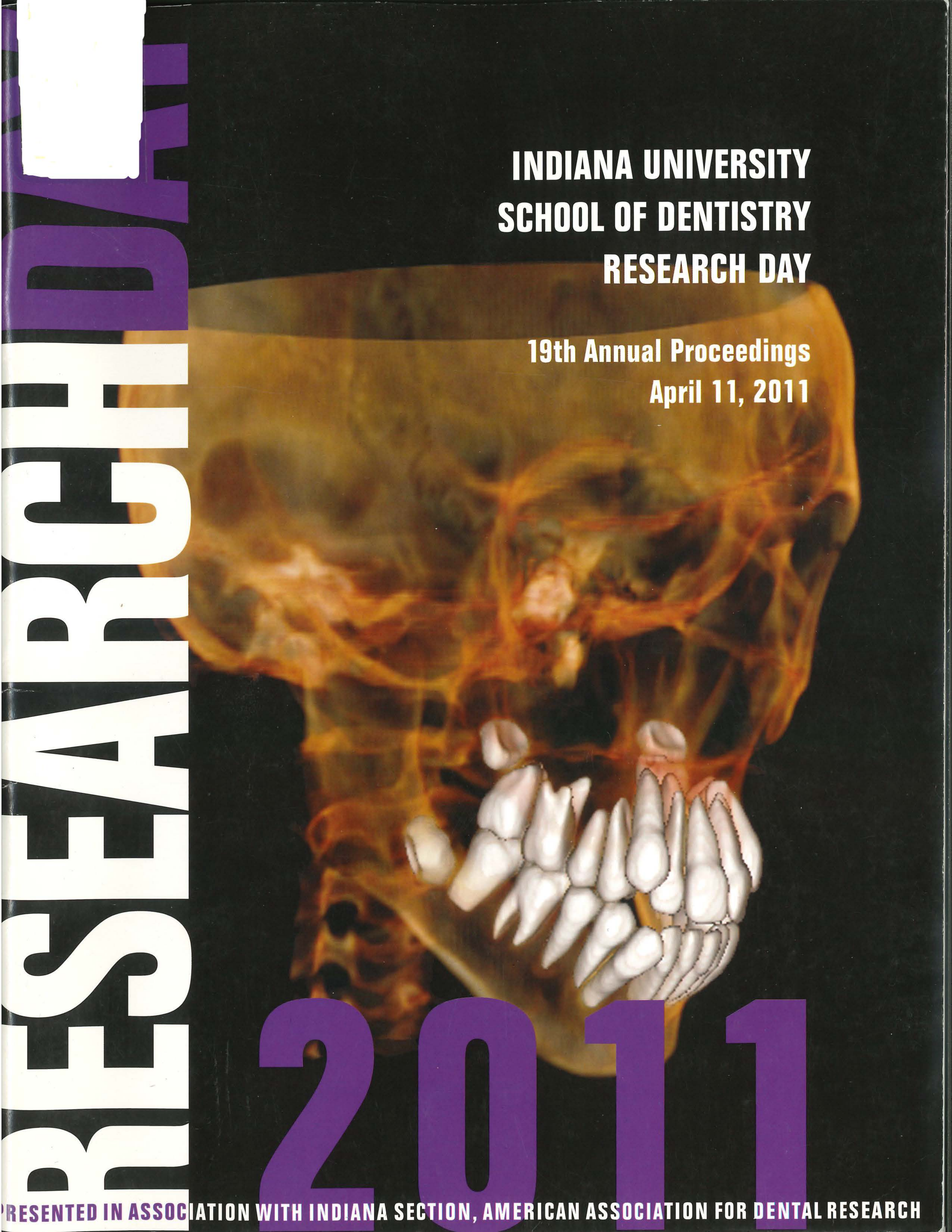
# INDIANA UNIVERSITY SCHOOL OF DENTISTRY RESEARCH DAY

19th Annual Proceedings
April 11, 2011



# RESEARCH DAY

# 2011

PRESENTED IN ASSOCIATION WITH INDIANA SECTION, AMERICAN ASSOCIATION FOR DENTAL RESEARCH

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# IUSD Research Day Proceedings
# Volume 19, 2011

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***On the Cover***: ***3D dental model superimposed on skull volume***. The 3D Imaging Lab at the Department of Orthodontics and Oral Facial Genetics uses the most advanced 3D imaging systems for many research and clinical applications. These systems allow visualization of the true 3D anatomy of the patient for enhanced diagnosis and treatment planning. The department now also uses the most sophisticated orthodontic 3D surface imaging software for multi-model image fusion, offering the best applications for assessing the patient condition, treatment and surgery planning, analyzing airways, simulating outcomes and monitoring the progress.

Image provided by Ahmed Ghoneima, BDS, MSc, PhD, Visiting Assistant Professor of Orthodontics, Department of Orthodontics and Oral Facial Genetics, Indiana University School of Dentistry.


*Cover design by Mark Dirlam. Research Day monograph prepared by Barbara A. Gushrowski*

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
AMERICAN ASSOCIATION FOR DENTAL RESEARCH

INDIANA SECTION

INDIANA UNIVERSITY SCHOOL OF DENTISTRY, 1121 WEST MICHIGAN STREET, INDIANAPOLIS, IN

April 11, 2011

Dear Participants and Guests,

On behalf of the Organizing Committee, the Indiana Section of the American Association for Dental Research (INAADR) and Student Research Group (SRG), we welcome you to Indiana University School of Dentistry's 19th Annual Research Day. Research Day at IU was established in 1993 to represent our local oral health research community. It strives to provide opportunities for IUSD's researchers to present their work and knowledge for the continual progress of oral health research and dentistry.

The Indiana Section is the local link to the national (AADR) and international (IADR) organizations for dental research. The INAADR's objective is to promote the advancement of research in all sciences pertaining to the oral cavity, its adjacent structures, and their relation to the body as a whole. Improved knowledge leads to improved cooperation and communication in our ultimate focus, prevention and treatment of oral diseases.

The INAADR sponsors or co-sponsors one scientific seminar per month on average, and is an integral Research Day Sponsor. This event showcases advances in IUSD's basic and clinical dental research, with an emphasis on encouraging our students to present their work and fostering opportunities for research collaboration. The SRG's Student Research Fellowship program provides predoctoral students a stipend and funding for supplies that they use to conduct original research under the guidance of a faculty mentor on any project of interest. The student researcher has opportunities to travel to national and international conferences, such as the AADR and IADR annual meetings, to present their research as well as build the strong foundation of developing an evidence-based practice.

The highlight of this afternoon's program will be the keynote address given by Dr. Jeffrey L. Ebersole, the current president of the American Association for Dental Research.

We want to offer a special word of thanks to our event and award sponsors, and also to all of the exhibitors for their generous contributions and their commitment to IUSD's Research Day. The success of this event depends on their continual support. We encourage you to visit with our exhibitors and acquaint yourself with the latest and greatest in dental products and services they have to offer. We look forward to continuing our relationship with every one of our sponsors and vendors, all of whom make this exciting and important event possible.

Last, but not least, we thank all of you for participating in Research Day 2011. We hope that each of you will enjoy what promises to be an enlightening afternoon.

Sincerely,

Karen S. Gregson, PhD
President, Indiana Section of the AADR

Olga Isyutina
SRG President

## Research Day Organizing Committee

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Masatoshi Ando
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***Future Research Day Event:*** ***April 16, 2012***

# Program

## *Campus Center 4th Floor*

**Thursday, April 7**

| | | |
|---|---|---|
| 5:00 p.m. – 8:00 p.m. | Judging (Dental School) | |

**Monday, April 11**

| | | |
|---|---|---|
| 8:30 a.m. – 11:00 p.m. | Interschool Student Research Competition (CC 406) | |
| 12:30 p.m – 1:00 pm | Registration | |
| 1:00 p.m. | Opening Remarks<br>(CC 450A-B) | **Dr. John N. Williams Jr.**<br>Dean, IUSD |
| 1:15 p.m. | Welcome and Introduction<br>of Guests and Keynote Speaker | **Dr. Domenick T. Zero**<br>Associate Dean for Research, IUSD |
| 1:25 p.m. | Keynote Address | **Dr. Jeffrey L. Ebersole**<br>President-Elect, American Association<br>for Dental Research |
| 2:00 p.m. | Presentation of Awards | **Dr. Masatoshi Ando**<br>Indiana Section, AADR |
| 2:25 p.m. | Acknowledgment of Special<br>Sponsors and Announcements | **Dr. Karen Gregson**<br>President, Indiana Section, AADR |
| 2:30 p.m. – 4:00 p.m. | Commercial Exhibitions (CC 450C) | |
| | Interschool Presentations (CC406)<br>Research Presentations (CC 405, 409)<br>2:30- 3:10 p.m.: Posters 1-43<br>3:20 a.m.-4:00 p.m.: Posters 44-79; Clinical Case Reports 1-6 | |
| 4:00 p.m. | Removal of Posters | |

# Introducing the Keynote Speaker

**Jeffrey L. Ebersole, BS, Ph.D.**

Dr. Jeffrey Ebersole is associate dean for Research and Graduate Studies, Alvin L. Morris Professor of Oral Health Research, Director of the Center for Oral Health Research at the University of Kentucky, College of Dentistry, and Professor, Microbiology, Immunology, and Molecular Genetics at the University of Kentucky, College of Medicine.

He earned his Ph.D. in microbiology at the University of Pittsburgh School of Dental Medicine and his B.S. degree in biology at Temple University.

In 1980, Dr. Ebersole joined the faculty at Harvard School of Dental Medicine. He joined the faculty at University of Texas Health Science Center at San Antonio in 1985, and in 2000 joined the faculty at the University of Kentucky, College of Dentistry.

Dr. Ebersole's area of research is B cell biology, particularly focusing on antibodies in secretory immunity and periodontal immunology. He has had continuous NIH funding for nearly 30 years. His primary research emphasis is in the development, specificity and functional abilities of antibodies in the oral cavity These characteristics are also explored as potential diagnostic tools in oral diseases. The interrelationships of specific host immune responses and local inflammatory mediators/cytokines and innate immune responses are being examined *in vitro* and in both human and animal models of oral disease and pathogenesis.

His current projects include research on mechanisms of pathogenesis of oral pathogens particularly from the perspective of host, profiles of host local inflammatory/immune mediators and innate immunity in human and nonhuman primate periodontal disease, characteristics of oral infections/periodontitis related to HIV reactivation, and biologic mechanisms of oral infections and systemic disease relationships.

In 2000, Dr. Ebersole received the Basic Research in Periodontal Disease Award from the International Association of Dental Research and in 2010, the Mentor Award from the Center for Clinical and Translational Sciences.

Dr. Ebersole currently serves as President-elect of the American Association of Dental Research.

# Presentation

***SALIVARY BIODIAGNOSTICS IN ORAL AND SYSTEMIC DISEASES***

# Recognizing Excellence

## 2011 List of Awards

—UNDERGRADUATE STUDENTS—

Procter & Gamble Undergraduate Student Award

—PREDOCTORAL DENTAL STUDENTS—

American Dental Association/Dentsply International Student Clinician Award

Cyril S. Carr Research Scholarship

INAADR Hatton Travel Award

Interschool Dental Student Research Awards

Johnson & Johnson IUSD Student Research Group Award

Procter & Gamble Award for Excellence in Preventive Oral Health Care

—GRADUATE DENTAL STUDENTS—

Delta Dental Award for Innovation in Oral Care Research

INAADR Hatton Travel Award

Indiana Dental Association Best Clinical Case Report Award

Maynard K. Hine Award for Excellence in Dental Research

Shofu Dental Student Award

—POSTDOCTORAL FELLOWS—

Shofu Dental Post-Doctoral Fellow Award

—FACULTY—

Indiana University School of Dentistry Alumni Association Distinguished Faculty Award for Teaching and Distinguished Faculty Award for Research

# Poster Presentations
**2:30 p.m. to 3:10 p.m.**

## BEHAVIORAL SCIENCE

**P1** **Disparities in Racial and Ethnic Minorities' Access to Dental Care.** C. MUNDY[1],* E. A. MARTÍNEZ-MIER[2] ([1]Indiana University-Purdue University Indianapolis; [2]Indiana University School of Dentistry)

There is increased interest on research to define strategies to improve ethnic and racial minority group's access to dental services. Results from numerous studies propose issues other than finances are responsible for most minority populations not seeking dental care. The purpose of this study was to assess barriers that prevent African Americans and English speaking Latinos to access oral care. This information may be useful to inform future development of strategies to decrease those barriers. Using a survey, we evaluated differences in barriers based on race and ethnicity. Questions inquired about insurance coverage, area of residence, occupation, education level, race/ethnicity, and patient navigation skills have on one's access to dental care. 18 African Americans, 2 American Indians and 10 Latinos 18 to 41 years old answered the survey. Results show that the majority of respondents had an annual income below $35,000. Only 23% of the participants reported having dental insurance. Most participants (94%) also reported it had been 6 months or more since they had had a dental visit, with 30% of them never having visited a dentist. In contrast 66% reported having had painful aching somewhere in their mouth during the past year. 23% reported they had talked to a health professional, other than a dentist about their oral health. Based on our results we conclude that access to dental services in this sample was limited and that participants reported not being able to access certain needed services at times.

**P2** **Community-Based Participatory Research Addressing Oral Health Disparities in the US.** A. E. SOTO-ROJAS,* L. C. GALVEZ, G. J. SMITH, E. A. MARTÍNEZ-MIER (Indiana University School of Dentistry)

Latino populations have higher rates of dental caries and untreated decayed teeth. Objectives, the aim of this study was to gain a better understanding of the caries prevalence of a group of Latino children in Indiana, and their parents' dental knowledge. Methods, a community organization and researchers collaborated to conduct focus groups, key informant interviews, and dental exams. Children ages 6 to 13 were examined for dental caries using the ICDAS index. Focus groups' guiding questions were developed and refined with a random sample of participants. Questions inquired about oral health prevention, access to care, and beliefs and knowledge of causes of dental problems. Focus groups interviews were recorded and transcribed. Codification of answers was performed. Results were analyzed using content analysis methodology in which the frequency of answers was assessed and tabulated. Caries prevalence was calculated for both cavitated and non cavitated lesions. Results were analyzed both by community members and researchers. Results, 130 adults ages 25 to 44 participated in the focus groups. 157 of their children received a dental exam. Results showed that 82% of the children had at least one caries lesion and 65% had at least one cavitated lesion. Most participants reported understanding of the roles of brushing, sugared beverages, candy and other cariogenic foods in caries etiology. However, the majority was not familiar with the role of fluoride in caries prevention or were aware of the role of dental sealants, in spite of 56% of the children examined having sealants placed. Many traditional beliefs were associated to the use of chewing gum and early childhood caries. Conclusions, the information obtained indicated

there was great need for information and education to parents; it also guided the development of a community action plan that will include the development of a culturally appropriate oral health educational program. Supported by an RSFG grant and Pilot Grant Funds from IUPUI-BICCHEC Center.

## BONE BIOLOGY

**P3** **BMP-2 Peptide and the Osteogenic Protein Production of MC3T3 Cells**. M. MAROPIS,* A. ALMARZA, P. KAMELIN, C. KUNKLE (Oral Biology and Bioengineering, University of Pittsburgh)

Bone Morphogenic Protein 2 (BMP-2) has been shown to promote the formation and regeneration of bone, but it is used in high concentrations. A BMP-2 derived peptide (KIPKASSVPTELSAISTYL) was developed to decrease concentrations, and was shown to increase bone matrix gene expression in-vitro in murine multipotent mesenchymal cells, and encouraging in-vivo results were also shown. However, the effect of the peptide on the early protein production was not studied. Objective: In our study we examined the role of BMP-2 peptide in the mineralization of preosteogenic MC3T3 cells by studying protein production at the early time points of 1 and 6 days. Methods: The peptide was absorbed into 24-well plates at a concentration of 0.2, 1, and 2 mg/ml in 200 ul of PBS and allowed to evaporate overnight. All results were compared to no peptide control, and analyses were performed on the media. Results: No detectable levels of osteocalcin and collagen were found, and all groups had a similar stain intensity of von Kossa at the end of culture. The presence of peptide also had no impact on alkaline phosphatase production. All peptide concentrations resulted in a significant increase in osteopontin production at Day 1 when compared to control ($p<0.05$). Both the 1 and 2 mg/ml concentrations showed higher protein content at Day 6 than control and 0.2 mg/ml ($p<0.05$). Conclusion: In summary, the peptide has a powerful osteogenic effect on the early protein production of MC3T3 cells. Future studies will determine the effect of the peptide on mineralization in 2D at longer time points, and in 3D novel polymeric scaffolds.

**P4** **Engineering of Periosteum Using Cell Sheets**. G. A. SHAH[1,3],* F. N. SYED-PICARD[1,2,3], B. J. COSTELLO[1,3,4], C. SFEIR[1,2,3] ([1]Center for Craniofacial Regeneration; [2]Department of Bioengineering; [3]Department of Oral Biology; [4]Department of Oral & Maxillofacial Surgery, University of Pittsburgh)

Objectives: Periosteum is a fibrous connective tissue investing the outer surface of bone, and as a source of osteoblasts, plays an integral role in the bone healing process. In this study, tissue engineered constructs were developed by wrapping bone marrow stromal cell (BMSC) sheets around calcium phosphate (CaP) cement in an attempt to create periosteum-like tissue. These constructs were implanted subcutaneously into immunocompromised mice, and the subsequent formation of bone and periosteum-like tissue assessed. Methods: BMSC sheets were grown on tissue culture plates and then wrapped around CaP cements. Four types of constructs were formed. Group 1: CaP cement alone. Group 2: CaP cements into which BMSC were pipetted and then incubated for two hours to allow cell attachment before implantation. Group 3: BMSC sheets wrapped around calcium phosphate cements. Group 4: CaP cements with BMSCs pipetted onto the cement followed by a BMSC sheet layered around the construct. These four groups were implanted subcutaneously into immunocompromised mice for 8 weeks. Results: Immunohistochemistry was performed on each of the four experimental groups to observe expression of bone proteins DMP-1, BSP and Col I, or periostin, a periosteum-specific protein. There was no expression of these proteins in samples without cells, however all cell-sheet wrap and cell-sheet wrap + injected cell samples showed marked expression of proteins, particulary

periostin. The expression of periostin and other proteins was greater at the cell-sheet/CaP pellet interface than within the center region of the CaP cement, suggesting the development of a periosteum-like tissue. Conclusions: These results suggest that the combination of a BMSC tissue sheet and CaP scaffolding induces the formation of a periosteum-like tissue. This technology could accelerate bone regeneration when compared to strategies employing CaP scaffolding alone. Further analysis and studies are warranted.

**P5 BENS, a Novel Regulator of Bone Cell Survival and Wound Healing.** N. LABBAN,* F. SONG, J. CAMERON, A. BRUZZANITI, P. REDWOOD, D. MILNER, L. J. WINDSOR (Indiana University School of Dentistry)

Each year, more than 1.5 million osteoporosis related fractures occur. Significant morbidity, mortality and health care costs are associated with these fractures. Traditional medicines have been used for thousands of years to treat various ailments, including bone/tissue healing. In this study, we used molecular, cell-based and in vivo approaches to investigate the bone-healing effects of a traditional nutritional supplement, which we have tentatively named BENS (Bone Enhancing Nutritional Supplement; US patent pending). To investigate the effects of BENS on cell survival, a human-derived osteosarcoma cell line, MG63, and primary human gingival fibroblast cells (HGFs) were incubated in a serum free media, with and without 0.8 mg/ml of BENS for up to 30 days. Microscopic examination revealed a significant increase in the survival of BENS-treated cells after 30 days, compared to controls, and these cells retained their ability to proliferate when serum was reintroduced into cultures. To begin to determine the mechanism of action of BENS, we cultured cells with BENS for 5, 10 and 15 days and examined changes in gene expression using Microarray Illumina Technology, which simultaneously evaluates the expression of 46,000 human genes. Of interest, mRNAs of multiple bone and connective tissue proteins were up-regulated in BENS-treated groups. Specifically, the mRNA for type I collagen alpha I chain was increased 23-fold in the HGFs. In addition, genes associated with the Wnt pathway were significantly enhanced ($p<0.05$) in both cell types. The Wnt pathway plays critical roles in cell growth, development and differentiation of multiple tissues such as bone/connective tissues. We next used an in vivo Xenopus system to evaluate the ability of BENS to promote bone healing in a non-critical size segmental bone defect model. Each frog had a small segment of the anterior hemisection of the tarsus bone (10% or less of the total tarsus bone length) excised to serve as a fracture model. The frogs were divided into three groups and given subcutaneous injections of either phosphate-buffered saline or BENS (3 mg/ml or 6 mg/ml) once daily for 30 days. The frogs were then euthanized, and the hindlimbs were cryosectioned and stained with hematoxylin/eosin. The cartilage mass formed along the cut tarsus in both of the treated groups was significantly increased in comparison with cartilage mass formed in the controls. The periosteal cartilage extended almost completely along the length of the fractured tarsus and some blood vessels could be observed penetrating the cartilage bridging the bone gap in treated groups. The results suggest that BENS has the ability to promote cell survival, which could extend the length of time that these cells are actively expressing bone/connective tissue proteins. In addition, BENS enhanced the gene expression for several bone and connective tissue biomarkers, which could explain its ability to promote tissue healing in the in vivo model. Our findings suggest BENS could be developed as a therapeutic anabolic agent for bone healing.

## CARIOLOGY

**P6 ICDAS Assessment of Caries Incidence and Progression over 40 months.**
A. FERREIRA ZANDONÁ[1],* E. SANTIAGO[2], G. J. ECKERT[3], D. T. ZERO[1] ([1]Indiana University School of Dentistry; [2]University of Puerto Rico, [3]Indiana University School of Medicine)

The objective of this study was to assess dental caries incidence and progression in a group of children enrolled on a 4 year longitudinal study in rural schools in the Commonwealth of Puerto Rico by tooth surface over a 40 month period using the International Caries Detection and Assessment System (ICDAS-II). 384 children provided informed consent and were examined with the ICDAS at baseline and 4 month intervals for 40 months. The enrolled children (49% female, 51% male) ranged from 5-13 yrs old (mean 9.27) and were mostly Hispanic (90%). Focusing on permanent teeth without fillings at the baseline exam, incidence and progression of lesions varied according to severity scores. Progression was defined as worsening ICDAS score and/or placement of a filling. ICDAS scores 0 were the least likely to change, with only 12% progressing to any type of lesion. 36% of surfaces with ICDAS score 1 experienced progression, almost equally likely for surfaces called 'active' (37%) and 'inactive' (28%). Progression for ICDAS 2 occurred for 16%, 18% for active and 7% for inactive. Progression for ICDAS scores 3-6 were 24%, 50%, 33%, and 51%. Surface type also played a role in both incidence and progression. New lesions as well as progression to cavitation or fillings with most likely on occlusal fissures, buccal pits, and lingual grooves. Although incidence of mesial (8%) and distal lesions (5%) was low, lesions present on these surfaces at baseline were highly likely to progress (37% for mesial, 44% for distal). Caries lesions incidence and progression are dependent on surface and initial ICDAS severity score. Supported by NIH/NIDCR RO1DE017890-04

**P7 Early Detection of Caries Using ICDAS and E-Learning**. N. SADEGHI[1],* G. J. ECKERT[2], A. FERREIRA ZANDONA[3] ([1]Purdue University; [2]Indiana University School of Medicine; [3]Indiana University School of Dentistry)

The ability of a dental student to detect dental caries at an early stage is critical to the success of their education in the dental field. At Indiana University School of Dentistry (IUSD) a caries detection lab has been offered to dental students as part of their predoctoral training since 2003. The International Caries Detection and Assessment System (ICDAS) has been used as the bases for the education of first year dental students on detection of caries in these labs since 2004. ICDAS is a visual scoring system (0-6) that scores caries lesions severity. The objective of this study was to determine whether the methodology assisted students in determining presence or absence of caries lesions and severity based on their performance on a practical exam and the histological assessment of the teeth used in the practical exam. All students received a lecture and then attended a caries detection lab where they experienced detecting caries on natural teeth mounted on manikins in a Phantom Head. They also had access to two e-learning programs, the ICDAS e-learning and an in house e-learning on caries detection. Students' test responses (sound, incipient or cavitated lesion) to the practical exam from DDS classes of 2010, 2011 and 2013 were recorded. The selected surface on the teeth (N=26) was scored by two independent examiners. The teeth used in the practical for DDS classes of 2011 and 2013 were sectioned in the center of the lesion (or the selected surface). Sections were examined and imaged with Nikon SM 1500 microscope for presence of caries using Ekstrand et al (2007) criteria. Spearman correlation of ICDAS with histology was 0.92. Spearman correlation of DDS Classes of 2011 and 2013' scores with histology was 0.65-0.72 . Spearman correlation of DDS Classes of 2011 and 2013' scores with ICDAS scores was 0.65-0.76. Weighted Kappa

scores (all DDS classes) with ICDAS were 0.61-0.65. All classes were significantly different from each other for all 3 outcomes ($p<0.001$) using Wilcoxon Rank Sum tests. The results indicate that this limited exposure to ICDAS and practical experience is effective on teaching students how to differentiate sound surfaces from incipient and cavitated lesions.

**P8 Smoking Associated with Tooth Loss – 2006 BRFSS data analysis**. R. L. GREGORY* (Indiana University School of Dentistry)

Objectives: We have recently reported upregulation of *Streptococcus mutans* growth, biofilm formation and virulence factors, such as antigen I/II, when grown with physiologically-relevant concentrations of either cigarette smoke extract or nicotine. The purpose of this study was to analyze the relationship between smoking and the number of teeth removed due to caries and periodontal disease in three age groups. Methods: Data from the 2006 Behavioral Risk Factor Surveillance System database (2006 BRFSS) were used for analysis. The current analyzed sample consisted of a non-institutionalized U.S. population, including a total of 314,986 subjects, 18 years or older with complete key variables. An ordinal logistic regression model was constructed including confounders such as demographic and socioeconomic information, behavioral health risk factors (e.g., smoking status) and general health status (e.g., diabetes and BMI). Results: Among this population, only 46.3% had all teeth remaining, and 8.4% were edentulous. About 52.8% were non-smokers and 18.8% were current smokers. The relationship between smoking status and tooth loss was statistically significant in this population. Generally, current daily smokers were at a significantly higher ($p<0.05$) risk of having teeth removed than current occasional smokers or former smokers (2.95, 1.96, and 1.64 fold increase over subjects that have never smoked, respectively). This trend remained significant in all three age groups. Moreover, middle-aged adults (35-64 years) who were smoking every day were at significantly greater risk than younger (18-34 years) and older (65 years or older) counterparts (3.18, 2.58, and 2.59, respectively). Conclusions: These results confirm that smoking is an important risk factor for tooth loss in younger, middle-aged and older groups. Current smokers are most at risk, especially for the middle-aged group. Supported, in part, by the Indiana University School of Dentistry Ph.D. Student Research Fund.

**P9 Effect of Strontium and Zinc on the Demineralization of Enamel.** R. GASAWAY,* F. LIPPERT (Indiana University School of Dentistry)

The roles of zinc and strontium in the caries process are poorly understood. The objective of this study was to investigate the effects of zinc and strontium on enamel de- and remineralization. Sound enamel specimens (n=10) were demineralized (lactic acid solution, pH 5.0) in the presence of 5mM zinc or strontium for 24h and then remineralized (artificial saliva, pH 7.0) for 70h. Specimens demineralized in the presence of 5mM calcium served as a control. A Vickers microindenter was used to determine changes in surface hardness (VHN) throughout the experiment. Data were analyzed using ANOVA ($p<0.05$).

| | VHN (sound) | VHN (demineralized) | VHN (remineralized) |
|---|---|---|---|
| Zinc | 323±18 A | 274±17 A | 284±16 A |
| Strontium | 321±20 A | 71±14 B | 218±16 B |
| Calcium | 321±20 A | 251±41 A | 290±23 A |

Data are means ± standard deviations. Significant differences are highlighted by different letters. In addition, a zinc dose-response experiment was carried out. Specimens were demineralized in the presence of 1, 2, 3 or 5mM zinc.

| Zinc | VHN (sound) | VHN (demineralized) | VHN (remineralized) |
|---|---|---|---|
| 1mM | 323±15 A | 199±27 B | 209±23 C |
| 2mM | 323±13 A | 251±23 A | 257±24 B |
| 3mM | 323±14 A | 266±34 A | 262±26 B |
| 5mM | 323±18 A | 274±17 A | 284±16 A |

No differences were found between the effects of zinc and calcium. However, demineralization in the presence of zinc somewhat retarded remineralization. Strontium showed only very little effect on protection from demineralization, but did not negatively affect remineralization. The zinc dose-response experiment demonstrated that even very low zinc concentrations showed a protective effect, which, however, was paired with a diminishing remineralization effect. In conclusion, zinc and strontium vary in their effects on enamel de- and remineralization in comparison to calcium. Supported by IUPUI LHSI.

**P10** **Development of a Standard Fluoride Analytical Method for Dental Plaque.** E. A. MARTÍNEZ-MIER,* C. B. BUCKLEY, P. CHANDRAPPA, A. E. SOTO-ROJAS (Indiana University School of Dentistry)

Fluoride diffusion analysis releases and concentrates free and bound fluoride. It is the preferred method for samples in which fluoride is in covalent or complexed form. Available diffusion techniques release fluoride at a pH of 1, which imitates the stomach pH. These methods may not have biological relevance when analyzing fluoride in dental plaque, since the oral environment does not reach such low pH. This study aimed at assessing the analytical precision and trueness of a newly developed diffusion analysis method to analyze dental plaque at a pH of 4. Initially, four combinations of reagents were tested for pH. These included different types and concentrations of acids and buffers. Once the target pH was obtained, the identified combination (2.0 ml DIH2O + 1.0 ml standard + 1.0 ml HMDS-saturated 0.00005M SO4) was used to analyze 15 NIST-traceable fluoride standards (0.02 µg F/g) and 44 plaque samples. Using a goal of 5% within-laboratory precision, the estimated standard deviation needed to exceed 0.0048 µg F/g to conclude that the measurement was not as precise as required. The trueness of the measurements was assessed based on the confidence interval. The standard deviation did not reach 5% level (0.0029 µg F/g) and the confidence interval was 0.0191-0.024 µg F/g, which in this case contained the true measurement value. Therefore, results were within ISO acceptable limits for precision and trueness. ICC for repeated measures was 97. As expected, significant differences were found among the mean values obtained using the pH 4 and 1 methods for plaque samples (65.37 ± 28.24 µg F/g vs. 0.28 ± 0.34 µg F/g). The method, which simulates biologically-relevant oral pH conditions, was able to render precise and true values when analyzing samples of known concentration and repeatable values when analyzing unknown concentration samples.

**P11** **The Effect of FOTI Light Output on Proximal Caries Detection.** E. McCREA[1],* M. ANDO[1], G. J. ECKERT[2], A. FERREIRA ZANDONA[1] ([1]Indiana University School of Dentistry; [2]Indiana University School of Medicine)

Background: Bite-wing radiographs are the standard for proximal surface detection of carious lesions, however, Fiber Optic Transillumination (FOTI) has been reported as a valuable supplement for detection of proximal caries. Several new FOTI devices are available with distinct characteristics such as light output and tip diameter. Objectives: Compare the effect of light output and tip diameter of three fiber optic devices (FOTI, Schott Fibre Optics; DIAlux,

KaVo; and Microlux Transilluminator, AdDent) in the detection of proximal caries. Materials and Methods: Sixty extracted unrestored posterior teeth stored in 0.1% Thymol, representing all scores of the International Caries Detection and Assessment Criteria (ICDAS 0-6) on proximal surfaces were selected, mounted on models with proximal surfaces in contact and placed in Phantom Heads for all exams. Three trained examiners performed an ICDAS exam followed by all fiber optic exams (0-6 scale used) two separate times. The teeth were then hemi-sectioned through the center of the lesion and analyzed under a stereomicroscope (2X) using a 0-4 scale (Ekstrand et al, 1987). Intra-class correlation coefficients (ICCs), sensitivity, specificity and area under the curve (ROC) were obtained for all methods. Results: Intra-and inter-examiner agreement for all FOTI devices were high (0.93-0.99 and 0.86-0.92 respectively). The FOTI devices were highly correlated with each other (0.88-0.92), with ICDAS (0.79-0.84), and with Histology (0.77-0.79). The area under the ROC curve was high for all FOTI devices (0.86 to 0.88), with no significant differences among them ($p>0.24$). Microlux had significantly higher specificity than Schott FOTI 1mm 3000K ($p=0.0013$), with no other significant differences in specificity among the FOTI devices. There were no differences among FOTI devices for sensitivity ($p>0.10$). We concluded that the performance of all FOTI devices was equivalent irrespectively of light output or tip diameter.

**P12 Determining the Association between Fluoride Content and Clinical Fluorosis Severity.** D. B. SHONE[1],* E. A. MARTÍNEZ-MIER[1], A. E. SOTO-ROJAS[1], H. EGGERTSSON[2], C. BUCKLEY[1], S. OFNER[3] ([1]Indiana University School of Dentistry; [2]University of Iceland; [3]Indiana University School of Medicine)

Dental fluorosis is a hypomineralization of the enamel caused by excessive fluoride during tooth formation. Disagreements have been reported among studies that have aimed to find an association between dental fluorosis severity and fluoride content within enamel. Objective: The aim of the current study was to compare fluoride content to clinical visual appearance based on the Thylstrup-Fejerskov Index (TFI) of fluorosis. Calibrated examiners selected 30 teeth from each of the following groups: TFI 0 (sound), TFI 1, 2, and 3. From each of the 120 teeth, a 2x2mm enamel square was cut to analyze fluoride content at 30, 60, and 90µm. At each depth level, enamel powder was collected using a micro-sanding technique, dissolved in 0.5M $HClO_4$, and fluoride levels were determined using a fluoride ion-specific electrode and Orion pH/ion meter. An ANOVA model with a Sidak adjustment was used to compare mean fluoride content among groups. Mean and SD fluoride concentrations (ppm) were found to be as followed (listed as 30µm, 60µm, 90µm): TFI 0 (717.8±582.8, 280.7±208.8, 182.8±97.1); TFI 1 (1231.2±517.8, 503.0±189.1, 390.9±159.7); TFI 2 (1150.4±576.4, 445.1±287.3, 324.4±186.1), and TFI 3 (1736.5±949.6, 655.0±473.7, 478.2±295.0). It was observed that teeth characterized as having some form of fluorosis (TFI groups 1-3) had significantly larger mean fluoride concentrations ($p<0.0001$) than those characterized as TFI 0. Furthermore, teeth from TFI group 3 had higher values ($p=0.0442$) than those from TFI group 2 at 30µm. All other comparisons were not significant. Conclusions indicate that fluorosed teeth had higher fluoride content than sound teeth, but there was not a difference in fluoride content among different fluorosis severities.
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**P13 Dose-Response Effect of Strontium and Fluoride on Caries Lesion Reminerlization.** G. YASSEN[1],* F. LIPPERT[1], G. J. ECKERT[2], J. EDER[1], A. FERREIRA ZANDONA[1] ([1]Indiana University School of Dentistry; [2]Indiana University School of Medicine)

Objectives: To investigate dose-response effects of Strontium (Sr) and Fluoride (F) in combination on the remineralization of early caries lesions in vitro. Methods: Artificial caries-like lesions were formed in 135 bovine enamel specimens. Lesion severity was analyzed using quantitative light-induced fluorescence (QLF) and transverse microradiography (TMR). The specimens were then randomly assigned into 9 treatment groups based on lesion volume after demineralization, as measured by TMR. Treatment groups were based on a 3×3 factorial design (0/0.05/0.1ppm F and 0/10/15ppm Sr). Lesions were remineralized at 37°C for 14 days in artificial saliva (1.5mM CaCl2, 0.9mM KH2PO4), which was supplemented or not with NaF and/or SrCl2×6H2O. The extent of lesion remineralization was assessed using QLF and TMR. Data were analyzed using two-way ANOVA. Results: For the TMR analysis, a trend of significance was observed between the two-way interaction of Sr and F ($p=0.06$), indicating that the Sr comparisons depend upon the F level and vice versa. Lesion remineralization in the 10ppm Sr + 0.05ppm F group was marginally higher than in the 0.05ppm F group ($p=0.055$) and significantly higher than in all other groups. However, the 10ppm Sr + 0ppm F group exhibited significantly less remineralization than the control group ($p=0.048$). For the QLF analysis, although inter-group differences were not the same as for the TMR analysis, the two-way interaction between Sr and F was significant ($p=0.0001$). No significant difference was observed between 10ppm Sr + 0ppm F and 15ppm Sr + 0ppm F groups ($p=0.19$) . However, $\Delta\Delta F$ in 10ppm Sr + 0ppm F group had significantly less change than all other groups. The QLF measurement was moderately correlated with TMR mineral loss ($r=-0.37$). Conclusion: There was an interaction between F and Sr when they were used in conjunction at specific concentrations.

## CELL BIOLOGY

**P14 Kalirin Knockout Causes Osteoporosis in Mice by Increasing Osteoclast Activity.** S.HUANG[1],* P. ELENISTE[1], C. CARTER[1], R.E. MAINS[2], M. ALLEN[3], A. BRUZZANITI[1] ([1]Indiana University School of Dentistry; [2]University of Connecticut; [3]Indiana University School of Medicine)

The guanine nucleotide exchange factors (GEFs) regulate the conversion of Rho-GTPases from active (GTP-bound) to inactive (GDP-bound) conformations. Few Rho-GEFs have been studied for their specific role in OCs and OBs, despite their known role in motility, cytoskeletal reorganization and actin turnover in other cells. Kalirin is a recently identified Rho-GEF belonging to the family of Dbl-homology (DH) proteins which leads to the activation of the Rho-GTPases Rac1 and RhoG. Kalirin has been shown to play a role in cytoskeletal remodeling, migration and dendritic spine formation/extension in neuronal cells, but its function in non-neuronal cells is largely unknown. Western blotting and real time PCR confirmed that Kalirin is expressed in mouse and human OCs and in mouse OBs, suggesting it may play a role in regulating bone cell function and therefore bone mass in vivo. Kal-KO mice were generated by crossing Kal-flox mice with germline-expressing Hprt-Cre mice, resulting in progeny that lack Kalirin expression in all tissues/cells (global KO). We used micro-CT to examine the bone phenotype of Kal-KO of 14 week old female mice. Our studies demonstrate that deletion of Kalirin leads to a significant 45% decrease in the ratio of bone volume/trabecular volume in the distal femur and vertebrae ($n=6$ mice, $p<0.05$). We also measured static bone parameters which revealed a 38% and 26% decrease in trabecular number and thickness, respectively in Kal-KO

mice compared with wild-type (WT) mice. We next determined the role of Kalirin on OC differentiation and function in vitro. OCs were generated from the bone marrow of WT or Kal-KO mice by differentiation with receptor activator of NF-kappaB ligand (RANKL) and mouse colony stimulating factor. After 7 days, OC bone resorbing activity was determined using an in vitro "pit" assay which relies on the ability of mature OCs to degrade dentin. Resorption pits were stained with toluidine blue and quantified microscopically using Bioquant 7.0 software. OC number was determined by counting multinucleated cells expressing tartrate-resistant acid phosphatase. These studies demonstrate that OCs lacking Kalirin exhibit a marked increase in bone resorbing activity on dentin, compared with WT OCs. In addition, OC number was significantly increased in cultures generated from the bone marrow of KO mice, compared to WT mice, suggesting OC differentiation was accelerated. To determine if Kalirin also played a role on OB function, OBs were generated from the calvaria of 2 day old neonates and differentiated for 7 days with ascorbic acid and beta-glycerolphosphate. The media was replaced and conditioned media was collected after 24 hrs and assayed for the level of secreted RANKL and osteoprotegerin (OPG), two cytokines essential for controlling OC differentiation in vivo. The results revealed that the ratio of RANKL/OPG was increased 3-5 fold in Kal-KO OBs compared to WT OBs, which would lead to increased OC differentiation. Taken together, our studies demonstrate that Kalirin is a key regulator of bone mass in vivo and that Kalirin's effects on bone are due to cell autonomous defects on OB and OC function, as well as the uncoupling of the bone remodeling process. This work was supported by a Postdoctoral Fellowship Award Grant and funds provided by the Indiana University School of Dentistry to Dr Bruzzaniti.

## COMMUNITY DENTISTRY

**P15 Differences Between Volunteer and Non-Volunteer Dentists in Northeastern Indiana.** T. KIMMEL[1],* G. J. ECKERT[2], K. YODER[1] ([1]Indiana University School of Dentistry; [2]Indiana University School of Medicine)

This study compares differences between dentists who choose to volunteer to see patients of safety net clinics/programs and those who do not in Northeastern Indiana. Identifying key demographic differences between volunteers and non-volunteers, as well as determining the key reasons why dentists choose to volunteer or not, will aide directors of safety net clinics in focusing their recruiting efforts on only the individuals shown to be more likely to volunteer. Directors will also be able to then develop improvements to their volunteer programs to make them more appealing to local dentists. A paper survey was mailed to 297 general dentists and specialists in the Indiana counties of Allen, Adams, Dekalb, Huntington, Jay, LaGrange, Noble, Steuben, Wells, and Whitley. The survey sought demographic data (year of graduation, dental school attended, professional associations). The survey asked about the dentists' volunteer activities (where they volunteer their services, how many hours they volunteer, how many patients they see). The survey ended by inquiring as to the reasons why the dentist either chooses to or not to volunteer their services through local safety net clinics or programs. Of the 297 dentists included in the survey, 134 (45%) responded. When comparing the two groups (dentists who volunteer versus those who do not), it was determined that the dentists who identified themselves as volunteers also identified themselves as members of one or more professional associations ($p<0.05$). When asked about interest in receiving a tax break or continuing education credit for volunteer services rendered, the dentists that identified themselves as volunteers were also more likely to be interested in receiving such benefits ($p<0.05$). The survey also demonstrated ($p<0.05$) that those dentists that identified themselves as volunteers also, on average, indicated an earlier graduation year than those who did not volunteer. This indicates that dentists that volunteer are more likely to have been in practice longer and to be more established than those dentists who do not volunteer. Dentists that

choose to volunteer their time gave many reasons for doing so, including a sense of duty ("right thing to do"), to fulfill a need in the community, and for religious reasons. Those dentists that did not volunteer had many reasons for not doing so, including being too busy, not having the financial resources to volunteer, not interested in volunteer work, and wanting to be able to select their own charity cases. Thus, the survey was able to elucidate several important differences between those dentists that volunteer to see patients of safety net clinics/programs and those dentists that do not. This information will be of use to directors of safety net clinics as they work to make their volunteer programs more appealing to local dentists.

## CRANIOFACIAL BIOLOGY

**P16 Effects of Fluoride on Condylar Growth and Density.** S. ELSAHY,* S. LIU, J. SUN, P. DUNN-JENA, K. T. STEWART (Indiana University School of Dentistry)

Orthodontists commonly use functional appliances to stimulate condylar growth by displacing the mandible forward when treating patients with a mandibular deficiency. Increased administration of dietary minerals, such as fluoride, has been shown to increase condylar thickness and density. The cumulative effects of condylar displacement and increased fluoride concentrations have not been previously investigated. Therefore, the objective of this study was to evaluate the effects of increased fluoride administration during lateral displacement of the condyle in a rat model. Thirty-two, 4-week old rats were fitted with a custom maxillary acrylic appliance, which caused their mandibles to permanently shift laterally. All rats were placed on a soft diet and were randomly assigned to two groups. One group received distilled water (control), while the other received distilled water with 100 ppm NaF. For 2 weeks the animals were allowed to eat and drink ad libitum. A paired T-test was used to assess differences between the displaced and non-displaced condyles. A Mann Whitney U test was used to evaluate differences between condyles exposed to distilled water or distilled water with 100 ppm NaF. Significant differences in condylar length, bone volume, bone surface, mineralization, porosity, and trabecular connectivity were observed ($p \leq 0.05$) between the distracted and non-distracted condyles, but this difference was independent of the water consumed. We concluded that lateral displacement of the mandible results in significant changes of the distracted condyle. Increased fluoride administration, however, did not produce a significant difference in condylar morphology or mineral composition. Funded by IUPUI Support for the Recruitment of Under-Represented Faculty (SURF) and Indiana University School of Dentistry.

## DENTAL MATERIALS

**P17 Mechanical Properties of Novel Calcium Phosphate Resin.** L. AL DEHAILAN,* T. G. CHU (Indiana University School of Dentistry)

Tricalcium phosphate (TCP) is a new calcium phosphate releasing material that has shown strong potential for remineralization applications (Karlinsey 2009). The objective of this study was to investigate the possible use of TCP filled composite resin as a tooth restorative material. The material was tested for compressive strength, modulus, degree of conversion, depth of cure, hardness and viscosity. The TCP composite was prepared using a monomer mixture of 34.3 wt% of EBPADMA, 34.2 wt% of HmDMA, and 30.5 wt% of HEMA. TCP provided by Indiana Nanotech, was added to the mixture at different levels (30, 40, 50 and 60 wt %). A universal testing machine was used to measure the compressive strength and modulus. FTIR was used to measure degree of conversion. Depth of cure was determined according to the ISO standards for dental resin 4049. Knoop hardness numbers were obtained by a microhardness tester. The viscosities of the experimental resin were determined in a viscometer. A p=0.05

significance level was used for all the tests. Composites with 30% TCP showed the highest compressive strength and hardness values. Also this group showed the lowest degree of conversion. Composites with 60% TCP showed the highest degree of conversion. However, this group showed the lowest compressive strength, depth of cure and hardness. Composites with 50% filler showed the highest compressive modulus. Composites with 40% filler showed higher viscosity values than composites with 30% filler. Increasing the filler level significantly reduced the compressive strength, hardness and depth of cure but increased the degree of conversion. Composites with the least filler (30%) had the highest compressive strength, depth of cure and hardness. It can be concluded that TCP filled resin used in this study cannot be used as restorative material.

**P18 Ion Release and Water Sorption of Novel Calcium Phosphate Resin.** A. ALZAIN,* T. G. CHU (Indiana University School of Dentistry)

Tricalcium phosphate (TCP)-filled restorative materials were introduced as an alternative to amorphous calcium phosphate (ACP). b-TCP combined with fumaric acid can formulate a material that has a greater potential to remineralize tooth structure compared to other TCP compounds (Karlinsey, 2009). The objective of this study was to characterize the concentrations of calcium and phosphate ion released, and the water sorption (WS) from TCP-filled resin at different filler levels. An in vitro study was conducted by formulating resin composite using TCP provided by Indiana Nanotech as the filler mixed with Ethoxylated bisphenol A dimethacrylate (EBPADMA), Hexamethylene dimethacrylate (HmDMA) and 2-hydroxyethyl methacrylate (HEMA) as the resin matrix. One-hundred-sixty samples were prepared, 40 samples of each filler concentration (30-60%) by weight. From each filler concentration, 5 samples of each of the 8 time points (4h-21d) were immersed in 100-ml deionized water. Calcium and phosphate ions were measured using atomic absorption spectroscopy and light spectroscopy, respectively. Water sorption was calculated according to ISO4049 specification and diffusion coefficient was calculated. The significance level was p=<0.001. Calcium and phosphate release increased with increasing filler level. Moreover, WS results were high and failed to meet the ISO4049 specification requirement. Diffusion coefficient results were also high. In the 30-60% filler level; calcium release at 21-day ranged from 0.1684-1.2993mmol/L and phosphate ion release ranged from 3.42-20.70mmol/L. These results were reverse to the results by Skrtic, (Skrtic 2007). Water sorption was 86.1-180.2µg/mm3. One-way ANOVA for 21-day data revealed that there is a statistically significant difference in filler level, and two-way ANOVA revealed that there is a statistically significant interaction between time and filler level on the calcium, phosphate released and WS. We concluded that concentrations of calcium, phosphate released and WS were increased by increasing filler level. However, the material cannot serve as a restorative material due to high WS values.

**P19 Effect of Mini Implant Screws on Osteoclast and Osteoblast Differentiation.** T. DODGE[1],* H. THERIAC[1], C. CARTER[2], K. SEMPSROTT[3], H. LARGURA[2], S. LIU[2], A. BRUZZANITI[2] ([1]Purdue School of Engineering and Technology; [2]Indiana University School of Dentistry; [3]Purdue School of Science)

Mini implant screws (MIs), typically made from a titanium alloy, have recently seen increased use as orthodontic anchors in patients with malocclusion. However, the success rate of these screws is only 75% because of breakage during loading and lack of osseointegration. Lack of integration can be attributed to the combined activity of osteoclasts (OCs) and osteoblasts (OBs) in the remodeling process that occurs around MIs after implantation. A potential replacement material, stainless steel (SS), has increased stiffness and can withstand loading forces better, but its osteogenic or anti-resorbtive properties have not been well established. A

promising method of increasing osseointegration is using bone morphogenic protein 2 (BMP-2) to coat the implant. BMP-2 is widely known to increase OB differentiation, leading to bone growth. The objective of this study is to explore the effect of SS and titanium MIs, along with BMP-2, on OB and OC differentiation in vitro. We hypothesize that both metals will inhibit OC differentiation when compared to a negative control. In addition, we predict the metals will have a negligible effect on OB activity and that BMP-2 will greatly increase OB differentiation. In contrast, BMP-2 will have little effect on OC activity. To assess the effects of the metals and BMP-2 on OC activity, OC precursors were isolated from wild-type C57/bl6 mice OC precursors were isolated from the bone marrow of C57/bl6 mice and differentiated with macrophage colony stimulating factor and receptor activator of NF-kappaB ligand for 7 days in the presence of 150 µm thick sections of either SS and titanium MIs. Cell cultures also contained different concentrations of BMP-2 (2 ng/mL, 5 ng/mL, or 10 ng/mL). On day 7, each plate was stained for tartrate-resistant acid phosphatase, a marker of mature osteoclasts and OC number was counted. OB precursors were isolated from the calvaria of 3 day old mouse neonates and differentiated for 7 days with ascorbic acid and B-glycerolphosphate. OB activity was determining using a qualitative alkaline phosphatase activity assay was tested by examining the differentiation of mouse stromal-derived osteoblast cells in (a marker of mature OBs). Our studies reveal OB differentiation was unaffected by the presence of either SS or titanium metals, while OC activity was significantly inhibited by SS but not significantly affected by titanium. Our findings also confirm the osteogenic effect of BMP-2 on OB activity, and demonstrate that BMP-2 has little effect on OC activity. These findings suggest that metals made of SS may offer greater long-term stability than Ti implants due to decreased OC activity. Moreover, SS MIs coated with BMP-2 may be more stable in vivo because of increased OB-mediated osseointegration around the implant. This study was sponsored by the Indiana University-Purdue University Indianapolis Multidisciplinary Undergraduate Research Institute and by funds provided by the Indiana University School of Dentistry to Dr. A. Bruzzaniti

**P20 Role of Osteoclasts in the Biocorrosion of Metal Implants.** H. THERIAC[1],* T. DODGE[1], H. LARGURA[2], A. HARA[2], S. LIU[2], A. BRUZZANITI[2] ([1]Purdue School of Engineering and Technology; [2]Indiana University School of Dentistry)

Mini implants (MIs), typically composed of stainless steel (SS) or titanium alloy (Ti), have recently emerged as superior alternatives to traditional dental and orthopedic implants. When a metal implant is inserted into bone, a process called bone remodeling is triggered near the implant. Bone remodeling involves the activity of osteoblasts (OBs), which produce new bone tissue, and osteoclasts (OCs), which degrade and digest bone. OCs degrade bone by acidifying the extracellular environment and secreting hydrolytic enzymes that degrade the extracellular matrix. However, the acidification of the extracellular environment can potentially lead to the biological corrosion of metal implants after implantation. This may have important consequence such as cell toxicity, decreased osseointegration of the implant, and implant loosening. The objective of this study is to determine if implants made from Ti are more resistant to OC-mediated biocorrosion than stainless steel (SS) implants. We hypothesize that biocorrosive activity by OCs will be greater on SS than titanium. To assess the biocorrosive effects of OCs on SS and Ti, the top face of 150 µm thick sections of each metal were scanned using a Proscan 2000 Scantron to provide accurate three dimensional surface measurements of the metals before introduction of OCs. OC precursors were isolated from the bone marrow of C57/bl6 mice and differentiated with macrophage colony stimulating factor and receptor activator of NF-kappaB ligand for 7 days in the presence of either SS or Ti metals. The metals discs were then removed and rescanned with the Proscan Scantron and changes in the surface measurements before and after OC growth was calculated. OCs were fixed and stained for tartrate-resistant acid phosphatase, a marker of mature OCs, and counted. Our preliminary

findings revealed that the surface roughness of SS was reduced to a greater extent than Ti metals. OC number was also reduced in cultures containing SS compared with Ti. These findings suggest SS may be more susceptible to OC-mediated biocorrosion than Ti-based metal implants. Although the physiological implications are unclear, we speculate that sustained corrosion of SS can negatively affect the long-term stability of implants in vivo.

**P21 A PQAS-Containing Glass-Ionomer Cement for Improved Antibacterial Function.** Y. WENG[1], X. GUO[1], R. L. GREGORY[2], D. XIE[1],* ([1]Department of Biomedical Engineering,Purdue School of Engineering and Technology, Indiana University-Purdue University Indianapolis; [2]Indiana University School of Dentistry)

The novel non-leachable poly(quaternary ammonium salt) (PQAS)-containing antibacterial glass-ionomer cement has been developed. Compressive strength (CS) and *S. mutans* viability were used as tools for strength and antibacterial activity evaluations, respectively. All the specimens were conditioned in distilled water at 37 oC prior to testing. Commercial glass-ionomer cement Fuji II LC was used as control. With PQAS addition, the studied cements showed a reduction in CS with 25-95% for Fuji II LC and 13-78% for the experimental cement and a reduction in *S. mutans* viability with 40-79% for Fuji II LC and 40-91% for the experimental cement. The experimental cement showed less CS reduction and higher antibacterial activity as compared to Fuji II LC. The long-term aging study indicates that the cements are permanently antibacterial with no PQAS leaching. It appears that the experimental cement is a clinically attractive dental restorative that can be potentially used for long-lasting restorations due to its high mechanical strength and permanent antibacterial function.

**P22 Impact of Oral Bacteria on Dental Resin Materials.** H. SHIH,* K. S. GREGSON, R. L. GREGORY (Indiana University School of Dentistry)

Objective: Previously, we found that exposure to bacteria causes degradation of ester bonds in dental resin materials. Hence, this project was to determine the mechanical and surface properties of dental resin materials after incubation with three strains of oral bacteria: *Streptococcus mutans, Streptococcus gordonii* and *Streptococcus sanguis*. Methods: Dental resin material was fabricated into flexural strength specimens and stored for 6 weeks in Tryptic Soy broth supplemented with 1% sucrose (TSBS) cultures of *S. mutans*, *S. gordonii*, *S. sanguis*, sterile saline or TSBS at 37°C in 5% CO2. Flexural strength, scanning electron microscopy (SEM) and Knoop Microhardness Tests were done weekly to determine changes to the material properties. Results: *S. sanguis*, TSBS and saline significantly ($p<0.05$) promoted polymerization in the material matrix by increasing the average peak stress while *S. mutans* and *S. gordonii* did not increase peak stress to the resin. This was supported by the surface topology observed in the SEM. Lastly, microhardness significantly ($p<0.05$) increased over six weeks for specimens stored in saline. Conclusions: *S. mutans* and *S. gordonii* oral bacteria have a negative impact on dental resin materials compared to the inert saline and TSBS and the non-cariogenic *S. sanguis*. This research indicated that microleakage associated with the cariogenic *S. mutans* from dental restorations made from these materials can contribute to restoration failure, secondary caries and the need for endodontic treatment. Supported, in part, by the Indiana University-Purdue University Indianapolis MURI, LHSI and CTEE programs.

**P23 TEGDMA Induction of Apoptotic Proteins in Pulp Fibroblasts.** G. BATARSEH,* L. J. WINDSOR, K. S. GREGSON (Indiana University School of Dentistry)

Monomers like triethylene glycol dimethacrylate (TEGDMA) leach from dental composites and adhesives due to incomplete polymerization or polymer degradation. The release of these monomers causes a variety of reactions that can lead to cell death. This death can be either necrotic that is characterized mainly by inflammation and injury to the surrounding tissues, or apoptotic that elicit little inflammatory response, if any at all. TEGDMA-induced apoptosis in human pulp has been reported recently. However, the molecular mechanisms and the apoptotic (pro and anti) proteins involved in this process remain unclear. The objective of this study was to determine the apoptotic proteins expressed or suppressed during TEGDMA-induced apoptosis. Human pulp fibroblasts (HPFs) were treated and incubated for 24 hours (hrs) with different TEGDMA concentrations (0.125-1.0 mM). Cytotoxicity was determined using the cytotoxicity Detection KitPLUS (Roche Applied Science, Mannheim, Germany). TEGDMA was shown to cause cell cytotoxicity on the cells at concentrations of 0.50 mM and up. The highest concentration with no significant cytotoxicity was 0.250 mM TEGDMA. Cells were incubated with or without (0.250 mM TEGDMA) for 6 and 24 hours. Cell lysates were then prepared and the protein concentrations determined using the Bradford protein assay. A Human Apoptosis Array kit (Bio-Rad Hercules, CA, USA) was utilized to detect the relative levels of 43 apoptotic proteins. The initial results indicated that there was an increase in the expression of the pro-apoptotic proteins such as Bcl-2-associated death promoter (BAD), caspase 3 and tissue necrotizing factor-α (TNF-α). There was also a slight increase in the expression of the anti-apoptotic proteins such as insulin growth like factor- 2 (IGF-2) and (IGF-4). These results show that TEGDMA has an effects on the pro- and anti- apoptotic protein expression.

**P24 Properties of a Low Shrinkage Resin Composite.** J. P. JANSEN,* M. MACPHERSON, J. PLATT (Indiana University School of Dentistry)

Resin composites have many uses in restorative dentistry..A resin composite is composed of four major components: organic polymer matrix, inorganic filler particles, coupling agent, and the initiator-accelerator system. In an effort to reduce polymerization stress, a novel high molecular weight DuPont molecule, DX-511 has been used as a matrix oligomer (Kalore®, GC America, GC). The purpose of this study was to evaluate the flexural strength, depth of cure, and surface hardness of a nano-filled resin composite based on DX-511compared to four commercially available materials. Materials evaluated in this study were EsthetX HD (Dentsply, EX), Premise (Kerr, PR), Filtek Supreme (3M, FS), TPH3 (Dentsply, TP), & GC. Equipment used included the FTIR, Knoop Hardness Tester, and universal testing machine using protocols consistent with routine materials testing at Indiana University School of Dentistry. A previous study evaluated the volumetric polymerization shrinkage and polymerization contraction stress. When compared to EX, PR, FS, & TP, GC exhibited a lower level of contraction stress, less shrinkage, and higher hardness values. Depth of cure for GC was very similar to the values exhibited by EX, PR, & FS. However, the flexural strength of the material was less than EX, FS, & TP. Using DX-511 in the matrix provided for lower contraction stress, lower volumetric shrinkage, & increased bottom/top Knoop hardness ratio, but a lower flexural strength when compared to four commercially available materials. Partially supported by the Indiana University School of Dentistry Research fellowship and GC America.

**P25 Dental Shear Bond Strengths of a Self-Adhering Flowable Composite.**

C. MCCREA,* J. PLATT (Indiana University School of Dentistry)

The purpose of this study was to compare the enamel and dentin shear bond strengths (SBS) of a new self-adhering flowable composite (Vertise Flow, VF, Kerr) against an etch & rinse flowable composite from the same manufacturer (Premise Flowable, PF). The mid-coronal dentin or the facial enamel of recently extracted human molars were flattened and finished through 600 grit SiC. Four groups (VF enamel, VF dentin, PF enamel, and PF dentin; n=20) were prepared. For PF, 37.5% phosphoric acid etch was applied to the enamel or dentin surface for 15 seconds, rinsed with deionized water, and then dried with canned air. Optibond Solo Plus was actively applied for 15 seconds followed by a 20 second cure. For VF, a thin layer of material was brushed with moderate pressure on the enamel or dentin surface for 15 seconds, and was then cured for 20 seconds. Composite cylinders (4 mm diameter x 2 mm high) of each material were created with the aid of a positioning jig and polyethylene tubing. All materials and each cylinder were polymerized with a LED light (L.E.Demetron I, Kerr) verified to have an output greater than 700 mW/cm2. A 40 second exposure was accomplished from the top of the cylinder. SBS of both materials was determined 24h after fabrication using a Universal Testing Machine (MTS, Chicago, IL) retrofit with MTS Sintech ReNew and equipped with the software package TestWorks 4. A circular knife-edge was positioned at either the enamel-cylinder interface or dentin-cylinder interface, and a load applied until fracture at a cross-head speed of 1 mm/min and the maximum load was divided by the cross-sectional cylinder area to determine SBS in MPa. Results were evaluated with Two-Way ANOVA and when needed All Pairwise Multiple Comparison Procedures (Holm-Sidak method) with α=0.05. Mean SBS values for VF and PF on enamel were 2.48 ± 1.38 and 13.82 ± 3.09 MPa, respectively, while on dentin the mean SBS values for these materials were 3.56 ± 1.22 and 11.81 ± 2.97 MPa, respectively. PF exhibited a much higher mean SBS than VF when bonded to either enamel or dentin. Failure modes in all groups were predominantly at the adhesive-tooth structure interfaces. The interaction between both materials and tooth structure was found to be statistically significant (P=0.006). No statistical significance was found when comparing the SBS of VF to enamel and VF to dentin. However, a statistically significant difference was found between the SBS of PF to enamel and PF to Dentin. The comparisons of PF to enamel and VF to enamel, as well as PF to dentin and VF to dentin in reference to SBS were also found to be statistically significant.

Shear Bond Strength (MPa)
Capital superscript letters = statistically similar groups in rows; lower case in columns.

| **Material** | **Enamel** | **Dentin** |
|---|---|---|
| VF | $2.48 \pm 1.38^{Aa}$ | $3.56 \pm 1.22^{Aa}$ |
| PF | $13.82 \pm 3.09^{Ab}$ | $11.81 \pm 2.97^{Bb}$ |

The results indicate that the SBS of Vertise Flow to both enamel and dentin was significantly inferior to that of Premise to both enamel and dentin.

**P26 Microtensile Bond Strength of Repaired Aged Silorane Resin Composite.**

J. PALASUK,* J. PLATT (Indiana University School of Dentistry)

A silorane based resin composite, Filtek LS, has been introduced to overcome the polymerization shrinkage of the methacrylate based resin composite. The repair of silorane resin composite may hold clinical advantages. The objecive of this laboratory study was to

compare the repaired microtensile bond strengths of aged silorane resin composite using different surface treatments and either silorane or methacrylate resin composite. One hundred and eight silorane resin composite blocks (Filtek LS) were fabricated and aged by thermocycling between 8oC and 48oC (5000 cycles). A control (solid resin composite) and four surface treatment groups (no treatment, acid treatment, aluminum oxide sandblasting and diamond bur abrasion) were tested (n=12 blocks, 108 beams/group). Each treatment group was randomly divided in half and repaired with either silorane resin composite (Filtek LS/LS adhesive) or methacrylate resin composite (Filtek Z250/Single Bond Plus). After 24 hours in 37oC distilled water, microtensile bond strength testing was performed using a non-trimming technique. Data were analyzed by Weibull-distribution survival analysis. Failure mode was examined using optical microscopy (20X). Weibull-distribution survival analysis revealed that aluminum oxide sandblasting followed by silorane or methacrylate resin composite and acid treatment with methacrylate resin composite provided insignificant differences from the control ($p>0.05$). All other groups were significantly lower than the control. Failure was primarily adhesive in all groups. Aluminum oxide sandblasting produced microtensile bond strength comparable to the cohesive strength of silorane resin composite. After aluminum oxide sandblasting, aged silorane resin composite can be repaired using either silorane resin composite and the LS system adhesive or methacrylate resin composite and a methacrylate dental adhesive. Partial funding and materials support of this study was provided by Delta Dental Foundation and 3M ESPE.

## DIAGNOSTIC SYSTEMS

**P27 Dental Student Questionnaire Results for VELscope**. C. KRUSHINSKI,* C. MCCREA, T. BARRICK, S. ZUNT (Indiana University School of Dentistry)

Objective: The purpose of this study was to assess third and fourth year dental students' responses concerning the usefulness of the VELscope™, which is an FDA approved device for the detection of oral cancer , in the screening clinic at IUSD. Methods: Recruitment of dental students was voluntarily by electronic mail. Information concerning the usage of the VELscope™ was sent via electronic mail to third and fourth year dental students at IUSD. Following a one time use of the Velscope™, a twelve question questionnaire was made available in the screening clinic. Responses were summarized using and averages and percentages. Results: Twenty-seven dental students completed the VELscope™ questionnaire; twenty-three fourth year dental students and four third year dental students responded. On average, the students reported the VELscope™ examination took about six minutes. About one-fourth of the students did detect an oral lesion that they did not see with the incandescent (operatory) light. Responses showed over 85% answered "Agree" or "Strongly Agree" to questions asking if the Velscope™ was user friendly, a useful diagnostic tool, beneficial in the clinical practice of dentistry, enhanced their learning experience, and patients were receptive to the device. However, many students were "Undecided" or "Disagreed" whether the VELscope™ should be the standard of care in the dental practice or about purchasing a VELscope™ for their future private practice. Conclusions: Dental students seemed hesitant to try the VELscope™, as only twenty-seven completed the questionnaire. On average, students reported the VELscope™ examination took about six minutes. About one-fourth reported detecting an oral lesion that they did not see with the incandescent (operatory) light. Most third and fourth year students responding seemed to like using the VELscope™ and found it to be user friendly; however, many students were undecided or disagreed whether the VELscope™ should be the standard of care in the dental practice or about purchasing a VELscope™ for their future private practice. Study Number: EX 0912-22.

## EDUCATIONAL RESEARCH

**P28 Development of an Online Tobacco Cessation Module: Lessons Learned.**

L. COAN[1],* T. JANKE[2], L. ROMITO[1] ([1]Indiana University School of Dentistry; [2]Indiana University-Purdue University Indianapolis)

Based on their competency documents, the American Dental Education Association (ADEA) stresses the need for dental/dental hygiene graduates to have the knowledge and skills to address tobacco use and dependence. The project objectives were to develop and pilot an online, comprehensive tobacco education program for dental & dental hygiene students, and assess learning outcomes and student satisfaction. Faculty reflections on the experience were also documented. The project consisted of 9 Learning Units that were organized and delivered within Oncourse. Content areas included: I. About tobacco; II. Addiction; III. Tobacco and General Health; IV. Tobacco: An Oral Health Issue; V. Treatment of Tobacco Dependence; VI. Health Behavior Change; VII. Patient Evaluation; VIII. Developing a Quit Plan; IX. Tobacco Cessation Protocols for the Dental Team. The Learning Units consisted of sequenced pre-test assessments, narrated presentations, web-based learning activities, videos and post-test assessments. The project was piloted with 100 first year dental and 13 second year dental hygiene student volunteers. After completing all units, student satisfaction was evaluated by survey. Gains in student content knowledge ranged from 0.76% to 29.92%. Students reported satisfaction with the instructional program, and in particular, the formative active learning portions. Students reported frustration with technical difficulties in managing the online environment and with unit pieces not working as planned. Faculty lessons learned included: underestimating the time to develop, implement, evaluate and maintain the online module, and underestimating the difficulties in correcting technical problems. This study suggests that for tobacco dependence education, an online program is a viable educational methodology, provided time and technical support for faculty are available. The project was funded by an IUPUI Center for Teaching and Learning Curriculum Enhancement Grant.

**P29 Changing Use and Perception of Lecture Capture and Podcasting Technologies**

B. GUSHROWSKI,* L. ROMITO (Indiana University School of Dentistry)

Podcasting and lecture capture technologies were introduced at IUSD in the spring semester of 2009. Subsequently, a survey was taken of faculty and student perceptions of the value of podcasting, benefits and weaknesses of using these tools in instruction, and future use of the technologies. Forty-six faculty members completed the survey and results were presented at 2010 IUSD Research Day. In that survey, seven faculty indicated they were using the lecture capture and/or podcast software. A second survey was conducted of the faculty in Fall of 2010. Sixty-eight faculty members completed the survey with thirty-three faculty indicating use of the software; twenty use lecture capture only, three use podcasts only, twenty-eight use both methods of content delivery. In the initial survey none of those who implemented podcasts believed student grades improved compared to previous years, and only two out of the seven faculty who developed a podcast believed student learning was improved through the use of podcasts. In the 2010 survey, sixteen of the thirty-three faculty who answered this question believed student learning had improved as a result of podcasts. Twenty-one faculty members reported in the 2010 survey that they were considering making a podcast or using lecture capture in the future compared to twenty-three in the original survey. This study demonstrates a substantial increase in faculty use of the technologies over an 18 month period and a change in their perceptions of the value to students in using these pedagogical tools.

**P30 Effectiveness of Multiple Pedagogies in Tobacco Dependence Education with D1 Students.** D. ZAHL,* L. ROMITO, S. SCHRADER (Indiana University School of Dentistry)

Although dental professionals believe tobacco dependence education (TDE) should be provided, studies suggest they do not routinely offer therapies due, in part, to a lack of TDE training in the pre-doctoral curriculum. This study evaluated the effectiveness of multiple pedagogies in TDE with first year dental students (D1). The multiple pedagogies evaluated in this study were standardized patient (SP) objective structured clinical examinations (OSCEs), problem-based learning (PBL), and experiential learning. D1 students were randomly assigned to two different OSCE groups and were scheduled to participate in three TDE educational interventions. Group 1 was scheduled to participate in its SP OSCE prior to both groups' concurrent participation in the PBL case and experiential learning activity. Group 2 was scheduled for its SP OSCE after the interventions. The present study is concerned with the following research questions: 1) Does participating in a TDE SP OSCE increase student TDE knowledge? 2) Does participating in a TDE SP OSCE change student attitudes about preparedness and willingness to use TDE with future clinical patients? 3) Does participating in a TDE PBL case and related experiential learning activity increase student TDE knowledge? 4) Does participating in a TDE PBL case and related experiential learning activity change student attitudes about preparedness and willingness to use TDE with future clinical patients? 5) Does participating in a TDE PBL case and related experiential learning activity improve student SP OSCE performance? Prior to and after any educational intervention, the knowledge and attitudes of 101 D1 students were assessed using a 34-item instrument. Student OSCE performance was assessed by a SP instructor using a 15-item checklist. Between the two OSCE days, students were scheduled to participate in a TDE PBL case. A component of the PBL case included an experiential learning activity at a local substance abuse treatment hospital. The mean scores for the TDE knowledge and attitudes pre- and post- SP OSCE assessments were compared within groups using a paired t-test with a 95% confidence interval (CI) and between groups using an independent t-test with a 95% CI. There was a statistically significant increase in student knowledge from the pre-SP OSCE assessment to the post-SP OSCE assessment for group 1 [$t(51) = 7.510$, $p < .000$] and group 2 [$t(50) = 8.333$, $p < .000$]. There was a statistically significant increase in students' mean SP OSCE scores between group 1 and group 2 [$t(101) = 2.287$, $p = .024$]. Lastly, there was a statistically significant change in the mean student responses to 5 of 6 attitudinal questions from the pre- to post-SP OSCE assessment. The results suggest that SP OSCEs are a likely effective method for TDE instruction and the experience of engaging with a SP instructor may be impactful in shaping the attitudes of early dental students in wanting to further educate themselves about TDE and to subsequently provide oral health tobacco education to their future patients. Furthermore, student engagement in PBL cases and experiential learning opportunities may have some related impact on early dental students' communicative, interpersonal, and TDE counseling skills.

## ENDODONTICS

**P31 Efficacy of CBCT Images for the Evaluation of Periapical Lesions.** P. SHAH,* A. BALASUNDARAM, M. WHEATER (University of Detroit Mercy School of Dentistry)

Objective: To help endodontists select the correct treatment plan (surgical/non-surgical) based on periapical lesion size using the most appropriate imaging modality that will prevent significant morbidity (more so if the treatment is surgical) and reduce treatment costs significantly. Methods: Patients and x-ray procedure: This is a clinical research study involving observers

(endodontists) who compared intra oral Periapical (PA) x-rays and Cone Beam Computed Tomography (CBCT) images to make treatment decisions (surgical or non-surgical) on periapical lesions measuring more than three millimeters in size. Twenty patients with a carious tooth and symptoms of a periapical lesion presented to the Endodontists who participated in the study. Digital sensors were used to obtain PA x-rays and a conventional CBCT scanner for CBCT images. Image Viewing: Six observers were selected for viewing periapical and CBCT images and each image modality was viewed for the following criteria: a) Identify periapical lesion on image corresponding to particular study tooth (present/absent), b) Measure extent of periapical lesion at greatest dimension – length and diameter of lesion (with "ruler" in software tool), c) Assess degree of radiolucency (used already established PAI (Peri Apical Index) scores for conventional radiography and CBCT), d) Expansion of periapical cortical bone (Absent/Present), f) Destruction of periapical cortical bone (Absent/Present), and g) Treatment decision of particular study tooth. Results: Characteristics of each of the PA and CBCT images as scored by observers were compared using ANOVA statistics. The CBCT images were found to alter treatment decisions by more than 50% after the observers initially reviewed the PA images. Inter-observer and Intra-observer agreements were also calculated. Conclusion: CBCT images provide more information to help with treatment decisions. However, future studies with a larger sample size is recommended to advocate the use of CBCT as a routine imaging technique for making treatment decisions on endodontically treatable periapical lesions.

**P32 Molecular Detection of Cytomegalovirus and Epstein-Barr Virus in Irreversible Pulpitis and Apical Periodontitis.** E. REVELS,* C. DAEP, D. R. DEMUTH, S. CLARK (University of Louisville School of Dentistry)

Epstein Barr Virus (HHV-4) and Human Cytomegalovirus (HHV-5) are members of the herpesviridae family which potentially contribute to periapical pathosis in lesions of endodontic origin. A study by Li et al has detected these viruses in human periapical lesions through PCR analysis, however, these results have not been widely replicated. The goal of this study is to correlate herpes infection with the development of periapical inflammation. Following IRB and Human Studies approval, samples were anonymously collected from patients exhibiting signs of periapical inflammation. Samples that were included in the patient database included extracted teeth with an intact periapical lesion upon extraction and pulp samples with a diagnosis of irreversible pulpitis. DNA from the samples was extracted using QiaAMP DNA Mini Kit and the total DNA was measured using Nanodrop. PCR was used to detect for the HHV-5 pp65 lower matrix phosphoprotein and HHV-4 BLR F2 structural protein using specific primers previously reported by Li et al. The resulting PCR products were analyzed in 2% agarose gel and visualized under UV light. In this current study, we were unable to detect either HHV-4 or HHV-5 via PCR. The lack of products was not due to degraded genomic DNA as PCR products for human β-actin was observed in all the samples. Studies are underway to optimize the detection procedure for the viruses as well as increasing the total number of samples.

## HEALTHCARE SYSTEM

**P33 Dental Hygienists' Awareness and Support for Auxiliaries and Midlevel Providers.** J. SANDERS,* S. NARENDRAN (Community Dentistry, Case Western Reserve University)

Objective: The study investigated the scope and satisfaction of services provided by dental hygienists, their opinions and awareness of the existing auxiliary and proposed midlevel dental providers: Expanded Function Dental Auxiliary (EFDA), Dental Therapist (DT), and Advanced Dental Hygiene Practitioner (ADHP). Methods: The study sample consisted of all 676 dental

hygienists from Cuyahoga County, Ohio. Data were collected by a self-administered mail questionnaire which assessed the scope of services provided by dental hygienists, their levels of satisfaction in providing the services, and whom they think should be providing such services. The questionnaire investigated respondents' awareness and support for the aforementioned positions, and the responses to these items were assessed on a scale of 1 to 10 with 10 being the highest rating. Statistical analyses included both descriptive and analytical tests. Results: After two mailings, four weeks apart, sixty one surveys were returned as undeliverable and we received 158 completed surveys for an effective response rate of 26%. Prophylaxis and dental health education were the two most commonly performed services by the study sample: 121 and 111 times/month. While subjects were most satisfied in performing the above two services (9.2+/-1.6, 8.6+/-1.1) they were least satisfied with intra-oral bite registrations for diagnostic models and also with repair (5.2+/-4.4), construction and finishing of prosthetic devices (3.9+/-4.4). Respondents' ratings for knowledge levels were 2.8+/-2.6 for DT, ADHP 4.0+/-3.4, and EFDA 8.1+/-2.6; their level of support for EFDA was 7.7+/-2.9, ADHP 7.0+/-3.4, and DT 4.0+/-3.4. A significantly ($p<0.05$) higher proportion were supportive of EFDA (78%) compared to ADHP (64.7%) or DT (25.3%). Conclusions: Respondents were mostly in support of Expanded Function Dental Auxiliary, followed by Advanced Dental Hygiene Practitioner, and Dental Therapist; there is a need to improve respondents' awareness of the newly proposed midlevel providers.

**P34 Physicians' Attitudes Regarding Oral Health in Diabetes Care.** A. ARRUDA,* C. DEMKO (Community Dentistry, Case Western Reserve University)

Objective: To examine attitudes and behaviors regarding oral health and diabetes among Ohio physicians. Methods: A 46-item survey was mailed to a random sample of 700 family physicians from the medical board list in Ohio; 5-item scale measuring the perceived importance of oral health in diabetes care was constructed. Scale values were compared by respondent demographics, presence of an office diabetes registry, and charting behaviors. Non-parametric tests were used for statistical comparisons. Results: Seventy-six physicians in 29 Ohio counties from around the state responded; they averaged 23 years (4-60) in practice, were in private practice (68%), public clinics (16%) or hospitals (16%), and 38% were female. Respondents estimated between 4% and 90% of their patients to be diabetic and 63% maintained a registry of diabetic patients. The most prevalent behaviors were recommending an annual dental visit (81%), informing patients that periodontal disease can affect diabetic status (74%), and charting oral health information(67%). Fewer physicians reported asking patients if they have periodontal disease (14%), advice to brush/floss (38%), or discussing the effect of diabetes on oral health (39%). The Importance scale (median=18, range 6-25) differed by charting behavior ($p=.002$), satisfaction with oral health knowledge ($p=.034$) and attitudes about oral health in diabetes management ($p<.001$), but not by physician age, gender, diabetes registry or years in practice. 45% of physicians in public clinics believe their patients have access to oral health care compared to 86% in private practice. Few physicians believe patients understand the oral-diabetes link (16%). Conclusions: Attitudes about the importance of oral health in diabetes management are favorable and are associated with reported behaviors. Physicians discuss the impact of oral health on diabetes control, but are less likely to inform patients that their diabetes can affect their oral health. Dental professionals must share that message with patients and their medical colleagues.

## INFECTION CONTROL

**P35 Detection of *Helicobacter pylori* in Pretreated Dental Unit Waterlines.** A. NOLES,* A. SAJADI, D. M. GALLI (Indiana University School of Dentistry)

Introduction: Even with proper use of disinfectants, dental unit waterlines (DUW) favor the formation of microbial biofilms, which can harbor pathogens. The contaminated dental unit water can pose an infection risk to both patients and dental practitioners. *Helicobacter pylori* is a gastric pathogen. Its transmission by municipal water has been suggested as a source of infection. We recently detected *H. pylori* in Citrisil-treated water lines of 5 clinics of the Indiana University School of Dentistry but it is unknown how prevalent the problem is. Objectives: i) Determine the presence of *H. pylori* in DUW from additional sources, and ii) assess the impact of disinfectants on *H. pylori* detection. Methods: Segments of 2 cm length were collected from the water lines of 9 dental chairs from a clinic at the Regenstrief Institute and a private dental practice in Carmel, IN. DUWs in these locations had been treated with Sterilix and A-Dec ICX respectively. Biofilm bacteria were removed by scraping the inner walls of the tubes, washed, and lysed by boiling to extract DNA. Presence of bacteria in general and *H. pylori* in particular was assessed qualitatively by standard PCR using sets of oligonucleotides specific for bacterial 16SrDNA and the *H. pylori* urease A gene, respectively. DNA from *H. pylori* strain ATCC43504 served as a positive control. Results: Bacteria were present in all 9 water lines. 60% of the lines from the Regenstrief Institute and 50% of the lines from the private practice also tested positive for *H. pylori*. Conclusions: The treatment of DUW with disinfectants does not prevent colonization by *H. pylori*.

**P36 Prevalence of *Helicobacter pylori* in Dental Unit Waterlines.** A. SAJADI,* A. NOLES, D. M. GALLI (Indiana University School of Dentistry)

Introduction: Dental unit waterlines favor the formation of microbial biofilms, which can harbor opportunistic and obligatory pathogens. The contaminated dental unit water can pose an infection risk to both patients and dental practitioners. *Helicobacter pylori* is a gastric pathogen that in recent years has been found in drinking water in various geographical regions. Transmission by municipal water has been suggested as a source of infection for *H. pylori*. Objective: To determine the presence of *H. pylori* in biofilms obtained from dental unit water lines that use municipal water. Methods: Segments of 2 cm length were collected from the water lines of 30 dental chairs. Bacteria were collected by scraping the inner walls of the tubes, followed by washing of the cells in phosphate-buffered saline and resuspension in water. DNA was extracted via boiling of the samples. Presence of bacteria in general and H*. pylori* in particular was assessed by standard PCR using sets of oligonucleotides specific for bacterial 16SrRNA and the urease A gene, respectively. DNA from *H. pylori* strain ATCC43504 served as a positive control. Results: Bacteria were present in all 30 water lines with 23 of them also testing positive for *H. pylori*. Conclusions: *H. pylori* does colonize in biofilms located within dental unit water lines and thus may be transmitted to practitioners and patients. Supported, in part, by the Indiana University School of Dentistry Dental Student Research Fund.

## MICROBIOLOGY/IMMUNOLOGY

**P37 The Role of Iron in the Growth of the Dental Pathogen Aggregatibacter Actinomycetemcomitans.** P. JEFFERSON,* E. A. NOVAK, D. R. DEMUTH (Department of Periodontics, Endodontics, and Dental Hygiene, University of Louisville School of Dentistry)

Objective: The iron acquisition systems encoded in the genome of A. actinomycetemcomitans are tailored towards the specific survival strategies needed to survive in the oral biofilm. The objective of this project was to determine the preferred iron source of A. actinomycetemcomitans for growth. Methods: A. actinomycetemcomtians was grown in chemically defined media (CDM) with or without an iron chelator (dipyridyl (DIP)), and supplemented with various iron forms to determine growth. Results: The growth of A. actinomycetemcomitans was similar when grown in CDM with or without exogenous ferrous sulfate. However, the growth of A. actinomycetemcomitans was decreased in a dose-dependent manner when cultured in CDM without exogenous ferrous sulfate supplemented with DIP (CD/DIP). Furthermore, the growth of A. actinomycetemcomitans was restored when CDM/DIP was supplemented with hemin, ferric citrate, and ferric chloride, but not ferrous sulfate. Conclusion: A. actinomycetemcomitans is able to grow in CDM with or without an exogenous iron source, suggesting that the chemicals used to make CDM have trace iron contaminates. A. actinomycetemcomitans is able to utilize ferric chloride, ferric citrate, and hemin as iron sources, but not ferrous sulfate.

**P38 Cigarette Smoke Extract Promotes Porphyromonas gingivalis Biofilm Formation.** C. PATEL,* J. BAGAITKAR, C. AMORIN-DAEP, D. R. DEMUTH, D. E. RENAUD, D. A. SCOTT (University of Louisville School of Dentistry)

Introduction: Smokers are more susceptible to persistent infection by the periodontal pathogen Porphyromonas gingivalis. P. gingivalis persistence is dependent on interactions with other plaque bacteria, including the primary colonizer Streptococcus gordonii. Our previous studies have established that exposure to cigarette smoke extract (CSE) alters the expression of the major fimbrial antigen, FimA, which plays important roles in P. gingivalis adherence. Hypothesis: We hypothesized that CSE exposure promotes FimA-dependent P. gingivalis-S. gordonii biofilm formation. Methods: FimA and the minor fimbrial antigen, Mfa1, expression and surface accessibility to antibody on CSE exposure were determined by Western blot and ELISA. P. gingivalis-S. gordonii open-flow biofilms were visualized by confocal laser scanning microscopy. Microcolony height and numbers were quantified by z-stack analysis. The ability of the S. gordonii ligands for FimA and Mfa1 (GADPH and SspB, respectively) to inhibit CSE-induced biofilms was also assessed. Results: CSE exposure increased P. gingivalis-S. gordonii biofilms formation as indicated by an increase in microcolony height ($p < 0.05$). While total FimA protein and FimA surface availability were upregulated on smoke exposure (both $p < 0.01$), Mfa1 was not. However, a peptide representing the Mfa1 binding site on SspB completely eliminated CSE-induced biofilm formation, whereas GADPH abrogated microcolony formation by only 40%. Conclusion: CSE-induced P. gingivalis-S. gordonii biofilm formation concomitant with an upregulation of surface exposed FimA protein. FimA upregulation augmented CSE-induced, Mfa1-dependent dual species biofilm formation. Thus, CSE exposure promotes P. gingivalis recruitment into biofilms in an adhesin-receptor dependent fashion and may help explain increased P. gingivalis persistence in smokers.

**P39 Effect of Nicotine on *Streptococcus mutans* Sortase Activity.** M. LI,* R. HUANG, R. L. GREGORY (Indiana University School of Dentistry)

Introduction: *Streptococcus mutans* is the major etiological agent of human dental caries. Tobacco use has a documented effect on *S. mutans* growth and colonization. Sortase is used by many bacteria, including *S. mutans*, to facilitate the insertion of certain cell surface proteins if the protein contains an LPXTGX motif. Among these sortase-controlled proteins is the spaP gene product, antigen I/II. This study examined the indirect effect of nicotine on *S. mutans* sortase activity using wild-type NG8, and sortase-defective and sortase-complemented strains. Wild-type UA159 *S. mutans* was used as a control in all experiments to ascertain the effect of nicotine on sortase function. Briefly, the strains were incubated with various nicotine concentrations in growth, metabolism and biofilm formation assays using microplate technology. Materials and Methods: For planktonic growth curve assays, bacteria in Todd Hewitt broth (THB) with different nicotine concentrations were placed into 96-well microtiter plates. There were 3 replicates of each bacteria for each nicotine concentration. The growth curve of each bacteria was recorded by a microtiter-plate reader at 595 nm every 20 min for 21 h. To measure metabolic activity an XTT assay was used by growing each bacterium in THB containing sucrose (THBS), cells were placed into selected wells, and incubated 24 h with 5% CO2 at 37oC. The wells were washed three times with saline to remove non-adherent cells. THBS supplemented with nicotine (0-32 mg/ml) was added to wells containing biofilm and incubated at 37oC for an additional 24 h. After incubation, the treated biofilms were washed three times. The metabolic activity of the biofilms was determined by the addition of the XTT reagent and the absorbance at 490 nm was measured. Biofilm formation was established by crystal violet staining. Bacteria were grown in THBS in microtiter plate wells, and incubated 24 h. The wells were washed three times, fixed with 10% formaldehyde, washed again, and stained with 0.5% (w/v) crystal violet. The wells were washed again, isopropanol added to release the dye and the absorbance at 490 nm was recorded. Results: The bacterial growth curves indicate that, lower concentrations of nicotine up-regulated growth of the bacteria. Generally, nicotine accelerated the growth during lag and stationary phases, while the srtA- strain grew more slowly than wild-type NG8 during a prolonged lag phase. In addition, all biofilm bacteria had increased metabolic activity as the concentration of nicotine increased. One-way ANOVA indicates that UA159 and srtA- both had significantly increased ($p < 0.05$) metabolism at 2-16 mg/ml. NG8 has significantly increased metabolism at 4-16 mg/ml. The SrtA-complemented strain had significantly increased metabolism at 1-16 mg/ml, while the srtA- strain had higher metabolic activity than that of NG8 and the srtA-complemented strains. All strains had increased biofilm formation as a result of the increasing concentration of nicotine. UA159 produced the most biofilm formation, and had significantly increased biofilm at 2-8 mg/ml of nicotine. NG8 and srtA-complemented strains had more biofilm than srtA-, while NG8 had significantly increased biofilm at 0.5-8 mg/ml, however, the srtA-complemented strain did not produce as much biofilm overall as NG8. Conclusions: Lower concentrations of nicotine can increase the growth of *S. mutans*, biofilm formation, possibly a reflection of increased spaP expression, and the ability of metabolically active planktonic cells. Higher concentrations inhibit the growth of bacteria. We can hypothesize that nicotine and sortase regulate some of the metabolic pathways of *S. mutans* based on the XTT data.

**P40 Effect of Nicotine on Metabolism of Starved and Unstarved *Streptococcus mutans*.** J. RODENBECK,* R. L. GREGORY (Indiana University School of Dentistry)

Introduction: *Streptococcus mutans* is the major etiological agent of dental caries. Nicotine is the addictive ingredient present in most tobacco products that has been shown to have an effect on the growth and metabolism of oral bacteria, specifically *S. mutans*. This same bacterium has

been recently linked to heart disease. Smokers regularly introduce this chemical into their system which causes an increased growth and metabolic rate of the bacteria, thus increasing their chances for dental caries. This research worked to further qualify the increase of metabolic rates by subjecting the bacteria to nicotine in a starved environment, on the basis that humans do not constantly have nutrients available to oral bacteria. Materials and Methods: Metabolic rates of an established biofilm of *S. mutans* were measured through an XTT and menadione assay with a spectrophotometer. The unstarved bacteria were grown in a full concentration of TSBS while the starved were grown in a 1:10 dilution of TSBS with sterile saline in various nicotine concentrations (0.0, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, and 128.0 mg/ml).Results: The metabolic activity of *S. mutans* was increased in the presence of nicotine, and the metabolic activity of the starved bacteria increased as concentration of nicotine increased up to 16.0 mg/mL (0.033 OD) in which beyond that the metabolic rates were negatively affected. The unstarved bacteria demonstrated an increase in metabolism up to 32.0 mg/mL (0.422 OD) of nicotine and concentrations beyond that were also negatively affected. When the starved bacteria were subjected to a 1.0 mg/mL concentration of nicotine an average reading of 0.027 OD was observed compared to an average of 0.021 OD when no nicotine was added. The unstarved bacteria provided mean readings of 0.109 OD when no nicotine was added and a mean reading of 0.153 OD when 1.0 mg/mL of nicotine was added. The maximum metabolic activity for the starved bacteria was observed at 4.0 mg/mL (0.124 OD) and the mean was 0.086 OD at this nicotine concentration. The average was very similar at the 8.0 mg/mL concentration with a reading of 0.080 OD. The highest reading for the unstarved was 0.480 OD which was at the 16.0 mg/mL concentration which had a mean of 0.438 OD. The mean value for the unstarved 32.0 mg/mL concentration was very similar, 0.422 OD. The MIC for the unstarved and starved cells were 32 and 16 mg/ml, respectively. The metabolic rates on average increased with increasing concentration of nicotine. Conclusions: As the concentration of nicotine increased, the metabolic rates of *S. mutans* also increased. The high concentrations in which the bacteria were no longer metabolically active are very high and a normal smoker would not be able to reach these concentrations. However, as more nicotine is present in smokers these more metabolically active bacteria would be more likely to cause caries.

## MICROBIOLOGY/IMMUNOLOGY

**P41 The Effect of CSC, Nicotine and Dissolvable Tobacco on *Streptococcus mutans* Metabolism.** C. MCGOUGH,* R. L. GREGORY (Indiana University School of Dentistry)

Introduction: *Streptococcus mutans*, a common oral bacterium proven to be a causative agent of dental caries, has been shown to be more prevalent in the mouths of smokers versus non-smokers. In the oral cavity, *S. mutans* attaches to the enamel of teeth (via Antigen I/II), aggregates and forms a biofilm. Generally, biofilms have shown increased resistance to antimicrobial agents than free-floating, planktonic bacteria. By using an XTT metabolic activity assay, this study aimed to observe the metabolism of established *S. mutans* biofilms and planktonic bacteria when treated with varying concentrations of cigarette smoke condensate (CSC), nicotine and dissolvable tobacco products. Materials and Methods: An established *S. mutans* biofilm was subjected to media containing differing concentrations of nicotine, CSC and other dissolvable tobacco products to examine the metabolic activity of the biofilm with an XTT metabolic activity assay. Tryptic soy broth with 1% sucrose (TSBS) was inoculated with *Streptococcus mutans* UA159 and incubated overnight in 5% CO2 at 37oC. This 24-hour culture (10 ul) was added to the wells of a 96-well microtiter plate where biofilm formation occurred over a 24 hour span in 5% CO2 at 37ºC in the presence of increasing concentrations of nicotine, CSC, and dissolvable tobacco products: Orbs, Sticks and Strips, in TSBS. These biofilms were incubated at room temperature in the dark for 2 hours with XTT/menadione reagent. A

spectrophotometer read the metabolic activity of the biofilm and planktonic bacteria at 490 nm by the oxidation of XTT. Results: *S. mutans* biofilm demonstrated lower oxidation of XTT/menadione reagent with CSC, Orbs, Sticks and Strips when the concentration of each increased. The biofilm demonstrated a significant increase ($p < 0.05$) in oxidation of XTT/menadione with increasing concentrations of nicotine up to 8 mg/mL, after which the activity steeply declined. Planktonic *S. mutans* demonstrated significant increasing oxidation of XTT/menadione reagent as the concentration of CSC and tobacco products increased. Conclusion: Using 4 mg/mL of nicotine as a positive control and 0 mg/mL nicotine as a negative control; the metabolic activity of *S. mutans* biofilm demonstrates a negative correlation and the planktonic *S. mutans* has a positive correlation with the concentrations of CSC and dissolvable tobacco products. It can be concluded that other inhibitory chemicals found in CSC and tobacco products have an impact in decreasing the metabolic activity of the biofilm whereas planktonic bacteria do not experience the same inhibition. Acknowledgements: This work was funded by the Center for Research and Learning Life-Health Sciences Internship at Indiana University-Purdue University Indianapolis.

**P42 Effect of Nicotine on a Mixed Culture of *Streptococcus sanguis* and Streptomycin-Resistant *Streptococcus mutans*.** I. LEVITT,* R. L. GREGORY (Indiana University School of Dentistry)

Introduction: *Streptococcus sanguis* is a gram-positive, normal inhabitant of the oral cavity. Another gram-positive bacterium, *Streptococcus mutans* is a known causal agent of plaque and dental caries. *S. mutans* modifies the oral environment to make it less hospitable for other streptococcal strains by producing bacteriocins. It is well established that smokers have an increased rate of caries as a result of a higher proportion of *S. mutans* than *S. sanguis*. One of the important virulence properties of *S. mutans* is the ability to form biofilm on tooth surfaces. The aim of this study was to show that exposure of high concentrations of nicotine would provide *S. mutans* with a competitive advantage that would lead to an inverse relationship in which streptomycin-resistant (SR) *S. mutans* would overshadow the presence of *S. sanguis* possibly by binding to the majority of available tooth surfaces by increased bacteriocins. Materials and Methods: *S. mutans* UA159 was made resistant to streptomycin (*S. mutans*$^R$) by step-wise growth on Tryptic Soy Agar (TSA) with 1 mg/ml streptomycin. Tryptic soy broth with 1% sucrose (TSBS) media ranging from 0 to 1 mg/ml of nicotine was added to sterile six-well plates containing three hydroxyapatite disks (representing tooth enamel) per nicotine concentration and inoculated with overnight TSB cultures of *S. mutans*$^R$ and *S. sanguis* 10556. Plates were incubated overnight at 37°C with 5% CO2. The disks were aseptically rinsed with sterile saline to remove non-adherent bacteria and placed into a tube with sterile saline to dislodge biofilm. The biofilm was spiral plated onto 1 mg/ml streptomycin-containing TSA (to enumerate *S. mutans*) and 0 mg/ml streptomycin TSA (to enumerate both *S. mutans* and *S. sanguis*). Plates were incubated overnight at 37°C with 5% CO2 before being counted using an automated colony counter. Results: The data obtained suggests a dosage-dependent effect of nicotine on the growth of *S. mutans*. *S. mutans*$^R$ predominated over *S. sanguis* in the 0 mg/ml of nicotine control possibly as a result of *S. mutans* bacteriocin production. Moreover, a trend of increased *S. mutans*$^R$ was seen in 0.25 mg/ml of nicotine compared to the control. It is hypothesized that low levels of nicotine increased bacteriocin production which led to a predominance of *S. mutans*$^R$ over *S. sanguis*. Increasing concentrations of nicotine reduced the number of *S. mutans*$^R$ in a mixed biofilm culture most likely resulting from lysis. Conclusion: This study confirmed earlier results demonstrating that nicotine upregulated the ability of *S. mutans* to kill *S. sanguis* resulting in exclusion of *S. sanguis*. This provides further evidence of the role of nicotine in caries formation in smokers. Increased numbers of *S. mutans* would bind to available sites on tooth surfaces, creating a destructive biolfilm, leading to increased

demineralization of enamel. The dispersal properties of biofilm would allow it to spread to nearby surfaces and ultimately result in increased caries.

**P43 The Effect of Nicotine on *Streptococcus mutans* UA159 Bacteriocin Production Against *Streptococcus sanguis*.** K. S. HEEKE,* R. L. GREGORY (Indiana University School of Dentistry)

Introduction: *Streptococcus mutans* is the main etiological cause of dental caries, and it has been shown that individuals who smoke have increased dental caries. *S. mutans* produces bacteriocins, which inhibit the growth of similar strains or species. The objective of the present study was to determine if nicotine upregulates *S. mutans* bacteriocin production against a noncariogenic bacteria, *Streptococcus sanguis*. Previous research has shown that *S. mutans* and *S. sanguis* have an inverse relationship pertaining to their growth. In addition, it was shown using spectroscopy that nicotine upregulates *S. mutans* UA159 bacteriocin production against *S. sanguis*. If nicotine upregulates bacteriocin production, the study would give further evidence that nicotine may facilitate *S. mutans* colonization over *S. sanguis* on the tooth surface. Materials and Methods: Todd-Hewitt Broth (THB) containing either 0 or 4 mg/ml of nicotine was inoculated with *S. mutans* UA159. THB alone served as a control. The cultures were clarified by centrifugation, filter-sterilized and 300 ul of the supernatants were added to corresponding cultures of *S. sanguis* (ATCC 10556) in 2.7 ml Tryptic-Soy Broth (TSB). Each *S. sanguis* culture was incubated overnight in 5% CO2 at 37oC. The cultures were then diluted 1:5 in 0.9% NaCl. A LIVE/DEAD BacLight Bacterial Viability Kit for microscopy (Invitrogen) was used to stain the *S. sanguis* cells (3 ul of stain to 1 ml of bacterial culture). All samples were vortexed, sonicated, vortexed again, and then placed in darkness at room temperature for 15 min. Each sample was transferred onto glass microscope slides and viewed under a microscope at 1000X. All live, bright green fluorescent cells in 10 microscopic fields were counted. Student's T-test was used to find P values for statistical difference ($P<0.05$). Results: The data suggest that nicotine does, in fact, increase *S. mutans* UA159 bacteriocin activity against *S. sanguis*. The THB control sample had a mean of 98.9+27.75 *S. sanguis* cells, the 0 mg/ml nicotine sample had a mean of 61.1+24.04 cells and the 4 mg/ml nicotine sample had a mean of 49.2+11.9 cells/field. Nicotine at 4 mg/ml significantly upregulated ($P < 0.05$) *S. mutans* bacteriocin activity compared to the THB control. In addition, 4 mg/ml of nicotine significantly increased *S. mutans* bacteriocin activity compared to the 0 mg/ml nicotine supernatant. *S. mutans* with 0 mg/ml nicotine also demonstrated a significant increase in *S. mutans* bacteriocin activity against *S. sanguis* compared to the THB control. Conclusion: Incubation of *S. mutans* with 4 mg/ml nicotine significantly increases bacteriocin activity against *S. sanguis*. This supports our previous work indicating that nicotine increases *S. mutans* bacteriocin activity and provides additional evidence that *S. mutans* establishes a special niche in the oral cavity allowing for greater caries activity in smokers. Acknowledgements: This work was funded by the Center for Research and Learning Life-Health Sciences Internship at Indiana University-Purdue University Indianapolis.

**Presentations**
**3:20 p.m. to 4:00 p.m.**

**MICROBIOLOGY/IMMUNOLOGY**

**P44 The Genotypic and Phenotypic Traits of *Streptococcus mutans* Isolated Under Cariogenic Condition**. R. ARTHUR[1],* P. ROSALEN[2], R. MATTOS-GRANER[2], G. VALE[3], C. TABCHOURY[2] ([1]Indiana University School of Dentistry; [2]Piracicaba Dental School, University of Campinas, Brazil; [3]Faculty of Dentistry of Piauí, State University of Piauí, Brazil.)

Oral cavity harbors several *Streptococcus mutans* genotypes, which could present distinct virulence properties. However, little is known about the diversity and virulence traits of *S. mutans* genotypes isolated in vivo under controlled conditions of high cariogenic challenge. We sought to evaluate the genotypic diversity of *S. mutans* isolated from dental biofilms formed in vivo under sucrose exposure, as well as their acidogenicity and aciduricity. To form biofilms, six subjects rinsed their mouths with water or 20% sucrose solution 8 times/day for 3 days. *S. mutans* isolates collected from saliva and biofilms were analyzed for their genotypic identity by arbitrarily-primed PCR. The genotypes identified in the biofilms were evaluated based on their ability to lower the suspension pH through glycolysis and their acid susceptibility and F-ATPase activity. For glycolitic profile, genotypes were resuspended in KCl/MgCl2 solution, the pH was adjusted to 7.2, glucose was added to a final concentration of 55.6 mM and the pH was monitored through 3 h. The area under the curve of pH fall (min*pH) and total hydrogen ion concentration were calculated. For acid susceptibility assay, genotypes were resuspended in glycine buffer pH 7.0, 5.0 or 2.8 and immediately after (time zero), and after 30 and 60 min, aliquots were plated on BHI agar and numbers of colonies-forming-units were determined in each condition after 48h incubation. For ATPase activity, the genotypes were permeabilized with toluene and incubated with adenosine 5´-triphosphate (ATP) for 10 min and the ATPase activity was expressed as micromoles of phosphate released from ATP per gram of dry cell per minute of reaction. In total, 19 distinct *S. mutans* genotypes were identified in biofilm samples. Most of the subjects harbored only one genotype in saliva, which was detected in almost all biofilm samples at high proportions, but other genotypes were also found in lower proportions in biofilms. Genotypes isolated only from biofilms formed with sucrose had higher acidogenicity than those isolated only from biofilms formed with water ($p<0.05$). Genotypes from biofilms formed with sucrose exhibited a higher tolerance for acidic conditions at pH 5.0 and 2.8 after 60 and 30 min of incubation, respectively ($p<0.05$). No differences were found regarding F-ATPase activity. Although no differences in genotypic diversity were observed between biofilms formed with water or sucrose, our results suggest that biofilms formed under high cariogenic conditions may harbor more aciduric and acidogenic *S. mutans* genotypes, among a diverse group of strains colonizing the oral cavity.

**P45 β2 receptor Activation of *Streptococcus mutans*.** H. HIRSCH,* R. L. GREGORY (Indiana University School of Dentistry)

Nicotine upregulates *Streptococcus mutans* growth, leading to increased adherence to tooth surfaces and increased caries. At this time, specific nicotinic receptors on *S. mutans* have not been found; however evidence indicates *S. mutans* has β2 nicotinic receptors triggered by the amine group on nicotine providing a mechanism of nicotine entrance. The nicotinic agonists/antagonists acetylcholine, succinylcholine, turbocurarine, epibatidine, varenicline, and bupropion contain amine groups which could bind to the hypothesized β2 *S. mutans* receptor. Objective: Examine the effects of the various agents with/without nicotine on β2 receptors.

Methods: *S. mutans* UA159 was incubated for 16h in sucrose-free tryptic soy broth (TSB). Bacteria were grown to late-exponential phase (OD=0.8) and inoculated into a 96 well plate. Experimental conditions included 10uL of UA159 plus 1.0mg/ml of each agent with or without nicotine. The agents were added to test wells 30 min prior to adding nicotine. Bacterial growth was kinetically measured every 9 min for 12h at 600nm. Results: Bupropion and bupropion plus nicotine had 40-60% significantly lower Vmax, a longer lag time, and a longer time to maximum absorbance compared to UA159. Turbocurarine and turbocurarine plus nicotine had 20% significantly lower Vmax compared to UA159. Acetylcholine plus nicotine had 25% significantly higher Vmax compared to UA159. Conclusion: Bupropion and turbocurarine have an antagonist effect on *S. mutans*. The addition of nicotine caused a synergistic effect with acetylcholine and bupropion. Addition of acetylcholine enhances *S. mutans* growth while for bupropion, it inhibits growth. Growth is not significantly different for turbocurarine with or without nicotine, but is significantly different than UA159, suggesting no additive effect of nicotine. Due to the significant effects on growth, these agents demonstrate receptor binding similar to nicotine, further supporting the presence of β2 nicotinic receptors. Supported, in part, by the Indiana University School of Dentistry Dental Student Research Fund. The project described was supported by Award Number T14DE017284 from the NIDCR. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIDCR or the NIH.

**P46** **Effect of Honey on *Streptococcus mutans* Biofilm Formation.** H. NASSAR[1,2],* R.L. GREGORY[1] ([1]Indiana University School of Dentistry; [2]Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia)

Introduction: Honey has been used as a source of nutrients as well as a medicine since ancient times. The antibacterial properties of honey have been well documented. However, the specific antimicrobial mechanism of honey is still unclear. Among the possible mechanistic agents are the presence of inhibitory factors such as flavonoids and hydrogen peroxide, low pH, and high osmolarity due to its sugar concentration. Honey may have a similar antibacterial effect on *Streptococcus mutans* which is considered the main causative organism of dental caries. Very limited studies have investigated the effect of honey on *S. mutans*. The aims of this study was to investigate the effect of natural honey (NH) on *Streptococcus mutans* growth, viability, and biofilm formation compared to an artificial honey (AH) solution. Materials and Methods: AH contained the sugars at the concentrations reported for NH. NH and AH concentrations were obtained by serial dilution with tryptic soy broth (TSB). Several concentrations of NH and AH were tested for inhibition of bacterial growth and biofilm formation after inoculation with *S. mutans* UA159 in 96-well microtiter plates. Diluted aliquots from both honey groups were spiral plated and incubated for 48 h. An automated colony counter was utilized to determine the number of CFUs.Results: Overall, NH demonstrated significantly less ($p < 0.05$) bacterial growth compared to the AH at 50%, 25% and 12.5% concentrations. At 50% and 25%, both honey groups exhibited significantly less bacterial growth and biofilm formation compared to the TSB control. For bacterial viability, all tested levels were not significantly different from the TSB control except for the 50% NH. Conclusions: NH had significantly more inhibitory effect against bacterial growth and viability compared to AH. This study highlights the potential antibacterial properties of NH, and could suggest that the antimicrobial mechanism of NH is not due to its high sugar content. Acknowledgements: This work was partially funded by the Indiana University School of Dentistry Ph.D. Student Research Fund.

## ORAL BIOLOGY

**P47 Role of Kalirin in Signaling Events Leading to Osteoclast Resorption.** P. P. ELENISTE,* S. HUANG, M. SHIVANNA, L. DU, A. BRUZZANITI (Indiana University School of Dentistry)

Osteoporosis is a bone disease that affects millions of people worldwide and is characterized by low bone mass and structural deterioration of bone tissue, which increases the risk of bone fracture, frailty, morbidity and mortality. Excessive bone loss is caused by the activity of osteoclasts (OCs) which degrade the bone matrix. The specific aim of this study is to identify and characterize the signaling proteins in osteoclasts that regulate the bone resorbing activity of these cells. Kalirin, RHO GDP-GTP exchange factor (GEF), plays a key role in cytoskeletal organization of dendritic spine\synapse formation in hyppocampal neurons. We recently demonstrated that deletion of Kalirin in mice leads to osteoporosis in part due to increase OC differentiation and OC bone resorbing activity, however the mechanism of action of Kalirin in OCs and the signaling pathways that regulate OC bone resorbing activity is unknown. Dynamin is a GTP-hydrolyzing protein that functions in the early stages of endocytosis. Previously, we reported that dynamin stimulates OC bone-resorbing activity in a GTPase dependent manner. Dynamin exists as a monomer, dimer or tetramer in solution, and liposomes induce the tetrameric form of dynamin, which is essential for its role in endocytosis. However, the function of the monomeric or dimeric form of dynamin is unknown. In this study, we sought to determine if Kalirin's effects on OC bone-resorbing activity were mediated by controlling oligomeric form and GTPase activity of dynamin. We expressed wild-type dynamin and assembly-defective mutants (dynamin-I684K) in the presence or absence of Kalirin in the human embryonal kidney cells stably expressing the vitronectin receptor (293VnR). Dynamin was immunoprecipitated and its GTPase activity determined using a calorimetric GTPase assay in vitro. Our data revealed that the GTPase activity of wild-type dynamin was increased in the presence of Kalirin. However, Kalirin failed to stimulate the activity of the assembly mutant dynamin-I684K, suggesting that Kalirin potentially stimulates dynamin GTPase activity after its assembly and stabilizes in it in its dimeric form. Our data indicate that Kalirin potentially influences dynamin's basal GTPase activity and oligomerization which decreases dynamin endocytosis activity. The mechanism by which Kalirin accelerates and decelerates the GTP hydrolysis of dynamin and the oligomeric stability of dynamin in the presence of Kalirin needs to be further examined, but may be important for OC bone-resorbing activity.

**P48 Effects of Alendronate on Cells of the Oral Cavity.** J. JENKINS,* J. SUN, L. J. WINDSOR (Indiana University School of Dentistry)

Bisphophonates (BPs) are drugs used to treat cancer and osteoporosis. They have been implicated as a cause in Bisphosphonate-Induced Osteonecrosis of the Jaw (BRONJ), a disease that is characterized clinically by exposed necrotic bone in the mandible, maxilla and/or palate. As BPs accumulate in bone over time, patients who have been administered BPs for a prolonged amount of time (2+ years) are at risk for developing BRONJ. The goal of this study was to evaluate the effects of a commonly used bisphosphonate (alendronate) on the viability, proliferation, collagen degradation and expression of matrix metalloproteinase (MMP)-1, MMP-2, tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 in human gingival fibroblast cells (HGFs) and in a human osteoblast-like cell line (MG63). HGFs and MG63s were seeded in 6 well plates at a concentration of 75,000 cells per well and grown for 72 hours with and without 10-8 to 10-4 M alendronate. A water soluble tetrazolium (WST-1) assay kit and a lactate dehydrogenase (LDH) assay kit were used to determine the effects of alendronate on cell proliferation and viability, respectively. In addition, the cells were seeded into 6-well collagen

coated plates and grown for 3, 5 and 7 days to determine the effects of alendronate on collagen degradation. The alendronate concentrations used were 10-8 up to 10-6 M. Media from the collagen degradation assays was used for further protein assays. Zymography was used to determine alendronate's effect on secretory MMP-2 expression. MMP-2 was separated by electrophoresis in a gel impregnated with gelatin and the gels were incubated for 24 hours at 37º C. Gels were stained with Coomassie Blue to visualize MMP-2 activity. To test alendronate's effect on secretory MMP-2 activity, zymography gels with MMP-2 were incubated for 24 hours at 37º C in 10-8 to 10-2 M alendronate. Western Blotting was utilized to examine relative MMP-1, MMP-2, TIMP-1 and TIMP-2 expressions. The WST-1 assays showed significant ($p<0.05$) changes in cell proliferation at 10-5, 10-4 and 10-3 M for both cell types. LDH assays revealed significant cytotoxicity at concentrations of 10-5, 10-4 and 10-3 M for both cell types. No change in collagen degradation with the addition of alendronate at non-toxic levels (10-8 to 10-6 M) was observed. The zymography results showed no significant inhibition of MMP-2 until a concentration of 10-3 M alendronate was used. At 10-2 M alendronate, MMP-2 was almost completely inhibited. MMP-1 and -2 and TIMP-1 and -2 expressions were not found to be altered at non-toxic levels of alendronate. At levels found in the serum (10-8 to 10-6 M), alendronate was not found to alter HGF or MG63 viability, proliferation, collagen degradation, secreted amounts of MMP-1, MMP-2, TIMP-1 or TIMP-2 or have any adverse affect on MMP-2 activity.

## ORAL DISEASE PREVENTION AND DIAGNOSIS

**P49 Curcumin's Promoter Demethylating Effects: a Useful Chemopreventive Strategy.**

B. HAN,* Z. LIU, A.S. HOLPUCH, S.R. MALLERY (The Ohio State University)

Objective: Promoter hypermethylation represents one of the primary mechanisms for inactivation of tumor suppressor genes (TSGs). Clinical studies have shown that loss of TSGs, most notably $p16^{INK4a}$ and fragile histidine triad (FHIT), occurs relatively early in oral dysplastic lesions. Furthermore, inactivation of these key genes has clinical relevance, as loss of function correlates with progression of these dysplastic lesions to invasive oral squamous cell carcinoma (OSCC). Demethylation of aberrant promoter hypermethylation can restore gene expression and recover tumor suppressor function. Decitabine, a FDA-approved chemotherapeutic agent, effectively demethylates hypermethylated promoters. Long-term myelosuppression, however, limits decitabine's utility as a chemopreventive agent. An effective, yet less toxic demethylating agent would be more amenable for chemoprevention. Our labs recently demonstrated that curcumin, a plant-derived polyphenol, effectively demethylates promoter sites. Methods: Six oral keratinocyte cell lines (ranging from normal-dysplastic-metastatic) were screened to identify lines that had promoter methylation of $p16^{INK4a}$ and FHIT. Polymerase chain reaction (PCR) was used to examine $p16^{INK4a}$ and FHIT promoter regions. To determine whether promoter methylation suppressed protein levels, cells were treated with decitabine (2μM) for 72 hours, and $p16^{INK4a}$ and FHIT assessed by Western blotting. Results: Findings to date: (1) development and validation of a LC-MS/MS method to determine both global and regional methylation levels; (2) demonstration that curcumin induces global hypomethylation; (3) retention of the promoter regions of $p16^{INK4a}$ by all cell lines except SCC9; (4) constitutive expression of $p16^{INK4a}$ and FHIT proteins by normal, HPV16-transduced, and SCC4 cells; (5) Restoration of $p16^{INK4a}$ protein in SCC15 by decitabine treatment. Conclusion: Due to its decitabine-induced recovery, SCC15 is a model cell line for $p16^{INK4a}$. Studies are ongoing to identify additional cell lines with a decitabine-responsive i.e. methylated, FHIT promoter. Evaluation of curcumin's capacity to reduce $p16^{INK4a}$ promoter methylation is being accessed by LC-MS/MS. Supported by T32DE14320, R01CA129609, R21CA132138, R21CA135478

**P50 Evaluation of a Fenretinide Mucoadhesive Patch for Local Intraoral Delivery.** A. S. HOLPUCH[1],* K. G. DESAI[3], W. CHEN[2], M. P. PHELPS[1], B. HAN[1], G. A. SEGHI[1], Z. LIU[2], S. P. SCHWENDEMAN[3], and S. R. MALLERY[1] ([1]Oral and Maxillofacial Surgery, Pathology, and Anesthesiology, The Ohio State University; [2]College of Pharmacy, The Ohio State University; [3]Pharmaceutical Sciences, University of Michigan)

Chemoprevention represents a promising strategy for inducing regression/inhibiting the progression of oral epithelial dysplasia to oral squamous cell carcinoma. Vitamin A-like compounds, such as fenretinide, have been shown to exhibit desirable anti-cancer effects *in vitro*. Clinical trials evaluating the systemic delivery of these compounds, however, have been unsuccessful due to vitamin A-like toxicities and sub-therapeutic concentrations in the target tissues, i.e., oral epithelium. In contrast, local delivery of fenretinide could provide a pharmacological advantage by obtaining therapeutically relevant concentrations in the oral epithelium while minimizing systemic exposure. Objectives: To formulate and evaluate a mucoadhesive patch for intraoral delivery of fenretinide, a highly hydrophobic chemopreventive retinoid. Methods: Determination of fenretinide apparent solubility in the presence of various solubilizers was studied in simulated saliva (pH 6.8, 37°C). The mucoadhesive layer (carbopol-974P, hydroxypropyl methylcellulose) and drug-release rate-modulating layer (Eudragit® RL-PO) were prepared by a solvent casting method, and patches were prepared by attaching the mucoadhesive/rate-modulating layers onto the impermeable backing layer (Tegaderm™ film). Fenretinide distribution in Eudragit® film was evaluated by X-ray diffraction (XRD) and scanning electron microscopy (SEM). In vitro fenretinide-release was conducted in simulated saliva at 37°C under sink conditions. Fenretinide was quantified by HPLC at 365nm. A 10-day pharmacokinetic study evaluating 30min/day fenretinide patch-delivery to rabbit alveolar mucosa is ongoing. Results: Tween®-20 and 80, Brij-98, and sodium deoxycholate exhibited the highest fenretinide solubilization potential. Fenretinide loading efficiency in Eudragit® films was 90-98%. XRD and SEM suggested that fenretinide was in an amorphous form in the films. Fenretinide/Eudragit®-RL-PO films containing 20/40wt% Tween®-80/sodium deoxycholate, the leading formulation, exhibited 24 and 75% fenretinide release after 1 and 8h, respectively. Conclusion: Fenretinide solubilization and controlled-release from mucoadhesive patches was successfully demonstrated *in vitro*, and *in vivo* studies to evaluate the pharmacological advantage of patch-local delivery are ongoing. [F30DE020992, R01CA95901, T32DE014320]

**P51 Oral Disease Awareness Evaluation of Medical Professionals in the US.** O. ISYUTINA[1],* P. KHURANA[1], G. J. ECKERT[2], N. ISLAM[1] ([1]Indiana University School of Dentistry; [2]Indiana University School of Medicine)

The physician being the proverbial ‘healer' remains the first line of defense for detection of most oral conditions. The medical community should be acutely aware of oral pathological lesions as current data demonstrates that malignancies like oral squamous cell carcinomas have shown no prognostic improvements due to lack of early detection. Objectives of this study were to evaluate the awareness of oral conditions among medical professionals and their dental counterparts, and to design interactive lectures/ continuing medical education (CME) programs for medical students/ practitioners, and refresher courses for the dental group. An anonymous online survey was run at the Indiana University School of Medicine and School of Dentistry. The questions regarding ‘level of training', need of oral pathology course, quiz on ten clinical photographs with histories, and feedback box were included. Of the 654 respondents, correct responses were 3.7+/-1.7 (mean+/-standard deviation) for medical and 4.8+/-1.8 for the dental group. The dental group had significantly higher scores ($p<0.0001$). The correct responses increased with training for both medical ($p=0.03$) and dental groups ($p<0.0001$). Percentages

correctly identifying the conditions from the two groups were 38% dental / 5% medical for linea alba, 71%/54% for leukoplakia, 14%/28% for oral hairy leukoplakia, 53%/43% for benign migratory glossitis, 79%/45% for pyogenic granuloma, 44%/23% for Langerhans cell disease, 44%/42% for primary herpetic ulcer, 21%/19% for pemphigoid, 66%/69% for Crohn's disease, and 48%/43% for squamous cell carcinoma. Both groups had difficulty identifying oral conditions. As hypothesized, the dental group performed better than the medical group. The 'level of training' in both groups was a strong precursor to superior diagnoses. The medical group showed the need of well-structured oral pathology lectures and CME programs. Noteworthy was the feedback from the medical group affirming their lack in identification of oral conditions with seemingly obvious long term impact on oral health.

## ORTHODONTICS

**P52 Comparison of Airway Dimensions Between African American and Caucasian Patients.** L. GLUPKER[1],* A. GHONEIMA[1], G. J. ECKERT[2], K. S. KULA[1] ([1]Indiana University School of Dentistry; [2]Indiana University School of Medicine)

The nasopharynx plays a fundamental role in dentofacial morphology and nasorespiratory function. Objective: The aim of this retrospective study was to compare the nasopharyngeal and dentoskeletal dimensions between two ethnic groups. Methods: Following reliability studies, lateral cephalometric radiographs of 30 African American and 30 Caucasian subjects (> 16 yoa) that met inclusionary criteria were collected from the archives of a postgraduate orthodontic clinic. Parameters (26 nasopharyngeal, 12 anteroposterior and 8 vertical dentoskeletal) were measured manually and compared between the groups using ANCOVA, with age and sex as covariates. Dentoskeletal parameters were correlated with the nasopharyngeal parameters (Pearson's correlation). Statistical significance was set at $P < 0.05$. Results: Following adjustments for sex and age, significant differences between Caucasian and African American subjects were detected in multiple dentofacial measures (SNA, SNB, 1/SN, 1-MP, ANS-PNS, Go-Gn, LAFH%, SN-PP, AFH, angle of convexity) as well as such airway measures as the anteroposterior depth of the bony nasopharynx: Ba-ptm (P=.0017) and AA-PNS (P=.0379); height of the bony nasopharynx: Ho⊥ANS-PNS (P=.0359); thickness of soft tissue of the posterior pharyngeal wall: Ba-ad1 (P=.0056) and the anteroposterior dimension of the pharynx: Ba-S-PNS (P=.0343); Ba-S-ptm (P=.0245), and AA-S-PNS (P=.0379). Significant correlations (r = 0.50-0.64) existed between all anteroposterior nasopharyngeal measures and anterior cranial base length (N-S), maxillary length (Co-ANS), mandibular length (Co-Gn), and anterior facial height. Conclusion: There are significant differences in anteroposterior and vertical nasopharyngeal and dentoskeletal dimensions between Caucasians and African Americans >16 yoa. Several dentoskeletal measures showed significant correlation with nasopharyngeal parameters.

**P53 Accuracy of Slot Dimension within Sets of Orthodontic Brackets.** C. PEARCE,* P. BROWN, H. CHOI, W. WAGNER, W. BROWN (University of Detroit Mercy School of Dentistry)

Objective: This study measured the slot dimensions of different sets of orthodontic brackets. Methods: A set of brackets was defined as upper and lower 5-5 (second bicuspid to second bicuspid). Brackets representing both.022 and .018 slot sizes from a variety of manufacturers were measured. Approximately 100 brackets or five sets, from each of ten different bracket systems were investigated. A Clark Microhardness Tester-Model CM-700 and a Clark Instrument Automatic Reading System-Model CM-AR90 was used to digitally image, scale and measure the distal slot on each bracket to the nearest micron. Following standardization of the

imaging and measuring processes, observers achieved a correlation coefficient of 0.947. Results: The measures represent the greatest width or largest width wire, from the distal aspect of the bracket that could be inserted in the slot without causing the slot itself to deform. Conclusions: The results showed a variation in slot dimension ranging from 20% undersized to 16% oversized within the bracket systems. The ability to express torque uniformly in a given bracket system is likely to vary from set to set and within the set themselves. We acknowledge the companies who donated brackets.

**P54 Quantification of Clinical 3D Tooth Displacement.** S. LI[1],* J. CHEN[2], K.S. KULA[2], P. ELLIS[2], S. LIU[2] ([1]School of Engineering and Technology, Indiana University-Purdue University Indianapolis; [2]Indiana University School of Dentistry)

Quantification of three-dimensional (3D) tooth displacement is essential to evaluate orthodontic treatment outcomes. While using both Cone-Beam CT (CBCT) images and dental cast to quantify tooth movement have been proposed, the consistency of these two methods remains unknown. The objective of this study is to assess the consistency of these two methods for further developing a new technology that accurately quantify 3D tooth movement. Two maxillary dental casts and two CBCT scans were taken before and after canine retraction, respectively. Dental casts were scanned using OPTIX 400S® 3D laser scanner and CBCT images were processed using MIMCS® software. Iterative closest point (ICP), a geometric optimization algorithm, was used to best-fit the two digital models before and after treatment for cast and CBCT images, respectively. Cast digital models were automatedly aligned and superimposed on the palatal rugae region, whereas CBCT models on the anterior inner curve of the hard palate. On each superimposed model, 3D displacements of both maxillary canines were computed from the transformation matrix based on the difference between before and after treatment models. The six displacement components were expressed by translation and rotation relative to the canines of the pre-treatment models with its x-, y-, and z-axes parallel to the buccal, distal, and apical directions, respectively. The difference in translation between the two methods was 0.22mm (6.51% of the total translation) for the left canine and 0.04mm for the right (1.79% of the total translation). The difference in rotation between two methods was 0.99° (9.29% of the total rotation) for the left canine and 0.27° (5.94% of the total rotation) for the right one. Conclusions: The CBCT and dental cast 3D superimposition methods were consistent with each other because of clinically insignificant variations between them. Funding agency: NIDCR R01-DE-018668.

**P55 Assessment of Microdamage Caused by Mini-implants in Mandible and Maxilla.** J. PONDER[1],* K. WISEMAN[1],M. ALLEN[2], J. SUN[1], K. T. STEWART[1], E. CRUZ[1], P. DUNN-JENA[1], S. LIU[1] ([1]Indiana University School of Dentistry; [2]Indiana University School of Medicine)

Mini-implants (MIs) have recently become a popular skeletal anchorage device for a variety of orthodontic indications. However, studies have demonstrated poorer success rates among MIs compared to traditional dental implants. Furthermore, literature indicates that MI failure rates are higher in the mandible than in the maxilla. Our objective is to gain a better understanding of the biological mechanism of MI failure, and why more MIs are failing in the mandible than in the maxilla. This study investigated whether microdamage is associated with MI diameter, sites of insertion, or cortical bone thickness. The sample of thirty-six MIs consisted of 1.6 x 6mm (n=16) and 2.0 x 6mm (n=20) Dentos Inc. implants. Groups of four and five MIs were randomly assigned to the maxillary and mandibular quadrants, respectively, using an incomplete block design of two mongrel adult dogs. After sacrifice, MIs were inserted following full mucosa reflection and pilot hole placement. MI and surrounding bone were harvested, stained with Basic

Fuchsin, embedded in methyl methacrylate, sectioned, and ground to 200µm parallel to the MI axis. A Leica epifluorescence microscope and Bioquant software were used to measure cortical bone thickness, crack length, and crack count adjacent to the MI. Microdamage burden per surface length was calculated. Mann-Whitney test was performed to compare the differences between jaws and two sizes of MIs. Kruskal-Wallis analysis was used to compare measurements among insertion sites. Placement of implants in the mandible resulted in significantly more microdamage accumulation (0.980 ± 0.442µm/µm) compared to the maxilla (0.523 ± 0.299µm/µm, p≤0.001). In addition, larger cortical thickness measurements were found in the mandible (2526 ± 1318µm) than the maxilla (2391 ± 969µm). Implant diameter non-significantly (p=0.055) affected microdamage accumulation, with 2.0mm implants having a 47.2% higher level of damage compared to 1.6mm implants. There was also a non-significant trend for greater cortical thickness and microdamage burden in the most posterior region of the mandible. The results of this study show significantly greater microdamage burden in the dog mandible than maxilla following implant placement. There was a trend indicating that larger implant size caused increased microdamage, but this observation was statistically non-significant. It is possible that the greater torsion forces required to place implants into thicker mandibular bone may be causing greater microdamage, ultimately leading to clinical MI failure. Future studies should investigate the effect of torsion forces on microdamage, and the use of drilling pilot holes of various sizes to mitigate torsion. Funded by IUPUI Support for the Recruitment of Under-Represented Faculty (SURF) and Indiana University School of Dentistry.

**P56 Three-Dimensional CT Analysis of Airway Volume Following RME Treatment.** T. SMITH[1],* A. GHONEIMA[1], K. T. STEWART[1], J. BALDWIN[1], S. LIU[1], G. J. ECKERT[2], S. HALUM[2], K. S. KULA[1] ([1]Indiana University School of Dentistry; [2]Indiana University School of Medicine)

Rapid maxillary expansion (RME) is an orthopedic procedure prescribed for treatment of crossbites and transverse maxillary deficiencies. RME reportedly increases nasal width and volume, thereby lessening nasal resistance and improving nasal respiration. Objectives: The purpose of this retrospective study, utilizing 3-dimensional computed tomography, was to determine if RME causes a significant change in the airway (maxillary sinuses, nasal cavity, nasopharynx, oropharynx, hypopharynx, soft palate area, and soft tissue thickness). Methods: Following reliability studies, volumetric and soft tissue thickness parameters were compared using spiral CTs of 20 patients taken as preliminary and 3 month retention records following rapid maxillary expansion. Intraclass correlations (ICC) were performed on duplicate measures of 20 CTs. Comparisons between the pre and post RME measurements were made using nonparametric signed rank tests. Spearman correlation coefficients were calculated to evaluate the association between the pre-RME measurements and the changes as well as correlations among the volume, area, and thickness measurements. Statistical significance was set at $P < 0.02$. Results: ICC values were >0.91 for all reliability measures. Statistically significant increases pre- to post-RME were found in the nasal cavity volume, nasopharynx volume, MP-SN, S-PNS, N-ANS, ANS-Me, and N-Me. Changes in hypopharynx volume, soft palate area, soft tissue thickness (SST) CV2, CV3sa, and CV3ia were related negatively to the starting values – subjects with larger initial values showed less change. Statistically significant positive correlations existed between changes in: nasopharynx volume and maxillary right sinus volume, hypopharynx volume and SST CV4ia, SST CV2 and SST CV3ia, SST CV3sa and SST CV3ia, PP-SN and N-ANS, and ANS-Me and N-Me. A statistically significant negative correlation existed between nasal cavity volume and oropharynx volume, and PP-SN and S-PNS. Conclusion: Statistically significant increases in volume and area, as well as positive and negative correlations between changes can be found following rapid maxillary expansion.

**P57 Effects of Alcohol on the Force Degradation of Elastomeric Chains.** K. T. STEWART,* T. LARRABEE, A. TORRES-GORENA, A. E. SOTO-ROJAS, J. PLATT, S. LIU. (Indiana University School of Dentistry)

Elastomeric chain is a commonly used method in orthodontics to facilitate tooth movement and consolidate space. Rapid loss of orthodontically applied force results in inefficient tooth movement and the need for an increased number of appointments to reactivate the appliance. Various factors, including pH, temperature, and environment have been shown to cause varying effects on elastomeric chains. Literature has also shown that emersion of orthodontic modules in a 75% ethanol/water mixture causes detrimental structural and molecular modifications, but degradation in force levels after such an exposure has yet to be evaluated. Therefore, it was the objective of this study to investigate the effect of alcohol on the force degradation of elastomeric chains in vitro. Four hundred and fifty elastomeric chain specimens were randomly divided into five different test groups: distilled water (control), 14% alcohol/water mixture, 26.9% alcohol/water mixture, Cepacol®, and Listerine®. Each group of elastomeric chain was mounted on custom jigs and exposed to its respective solution for 60 seconds, twice a day, for 28 days. After exposure, the elastomeric chains were dipped in intermediate baths for 10 seconds, and then stored in separate water baths. During the study, the water baths were maintained at a constant temperature of 37°C and a pH between 6.00 and 7.00. All elastomeric chain force levels were measured with a digital force gauge, by the same blinded investigator. Force levels were measured at 6 intervals: initial, 24 hours, 1week, 2 weeks, 3 weeks, and 4 weeks. Significant differences in force degradation between the groups were observed using a 2-way ANOVA, at a significance level of 0.05. After 28 days, elastomeric chains exposed to 14% alcohol ($p=0.0001$), 26.9% alcohol ($p<0.0001$), Cepacol® ($p<0.0001$), and Listerine® ($p=0.02$) all demonstrated a statistically significant decrease in force degradation, when compared to the control group. We concluded that alcohol does increase force degradation of elastomeric chains, however, no linear relationship between alcohol concentration and force degradation was observed. Although we cannot accurately simulate the oral environment, alcohol still shows an effect on elastomeric chains that could have a clinical impact on orthodontic treatment.
Funded by IUPUI Support for the Recruitment of Under-Represented Faculty (SURF) and Indiana University School of Dentistry.

**P58 Rotational Tendency Experienced by a Lateral Incisor During Space Closure.** D. WU,* S. ISIKBAY, J. CHEN (Indiana University School of Dentistry)

The objective of this study was to measure the initial forces and moments experienced by a lateral incisor bracket (LI) during space closure using combinations of two power chain (PC) engagements (around the whole bracket, or the wings adjacent to the space) and three ligation methods (no ligature ties, ligature tie around the whole bracket, or the wings adjacent to the space) in order to identify the best combination for reducing rotation. A custom-made dentoform with space distal to LI representing an ideal maxillary arch was mounted on an orthodontic force tester with a load cell attached to the right LI separated from the rest. A stainless steel (SS) 0.016” archwire on 0.018” fixed appliances was used for each testing. A 0.010” SS ligature tie was used to ligate the LI as needed. Three loops of elastomeric PC were used to initiate space closure from the LI to the next distal tooth. Six force and moment components were recorded for each combination. Each combination was tested ten times for assessing variations. Analysis of variance was performed to evaluate the effects of the combination on two key components, the distal force and the rotation moment. There were no statistical differences between forces along the mesial-distal axis indicating consistent forces applied by PC ($p>0.005$). There were statistical differences ($p<0.005$) between moments around the incisal-apical axis indicating differences in rotational tendency. The rotation moment of the combination with PC around the

whole bracket with a ligature tie was one-fourth of that of PC on the wings adjacent to the space with no ligature tie. The results suggest that a clinician attempting to reduce rotational tendency during space closure should consider placing the PC around the whole bracket and a ligature tie either around the whole bracket or only around the wings adjacent to the space. Funded by an IUSD-grant.

**P59 Do Occlusal Contact Detection Products Alter the Occlusion?** R. HELMS[1],* G. J. ECKERT[2], T. KATONA[1] ([1]Indiana University School of Dentistry; [2]Indiana University School of Medicine)

Many clinicians rely on occlusal contact detection products to identify high contacts and to equilibrate occlusions. Serious concerns about these products have stimulated constant investigations into marking reproducibility, accuracy and interpretation, but none have looked at their effects on the occlusion itself. The aim of this study was to assess if these products alter the occlusion that they purport to measure by determining if there are differences in the forces and moments experienced between occluding teeth with and without their presence. A pair of IPN Portrait 33° (Dentsply) molar denture teeth was placed into occlusion with the mandibular tooth supported by a load sensor and the maxillary tooth mounted onto a vertically sliding jig weighing 15.1 N. The three-dimensional force and moment components on the mandibular tooth were measured when the teeth were in direct tooth-to-tooth contact (control) and when the products were positioned between them. Seven products [Accufilm I, Accufilm II (Parkell Products), Articulating Silk (Whaledent), cotton roll, Rudischhauser Thick and Thin (Dental Articulating Paper), and T-scan (Tekscan)] were tested. (The cotton roll was included as a positive control.) All products showed differences from control with Accufilm I, T-scan and cotton roll rising to significance (ANOVA) with p-value <0.05.

**P60 Validation of a Tooth-PDL-Bone Complex for invitro Orthodontic Load Measurement.** Z. XIA[1], T. FU[1], J. CHEN[2] ([1]School of Engineering and Technology, Indiana University-Purdue University Indianapolis; [2]Indiana University School of Dentistry)

Objectives: Optimization of tooth movements and reduction of side effects during the orthodontic treatment require quantification of the orthodontic load systems. Periodontal ligament (PDL) affects the crown displacement introduced by the sliding mechanics, which needs to be simulated when the load systems are measured. Artificial materials imitating human PDL are needed for an in vitro measurement. The objective of this study is to seek and validate suitable materials to simulate human PDL based on the resulting crown displacement in response to an orthodontic force. Methods: An artificial tooth-PDL-bone complex (TPBC) was created. The complex was attached to a device consisting of a load cell, a linear actuator and a tooth adaptor. The device allows applying a force to the crown and measuring the resulting crown displacement. The TPBC has a socket hosting the tooth with a thin layer of silicon mixture for simulating the PDL. The following mechanical behaviors of the TPBC were tested, force-displacement relationship, time dependency of the force and displacement, loading and unloading, which affects the load measurements. Results: The mixture consisted of two types of silicones, 50% Gasket Sealant #2 and 50% RTV 587 Silicone, with a thickness of 0.3 mm simulated the TPBC well. With this mixture, the following mechanical behaviors of TPBC were validated by the results published previously. The behaviors tested included (a) force - displacement relationship, (b) stress relaxation, (c) creep, and (d) loading and unloading. These behaviors were consistent with results previously reported for biological PDLs from human, beagle, and pig. Conclusion: The TPBC can be used for in vitro orthodontic force measurement

of sliding mechanics. Acknowledgement: This research was partially supported by the NIH/NIDCR under grant #1R01DE018668.

## PEDIATRIC DENTISTRY

**P61 A Novel 3D Technology to Evaluate Root Canal Instrumentation.** A. AL HOSAINY[1],* S. LI[2], J. CHEN[2], J. DEAN[1], S. LIU[1] ([1]Mansoura University School of Dentistry; [2]Indiana University-Purdue University Indianapolis)

Proper root canal preparation is essential for the success of endodontic treatment in primary molars. Due to the high anatomic variations in the pulp and root canals, better understanding of their morphology and spatial changes following canal instrumentation can provide predictable treatment outcomes. Our goal was to develop a new method to quantify 3-dimenstional changes of primary teeth root canal before and after instrumentation. Methods: A SkyScan 1072 micro-computed tomography (micro-CT) was used to scan an extracted mandibular primary molar before and after root canal instrumentation. Two 3D digital models, before and after instrumentation, were rendered for the root and canal system using volume visualization software (CtAn). They were automatedly aligned and precisely superimposed using our custom-developed software to quantify changes in root canal space. Results: 3D morphologic features, such as, shape, cross section configuration and curvature of root canals, presence and characteristics of ismuths, accessory or lateral canals, apical foramen, and accessory foramina were successfully displayed. 2D parameters of canal cross sections, including area, perimeter, roundness, widest and narrowest diameter, and 3D canal volume and amount of dentin removed after instrumentation were calculated and reported in the table below.

| | | 2D | | | | | 3D | |
|---|---|---|---|---|---|---|---|---|
| | | Area | Perimeter | Roundness | Widest diameter | Narrowest diameter | Total volume | Total surface area |
| Before | Apical | 0.44 | 4.58 | 0.1 | 2.01 | 0.49 | 9.41 | 57.1 |
| | Middle | 1.50 | 6.77 | 0.2 | 2.80 | 1.00 | | |
| After | Apical | 0.47 | 4.74 | 0.1 | 2.02 | 0.49 | 10.43 | 59.8 |
| | Middle | 1.69 | 7.30 | 0.2 | 2.88 | 1.05 | | |

Unit= mm, area in $mm^2$, volume in $mm^3$; Amount of dentin removed = After Total volume - Before Total Volume = 1.2 $mm^3$

Conclusion: This newly developed technology provides a valuable tool to visualize 3D root canal morphology and quantify changes of root canal instrumentation and also provides an education model for future dentists.

**P62 Cariogenicity of Diets for Children Attending Head Start vs Daycares.** K. CZARKOWSKI,* J. E. KOWOLIK (Indiana University School of Dentistry)

This study reviewed and compared the dietary guidelines used in Early Head Start and Head Start groups with those for “at-home” care centers and purpose built kindergarten and daycare centers. This study examined the dietary habits of children in Head Start programs and child daycare centers according to food served throughout the school day. This study was limited to the ages of newborns through kindergarten, approximately 5 years of age. Survey questionnaires were distributed to daycare centers and the Head Start programs throughout the Indianapolis area. The data gathered from the questionnaires addressed the types of food consumed by children at school and any type of oral hygiene program implemented in the setting to teach children about tooth brushing and avoiding the consumption of cariogenic foods in their daily dietary habits. Using a Mantel-Haenszel chi-square test for the age comparison,

and Fisher's Exact Tests for all of the other comparisons; the statistically significant differences were: Head Start children were older, with a higher % of tooth cleaning, and a lower % of vegetables consumed as snacks, a higher % of chocolate milk drunk, but a lower % of birthday/class celebrations. The results suggest that the diets of children attending Head Start vs. daycares are statistically similar. This study was supported by Indiana University School of Dentistry.

**P63 Ex-vivo Evaluation of Electronic Apex Locator Accuracy in Primary Molars.** A. AL HOSAINY[1],* S. LIU[2], J. DEAN[2], I. ELKALLA[1] ([1]Mansoura University School of Dentistry; [2]Indiana University School of Dentistry)

One of the most critical and controversial issues in pediatric endodontics is to determine root canal working length in primary teeth. As root resorption progresses in the primary teeth, the apical foramen undergoes constant changes in dimension, shape, and position relative to the apex, causing misleading radiographic interpretation. Progressive root resorption may result in a wider apical foramen that gives a false reading of traditional apex locators, despite the proven reliability and accuracy in determining root canal length of non-resorptive permanent teeth. The aim of this study was to assess ex-vivo accuracy of Dentaport ZX electronic apex locator to determine root canal length in primary molars with different stages of root resorption. 135 extracted mandibular primary molars (a total of 502 root canals) were divided into three groups: Group A (n=45; 158 canals), molars without apparent root resorption, group B (n=45; 174 canals), molars with only apical third root resorption; group C (n=45; 170 canals), molars with more than one third but less than half root resorption. A K-type file was inserted into the root canal until visualizing the file tip at the major foramen. A stopper was held against the molar cusp tip. Actual canal length was obtained from the tip to the stopper using an endometer. The molars were embedded in alginate, serving as conducting medium, to simulate oral environment. Dentaport ZX electronic apex locator was used to measure canal length with the same K-type file. Within each group, the differences between actual and electronic measurements were compared using t-test and correlations between them were evaluated using Pearson's correlation test. Results: There was no statistically significant difference between the actual and the electronic measurements. Correlations between the two measurements were significantly high (0.968-0.996). Conclusion: The Dentaport ZX electronic apex locator was able to determine the root canal length of primary molars with high accuracy regardless of the stage of root resorption present.

**P64 Pediatric Dental Referral: Current Methodology and Resources Used by Pediatricians.** K. H. LUDWIG,* J. E. KOWOLIK (Indiana University School of Dentistry)

The purpose of this study was to evaluate the current referral methodology and resources employed by pediatricians when making dental referrals for children. A questionnaire was distributed amongst the 554 members of the Indiana chapter of the American Academy of Pediatrics (INAAP). This explored the current referral methodology; types of resources used by pediatricians and staff when making referrals; preferred attributes of dentists; and reported barriers to referral. It was distributed by email and was completed using an online survey. Data was collected and chi-square tests were used for comparisons. Responses were summarized using frequencies and percentages. Results, n=42, show 48.8% of participants make referrals for over 80% of their patients with 90% giving specific contact information when making referrals. Pediatricians were the sole provider of referral information 43% of the time. Participants reported that patients insured with Medicaid (50%), young patient age (33%), and a lack of information about available dentists (31%) were more often barriers to referral than knowing whether or not a referral is warranted (7%). Just over half of respondents believed their

referrals resulted in regular dental care in greater than 60% of their referrals. Participants stated they would refer to a dentist they did not know personally (88%) and would benefit from being provided a referral resource listing age of patients and payment types accepted (93%). Results did not show any difference in type of referral resource used or use of staff to provide referral information on both the percent of patients referred and the perceived success of the referral. Despite making a large number of referrals and providing specific contact information, pediatricians struggle in referring young patients and those insured with Medicaid, and they perceive that their referrals are often unsuccessful in patients receiving regular dental care.

## PERIODONTICS

**P65 The Responses of Human Neutrophils to Tobacco Smoke Components.**

N. AL-SHIBANI,* I. DKEIDEK, L. J. WINDSOR (Indiana University School of Dentistry)

The relationship between tobacco smoking and periodontal disease has been well documented. There is a large body of scientific evidence that smokers have an increased risk, incidence and severity of periodontal disease as evident by increased gingival recession, tooth loss, and periodontal destruction. Tobacco consists of about 6700 compounds and almost 4000 have been identified in tobacco smoke. These compounds include known carcinogens, toxic heavy metals, and many untested chemicals. Evidence has implicated neutrophils as the primary mediators of the host response against proliferating pathogenic microorganisms during periodontal disease through the oxidative mechanism by producing reactive oxygen species (ROS) which are primarily released to kill the bacteria. However, the extracellular release of ROS also results in collateral damage of the surrounding tissues. Many studies in the literature have focused on the adverse effects of nicotine on both cell-mediated and humoral immune responses, as well as on its effects on various cells of the body including neutrophils, epithelial cells, and fibroblasts, but minimal studies have evaluated the effects of other components of tobacco smoke on the neutrophils. The goal of this study was to evaluate the cytotoxicity of different components of tobacco smoke (2-naphthylamine, hydroquinone, acrolein, and acetyldehyde) on neutrophils as compared to nicotine and cigarette smoke condensate (CSC). Cell toxicity was determined by measuring the membrane damage of 2-naphthylamine, hydroquinone, acrolein, acetyldehyde, nicotine and CSC-treated cells utilizing dose responses. Neutrophils (22 × 106 cell/ml) were treated with the chemicals at various concentrations for 2 hours. Membrane damage was assessed using a cytotoxicity detection kit plus (Roche Applied Science, Indianapolis, IN) to establish the levels of lactate dehydrogenase released into the media by the cells. The cytotoxicity values for 2-naphthylamine was not significant at 0.3 mM (p value = 0.87), 0.6 mM (p value = 0.8), 1.25 mM (p value = 0.42), 2.5 mM (p value = 0.1), but was statistically significant at 5 mM (p value = 0.04), while the cytotoxicity values for hydroquinone was not significant at 0.3 mM (p value = 0.89), 0.6 mM (p = 0.414) or at 1.25 mM (p = 0.109), but was statistically significant at 2.5 mM (p value < 0.001). Both acrolein and acetyldehyde had similar results in that of neutrophils at any of the tested concentrations. For nicotine, the cytotoxicity was statistically not significant for 25 µg/ml (p = 0.1000), 50 µg/ml (p = a 0.194), and 100 µg/ml (p = 0.098), but was statistically significant at 200 µg/ml. For CSC, the cytotoxicity was statistically not significant for 25 µg/ml (p = 0.103), and 50 µg/ml (p = 0.017), but was statistically significant at 100 µg/ml. The results show the the maximal doses that can be used for these different chemicals on neutrophils for determining the reactive oxygen species production in response to these different components of tobacco smoke.

**P66 Cervical Enamel Projections and Pouch-Like Opening in Mandibular Furcations.** S. BLANCHARD[1],* G. DERDERIAN[2], V. JOHN[1], D. NEWELL[1] ([1]Indiana University School of Dentistry; [2]Marquette University School of Dentistry)

Cementoenamel projections (CEP) have been listed among the tooth associated risk factors that could lead to isolated furcation defects around molar teeth. This is more likely when Grade III CEPs are present. However the histological aspects of the CEP interface with regards to presence or absence of cementum over the enamel projection within the furcations has not been well described. This study was initially undertaken to evaluate this relationship. Thirty five mandibular molars with Grade III CEPs were evaluated for the presence of cementum covering these areas by stereomicroscopy (SM), light microscopy (LM) and scanning electron microscopy (SEM). Sixteen teeth (45.7%) appeared completely covered by cementum under examination with a dissecting microscope. SEM evaluation showed the presence of a narrow pouch-like opening between cementum and enamel in 15 of the 16 teeth (93.8%). LM evaluation confirmed the presence of the pouch along with some indication of residual degenerated mesenchymal tissue within the defects. SEM evaluation showed the presence of globular bodies in this pouch. Our study is the first to report the existence of this pouch-like opening between the enamel and cementum in mandibular molars with Grade III CEPs. The clinical significance of this finding has yet to be determined but microbial colonization of this opening may contribute to periodontal attachment loss in these furcal regions.

**P67 Pyrosequencing Reveals Significant Associations Between Ethnicity and the Subgingival Microbiome.** M. R. MASON[1],* V. JOSHI[2], H. FISCHBACH[1], P. S. KUMAR[1] ([1] The Ohio State University College of Dentistry; [2] Department of Periodontics, Maratha Mandal Dental College, Belgaum, India)

Introduction: It is known that the composition of the subgingival microbiome is influenced by several host-associated colonization factors; however, the contributions of ethnicity to bacterial colonization have been little explored. Since it is known that periodontal diseases have a racial and ethnic predilection, it is important to examine the effect of ethnicity on bacterial colonization in health. Objective: To compare the subgingival microbial profiles of periodontally healthy subjects belonging to four different ethnicities using next generation sequencing for bacterial identification and characterization. Methods: 100 periodontally healthy subjects of Caucasian (n=25), African-American (n=25), Hispanic (n=25), and Chinese (n=25) ethnicities were recruited. All subjects were adults without history of systemic disease, pregnancy, and recent or prophylactic antibiotic use. Subgingival plaque samples were collected, DNA isolated, and massively-parallel titanium bacterial tag-encoded FLX amplicon pyrosequencing was performed. Chimera-depleted sequences were compared against a locally-hosted curated database for bacterial identification. Shannon Diversity index and total number of species were computed. Non-parametric tests were used to compare bacterial levels between ethnic groups. Results: No differences were observed in the total number of species between ethnic groups. African Americans demonstrated significantly lower bacterial diversity than the other three ethnicities. African-Americans biofilms contained significantly more *Streptococcus* and less *Prevotella* genera than the other three ethnicities. The genera *Treponema* and *Porphyromonas* were detected in fewer African-Americans and Caucasians than Hispanics and Chinese. The genus *Neisseria* was signifcantly more prevalent in Chinese than the other three ethnicities. Conclusions: There is a significant association between ethnic preference and the bacterial composition of the health- compatible subgingival biofilm. However, the relative contributions of genetics and shared environment remain to be investigated. This research was supported by the Ohio State College of Dentistry CTOC T32 DE 0143220 training grant.

**P68 EGCG Inhibits Inflammatory Effects of Nicotine in Gingival Epithelium.** J. SHANGO,* A. KOKOIY, M. WHEATER (University of Detroit Mercy School of Dentistry)

Gingival tissues are exposed to nicotine as a result of tobacco use. It is known that nicotine can upregulate secretion of cytokines including IL-6 and IL-8 in gingival epithelial cells and fibroblasts. Objective: To determine if epigallocatechin gallate (EGCG), a major catechin component of green tea, will suppress nicotine-induced cytokine expression in cultured gingival epithelial cells. Methods: Primary human gingival epithelial cells were purchased from Zen-Bio (Research Triangle Park, NC). Confluent cells were treated for 24 hours with 0.1 mM nicotine with or without 10 µg/ml LPS or 10 µg/ml TNF-α in the presence or absence of 10 µg/ml EGCG. After 24 hours of treatment, culture medium samples were assayed for IL-6, IL-8, or IL-10 concentrations using ELISA. Results were standardized to total cell number using crystal violet blue staining. In separate experiments cells were treated for 24 hours as described and assayed for cytotoxicity using a colorimetric LDH protocol. Statistical analysis was completed using ANOVA with probability set at $p \leq 0.05$. Results: EGCG significantly inhibited cytotoxicity induced by nicotine, nicotine/LPS, and nicotine/LPS/TNF-α ($p \leq 0.05$ for all comparisons). EGCG significantly suppressed IL-8 secretion, but significantly increased IL-6 and IL-10 secretion, in gingival epithelial cells treated with combinations of nicotine, LPS, and TNF-α ($p \leq 0.05$ for all comparisons). Conclusions: In tobacco users, a combination of nicotine and bacterial infection (LPS) or inflammatory reactions (TNF-α) result in increased cytokine production. This in turn may contribute to increased breakdown of periodontal tissues. This study suggests that tea catechins such as EGCG function as effective natural anti-inflammatory compounds in the oral cavity, functioning to suppress pro-inflammatory cytokines and/or TNF-α. This information could be used to support the use of tea as a natural anti-inflammatory agent to potentially suppress the progression of gingivitis and peridontitis in tobacco users.

**P69 Calcium Sulfate Versus Freeze Dried Bone Allograft for Ridge Preservation.** S. TOLOUE[1],* G. J. ECKERT[2], M. KOWOLIK[1], V. JOHN[1], S. ZUNT[1], A. BRUZZANITI[1], S. BLANCHARD[1] ([1]Indiana University School of Dentistry; [2]Indiana University School of Medicine)

Orthodontists commonly use functional appliances to stimulate condylar growth by displacing the mandible forward when treating patients with a mandibular deficiency. Increased administration of dietary minerals, such as fluoride, has been shown to increase condylar thickness and density. The cumulative effects of condylar displacement and increased fluoride concentrations have not been previously investigated. Therefore, the objective of this study was to evaluate the effects of increased fluoride administration during lateral displacement of the condyle in a rat model. Thirty-two, 4-week old rats were fitted with a custom maxillary acrylic appliance, which caused their mandibles to permanently shift laterally. All rats were placed on a soft diet and were randomly assigned to two groups. One group received distilled water (control), while the other received distilled water with 100 ppm NaF. For 2 weeks the animals were allowed to eat and drink ad libitum. A paired T-test was used to assess differences between the displaced and non-displaced condyles. A Mann Whitney U test was used to evaluate differences between condyles exposed to distilled water or distilled water with 100 ppm NaF. Significant differences in condylar length, bone volume, bone surface, mineralization, porosity, and trabecular connectivity were observed ($p \leq 0.05$) between the distracted and non-distracted condyles, but this difference was independent of the water consumed. We concluded that lateral displacement of the mandible results in significant changes of the distracted condyle. Increased fluoride administration, however, did not produce a significant difference in condylar morphology or mineral composition. Funded by IUPUI Support for the Recruitment of Under-Represented Faculty (SURF) and Indiana University School of Dentistry.

## REGENERATION

**P70 The Homology of EVI5 and ABK Sequences among Animals.** C. SWIHART,* Y. LIU, F. SONG (Indiana University School of Dentistry)

Amphibian Axolotl (Ambystoma mexicanum) can regenerate its limb after amputation. During the early stage of regeneration, a G2 maintaining protein, ecotropic viral integration site 5 (EVI5), has dramatically increased, which suggests the delay of cell entering mitosis. Aurora B Kinase (ABK) assists to degrade EVI5, which moves cell from G2 to mitosis. Unfortunately, sequence information of either EVI5 or ABK was unknown for axolotl, which hinders future study on their roles in axolotl limb regeneration. The cDNA sequences of ABK and EVI5 from human, mouse, rat, and xenopus were then collected from GeneBank database and compared utilizing ClustalW software. Total RNA was extracted from axolotl tissue for degenerate reverse transcriptase polymerase chain reaction (RT-PCR). GADPH was used as an internal control. The RT-PCR products were analyzed by two direction sequencing. After comparison, 342 bp of nucleotides from EVI5 and 1035 bp from ABK were highly conservative among animals and used as templates to design primers for degenerate RT-PCR. The sequencing analysis on axolotl RT-PCR products identified 166 bp and 261 bp nucleotides for EVI5 and ABK, respectively. This study provided us the partial sequence information of axolotl EVI5 and ABK, which advance the study on their roles in regeneration. This study is supported by IUPUI Life-Science Health Internship Program, the IUSD Start-up Grant and IUPUI Research Support Funds Grant (RSFG) to F. Song

**P71 The Comparison on cDNA Sequence of Early Mitotic Inhibitor 1.** J. ELIKOFER[1],* F. SONG[2] ([1]Indiana University School of Science, Department of Biology; [2]Indiana University School of Dentistry)

Regeneration of oral tissues through chemical induction may provide exciting new treatment options for acute and chronic oral conditions and/or diseases. Elucidation of molecular mechanisms by which regeneration capable organisms, such as urodele salamander axolotl, regenerate their tissues will play a key role in the development of treatments. Preliminary studies have shown the significant up-regulation of cell cycle regulatory protein, ecotopic viral integration site 5 (EVI5), during salamander limb regeneration. EVI5 functions as a regulator of cell cycle progression through the stabilization of early mitotic inhibitor 1 (Emi1). However neither the sequence of EVI5 nor that of Emi1 is available in axolotl for further investigation. The objective of this study was to compare Emi1 sequences cross-genus to determine a highly conservative region which can be used in degenerate PCR technique to identify the Emi1 sequence in axolotl . Emi1 cDNA sequences of humans, zebrafish, frog, yeast, mouse, chicken, and cattle were collected from GenBank database and their sequences were compared utilizing ClustalW2 software. The sequence alignment scores cross-genus ranged from 49% to 87%. The comparison between mammalian and amphibian maintain low scores, 57%-73%, while the scores within the same genus were higher than 80%. Xenopus family shared 86% to 90% similarity on their Emi1 cDNA sequences. Given this data, we propose that relatively high sequence homology will be shared between Xenopus and Salamandra. Xenopus Emi1 cDNA can provide a relatively accurate template for the development of primers for axolotl used in degenerate PCR study. This study is supported by IUSD Start-up Grant and IUPUI Research Support Funds Grant (RSFG) to F. Song.

## SALIVARY RESEARCH

**P72 Characterization of the Salivary Proteome/Peptidome in Diabetics and Healthy Controls.** J. SALMERON,* R. JUREVIC, K. LUNDBERG (Case Western Reserve University)

The constellation of oral effects of diabetes includes xerostomia, increased susceptibility to infection, complications in wound healing, increased incidence of periodontal disease, and other defect associated with epithelial barrier function. Proteomic and peptidomic analysis of saliva could lead to the identification of potential biomarkers for both diagnosis and monitoring of disease progression and therapeutic outcomes. Objectives: Expression proteomics analysis using a label-free approach was performed on whole un-stimulated saliva obtained from affected persons and controls. Methods: A total of 3 saliva samples per subject were prepared from each group, which consisted of 20 individuals and was fractionated using a 3K cut off filter to isolate the peptidome from the proteome. Samples were prepared for Lys-C digestion. Digests were analyzed by LC/MS/MS via capillary liquid chromatography and a LTQ-FT. Automated differential quantification of peptides was accomplished using Rosetta Elucidator. Peptide and protein identifications were integrated with these quantifications and used for statistical analysis via one way ANOVA. Results: Utilizing label free protein expression enabled effective fractionation of a complex sample and robust protein quantification, leading to the identification and quantification of approximately 130 proteins and peptides. Conclusion: Further analysis may uncover a relationship between some of these proteins and peptides with the diabetic condition. Supported by grants from NIH/NIDCR: 1 K23DE016110-1, P01DE019759-01

## TOBACCO

**P73 Effect of Nicotine on Growth and Metabolism of *Streptococcus mutans*.** R. HUANG,* M. LI, R. L. GREGORY (Indiana University School of Dentistry)

Dental caries is one of the world's most prevalent diseases, and *Streptococcus mutans* is known as the chief pathogen for caries. The association between tobacco smoking and periodontal diseases is well known, but the association between tobacco and caries is little investigated. Nicotine is the most important component in tobacco, therefore, research on the effect of nicotine on *S. mutans* is necessary. Seven *S. mutans* strains were used in the present study: UA159 (ATCC 700610), UA130 (ATCC 700611), 10449 (ATCC 25175), A32-2, NG8, LM7, and OMZ175. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), planktonic cell growth, biofilm formation, and biofilm metabolism of the seven *S. mutans* strains treated with different concentrations of nicotine were investigated. For MIC/MBC experiments, a two-fold dilution method was used. For planktonic cell growth, the absorbance of 24-hour cultures were recorded. For biofilm formation, 24-hour biofilms were stained with crystal violet and the absorbance of extracted crystal violet was recorded. For biofilm metabolic activity, an XTT method was used. The MIC and MBC were 16 mg/ml (0.1 M/ml) and 32 mg/ml (0.2 M/ml), respectively, for all the *S. mutans* strains. Although there were variations between strains, the planktonic growth trends of *S. mutans* were the same, that was significantly upregulated ($P<0.05$) by low nicotine concentration treatment, but growth was significantly repressed by high nicotine concentration treatment. Most of the strains significantly increased biofilm formation in a nicotine-dependent manner, but at 8.0 mg/ml strains 10449 and OMZ175 biofilm formation was significantly decreased. For the biofilm metabolic activity test, all the strains significantly increased metabolism in a nicotine-dependent manner, except at nicotine concentrations of 32.0 mg/ml. Nicotine enhances *S. mutans* planktonic growth, biofilm formation, and biofilm metabolic activity. These results suggest smoking can increase the

development of caries. This work was partially funded by the Indiana University- Purdue University Tobacco Cessation and Biobehavioral Group, and Indiana University School of Dentistry Ph.D. Student Research Fund.

**P74 Effects of Orbs® on p53 and p21 in Human Gingival Fibroblasts.** H. MOTEVASELOLHAGH,* K. BONTRAGER, W. ZHANG, N. AL-SHIBANI, L. J. WINDSOR (Indiana University School of Dentistry)

Tobacco companies are promoting alternative smokeless and spit-less tobacco products as a result of the bans on smoking in public places. Camel Orbs® comes in a twisted stick (Mellow Sticks) or as film strips that dissolves on the tongue (Fresh Strips). These strips and sticks contain some of the same harmful chemicals as other tobacco products such as cigarettes and chewing tobacco. Tobacco has been shown to affect the proliferation of human gingival fibroblasts (HGFs), as well as to induce inflammatory cytokines/growth factors. Tobacco products have also been shown to alter cell cycle checkpoint proteins such as p53 and p21, which are tumor suppressors. The goals of this project were to investigate the effects that extracts of Camel Orbs® have on the proliferation, cytotoxicity, and p53 and p21 expression of HGFs. The Orbs® strips and sticks was extracted by incubating 1 g/ml of product in distilled water by placing them on a shaker at 37 C0 for 1 hour. Human gingival fibroblasts were seeded in 6 well plates at a concentration of 75,000 cells per well. They were then exposed to different concentrations (0.2 g/ml, 0.1 g/ml, 0.05 g/ml, 0.025 g/ml and 0.0125 g/ml per well) of the extracts diluted in serum free media for 72 hours. Cell proliferation and cell cytotoxicity were determined by WST-1 assays and lactate dehydrogenase released into media utilizing a Cytotoxicity Detection Kit Plus, respectively. The highest concentrations of the extracts that did not result in significant cell death or decrease in proliferation compared to the control group was selected in this study. The cells were incubated at these concentrations to determine their effects on the expression of p53 and p21 by Western blot analyses. Extracts of these tobacco products altered p53 and p21 protein levels. The data for this experiment is still being analyzed, and the results will be updated. This work was supported by IUPUI Life-Health Sciences Internship Program and IUSD Tobacco Cessation and Biobehavioral Group.

**P75 CSC Affects the Reparative Ability of Human Dental Pulp Cells.** Y. LIU[1,2], * M. PATEL[3], E. GROW[4], N. SANTOSH[1,5], J. SUN[1,6], W. ZHANG[1,6], F. SONG[1,5,6] ([1]Indiana University School of Dentistry; [2]State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, P. R. China; [3]Department of Biology, Indiana University College of Arts and Sciences; [4]Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis; [5]Center of Regenerative Biology & Medicine, Indiana University; [6]Tobacco Cessation and Biobehavioral Group)

Smoking has been shown to be related to dental caries, but the mechanism underneath is not clear. The aim of this study was to investigate the effects of the cigarette smoke condensate (CSC) on the remodeling and reparative ability of human dental pulp cells in order to understand the mechanism of the caries associated with tobacco usage. The cell proliferation and cytotoxicity of CSC were evaluated by the Water-Soluble Tetrazolium-1 (WST-1) Assays and Lactate Dehydrogenase (LDH) Assays, respectively. The low (25 ug/ml) or high (200 ug/ml) concentration of CSC was applied to human dental pulp cells and their effects on the MMP activity, expression levels of dentin associated proteins, and multiple cytokines expression were detected by zymography, reverse transcription polymerase chain reaction (RT-PCR), and cytokine array, respectively. The results showed that CSC increased the expression of both pro- (1.7 folds, $P<0.05$) and active MMP-2 (46 folds, $P<0.05$) released in the conditioned media,

while decreased the mRNA expression of the major dentin associated proteins down to 0.7 fold. The expression levels of cytokines such as interleukins, Growth Regulated Oncogene proteins, monocyte chemoattractant protein, and tumor necrosis factor-alpha (TNF-α), which involved in the immune responses and inflammatory regulations, were also altered by CSC. It is suggested that CSC disturbed the balance of dentin matrix accumulation and degradation by inhibiting the immune defense, suppressing the expression of dentin matrix proteins, and enhancing the activity of MMP-2, the extra cellular matrix degrading enzyme. Once the balance was broken, the dentin destruction was facilitated, and this might contribute to dental caries. Supported by the Indiana University School of Dentistry Start-up Fund to F. Song, by the Chinese Scholar Fellowship from Chinese Government Scholar Administration to Y. Liu

**P76 An Evaluation of Tobacco-Related Continuing Education Courses for Dental Providers.** R. H. LOVE III,* L. M. ROMITO (Indiana University School of Dentistry)

Similar to diabetes mellitus and cardiovascular disease, tobacco dependence is considered a chronic condition which can affect dental patient management and oral health status. The purpose of this study was to quantify tobacco-related Continuing Education (CE) courses offered to dental professionals and compare their numbers to the number of CE courses concerning dental management of diabetes and concerning cardiovascular disease. Titles and descriptions of CE courses offered by all U.S. dental schools, the annual session of the American Dental Association, and the 50 U.S. state dental associations between January 1, 2008 and January 1, 2010 were searched for specific terms related to tobacco, systemic cardiovascular diseases and diabetes. Each CE course which met the search criteria was recorded in a spreadsheet along with the title of course, presenter, sponsor, credit hours, and location. Preliminary results obtained from 18 state dental associations and 10 U.S. dental schools indicate that 8 courses were held on diabetes, 10 courses on cardiovascular disease and 7 on tobacco-related topics. These results suggest that of the more than 1100 CE courses reviewed thus far which were offered to dental professionals during the two-year period, a very small percentage were available in these topic areas. However, the number of tobacco-related CE offerings appears to be similar to the number of CE courses focusing on the dental management of patients with cardiovascular disease and diabetes mellitus. The study was supported, in part, by the Indiana University School of Dentistry Dental Student Research Fund.

**P77 Promotional Practices for Camel Dissolvable Tobacco in a U.S. Test Market.** L. COAN[1], M. K. SAXTON[2], L. ROMITO[1],* ([1]Indiana University School of Dentistry; [2]Kelley School of Business, Indiana University-Purdue University Indianapolis)

Tobacco companies are developing and marketing new smokeless products to maintain their consumer base. This study assessed the retail promotional strategies and public perceptions for RJR's new line of dissolvable tobacco branded Camel Orbs, Strips and Sticks. A field audit was conducted of retail stores (N=81) in the Indiana test market. Data included: store type, location, product placement, forms & flavors, price, promotions, and advertisement types, locations and messages. Consumer surveys assessing awareness and perceptions of the products were also conducted. The product line was carried by 46% (N=37) of stores, most frequently by gas stations (100%) and convenience stores (75%). Most stores (84%) carried all 3 forms. The Dissolvables were displayed with other smokeless products (70%), cigarettes (25%) or candy (5%). Prices ranged from $3.59 -$4.19 per unit pack. Most stores carried at least 1 promotional item. Free samples were offered in 14% of stores. In 84% of stores, ads were located right next to the products. Ad messages included: "Dissolvable Tobacco" (60%), "Free Trial" (24%), "Special Price" (24%), "What's Your Style?" (22%), "Now Available" 11%). Product awareness was reported by 42% of respondents (N=243), and trial by 3%. While consumer interest was

low, younger respondents (<40 years) were more familiar with Camel Dissolvables (60% vs. 45% for those >40 years, p<.01); males, as well as current and former smokers had higher rates of interest and trial of Camel Dissolvables. Current retail promotional strategies appear to target existing smokers and support dual tobacco use.

**P78 Dissolvable Tobacco Effects on Growth and Adherence of *Streptococcus mutans*.** B. SCHEER,* R. L. GREGORY (Indiana University School of Dentistry)

Objectives: Smokeless tobacco use and periodontal disease and oral cancer have been linked. Tobacco's role in dental caries, however, is less well-defined. The aim of this research was to investigate any role that smokeless dissolvable tobacco (DT) may have in the etiology of dental caries by investigating the effect that novel DT products have on growth and sucrose-dependent adherence (SDA) of *Streptococcus mutans* UA159. *S. mutans* UA159 was incubated with DT extracts in order to assay these virulence factors. Methods: Extracts of Camel Sticks, Strips and Orbs DT products were used. *S. mutans* was incubated overnight in tryptic soy broth (TSB) with DT extracts and sucrose-independent growth was monitored using a microplate reader every 10min for 12h. *S. mutans* was also incubated overnight in TSB containing sucrose with DT extracts to measure SDA by determining the proportion of tightly-adhered biofilm to planktonic cells. Results: The sucrose-independent growth assay demonstrated that 4mg/ml nicotine completely inhibited *S. mutans* growth. DT products overall significantly ($p<0.05$) delayed *S. mutans* growth (increased lag time ranging from 11.3-28.2%), with the sticks and orbs having the strongest effect. However, they increased maximum velocity of the log phase (9.7 to 25.6% increase) and increased maximum amount of growth (4.9-27.5% increase). The SDA assay indicated that nicotine significantly increased biofilm formation while the DT products inhibited biofilm (27.3-36% decrease) and enhanced planktonic growth (5.9-23.7% increase). Conclusion: These results provide the first evidence for the effect on oral bacteria of these new DT products promoted to reduce harm. The data indicates significant alterations in *S. mutans* sucrose-independent and –dependent growth and attachment to smooth surfaces suggesting that these effects may contribute to changes in individuals who use the DT products. Supported, in part, by the Indiana University School of Dentistry Dental Student Research Fund.

**P79 Cigarette Smoke Condensate and Nicotine on Collagen Degradation.** W. ZHANG,* M. FANG, F. SONG, L. J. WINDSOR (Indiana University School of Dentistry)

Introduction: The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, which can digest the extracellular matrix. The tissue inhibitors of metalloproteinases (TIMPs) are important endogenous MMP inhibitors. TIMP-2 serves as a bridging molecule for the binding of pro-MMP-2 to active MMP-14 on the cell membrane. This trimolecular complex results in pro-MMP-2 activation by MMP-14. The MMPs have been shown to be involved in periodontal disease. Risk factors for periodontal disease include smoking. Cigarette smoke condensate (CSC), the particulate matter of cigarette smoke, is comprised of thousands of chemicals. Nicotine is one of the active components in tobacco. Objective: This study compared the effects of the CSC and nicotine at the level in CSC on the collagen-degrading ability of human gingival fibroblasts (HGFs) and the expression of MMP-2, MMP-14 and TIMP-2. Materials and methods: HGFs were cultured from explants of clinically healthy gingival tissue removed from a non-smoking patient undergoing crown-lengthening surgery. HGFs were seeded as single colonies in 6-well Type I collagen coated plates and exposed to 100 µg/ml (2.4 µg/ml nicotine) CSC or 2.4 µg/ml nicotine. After three days, the conditioned media was collected and membrane proteins were extracted for gelatin zymography and western blot analyses. The mRNA levels of MMP-2, MMP-14 and TIMP-2 were measured by reverse transcription-polymerase chain reaction. All the experiments were repeated 3 times.

Statistical analyses were performed by a One-way Analysis of Variance (ANOVA) and the Tukey's test. Results: 100 µg/ml CSC increased the collagen degrading ability of HGFs (1.57±0.48 fold, p=0.011) when compared to the control. Furthermore, CSC increased TIMP-2, MMP-14 and active MMP-2 in the membrane extracts (1.56±0.17 fold, p=0.003; 1.57±0.19 fold, p<0.001; and 1.82±0.73 fold, p=0.019, respectively), as well as mRNA levels of MMP-2, MMP-14, and TIMP-2 (1.62±0.07 fold, p=0.10; 0.90±0.01 fold, p=0.257; and 1.65±0.02 fold, p=0.09, respectively). In addition, CSC increased the active form of MMP-2 in the conditioned media (1.36±0.18, p=0.043). However, 2.4 µg/ml of nicotine alone had little influence on the collagen degrading ability of the HGFs (1.01±0.29 fold, p=0.99), as well as on the membrane protein levels of proMMP-2, active MMP-2, MMP-14, and TIMP-2 (1.10±0.19 fold; p=0.62; 1.09±0.031 fold, p=0.79; 1.04±0.12 fold, p=0.92; and 1.30±0.48 fold, p=0.53, respectively) and on the mRNA levels of MMP-2, MMP-14, and TIMP-2 (1.62±0.07 fold, p=0.10; 0.90±0.01 fold, p=0.257; and 1.65±0.02 fold, p=0.09, respectively). Summary and Conclusions: CSC may increase HGF-mediated collagen degradation by affecting membrane associated MMPs/TIMPs and that the level of nicotine in the CSC may only play a limited role in this process. This project was supported by the IUPUI Tobacco Cessation and Biobehavioral Group.

## CARIOLOGY

**P80 Thermal Studies of 960-nm Diode Laser Irradiation on Human Enamel.** E.K. KOHARA[1],* I.T. KATO[2], D.M. ZEZELL[2], N.U. WETTER[2] ([1]Indiana University School of Dentistry; [2]Center for Lasers and Applications, Instituto de Pesquisas Energéticas e Nucleares, Universidade de Sao Paulo, Brazil)

Dental enamel becomes more resistant to caries after chemical and physical alterations induced by laser irradiation. The aim of this study is to demonstrate that 960-nm diode laser irradiation can increase the temperature in enamel surface without pulp tissue damage. For superficial temperature measurements, a thermal camera and a quick-response thermocouple were used. To register intrapulpal temperature, a type-K thermocouple was positioned inside the pulp chamber of a lower incisor with endodontic access. SEM analysis was also done in the irradiated surfaces of enamel. Laser parameters used in this study were: peak power of 6.5 W, quasi-continuous mode, pulse duration of 5 ms, repetition rate of 10Hz, and intensity of 2.29kW/cm$^2$. In intrapulpal investigation, enamel samples were irradiated during 10 s (100 pulses), and for surface measurement one single pulse was applied on enamel surface. Samples were covered with charcoal powder prior the irradiation. Enamel surface registered temperature rises between 74.9 °C and 110.0 °C, whereas intrapulpal heating was 2.58 °C. SEM showed some superficial changes in the irradiated area. The results showed that 960-nm laser irradiation can promote morphological changes in the enamel surface, with safe parameters to the pulp tissues. Grants CAPES/Procad (0349/054), FAPESP (95/9503-5).

## CLINICAL CASE REPORTS

### AESTHETIC DENTISTRY

**CC1 Conservative Cast Gold Inlay Restoration on a Maxillary First Premolar.**
N. RORICK,*T. CARLSON, S. CHO (Indiana University School of Dentistry)

The use of gold as a restorative material has many advantages such as longevity, wear, imperceptible margins, and similar coefficient of thermal expansion to that of tooth structure. Additionally, unlike amalgam, gold does not discolor the teeth. Dr. R.V. Tucker has developed a unique technique for conservative cast gold restorations. A study reported excellent longevity in the restorations done with this technique. The purpose of this report is to present a clinical case in which a discolored and structurally compromised maxillary first premolar was restored with a conservative cast gold restoration with imperceptible margins. A 56 year-old female patient presented with a 25 year-old existing distal-occlusal amalgam on tooth #12 which had a crack on the mesial marginal ridge and significant discoloration due to the amalgam restoration. The R.V. Tucker conservative cast gold technique was utilized to fabricate a cast mesial-occlusal-distal Type II gold inlay. The existing DO amalgam and liner were removed. Next, the secondary caries was removed. Temporary composite buildup was used to block out the undercuts. The preparation was extended to include the mesial box. The preparation was completed with proper path of draw, retention and resistance form. A gingival bevel was placed. The walls of the preparation were meticulously smoothed with hand instruments and discs. An internal bevel was made on the mesial and distal boxes to assist in seating during cementation. Cord was placed and an impression was obtained with a check-bite tray. Dies were fabricated using the dowel pins. The inlay was waxed, invested, and cast. The restoration was cemented with zinc phosphate cement, finished, and polished so the margins were imperceptible to the explorer tip. The conservative cast gold inlay using Dr. R.V. Tucker's principles for conservative cast restorations produced a restoration that will not discolor the tooth, while restoring form and function.

### DIAGNOSTIC SYSTEMS

**CC2 Application of Cone Beam Computed Tomography (CBCT) in Endodontics**.
H. IQBAL,* K. J. SPOLNIK, M. M. VAIL (Indiana University School of Dentistry)

Cone-beam computerized tomography (CBCT) has existed since the last 1990s. However, recent technological advances have made cone-beam computed tomography (CBCT) a feasible option especially in the field of endodontics. Cone-beam technology uses a cone-shaped beam of radiation to acquire isometric voxels which allows for the identical length, width and depth in the projection. CBCT has the potential to revolutionize the specialty of endodontics with its increased accuracy and resolution, decreased radiation dosage, and low financial burden for the patients. This poster presentation aims to illustrate the various applications of CBCT in endodontics specifically in the diagnosis of apical periodontitis, assessment of root canal anatomy, and detection of root resorptions and fractures.

## ENDODONTICS

**CC3 Internal Root Resorption Repair with a Bioceramic Sealed Endodontic Stabilizer**.
B. BRASSEALE,* M. VAIL, K. J. SPOLNIK (Indiana University School of Dentistry)

Internal Root Resorption (IRR) is an inflammatory phenomenon that employs native odontoclasts to resorb intraradicular dentin by a mechanism similar to bone resorption. In permanent teeth the progressive erosion of intraradicular dentin is a pathologic process that can result in loss of root strength and eventual tooth loss if not intercepted by the Dentist. The following case report outlines a novel treatment of severe IRR of tooth #9 in a 56 year-old male. Surgery was performed to remove the resorptive lesion and root structure apical to lesion. A titanium endodontic stabilizer was then placed to restore adequate crown to root ratio and sealed with bioceramic repair material before grafting with demineralized freeze dried bone and resorbable membrane. The patient was followed for 9 months and demonstrated clinical and radiographic signs of healing. The result suggested that the superior biocompatibility, sealability, and handling characteristics of modern endodontic materials enable the clinical endodontist more predictable and reliable treatment options for treating and retaining teeth previously thought to be of a hopeless prognosis.

**CC4 Nonsurgical and Surgical Root Canal Therapy of a Maxillary Central Incisor**.
J. NAZZAL, K. J. SPOLNIK, M. M. VAIL (Indiana University School of Dentistry)

Hard tissue surrounding the dental pulp can take a variety of configurations and shapes. It is crucial to understand tooth morphology to achieve an unobstructed direct access to the root canal and apical foramina. The main objectives of root canal therapy are thorough shaping and cleaning of all pulp spaces and complete obturation of these spaces with an inert filling.1 Accessory canals are minute canals that extend horizontal, vertical, or lateral direction from the pulp to the periodontium. In the apical third 74% are found, 11% found in the middle third, and 15% in the cervical third.2 Accessory canals contain connective tissue and vessels but do not supply the pulp with sufficient circulation to form a collateral source of blood flow. Pathologically, they are significant because they serve as avenues for the passage of irritants from the pulp to the periodontium. The present case report illustrates the successful recognition and treatment of a maxillary central incisor with a lateral canal.

## PROSTHODONTICS

**CC5 Restoring the Vertical Dimension of Occlusion with Removable Partial Denture.**
S. S. BORZANGY,* J. A. LEVON (Indiana University School of Dentistry)

Removable partial dental prostheses (RPDP) are used to replace patient's missing teeth. They usually are considered in cases when the fixed dental prostheses cannot be fabricated. They are useful in restoring the patient's function, esthetic and phonetics. The objective of this clinical report is to describe a clinical technique to restore lost vertical dimension of occlusion with overlay RPDP. A 68 year old female patient presented to graduate prosthodontic clinic requesting the restoration of her missing teeth with fixed and removable dental prostheses. The patient presented with maxillary Kennedy Class II modification 1 and mandibular Kennedy Class III. She lost her PFM 4 unit fixed dental prosthesis # 3-6 and teeth # 14,15,16, 29,30 were missing. Her upper right segment was supererupted and hitting tooth # 31. There are several restorations including amalgams and gold crown # 19. The patient vertical dimension of

occlusion (VDO) was collapsed because she lost her posterior support “Turner Class I”. After oral hygiene assessment and caries control, surveyed porcelain fused to metal (PFM) crown was fabricated on tooth # 31. Then, upper and lower removable interim partial prostheses were fabricated to restore the patient’s VDO. After that upper and lower overlay RPDP were fabricated as final prostheses. The use of overlay RPDP is a viable option to restore the vertical dimension of occlusion.

**CC6 CAD/CAM Implant Overdenture Bar with Locator Attachments; Case Report.**

M. HAJJAJ,* J. A. LEVON (Indiana University School of Dentistry)

Edentulous patients with a severely resorbed mandible often experience problems with their dentures. Endosseous dental implants have been used successfully to improve the retention and stability of removable dentures. Rigid prosthesis anchorage with milled bars is associated with fewer prosthodontic maintenance efforts than overdentures with resilient denture stabilization. The objective of this clinical report is to describe the fabrication technique of CAD/CAM implant overdenture bar with locator attachments. A 70 year old white Caucasian female patient presented to the graduate prosthodontic clinic at Indiana University School of Dentistry requesting replacement of her old dentures. The patient was complaining of loose and unstable lower denture. The patient had 3 Branemark implants in the anterior mandible with 2 Spheroflex attachments. The patient was treatment planned to have an implant supported lower denture with locator overdenture bar and an upper conventional denture. Master impression was made with polyvinyl siloxane impression material using open tray impression coping splinted with Duralay resin. Accuracy of master cast was checked via verification jig made of Triad resin. Complete teeth set up was made, and occlusion, esthetics and phonetics were evaluated. The master cast was then scanned using Nobel Procera Conoscopic Scanner and the bar was designed using computer software. The bar was milled in titanium and 3 locator abutments were tapped and threaded in. The milled bar was tried in and periapical x-rays were taken to verify complete passive seating. Later, the master cast with the bar was duplicated, and the denture was processed and delivered. The patient was very comfortable and satisfied with the esthetic and functional outcomes of the treatment. The use of CAD/CAM implant overdenture bar with locator attachments is considered a viable treatment option to improve retention, support and stability for overdenture in challenging cases.

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## MONOGRAPH ADDENDUM

## NINETEENTH ANNUAL RESEARCH DAY 2011

**P80 Thermal Studies of 960-nm Diode Laser Irradiation on Human Enamel.** E.K. KOHARA[1],* I.T. KATO[2], D.M. ZEZELL[2], N.U. WETTER[2] ([1]Indiana University School of Dentistry; [2]Center for Lasers and Applications, Instituto de Pesquisas Energéticas e Nucleares, Universidade de Sao Paulo, Brazil)

Dental enamel becomes more resistant to caries after chemical and physical alterations induced by laser irradiation. The aim of this study is to demonstrate that 960-nm diode laser irradiation can increase the temperature in enamel surface without pulp tissue damage. For superficial temperature measurements, a thermal camera and a quick-response thermocouple were used. To register intrapulpal temperature, a type-K thermocouple was positioned inside the pulp chamber of a lower incisor with endodontic access. SEM analysis was also done in the irradiated surfaces of enamel. Laser parameters used in this study were: peak power of 6.5 W, quasi-continuous mode, pulse duration of 5 ms, repetition rate of 10Hz, and intensity of 2.29kW/cm$^2$. In intrapulpal investigation, enamel samples were irradiated during 10 s (100 pulses), and for surface measurement one single pulse was applied on enamel surface. Samples were covered with charcoal powder prior the irradiation. Enamel surface registered temperature rises between 74.9 °C and 110.0 °C, whereas intrapulpal heating was 2.58 °C. SEM showed some superficial changes in the irradiated area. The results showed that 960-nm laser irradiation can promote morphological changes in the enamel surface, with safe parameters to the pulp tissues. Grants CAPES/Procad (0349/054), FAPESP (95/9503-5).

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## EXHIBITOR

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***P69 Calcium Sulfate Versus Freeze Dried Bone Allograft for Ridge Preservation.** S. TOLOUE[1],* G. J. ECKERT[2], M. KOWOLIK[1], V. JOHN[1], S. ZUNT[1], A. BRUZZANITI[1], S. BLANCHARD[1] ([1]Indiana University School of Dentistry; [2]Indiana University School of Medicine)

It has been well documented that there is significant ridge resorption following tooth extraction. Many materials have been used for preservation of post-extraction ridge dimensions but many practitioners utilize freeze dried bone allograft (FDBA) as their preferred material of choice. FDBA has been well documented as an effective ridge preservation material however patients may have concerns with FDBA over the potential for disease transmission or religious issues. Calcium sulfate has been used as a bone substitute for over 100 years due to its abundant availability, low cost and resorbability. It has been shown to be safe, biodegradable and osteoconductive. The objective of this study is to evaluate the effectiveness of *DentoGen*® (calcium sulfate) in preserving post extraction ridge dimensions compared to FDBA. Thirty consecutive extraction sites from non molar, single rooted sites were selected that met the inclusion criteria for the study. Post extraction clinical measurements of ridge dimensions were made by 2 examiners with the use of a pre-fabricated stent and dental calipers. The sites were then divided randomly into the test group (calcium sulfate) or the control group (FDBA). Patients were recalled after 3 months, sites were reentered and clinical measurements were again made. A trephine bone core was harvested and sent for histomorphometric analysis. A total of 21 subjects with 41 potential sites were recruited to this study (IRB approval # 1003-56). Following extraction, 29 sites met the inclusion criteria. To date, 10 test sites and 10 control sites have been evaluated. There was no significant change in vertical ridge height pre to post surgery within the test and control groups (0.53 $\pm$ 1.63 mm, 0.35 + 1.13 mm, respectively). There was a significant decrease in buccal-lingual ridge width within both groups, (-1.23 $\pm$ 1.14 mm test group. 0.93 $\pm$ 0.94 mm control group) but no significant differences between each group. Despite the decrease in ridge width for both groups, the overall preservation was maintained at 83% and 87% which is consistent from previous reports on socket preservation. There was no significant difference in the preservation performance between the two treatment groups for both ridge width and vertical height. Histological samples are currently being analyzed. Clinically, the cores taken from test sites appeared to have a more homogeneous solid texture where cores taken from the control sites appeared to have residual graft material visible. Results suggest no statically significant differences between the use of calcium sulfate versus FDBA in preserving post extraction ridge dimensions. These results indicate that calcium sulfate will be as effective as FDBA in preserving post extraction ridge dimensions. Supported by Orthogen LLC.

***The abstract contained in the print monograph is incorrect. Above is the correct abstract for Dr. Toloue's presentation.***


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