INDIANA UNIVERSITY
SCHOOL OF DENTISTRY
RESEARCH DAY

Eighteenth Annual Proceedings

April 12, 2010

Presented in Association with

Indiana Section, American

Association for Dental Research

RESEARCH DAY
Indiana University School of Dentistry
Eighteenth Annual Proceedings
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Contents

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
AMERICAN ASSOCIATION FOR DENTAL RESEARCH

INDIANA SECTION

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

April 12, 2010

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 18th 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.

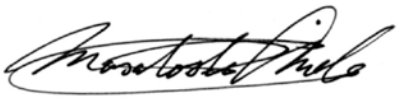
The INAADR sponsors or co-sponsors one scientific seminar per month on average, and is an integral Research Day Sponsor. This event showcases advances in IUSD's basic and clinical dental research, with an emphasis on encouraging our students to present their work and fostering opportunities for research 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. David T. Wong, 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 2010. We hope that each of you will enjoy what promises to be an enlightening afternoon.

Sincerely,



Masotoshi Ando, DDS, PhD
President, Indiana Section of the AADR

Heather N. Hirsch
SRG President

Program
Campus Center 4th Floor

Thurs., April 8

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

Mon., April 12

10:30 a.m. – 12:30 p.m. Interschool Student Research Competition (CC 406)

12:30 p.m – 1:00 pm Registration

1:00 p.m.	Opening Remarks (CC 450A-B)	**Dr. Lawrence I. Goldblatt** Dean
1:05 p.m.	Welcome to IUPUI	**Dr. Kody Varahramyan** Vice Chancellor for Research, IUPUI
1:15 p.m.	Welcome and Introduction of Guests and Keynote Speaker	**Dr. Domenick T. Zero** Associate Dean for Research, IUSD
1:25 p.m.	Keynote Address	**Dr. David T. Wong** President, American Association for Dental Research
2:00 p.m.	Presentation of Awards	**Dr. Judy Chin** **Dr. Andréa Ferreira Zandoná** Indiana Section, AADR
2:25 p.m.	Acknowledgment of Special Sponsors and Announcements	**Dr. Masatoshi Ando** President, Indiana Section, AADR

2:30 p.m. – 4:00 p.m. Commercial Exhibitions (CC 450C)

Interschool Presentations (CC406)
Research Presentations (CC 405, 409)
2:30- 3:10 p.m.: Posters 1- 42
3:20 a.m.-4:00 p.m.: Posters 43 - 80; Clinical Case Reports 1-4

4:00 p.m. Removal of Posters

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

Keynote Speaker
David T. Wong DMD, DMSc

Dr. Wong is Felix & Mildred Yip Endowed Professor and Associate Dean of Research at the UCLA School of Dentistry. He is also the Director of the UCLA Dental Research Institute (DRI). Dr. Wong is an active and leading scientist in oral cancer and saliva diagnostics research. He has authored over 180 peer reviewed scientific publications. His research is funded by the NIH since 1986. He directs the UCLA Collaborative Oral Fluid Diagnostic Research Center, the UCLA comprehensive T32 Clinical Research Training program as well as the Laboratory of Head & Neck Oncology Research. He chaired the NIDCR Special Grant Review Study Section from 2002-2005. Currently he is a member of the NIH CSR Cancer Genetics Study Section, a fellow of the American Association for the Advancement of Sciences (AAAS), member of the ADA Council of Scientific Affairs and the president-elect of AADR.

Keynote Presentation
Salivary Diagnostics for Oral & Systemic Diseases

Saliva has long been considered a “mirror of the body” that reflects the state of a person’s overall health. A wide range of systemic diseases, such as diabetes and Sjögren’s syndrome, have oral manifestations that dentists can encounter in patients at various stages of disease development. Dentists are therefore ideally situated to monitor and treat oral disease progression, impaired salivary status, and various oral complications associated with systemic conditions.

In recent years, sparked by the investment from the National Institute of Dental & Craniofacial Research (NIDCR), saliva has attracted widespread interest as a diagnostic medium for rapid, point-of-care testing. The advantages of using saliva for disease diagnostics include ease of access, noninvasive sample collection, increased acceptance by patients, and reduced risks of infectious disease transmission. Oral samples are readily accessible as whole saliva or by sampling secretions from specific glands or gingival crevicular fluid.

Advances in the science of salivary diagnostics will lead to identification of disease signature patterns of candidate biomarkers and/or confirmation of genetic susceptibility for some conditions. The speed and scope of available tests are also likely to increase. As salivary diagnostic applications advance, dentists are encouraged to take leadership roles in integrating the tests and related technologies into clinical practice, consistent with the best available scientific evidence.

Research Day Award Categories

Undergraduate Students

The Procter & Gamble Undergraduate Student Award. This award is given by **Procter & Gamble** to the undergraduate student (non DDS) including dental hygiene students, who presents the best poster or table clinic on dental related research. The winner receives a monetary award, and the recipient's name is added to a plaque on permanent display in the dental school library.

Predoctoral Dental Students

The American Dental Association/Dentsply International Student Clinician Award. The winner of this award receives an expense-paid trip to the ADA's Fall Annual Session. First-, second-, and third-year dental students are eligible for the competition, which is supported by **Dentsply International,** of York, Pa. The award goes to the best table clinic or poster prepared by a member of the DDS class of 2009, 2010, or 2011. The recipient is presented with a personal plaque, and his or her name is added to a plaque on permanent display in the IUSD 3rd Floor Clinic. The winner's presentation will be entered in the national competition (in table clinic format) during the ADA's scientific session next fall.

Cyril S. Carr Dental Student Research Scholarship. This scholarship honors the memory of **Cyril S. Carr**, a 1916 graduate of the Indiana Dental College who was a dental practitioner in Indianapolis for half a century. All predoctoral dental students who have been engaged in research projects at the dental school are eligible for the Carr scholarship. Presentations and publications will be factors in selection of a winner. The recipient is selected by the **IUSD Research Committee.**

Interschool Dental Student Research Awards. Supported by **INAADR**, this award is given to any dental student from participating dental schools who presents the best poster or table clinic emphasizing dental research and has contributed over the years to dental research. Three students from each of the invited dental schools will compete for the **Interschool Traveling Research Award** against the other traveling schools as well as for **Interschool Research Award** against each other and three IUSD dental students identified in the pre-Research Day judging that occurs the night before. The winner receives a monetary award.

Johnson & Johnson Listerine Student Research Group Award. Supported by **Johnson & Johnson** and the **IUSD Student Research Group**, this award is given to any dental student who presents the best poster or table clinic emphasizing dental research and has contributed over the years to dental research while at IUSD. The winner receives a monetary award, and the recipient's name is added to a plaque on permanent display in the dental school library.

The Procter & Gamble Dental Student Award for Excellence in Preventive Oral Health Care. This award is given by **Procter & Gamble** to the dental student who presents the best poster or

table clinic emphasizing prevention of oral disease. The winner receives a monetary award, and the recipient's name is added to a plaque on permanent display in the dental school library.

Graduate Dental Students

The Indiana Dental Association Best Clinical Case Report Award for M.S.D. and M.S. students. This award has been established in 2009 by the **Indiana Dental Association** to recognize any M.S.D. and M.S. student presenting the most outstanding clinical case presentation in a poster or table clinic format. The case report demonstrates proficiency, skill, and expertise in the proper management of dental care. Case reports also allow assessment of proper clinical decisions that are supported by evidence-based published literature or for which valid justification is provided by the author. The winner receives a monetary award.

Maynard K. Hine Award for Excellence in Dental Research. Established in 1988, this award honors the late Maynard K. Hine, a professor of periodontics who served as dean of dentistry from 1945 to 1968 and as first chancellor of IUPUI from 1969 to 1973. The Hine award is supported by **Procter & Gamble** and sponsored and administered by the Indiana Section of the American Association for Dental Research. The winner receives a certificate and three years paid membership in the AADR. The recipients' name also is engraved on a plaque that is permanently displayed in the dental school library.

Trident Award for Innovation in Oral Care Research. Supported by **Cadbury Schweppes Americas Confectionary**, this award is available for all Ph.D. graduate students presenting a poster at Research Day. The award recipient will receive a certificate and a monetary award.

Wrigley Student Award. Supported by **Wrigley**, this award is available for all M.S.D. and M.S. graduate-level students presenting a poster at Research Day. The award recipient will receive a certificate and a monetary award.

Postdoctoral Fellows

Sunstar Americas Post-Doctoral Fellow Award . This award is being supported in 2010 for the first time by the **Sunstar Americas Inc.** and is available for all Postdoctoral fellows presenting a poster at research day. The research must have been conducted while a postdoctoral fellow and the researcher may not hold any faculty position for longer than a year after completing their Post-doctoral research. The award recipient will receive a certificate and a monetary award.

Faculty

IU School of Dentistry Alumni Association Distinguished Faculty Awards for Teaching and Research. These time-honored awards are given by the **Indiana University School of Dentistry Alumni Association**. They serve as an annual salute from the dental school's alumni to faculty members who have demonstrated exceptional talent as researchers and teachers. Candidates are selected by a committee of the IUSD Alumni Association's Board of Directors. Each recipient is presented with a plaque and a monetary award.

Research Day Organizing Committee

Masatoshi Ando, Chair
William Babler
Judith Chin
Sopanis Cho
Pamela Clark
Jeffrey Dean
Mark Dirlam
Andréa Ferreira Zandoná
Dominique Galli
Richard Gregory
Karen Gregson
Barbara Gushrowski
Paul Hancock
Heather Hirsch
John Justice
Luciana Kano-Wilson
Sean (Shih-Yao) Liu
Marilyn Richards
Adam Smith
Domenick Zero

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Past President: Burak Taskonak

Officers
IUSD Student Research Group

President: Heather Hirsch
Secretary: David Wagner
Faculty Advisers**:** Richard Gregory, Jeffrey Platt

Future Research Day Event: ***April 11th, 2011***

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

SCOTTSBURG MIDDLE SCHOOL

P1 Bad Breath.
J. ROMERO,* L. HOWSER,* M. KRAFT,* P. CLARK

This study was to determine the causes of bad breath. Information was gathered using the internet. Causes of bad breath include: using tobacco based products, not brushing properly, eating strong smelling foods, using certain medications, having bacteria in the mouth (Halitosis). Ways to prevent bad breath include: drinking water, chewing sugarless gum, brushing your teeth regularly, flossing, eating foods like yogurt, drinking orange juice, and herbs. Bad breath is common, but with good oral hygiene everyone can do something about it. Supported by Hoosier Uplands South Central Indiana Area Health Education Center.

P2 Tap Water vs. Bottled Water.
J. OAKS,* N. CAMPBELL,* S. BOWYER,* P. CLARK

This study was to determine whether bottled water of tap water is better for you. Research was done on the internet. The benefits of bottle water are: it's safe from bacterial infections; it is portable and tastes good. The benefits of tap water are: it contains fluoride, it's inexpensive, it is easily available, and it is regularly checked for purity. The drawbacks of bottled water are: it is expensive, it is acidic, it has no fluoride, and chemicals from the bottle can get into the water. The drawbacks of tap water are: inadequate purification, and contamination from pipes. We have found that tap water is better for your oral health, general health and your wallet. Supported by Hoosier Uplands South Central Indiana Area Health Education Center.

P3 To Swish…And What To Swish.
I. HUNEFELD,* C. AMICK,* P. CLARK

This study compares the benefits and disadvantages of commercial mouthwashes, all-natural oils and hydrogen peroxide and which options are best. Research was done on the internet. Benefits include: fighting bad breath, relieving mouth irritations, removal of debris, reduction of plaque, and reduction of gum disease. Disadvantages include: overuse, dehydration due to alcohol content, overdose due to swallowing, staining, mouth and tongue irritation or numbness, taste disorder, retention of sodium, sensitivity of tooth roots, swollen glands. Best options may vary depending on the desired result, but it is important to select a product with the ADA Seal. Supported by Hoosier Uplands South Central Indiana Area Health Education Center.

BEHAVIORAL SCIENCE

P4 Comparison of Children's Behavior Following Hospital-Based GA vs. Office-based GA.

M. RASCHE,* J. WEDDELL, B. SANDERS, J. JONES, M. SAXEN, A. TOMLIN

Indiana University School of Dentistry

The objective of the investigation was to determine if there were differences in patient behavior at recall visits following dental treatment performed under hospital-based general anesthesia vs. office-based general anesthesia. Retrospective chart auditing was performed in a pediatric dental office of patients who presented before the age of 36 months for an initial exam and were diagnosed with early childhood caries. Following the initial exam, the patients included in this study were treated under hospital-based GA or office-based GA. These patients were followed to determine their behavior at the 6, 12, and 18 month recall appointments. Preliminary analysis indicates positive behavior at the 6 month recall in 45% of hospital-based GA patients and 73% of office-based GA patients. The results suggest that there were no significant differences between groups for age, sex, behavior at initial exam, behavior at the 6 month recall. Significant differences were found between groups for number of treated teeth and race. Study Number: EX1002-16.

P5 Do Pediatricians Motivate Caregivers to Seek Dental Care for Children?

K. LUDWIG[1],* G. J. ECKERT[2], J. KOWOLIK[1]

[1] Indiana University School of Dentistry; [2] Indiana University School of Medicine

Objective: The purpose of this study was to evaluate the sources of information that motivate caregivers to seek dental care for their young children and to determine the role of pediatricians as a source. **Methods**: A questionnaire was given to caregivers in their first year of seeking dental care for a child under 5 years of age. This evaluated the significance of different sources of oral-health information, at-home oral-care practices, self-report of race/ethnicity, method of financing, and level of education. It was distributed in four clinic settings: 2 general dental practices, one accepting Medicaid and the other not; a pediatric dentist; and a corporate clinic primarily treating Medicaid patients. Responses were summarized using frequencies and percentages. Chi-square and Kruskall-Wallis tests were used for comparisons. **Results**: Responses (n=80) showed caregivers received information from a total of 9 different sources, averaging 1.7 sources per caregiver. Pediatrician (25%), Dentist (24%) and Family/Other (29%) were often named the most motivating source. Pediatricians provided information to 38% of the participants and were identified as the most motivating 67% of the time. Of those not receiving information from pediatricians, 44% named pediatricians as a source they would prefer. Caregivers were more likely to have been given information by the pediatrician if white than non-white (p=0.0439) and if older caregiver age (p=0.0361). Across all racial and demographic subgroups, participants placed a high value, 4.5 of 5, on the information supplied by pediatricians. **Conclusions**: Caregivers receive oral health information from a variety of sources. They place a high value on information supplied by pediatricians, commonly reporting

this to be the most motivational source. In an attempt to reduce dental disease in young children especially in underserved demographic groups, pediatricians should increase their efforts to supply oral-health information to caregivers advising a dental visit on the first birthday.

P6 Racial and Ethnic Disparities in Dental Access to Care and Use of Services.
C. MUNDY,* E. A. MARTÍNEZ-MIER

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 that financial issues are not the primary reason that most minority communities do not seek dental care. Based on this information, research still needs to be conducted to investigate other factors for lack of utilization of services in order to develop strategies to improve minorities' chance of seeking dental care. The purpose of this study is to gather information on barriers that prevent African Americans and English speaking Latinos to access dental 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. Eighteen African American participants and four Latinos answered the survey. 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. Preliminary results show that most respondents answered they could not get help with language translation and lack of transportation when they were sick. Most participants also reported it had been 6 months or less since they talked to a doctor and in the past 6 months they had had not had a dental visit. 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.

CARIOLOGY

P7 Beverage Consumption and Dental Caries of Individuals Living In Rural Mexican Communities.
B. CARLTON,* A. HOLT, E. A. MARTÍNEZ-MIER, G. MAUPOME-CARVANTES

Indiana University School of Dentistry

In more recent years, the oral health of individuals living in underserved communities has sparked the interest of many researchers in the oral health field. As part of a larger study that is investigating the effect rural settings have on the diet and in turn the oral health of individuals, the current project determined beverage intake and dental caries experience of individuals living in the rural, Mexican communities located within the central state of Hidalgo. Data collected as part of a service-learning program over the last eight years were analyzed for this project. All consented subjects were given an oral hard and soft tissue exam by dental students

to determine caries prevalence. Beverage consumption was assessed in a subset of participant via questionnaire. This subset of participants was also examined by investigators who used the International Caries Detection and Assessment System (ICADS). Results are presented for this subset of subjects. 4369 subjects participated in the service learning program which has taken place since 2002 and continues to be ongoing. Many of the subjects were under the age of 18 which constituted 52% of all participants. 406 subjects answered a beverage questionnaire and received an ICDAS exam. Most subjects exhibited some form of dental caries with the mean number of teeth with cavitated carious lesions ranging from 2.1 to 4.5. Dental caries ranged from very mild to more severe cases, with ICDAS severity scores ranging from 33.6 to 71.9. 100% of the subjects reported consuming sugared beverages at least occasionally. It is concluded that in this subset of participants from Mexican rural villages sugared beverage intake is extremely high and dental caries prevalence is high with many children presenting severe caries. Study supported by IUPUI MURI program and BiCCHEC Signature Center.

P8 Comparison Between Single and Multiple-species Microbial Caries-model Through Surface Characterization.

T. RIBEIRO[1],* M. FONTANA[2], G. J. ECKERT[1], D. ZERO[1], M. ANDO[1]

[1] Indiana University School of Dentistry; [2] University of Michigan School of Dentistry

The objective of this study was to compare the surface characteristics of demineralized enamel developed by five species [Group-1: *Streptococcus mutans* TH16 (serotype c), *Lactobacillus casei* (ATCC 7469), *Actinomyces naeslundi* (WVU 626), *Streptococcus parasanguis* (FW 213) and *Streptococcus salivarius* (strain 196)] and one species (Group-2: *Streptococcus mutans* TH16) cultures using microbial caries-model. Sixteen ground/polished 3x3x2mm human enamel blocks were equally divided into two groups. Specimens were demineralized for seven days. After demineralization, measurements were obtained by Optical Reflectometry [Reflection (Amplitude, %)], Surface Profilometry [Roughness (Ra, µm)], and Quantitative Light-induced Fluorescence [QLF (fluorescence loss, %)] with dehydration. At baseline, surface was hydrated. Fluorescence images were acquired at 1-second interval for ten seconds. During image acquisition, specimens were dehydrated with continuous compressed air. Change in fluorescence radiance per second (ΔFD) was obtained. After measurements, specimens were sectioned (100µm) and analyzed with Transverse Microradiography [TMR: lesion depth (LD: µm) and mineral loss (IML: vol%×µm)]. Means and standard deviations of TMR, Reflection and Roughness (Table 1) and those of ΔFD at each second (Table 2) are shown. There were no significant differences ($p>0.05$) between the Table 1 groups. In ΔFD at each second, there was difference ($p\leq0.05$) between groups. This study suggested possibly different characteristics of the demineralized enamel surface developed by single and multiple-species microbial caries models. Supported by NIH/NIDCR R21 DE018390-01A2.

P9 Detection of Secondary Caries with ICDAS, X-rays and Laser Fluorescence.

O. CAPIN[1],* M. DINIZ[2], G. J. ECKERT[3], A. FERREIRA ZANDONÁ[1]

[1] Indiana University School of Dentistry; [2] Araquara Dental School, Brazil; [3] Indiana University School of Medicine

Replacement of dental restorations accounts for around 75% of all operative work, and caries at the margins of restorations (CARS) is frequently a reason given by dentists for replacing restorations. Accurate detection of CARS is difficult with conventional techniques unless the lesion is relatively advanced and a significant amount of tissue has been lost. New methods to aid clinicians in the detection of CARS have been investigated. The objective of this study was to evaluate the validity and reproducibility of the International Caries Detection and Assessment Criteria (ICDAS), digital bite-wing radiographs (BW) and two infra-red laser fluorescence devices (DIAGNOdent (DD) and DIAGNOdent Pen - (DDPen); KaVo, Germany) for detecting caries around occlusal amalgam restorations. Ninety posterior permanent teeth with occlusal amalgam restorations varying from intact to cavitated margins were used. One occlusal site per tooth was selected and a photograph taken to identify the site. The teeth were mounted in dental models and placed in a phantom head. Two trained examiners assessed the teeth twice observing an one-week interval using: ICDAS, BW, DD and DDpen. Teeth were sectioned and evaluated under a Microscope (Nikon Micro photo – FXA, Japan) using a 10x magnification, a digital camera (Nikon Digital Camera DXM 1200, Japan), and an image capturing software (Nikon ACT – program version 2-62). Repeatability was assessed by intraclass correlation coefficients (ICCs). Specificity, sensitivity, accuracy, area under the ROC curve and Spearman correlation of each method with histology scores were estimated using bootstrap analyses. ICCs for intra-examiner repeatability were high for ICDAS (0.94-0.96), DD (0.89-0.97) and DDpen (0.91-0.97), except for BW (0.59-0.93). Inter-examiner repeatability was acceptable for ICDAS (0.83), BW (0.75), DD (0.88) and DDpen (0.89). Spearman correlation coefficients between the methods and histology scores varied from poor to moderate (DDpen=0.36< DD=0.37< BW=0.46< ICDAS=0.58). DD and DDpen had higher specificities (0.92) followed by BW (0.88) and ICDAS (0.75). For enamel caries lesion the sensitivity for ICDAS was 0.2 (D1) and 0.59 (D2) followed by DDpen 0.2 (D1) and 0.22 (D2), with DD having the lowest scores 0 (D1) and 0.9 (D2). For caries lesion in dentin ICDAS had the best sensitivity 0.61(D3) and 0.88 (D4) followed by BW 0.11(D3) and 0.68(D4) with DD having the lowest score 0.29 (D3) and 0.14(D4). The area under the ROC curve (Az) varied from 0.59 (BW) to 0.70 sensitivity overall (0.63) and BW and DD and DDpen had very low sensitivities (0.15-0.28). ICDAS visual criteria showed superior reproducibility and validity to detect occlusal caries lesions around amalgam restorations in permanent teeth, while, DD and DDpen presented the poorest capability to detect these lesions.

P10 Determination of Plaque and Plaque Fluid Fluoride Content in Situ.

E. A. MARTÍNEZ-MIER,* C. BUCKLEY, P. CHANDRAPPA, S. A. KELLY, D. T. ZERO

Indiana University School of Dentistry

Fluoride levels in plaque and plaque fluid are directly related to its anticaries effects. Therefore, models that incorporate these parameters while testing the anticaries potential of products would be valuable. The current project aimed at modifying an in situ model to measure plaque and plaque fluid fluoride with sufficient sensitivity and repeatability. 22 previously-consented subjects participated in a two-treatment, examiner-blind study. A modification of the Koulourides model with partially-demineralized enamel specimens covered with gauze was used. Two days before each leg, subjects received a dental cleaning. Under supervision, subjects brushed for one minute using 0 or 1100 mg F/mg F toothpaste. Then, subjects brushed twice daily for two weeks with their assigned toothpaste for one timed minute. At their next visit, plaque samples were obtained from the gauze. Several modifications to the collection and analysis techniques were tested. Plaque and plaque fluid fluoride were analyzed using modifications to the microdiffusion method and directly using a microelectrode. Plaque samples analyzed directly were acidified prior to analysis. Average (±SD) plaque fluid fluoride was 0.25 ± 0.12 mg F/mg for the 0 mg F/mg group, and 2.50 ± 0.82 for the 1110 mg F/mg group. Average (±SD) plaque fluoride was 2.03 ± 1.57 and 16.24 ± 14.28 mg F/mg, for the 0 mg F/mg group, measured directly and by diffusion; it was 41.23 ± 55.60 and 252.97 ±254.72 mg F/mg for the 1110 mg F/mg group. All differences between the 0 and 1100 mg F/mg groups were statistically significant ($p<0.01$). ICCs for repeated analysis ranged from 0.97 to 0.99. It is concluded that the technique rendered repeatable values when analyzing samples of unknown concentration and differentiated between groups exposed to different fluoride concentrations.

P11 Development of a Synthetic Orange Juice Surrogate for Erosion Studies.

T. SCARAMUCCI[1],* A. HARA[2], S. FERREIRA[1], I. AOKI[3], M. SOBRAL[1], D. ZERO[2]

[1] University of São Paulo School of Dentistry, São Paulo, Brazil; [2] Indiana University School of Dentistry; [3] University of São Paulo - Polytechnic School – Chem. Eng. Dept, São Paulo, Brazil

The composition of orange juices can vary over time and according to the origin of the fruit. Although citric acid solution (0.05M, pH3.8) is often used as orange juice substitute in dental erosion studies, it is unknown whether it can adequately represent the natural juice. The objective of this study was to create a synthetic solution and evaluate its ability to serve as a surrogate for natural orange juices by verifying its erosive potential. A synthetic solution (SJ) was formulated based on the concentration of main chemical components of natural orange juices. Its erosive potential was compared against two commercially available orange juices: Minute Maid® (MM) and Florida Natural® (FL) and 0.05M citric acid, pH 3.8 (CA), using an in vitro erosion-remineralization cycling model. Forty enamel and 40 root dentin specimens (4x4mm) were embedded in acrylic resin, ground flat and polished. Adhesive tapes were placed on the polished surface leaving a central exposed area of 4x1mm. The specimens were randomly assigned to the experimental groups (n=10) and immersed in the respective solutions for 5min, 6x/day for 5 days. Between the immersions and overnight they were stored in

artificial saliva. Enamel specimens were analyzed by surface Knoop microhardness (50g, 15s) and optical profilometry, while dentin specimens were analyzed only by optical profilometry. Means (standard-deviations) of surface microhardness change (SMC), in Knoop numbers; and surface loss, in µm.

Analysis	SJ	MM	FL	CA
Enamel SMC	-199.6(20.2)[a]	-202.2(17.3)[a]	-260.3(25.2)[b]	-283.0(10.4)[b]
Enamel loss	-0.53(0.13)[b]	-0.28(0.15)[b]	0.77(0.39)[a]	-2.79(0.61)[c]
Dentin loss	-4.66(0.63)[a]	-5.07(0.71)[a]	-7.11(1.63)[b]	-11.28(0.85)[c]

Different superscript letters indicate significant differences, in rows. High variation was observed between the two natural juices. Overall, the citric acid was the most erosive solution. The synthetic juice showed to be an adequate surrogate, but only for one of the orange juices tested. Supported by IUSD-Professional Development Program.

P12 Effect of Gap Geometry on Secondary Caries In Vitro.

H. NASSAR[1],* C. GONZÁLES-CABEZAS[2]

[1] Indiana University School of Dentistry; [2] University of Michigan School of Dentistry

The relationship between gap size and the rate of secondary caries development has been studied in details. However, the effect of gap geometry on the rate of development of secondary caries has not been studied previously. The objective of this study was to investigate the effect of the geometry of the space between the restoration and the dentinal wall of the tooth on the development of secondary caries. Tooth-resin-matrix composite specimens were mounted on custom-made gap-model stages. Specimens were divided into the following four groups (n=10): group 1 with 30µm gap throughout both enamel and dentin, group 2 with 30µm enamel gap size and 530µm dentinal gap, group 3 with 525µm gaps in both enamel and dentin and group 4 with 525µm and 1025µm gaps in enamel and dentin, respectively. Specimens were attached to plastic Petri plates, gas-sterilized and then incubated in a microbial caries model with *S. mutans* TH16 in (1% sucrose tryptic soy broth for 1 h, 4 times/day, and with a buffer solution for the rest of the day). After 8 days of incubation, tooth specimens were sectioned and stained with a rhodamine B solution. Digital images were taken under a confocal microscope and analyzed for lesion size at the enamel outer lesion (EOL), enamel wall lesion (EWL), dentin wall lesion next to the DEJ (DWL-A) and dentin wall lesion at 750µm from the DEJ (DWL-B). No difference in EOL size was found between the groups. DWL-A and -B were larger in group 3 than groups 1and 2. Larger DWL-B was found in group 3 than group 4. The results suggest that the presence of additional space at the dentinal wall area did not affect secondary caries development as long as the enamel gap was small. However, with enamel gaps of ≈500 µm, the presence of the additional gap space at the dentinal wall led to the development of smaller dentinal wall lesions at the deeper parts of the simulated cavity. In uniform gaps, the size of the interface was positively correlated with size of the dentinal wall lesions. This study was partially funded by Delta Dental Foundation.

P13 Effect of Temperature and Media on Fluoride Release of Duraphat.

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The number and type of fluoride varnishes available in the market is rapidly growing, however, most of the new products have limited or no evidence of efficacy. While clinical evaluation is the ideal standard for evaluation, screening for their potential efficacy in vitro is a necessary preliminary step. Fluoride release pattern might be the most important factor determining their potential. Unfortunately, currently there is no standard in vitro test for its evaluation. The purpose of this study was to determine the effect of temperature and media on rate and quantity of fluoride released during specific time intervals by the fluoride varnish Duraphat. Consistent amounts of Duraphat were applied to custom-made molds (n=48). Each specimen was placed in either human saliva, artificial saliva, or deionized water with constant stirring (100rpm). The specimens were divided into two groups, one kept at room temperature (~25°C; RT) and the other incubated at oral cavity temperature (35.5°C; OCT). The specimens were transferred to fresh 2 mL aliquots of their assigned media at 1h, 4h, 8h, 24h, and 48h. Three samples from each media and time increment were tested for pH levels and stored in a freezer until fluoride analysis was performed. It was found that neither temperature nor media affected fluoride release during the first hour. After the first hour, however, fluoride release was in general significantly higher in human saliva regardless of the temperature. Temperature did not affect significantly the fluoride release in human saliva, but it had a significant effect in the other two media, particularly in deionized water.

Average Fluoride Release (ppm) per hour

	0-1hr		1-4hr		4-8hr		8-24hr		24-48hr	
	RT	OCT	RT	OCT	RT	OCT	RT	OCT	RT	OCT
Human Saliva	231	265	141	108	61	61	62	94	74	98
Artificial Saliva	232	231	59	54	36	30	15	17	10	30
DI Water	255	311	55	80	45	57	36	25	43	20

In conclusion, it was found that both incubation media and temperature can significantly affect the rate of fluoride release in Duraphat varnish.

P14 Effects of Additive Ingredients on Available Fluoride in Sodium Fluoride Dentifrices.

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Additional ingredients in dentifrices intended for extra benefits have been hypothesized to interfere with ionic fluoride release, hence potentially diminishing their caries preventive efficacy. Therefore, it was our objective to compare the amount of available fluoride in

dentifrices containing various additive ingredients to similar dentifrices that do not. Samples of regular, tartar control, and bleaching (whitening) dentifrices commonly available on the US market {Crest® (CS), Colgate® (CG), and Aquafresh® (AF); total of 9 different dentifrices} obtained from three different production lots were analyzed for soluble fluoride. Three samples from each sample were diluted 1:100 in d.i. water for 1 and 5 minutes and analyzed using a fluoride ion-specific electrode. A standard fluoride curve was similarly prepared and used for determination of the ionic fluoride content of each of the dentifrice slurries. Data were analyzed using ANOVA models. To determine if the dilution affected the results, a sample from each toothpaste was analyzed at a dilution 1:3 using the same protocol.

Results at 1:100 dilution:

	1 min F Available			5 min F Available		
	Mean		SE	Mean		SE
AF Advanced	1136.9	±	11.3	1093.8	±	15.2
AF Advanced 2x-Whitening	1106.5	±	3.0	1084.1	±	11.7
AF Tartar Control Whitening	1128.7	±	15.0	1095.3	±	19.1
CG Total	1075.1	±	15.1	1120.3	±	9.0
CG Total Whitening	1056.3	±	37.6	1102.8	±	17.4
CG Luminous	1102.6	±	26.8	1115.1	±	21.3
CS Cavity Protection	1081.9	±	61.5	1108.0	±	30.7
CS Vivid White	928.5	±	83.6	1060.8	±	216.8
CS Tartar Protection	1117.1	±	3.1	1094.0	±	12.1

Results at 1:3 dilution:

	1 min F Available			5 min F Available		
	Mean		SE	Mean		SE
AF Advanced	940.1	±	10.9	973.4	±	24.0
AF Advanced 2x-Whitening	1126.4	±	52.1	1100.6	±	15.1
AF Tartar Control Whitening	1118.4	±	17.6	1032.2	±	16.7
CG Total	967.4	±	15.3	1047.1	±	12.5
CG Total Whitening	909.4	±	50.8	972.8	±	3.4
CG Luminous	1207.1	±	17.9	1119.4	±	56.5
CS Cavity Protection	1135.8	±	13.4	1153.6	±	1.5
CS Vivid White	1042.9	±	56.4	782.2	±	23.1
CS Tartar Protection	1074.5	±	20.5	1076.4	±	14.7

There were no significant differences ($p>0.05$) among the groups. The analysis at 1:3 dilution produced similar results showing no consistent reduction of available fluoride in the pastes with additional ingredients. The amount of ionic F released by all the dentifrices evaluated was acceptable for US-standards. We concluded that the presence of additional ingredients in the dentifrices studied did not significantly reduce the amount of free fluoride.

P15 Effects of Fluoride on Fluoride Releasing Materials and Secondary Caries.

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Despite progress in improving the physical and chemical properties of fluoride releasing dental materials (FRDMs) over the last several decades, secondary caries remains one of the main reasons for clinical failure. The objective of this study was to see how daily addition of fluoride to FRDMs affects overall fluoride release from these materials during aging and in the prevention of secondary caries. 108 bovine teeth were divided into 3 groups and restored using resin modified glass ionomer (RMGI) Photac-fil, glass ionomer (GI) Ketac-fil, or a control resin, Z100. Specimens were aged for 30 days in deionized water, with half of each group being subjected daily for 2 min to an 1100 ppm NaF dentifrice slurry. Water was changed daily and saved for fluoride analysis. Following the aging period, specimens were subjected for 96 hours to demineralization in a lactic acid/carbopol solution, at 37^{O}C. The specimens were then sectioned and analyzed for secondary caries lesion size using Confocal Laser Scanning Microscopy (CLSM). Data collection and analysis are currently in process and are expected to be completed by March 20th, 2010. The fluoride and caries data are to be analyzed using 2-way analysis of variance (ANOVA), with 3 levels for the material factor and 2 levels for the aging factor.

P16 Evaluation of Caries Risk Assessment at IUSD.

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Caries Risk Assessment (CRA) is an essential element in the development of patient-centered caries management plans, and is part of Indiana University School of Dentistry (IUSD)'s clinics since 2001. Objectives of this study were to compare caries risk status and risk variables between four age-groups of adults at IUSD and to measure the use of CRA forms and compare this with the results of a 2004-study. 256 electronic Axium charts (May 2007-May 2009), distributed equally between the following age groups: 18-26, 27-35, 36-50, ≥ 51, were randomly chosen from all comprehensive care adult clinics, and evaluated for completion of CRA forms, reevaluation visits, caries risk factors, and management strategies. The percentage of patients with a risk assessment completed (29%) was significantly ($p<0.01$) lower than in the 2004-study (57%), while reevaluation percentages were similar with the 2004-study (55% vs. 45%, respectively). The risk status did not change for 61% of subjects and improved for 37%. DMFT and DMFS were significantly different among age groups ($p<0.0001$), with 18-26 year-olds significantly lower than the other groups, and 27-35 and 35-50 year-olds significantly lower than 50+ year-olds. Medical-behavioral risk factors (e.g. hyposalivatory problems) were more likely to be found in the 50+ age-group. Prescription F toothpaste was recommended differentially by age ($p=0.0428$), being used more frequently in 27-35 and 36-50 year-olds than in 50+ year-olds, and more frequently in 27-35 year-olds than in 18-26 year-olds. Also, in-office fluoride was recommended less often in 50+ year-olds than in any of the other age groups

(p=0.0113). While there are differences in risk factors and recommended management strategies by age group, integration of a risk-based caries program has not improved since 2004, even though it is an expectation for a CRA form to be completed for every patient. This study was funded with a grant from IUSD.

P17 Incidence of Secondary Caries on a Longitudinal Study Using ICDAS.
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Caries around restorations and sealants (CARS) are the major reason for restoration replacement and represent diagnostic challenges. International Caries Detection and Assessment System (ICDAS) has been increasingly accepted, but there is only limited information regarding its use for longitudinal CARS assessment. This study aims to evaluate the prevalence and incidence of CARS in a 32-month interval in 569 children enrolled in rural schools in the Commonwealth of Puerto Rico who followed longitudinally using ICDAS. Associations with CARS prevalence and incidence were performed using GEE methods applied to logistic regression. Out of 2953 restored surfaces examined at baseline, 298 surfaces (10%) had lesions ICDAS score≥1 and 121 surfaces (4%) had ICDAS score≥3. During the 32-month follow-up 12% of the surfaces developed a new lesion ICDAS score≥1 and 6% ICDAS score≥3. At baseline and 32 months 96% of the surfaces were restored with amalgam restorations, 2% with tooth-colored restorations and the remainder with provisional restorations and crowns. There was a significant difference ($p<0.01$) between the restoration type and the prevalence of CARS, with temporary fillings having a higher prevalence followed by tooth-colored restorations. Primary teeth had significantly higher incidence of CARS than permanent teeth ($p<0.001$). There were significant differences between surfaces ($p=0.0001$) for both prevalence and incidence, buccal surfaces having a higher prevalence and incidence when lesions ICDAS score≥1 were included and occlusal surfaces having a higher prevalence and incidence for lesions ICDAS score≥3. Following placement of a new restoration, 111 of 1080 surfaces (10%) developed a lesion ICDAS score≥1 around the restoration, with 92 lesions occurring within 1 year of placement and 21 having ICDAS score≥3. In conclusion, ICDAS allows detailed analysis of CARS prevalence and incidence. Supported by NIH/NIDCR RO1DE017890-01.

P18 In-vitro Detection of Caries Lesions Through a Clear Sealant.
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There is strong evidence for the effectiveness of sealants in preventing and arresting dental caries. In a previous study FONTANA et al. (2007) had concluded that different stages of caries lesion severity can be objectively quantified in vitro using Quantitative Light-Induced Fluorescence (QLF) after clear sealant placement, but it is unclear if this is true also for other clear sealants. **Objective**: To evaluate the effect of Helioseal Clear sealant placement on detection and quantification at one point in time of different stages of caries lesion severity using QLF. Method: Eighty permanent molars were selected using the visual International

Caries Detection and Assessment System criteria (ICDAS). Lesion severity scores ranged from 0-4 (10 teeth/category 1-4, and 40 teeth for category 0). Teeth were randomly numbered, and after brushing with water and drying for at least 10 seconds, baseline images were acquired using QLF. The occlusal surfaces were then sealed (Helioseal Clear, Ivoclar Vivadent) according to the manufacturer's instructions. A second QLF image was acquired of the sealed fissures. Images were analyzed for lesion average fluorescence loss, (DF [%]), size (S [mm2]), and ΔQ [%´mm2]. **Results**: As in previous studies, lesions appeared larger/more severe after sealant placement with significant ($p<0.05$) strong correlations between before and after sealant placement for all QLF measurements [$r=0.85$, 0.86, and 0.89 for ΔF, S and ΔQ, respectively]. Furthermore, higher QLF values were associated with higher visual ICDAS scores, regardless of sealant presence (e.g. the correlation between ICDAS score and ΔF before and after sealant placement was $r=0.70$ and $r=0.79$, respectively). Conclusion: Different stages of caries lesion severity can be observed and objectively quantified using QLF after Helioseal Clear sealant placement. This study was supported by a grant from Indiana University School of Dentistry.

P19 Lesion Progression evaluated by Fluorescence Image Analyses.

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Objective: To evaluate the Quantitative Light-induced Fluoresce (QLF, Inspektor Research Systems B.V., Amsterdam, The Netherlands) variables for lesions which progressed from International Caries Detection Assessment System (ICDAS) scores 0/1/2/3 to ICDAS 4/5/6. **Methods**: 569 children (5-13yrs) enrolled from a rural school district in Puerto Rico. One examiner observed occlusal and buccal surfaces of all erupted permanent molars, and lingual surfaces of upper molars using the ICDAS. The QLF images were acquired at the same time and later independently analyzed. For fluorescence image analyses, 28 lesions that progressed from ICDAS 0/1/2/3 at baseline to ICDAS 4/5/6 at 8months (Group-8M) and additional 35 lesions that progressed to ICDAS 4/5/6 between 8months and 12months (Group-12M) were selected. The QLF variables (QLFM); average fluorescence loss (ΔF [%]), size (S [mm2]), and ΔQ [%´mm2], were determined. Change-in-QLFM per month (DQLFM: DFM, DSM, DQM) was calculated. **Results**: For Group-8M, DF8M averages were 0.7, 2.0, 0.6, and 0.5 (ICDAS=0, 1, 2, and 3, respectively); DS8M were 0.3, 0.5, 0.1, and 0.2; and DQ8M were 25.0, 39.2, 8.0, 19.1. For Group-12M, DF12M were 2.3, 0.9, 0.6, and 0.7; DS12M were 0.3, 0.0, 0.0, and 0.2; and DQ12M were 20.1, 7.0, 4.8, and 14.5. The Group-12M lesions were also evaluated between baseline and 8 months, when the ICDAS did not find progression to ICDAS 4/5/6: DF12M-8 were 0.4, 0.1, 0.2, and 0.3; DS12M-8 were 0.0, 0.1, 0.0, and 0.1; and DQ12M-8 were 5.4, 2.8, 2.6, and 6.0. **Conclusion**: These results indicate that QLF variables show changes for lesions classified by ICDAS as progressing and appear to show less during a period when the lesions did not progress. This study was supported by NIH/NIDCR grant R01 DE017890-04.

P20 Reliability of Proximal Detection Devices Under Simulated Clinical Conditions.

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Objectives: This study's aim was to compare the use of visual examination (ICDAS II criteria), fiberoptic transillumination (FOTI, Schott Fibre Optics), digital imaging fiberoptic transillumination (DIFOTI®, Electro-Optical Sciences), DIAlux (DL, KaVo), and Midwest Caries Detection (Dentsply) for the detection of proximal caries. **Methods:** Eighty-one unrestored extracted posterior teeth representing the ICDAS II criteria (0-6) on proximal surfaces were mounted on 6 sets of manikins with 6 teeth mounted on each model (1 premolar and 2 molars per quadrant) with proximal surfaces in contact. These models were mounted on Phantom Heads prior to examination with all methods by 3 different examiners. Training was conducted on all methods prior to study initiation. The teeth were sectioned and histology was used as a gold standard. The following criteria were used with FOTI and DIALUX: 0-sound, 1-lesions confined to enamel, 2- large shadow visible (inner portion of enamel or at DEJ), 3-shadow in dentin, 4- > 4 mm in diameter; DIFOTI:0-no shadow, 1-light gray shadow, 2-dark gray shadow, 3-loss of tooth structure or translucent light surrounded by dark shadow; Midwest: 0-sound, 1-slow signal, 2-medium signal, 3-loud signal; Radiographs: 0-sound, 1-outer enamel, 2- inner enamel/outer dentin, 3- dentin, 4-pulp; and Histology: 0-sound, 1-outer half enamel 2-inner enamel/ outer dentin 3- middle third dentin 4- inner third dentin/pulp.
Results: Intra- examiner ICC was 0.92 for ICDAS, 0.91 for BW, 0.59 for MC, 0.93 for DL, 0.88 for FOTI, and 0.83 for DIFOTI. Inter-examiner ICC was 0.88 for ICDAS, 0.79 for BW, 0.47 for MC, 0.87 for DL, 0.87 for FOTI, 0.81 for DIFOTI. Specificity was highest for DL (0.92-1) followed by ICDAS (0.90-0.98) and BW (0.90-0.96); DIFOTI (0.9-0.96), FOTI (0.82-0.92) and MC (0.62-0.78). Sensitivity was highest for DIFOTI (0.75-0.84), followed by ICDAS (0.74-0.78) and DL (0.73-0.79); FOTI (0.71-0.76), MC (0.66-0.71) and BW (0.55-0.60). **Conclusions:** Methods based on transillumination performed better overall than other methods. The combination of a visual exam with a method based on transillumination may provide the best methodology for detection of proximal caries.

P21 Sealant Retention: A Comparison of Placement Providers in the Pediatric Clinic.

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Long term retention of pit and fissure sealants is essential for their success. Teeth that have a partially lost or fractured dental sealant are 25% more likely to experience dental caries than intact sealed teeth. The objective of this study was to compare retention rates of sealants placed by dental hygiene students to those placed by 3rd and 4th year dental students. A retrospective analysis of electronic health records of patients who had dental sealants placed between October 1st 2005 and September 31st 2007 in the undergraduate pediatric dental clinic was examined. Of the 2843 sealants placed, 2725 were by DDS students while 118 were placed by dental hygiene students. 16% of those placed by DDS students needed replaced within two years of original placement while 6% of those placed by hygiene students needed replaced in

the same time frame. The difference is statistically significant (p=0.0419 using a logistic regression, which included a term for student group and random effects to account for the correlations between sealants within a subject and for the correlations between sealants placed by the same student. The results suggest that dental sealants placed by dental hygiene students have a lower failure rate than those placed by dental students.

P22 Students' Perception of Clinical Preparedness to Detect Possible Carious Lesions.

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Objective: Curriculum evaluations are a critical requirement for schools, but student input is not typically considered when structuring the curriculum. The purpose of this study was to obtain information related to pre-doctoral dental students' perceptions of their clinical preparedness based on the first two years of their curriculum. **METHOD**: The survey was administered toward the end of the spring term to the second-, third-, and fourth-year students of each class. Student responses were anonymous and participation was not mandated. Students were asked to rate their confidence on a scale of 1 to 5 in providing patient care in eight different fields. Second-year students were asked to complete the survey as, "now that you are about to enter clinic, how prepared do you feel" while third- and fourth-year students were asked to complete the survey as, "now that you have had experience in the clinic, how has your preclinical experience prepared you for treating patients." The survey was administered each year from 2003-2009. **Results**: On average, 27.5% of second-year students report a lack of confidence in detecting caries radiographically, and 23% reported the same lack of confidence in detecting caries clinically. Despite more clinical experience and teachings, third year-students also appear to show less than optimal confidence: 24% on average reported lacking confidence in detecting caries in a radiograph and 27% clinically. As expected, these figures improved in the senior year: 12.5% by radiographs and 11% clinically. **CONCLUSION**: The data indicate that among a population of second-, third-, and fourth-year dental students, there is a level of discomfort in clinical preparedness related to identifying caries radiographically and clinically. Although this discomfort decreases overtime, exploring methods to further improve students' confidence may be warranted.

P23 The Effect of FOTI Light Output on Proximal Caries Detection.

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Background: Bite-wing radiographs are the standard for detection of carious lesions on proximal surfaces, however, Fiber Optic Transillumination (FOTI) has been reported as a valuable supplement for detection of proximal caries. Several new FOTI devices have been introduced recently 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. Teeth were cleaned and mounted on five sets of models with six teeth mounted on each model with proximal surfaces in contact. Models were placed in a Phantom Head and a trained examiner performed an ICDAS exam (used as a reference for the fiber optic exams). On a separate occasion, the same examiner performed all fiber optic exams (each at least 96h apart), following manufacturer's instructions, with lights off, and models placed in a Phantom head. Assigned surfaces were scored on a 0-6 scale, relying on the ICDAS scores to differentiate scores 2 and 3. **Results**: There was complete agreement between FOTI using standard output (Exposure value of 9.7 @ 3000K and 2,079 lux with 1.0mm tip) FOTI using maximum output (Exposure value of 10.8 @ 3300K and 4,457 lux with 1.0mm tip) (wk=1.00). There was high agreement between FOTI using standard or maximum output (with a 1.0mm tip) and DiaLUX (Exposure value of 12.2 and 11,763 lux with 3.0mm tip) (wk=0.93); as well as Microlux (Exposure value of 14.8 and 71,315 lux with 3.0mm tip) (wk=0.93); and between DiaLUX and Microlux (wk=0.89). Correlations for all combinations ranged from 1.00-0.93. **Conclusion**: Although agreement between DiaLUX and Microlux was high among themselves and with FOTI, these devices classified fewer surfaces with scores 1 than FOTI devices. These results indicate that fiber optic tip diameter has little influence on caries detection, but light output may have more influence on sensitivity.

P24 Time-dependent Changes in Transparent Sealants and Their Effects on QLF.

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Objectives: The purpose of this study was to evaluate one year post placement changes in Helioseal Clear Chroma sealant, including changes in both sealant thickness and Quantitative Light-Induced Fluorescence measurements. **Methods***:* Impressions of 228 one month and one year post-sealant first molars of children between seven and ten years of age were made with a vinyl polysiloxane material and poured with die stone. The fabricated models were scanned and analyzed with Proscan 2000A software in order to calculate an average and maximum change in sealant thickness over one year. QLF images were analyzed for average fluorescence loss (DF [%]), size (S [mm^2]), and ΔQ (DF×S [%´mm^2]). The data were combined with average and maximum QLF values for each corresponding tooth and analyzed using correlation coefficients. **Results**: 90 impressions were used for analysis. The mean average sealant thickness change was determined to be 94µm, while the mean maximum sealant thickness change was 290µm. There was no correlation ($r < 0.01$) between sealant thickness change and caries lesion monitoring (change from one month to one year) as measured by QLF. It was also determined that there were no significant differences in change in sealant thickness between ages ($p=0.76$ for maximum thickness change, $p=0.18$ for average thickness change); sealants with a larger baseline maximum thickness had a larger maximum change in sealant thickness overtime; sealants that clinically failed by one year had significantly larger changes in average sealant thickness than those that did not ($p=0.0258$). **Conclusions**: The mean average annual wear rate of Helioseal Clear Chroma sealant was calculated to be 94µm. Changes in sealant thickness do not influence the effectiveness of sealed lesion monitoring using QLF. It was also concluded

that with increased sealant thickness more wear was experienced. This study was supported by NIH grant R21 DE018115-01.

P25 Variation in Dental Caries Management Decisions of U.S. Dental Students.
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Caries management is an important aspect of dental practice. However, caries risk assessment and management strategies beyond restorative care have not been well-integrated into the cariology curriculum in most U.S. dental schools. This can result in variation in students' management decisions and have significant health and economic consequences for patients and third-party providers. In 2001, Indiana University School of Dentistry (IUSD) began a Caries Management Program aimed at training students to become competent in managing dental caries. Variation in faculty management decisions had been previously evaluated, but there was no information on variation of students' decisions throughout the curriculum. The objective of this study was to evaluate the variation of caries management decisions of dental students (2^{nd}, 3^{th}, 4^{th}-year predoctoral; operative/preventive dentistry graduate) at IUSD. The study population consisted of 331 dental students. A survey was conducted electronically via surveymonkey.com®, an internet technology media. Voluntary participants were asked to access the survey through a given website using their computers. The survey involved questions concerning caries management decisions based on selected clinical and radiographic images and patient case-scenarios, with questions regarding choice of cavity preparations and dental materials. The response rate was 70.7%. 2^{nd}, 3^{th}, and 4^{th}-year students were significantly more likely to restore teeth that showed caries radiographically into dentin and those with an occlusal break in enamel compared to graduate students. 3^{th} and 4^{th}-year students were also more invasive in their restorative treatment threshold than the other students. 4^{th}-year students were less likely than the other groups to document and provide a treatment plan for white spot lesions in adults. Disparities exist between predoctoral and graduate students in treatment decisions for cavitated and non-cavitated carious lesions, with 3^{rd} and 4^{th} year students more similar in their treatment decisions and strategies. Funded by an IUSD-grant.

CELL SIGNALING

P26 Molecular Mechanism of Pyk2 Y402 Dephosphorylation by Dynamin2.
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Indiana University School of Dentistry

Osteoporosis is a bone disease that is caused by excessive bone resorption and affects hundreds of millions of people worldwide. Bone resorption is the process by which osteoclasts degrade the organic and inorganic material of bone. Non-receptor tyrosine kinase, pyk2, is highly expressed in osteoclasts. Mice with deletion of pyk2 have an increase in bone mass due

to impairment in osteoclasts function. It has been demonstrated that Tyr-Y402 phosphorylation of pyk2 is very important for osteoclasts spreading and bone resorption. We also established that the GTPase dynamin 2 (dyn2 (wt)) controls osteoclast bone resorption. Our group reported that dyn2 (wt) reduced pyk2 Y402 phosphorylation which then affects pyk2 function. This current study is to understand the process by which dyn2 (wt) regulates pyk2 dephosphorylation. Our findings demonstrated that pyk2 dephosphorylation is mainly attributed to dyn2 (wt) GTPase hydrolysis. Expression of dyn2 mutants that have reduced affinity to GTP and defective GTPase activity increased pyk2 Y402 phosphorylation. We have also found that that pyk2 phosphorylation is partially rescued in the presence of tyrosine phosphatase inhibitors. Our preliminary results indicate that the phosphatase might be PTP-PEST. By understanding the process that regulates pyk2 phosphorylation and its binding to dynamin, will lead to better identification of potential drug targets to treat bone related disease. Over the past few decades, bisphosphonate have played a significant role in the treatment of osteoporosis. Unfortunately osteonecrosis of the jaws has been recently described as a harmful side effect of bisphosphonate therapy. An alternative approach would be combination therapies were pyk2 inhibitors are paired with bisphosphonate as a way to boost the therapeutic potency and decrease side-effects.

P27 Regulation of Osteoclast Function and Signaling by Kalirins.

M. SHIVANNA,* L. DU, A. BRUZZANITI

Indiana University School of Dentistry

Osteoclasts are the primary bone resorbing cells and they arise from hematopoietic mononuclear cells in the bone marrow. Excessive osteoclast activity is known to result in decreased bone mass leading to osteoporosis. Pyk2, a tyrosine kinase, is highly expressed in osteoclasts. It is well established that Pyk2 plays a central role in linking the integrin avb3 activation to the formation of the actin ring/sealing zone that is essential for osteoclast function. Phosphorylation at Tyr-402 in Pyk2 is essential in the regulation of adhesion dependent cytoskeletal organization in osteoclasts. Our previous studies have shown that knockdown or deletion of Pyk2 leads to impaired osteoclast function. Kalirin, a member of the Dbl family of proteins, is a novel multifunctional RhoGDP/GTP exchange factor known to play a vital role in cytoskeletal organization in neuronal cells. The interaction of kalirin with Pyk2 in osteoclasts has not been investigated so far. This study was undertaken to identify whether kalirin associates with Pyk2 and regulates its phosphorylation as well as to characterize the role of Kalirin in osteoclasts. We used confocal microscopy to examine the expression kalirin in osteoclasts and found that it colocalized with actin and Pyk2 in osteoclasts, implicating its role in cytoskeletal organization and therefore osteoclasts function. Importantly, we found that osteoclasts isolated from kalirin KO mice had significantly reduced bone resorbing activity. Knockdown of kalirin also led to a decrease in bone resorption in osteoclasts. To examine the interaction of kalirin and pyk2 in vitro, we transiently expressed with Pyk2 and kalirin in 293VnR cells and cell lysates were subjected to co-immunoprecipitation followed by western blotting. We found that Pyk2 associates with Kalirin 7, a major isoform found in OCs, and dose-dependently inhibits Pyk2 phosphorylation at Y402. These results demonstrate that kalirin negatively regulates Pyk2 phosphorylation and thereby Pyk2-mediated signaling. The decreased

bone resorbing activity of osteoclasts lacking kalirin further suggests that kalirin is a regulator of osteoclast function and may be novel target for anti-resorptive therapies to treat osteoporosis.

DENTAL MATERIALS

P28 Evaluation of the Microhardness of Triclosan-incorporated Acrylic and Composite Resin.

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Indiana University School of Dentistry

This study was designed to investigate the microhardness of composite resin and temporary acrylic resin modified by the addition of Triclosan, an antimicrobial and antifungal agent. Polymethylmethacrylate (PMMA) resin (Jet Acrylic (Lang Dental Mfg. co., inc.)), modified by the addition of triclosan (Fluka Biochemika: Irgasan (Catalog Number: 72779-5G-F)) in concentrations of 0 wt% (control), 2.5 wt%, 5 wt%, and 10 wt%, was formed into tablets (2 mm deep x 6 mm diameter) for testing. Similarly, composite resin (laboratory-formulated) in the same concentrations was formed into tablets. The tablets of each group were subjected to Knoop hardness testing using a M-400 Hardness Tester and ACP-94 Digital Measuring Microscope. The load used on the indenter was a 50 gram force (gf) with indents made in the center and periphery of the discs. The findings indicated that there was a significant decrease in microhardness values of the 10% triclosan-incorporated composite resin group compared to that of the control composite resin group. There was no significant difference ($P = 0.550$) in microhardness values among the four concentration groups of triclosan-incorporated temporary acrylic resin groups. Increasing the triclosan concentration in composite resin significantly lowered the microhardness properties compared to that of the control group (0% triclosan in composite resin). Increasing the triclosan concentration (up to 10%) in temporary acrylic resins did not have a significant impact on microhardness properties of temporary acrylic resins. Funded by IUSD Grant.

P29 Microleakage: New Technique to Reduce Effects of Composite Polymerization Shrinkage.

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University of Detroit Mercy School of Dentistry

The gap filling method for reducing effects of composite shrinkage during polymerization was initially introduced by Sclafani and Wagner (2005). In the present study, a more clinically applicable material was used for the gap filling material. **Objectives**: To determine if use of the new gap filling technique and material reduce microleakage in Class I restorations. **Methods**: 21 class I cavities were prepared on extracted teeth. The material used for gap filling was an orthodontic adhesive (from the Phase II system, Reliance Orthodontic), the single-component adhesive was OptiBond Solo Plus (Kerr-Sybron), and the composite used for all samples was PermaFlo (Ultradent). Seven teeth were used for each of three treatment groups: (1) Control:

prepared with standard procedures (etch, adhesive-light cure, composite-light cure). (2) As with control but gap filling material was applied before the composite was placed. (Composite was light cured and then the gap filling material was allowed to chemically cure.) (3) No etch or adhesive, but with gap filling material. Specimens were thermocycled 1000 cycles. 0.5% Basic Fuchsin for 24hrs was used for microleakage staining. Cross sections were analyzed for depth of staining at margins by one investigator who was "blinded" to the treatment identification. ANOVA and Scheffe's statistical tests were used to determine if microleakage was significantly different for different treatments. **Results**: Group (2) had the lowest mean depths of dye staining (mean depth 596µm); however, it was not significantly different from group (1) (959µm) ($p=0.510$). Group (3) showed much higher staining depths (2242µm) and was statistically different than the other two groups ($p\leq0.001$). Conclusion: The gap filling technique in conjunction with standard composite placement procedures produced the lowest microleakage; however, it was not statistically different from the standard composite placement technique. Using the gap filling technique without etching or adhesive produced the highest microleakage.

P30 Resorbable PPF-Brushite Composites with Mechanical Properties Comparable to Trabecular Bone.

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The objective of this study was to characterize the mechanical properties and biocompatibility of a candidate bone repair material consisting of brushite cement reinforced with the biodegradable polymer, poly(propylene fumarate) (PPF). Brushite cement was prepared using a 1:1 monocalcium phosphate monohydrate:β-tricalcium phosphate molar ratio and deionized water in powder to liquid mass ratios (P/L) of 1.0 and 1.5. For reinforcement, the cement was saturated with PPF (Mp = 1,700 g/mol) mixed with the crosslinking monomer N-vinyl pyrrolidinone at a 4:3 mass ratio, 5 wt % benzoyl peroxide, and 0.1 wt % butylated hydroxytoluene. Crosslinking was achieved by heating at 80°C for 24 hours under vacuum. Mechanical properties of the PPF-brushite composites were evaluated in three point bending, and trends were correlated to the mass of polymer incorporated. To show potential for fabricating scaffolds for bone tissue engineering, 3D PPF-brushite scaffolds (P/L = 1.0) comprised of orthogonally intersecting beams were prepared by rapid prototyping and tested in compression. Finally, biocompatibility was evaluated by exposing murine mesenchymal stem cells to PPF-brushite disks (P/L = 1.0). After 24 h, the cells were trypsinized, incubated with propidium iodide and FITC conjugated anti-annexin V antibody, and then analyzed by flow cytometry to determine the percents of viable, necrotic, and apoptotic cells. At P/L = 1.0, polymer incorporation was 0.38 ± 0.03 mg/mm3 of brushite, and resulted in significant increases in mechanical properties. Flexural strength was increased from 0.75 ± 0.26 MPa to 12.40 ± 3.72 MPa, flexural modulus from 302.00 ± 139.28 MPa to 854.00 ± 312.49 MPa, maximum displacement from 0.074 ± 0.01 mm to 0.50 ± 0.09 mm, and work-of-fracture from 2.77 ± 0.99 J/m2 to 219.34 ± 83.4 J/m2 ($n = 5$; $p < 0.05$). Only modest improvements were seen at P/L=1.5, due to the significantly decreased polymer incorporation (0.19 ± 0.01 mg/mm3; $p < 0.05$). Large increases in strength were also seen for the 3D scaffolds, as PPF reinforcement

increased the compressive strength from 0.31 ± 0.06 MPa to 7.48 ± 0.77 MPa (n = 4; $p < 0.05$), which is comparable to trabecular bone. Finally, PPF-brushite composites showed good in vitro biocompatibility, as 96.00 ± 0.91% of the cells were viabile after 24 h of exposure (n = 3; $p > 0.05$ compared to negative control). In conclusion, PPF-brushite composites have excellent mechanical properties, show good biocompatibility in vitro, and may be an ideal material for bone tissue engineering.

DIAGNOSTIC SYSTEMS

P31 Magnification Effect on the Treatment Decisions of Occlusal Carious Lesions.

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There is little understanding on how magnification influences treatment decisions for carious lesions. Our previous work concluded that magnifications up to 5.5x did not significantly affect how caries lesions are visualized. **Objective**: To compare the effect of magnification on treatment decisions for occlusal carious lesions. **Methods:** Ninety-six permanent human molars were selected using the International Caries Detection and Assessment System II criteria-(severity scores 0-4) and mounted in eight "dentoform mannequin" models simulating clinical conditions, and assessed for treatment choices [none, preventive (fluoride/sealants), restorative] by 2 dentists and 1 dental student using 0x and three magnifications (2.5x, 3.5x, 5.5x). Forty-eight teeth were re-examined within 1-week. **Results:** Overall intra-examiner repeatability was acceptable (wk>0.6), but varied by examiner. It was lower without magnification for Examiner-1; acceptable regardless of magnification for Examiner-2; and acceptable regardless of magnification, with an increased repeatability with 3.5x and 5.5x magnification for Examiner-3. Inter-examiner agreement tended to be higher without magnification (wk>.65) than with magnification (wk>.49) for all examiners at all magnification levels. 0x resulted in significantly less invasive treatment than 3.5x and 5.5x for all examiners. For examiner 1, 0x resulted in significantly less invasive treatment than 2.5x, while there were no differences for examiner 2 and 3. 2.5x resulted in less invasive treatment than 3.5x for examiner 3, while there was no difference for examiners 1 and 2. 2.5x and 3.5x had significantly lower treatment than 5.5x for examiner 1 and 3, while there was no difference for examiner 2. Conclusion: Under the conditions of this study, it is concluded that magnification up to 5.5x can affect treatment decisions for occlusal caries lesions, with variations between examiners. Study funded by an AADR Fellowship.

EDUCATION METHODS

P32 Assessment of Biomedical Content Acquisition Performance through PBL Group Interaction.

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PBL group interaction, research and collaborative discussion should enable students to deepen their understanding of biomedical science content. However, anecdotal evidence suggests student learning and exam preparation may be largely driven by post-case individual study and the publicized Learning Objectives rather than PBL group interactions. To determine whether students were actually learning SABS content during PBL process activities, just prior to the Learning Objectives dissemination, we administered a quiz with a content item, and an application item as well as a survey item to determine students' role in group regarding the assessed topic. Results indicated that both Year 1 and Year 1 students scored higher on content recall test items than on application items**.** For Year 1 students, self-reported role in group regarding the tested SABS Learning Issue topic correlated with scores of 100% (r=0.78) and 0% (r=-0.97). For Year 2 students, self-reported role in group regarding the tested SABS Learning Issue topic correlated with scores of 50% (r=0.68) and 0 %(r=-0.78). Conclusion: Year 1 and 2 students performed better on test items assessing content recall rather than clinical application. Students who reported being more active in the PBL group process activities tended to have better assessment performance and were less likely to obtain a zero score.

P33 IUSD Faculty and Student Perceptions of Podcasting.

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Indiana University School of Dentistry

Podcasting is fast becoming the preferred mode of capturing and delivering audio and video course content. Proponents highlight the technology's ease of use, ability to suit differing learning styles, and acceptance and familiarity with the technology on the part of students. Skeptics ask for evidence that student learning is enhanced by podcasting and cite concerns about protecting their intellectual property. Meanwhile there is some confusion about the technology and how best to apply it in a dental education program. To obtain an assessment of faculty and student perceptions of podcasting technology, IUSD students and faculty were surveyed about their experiences with podcasting during the 2009 spring semester. Of total student respondents (n=109), only 30% had been in a course offering a podcast. Nearly all students with podcasts available reported viewing them. Podcasts were most frequently viewed two times. The majority of students preferred lecture-capture podcasts over pre-recorded forms. Only 17% of students believed podcasts alone are sufficient to review course material. Of total faculty respondents (n=46), 7 developed a podcast for use in spring 2009. Of the faculty who created and used podcasts, all but one found the technology easy to use. None of those who implemented podcasts believed student grades improved compared to previous

years, and only 2 out of the 7 faculty who developed a podcast believed student learning was improved through the use of podcasts. Twenty-three faculty members reported considering making a podcast in the 2009-2010 academic year. Proof of improved learning was reported as the primary incentive for faculty to develop and use podcasts. This study indicated that most IUSD courses do not incorporate podcasts. Students exposed to podcasts generally regard them as useful adjuncts to learning, while faculty are more skeptical of their utility in facilitating learning.

EXPERIMENTAL PATHOLOGY

P34 Effects of Alendronate on Human Osteoblast-like MG63 Cell.

J. SUN,* F. SONG, W. ZHANG, L. J. WINDSOR

Indiana University School of Dentistry

Alendronate is one type of bisphosphonate used to inhibit osteoclast activity in the treatment for osteoporosis, Paget's Disease, multiple myeloma or tumor-associated metastasis. According to pharmacokinetics studies of alendronate, its plasma concentration ranges from 10-8 to 10-7 M and its concentration in the bone can be up to 10-4 M. Reports of bisphosphonates-associated osteonecrosis of the jaw (ONJ) have raised great concern among patients and health care professionals. Currently the pathogenesis of bisphosphonates-associated osteonecrosis of the jaw remains unclear. One of the hypotheses is proposed to be due to a defect in jaw bone remodeling and wound healing as a result of bisphosphonates interference with normal bone cell functions and activity. The objective of this study was to examine the effects of Alendronate on matrix metalloproteinases and collagen degradation by human osteoblast-like MG63 cell. Human osteoblast-like MG63 cell was cultured and exposed to various concentrations of Alendronate (from 10-8 up to 10-4 M). Cell proliferation and cytotoxicity were evaluated by water-soluble tetrazolium-1 and lactate dehydrogenase, respectively. MG63-mediated collagen degradation was assessed on the 6-well plates precoated with a reconstituted Type I collagen. Conditioned media and membrane extracts were collected for zymography and western blot analyses of matrix metalloproteinases (MMPs). The results showed significant ($P<0.05$) changes in cell proliferation and cytotoxicity at concentrations of 10-5 and 10-4M. MG63 cells were capable of mediating type I collagen degradation. There is no statistic significance ($P>0.05$) among collagen degradation with (10-8, 10-7 and 10-6M) or without alendronate. Western blotting showed alendronate stimulation had no effect on MMP expression from MG63 conditioned media and membrane extracts. Initial results showed that alendronate affected MMP activity. Alendronate at concentrations higher than 10-5M had toxic effects on MG63, but alendronate with plasma concentration did not regulate MMP expression and had no effect on collagen degradation by human osteoblast-like MG63 cell.

P35 In-vivo Effects of Zoledronic Acid on Oral Mucosal Epithelial Cells.

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Osteonecrosis of the jaw is a serious complication of bisphosphonate treatment for which the pathophysiology is unknown. The purpose of this study was to investigate whether in vivo zoledronic acid (ZA) administration induces alterations in cell proliferation, apoptosis, and matrix metalloproteinase (MMPs) expression of oral mucosal epithelial cells. 1 year old skeletally mature dogs were either untreated or given high doses of intravenous ZA for three months. The doses of ZA were equivalent to those given to cancer patients (0.06 mg/kg), yet were administered two times more frequently (every 2 weeks) than compared to what is used clinically. Hard palate mucosal tissue was collected and assessed immunohistochemically for cell proliferation (proliferating cell nuclear antigen, PCNA), matrix metalloproteinase (MMP-2, MMP-9 and MMP-14) expression, and apoptosis (caspase 3 and TUNEL). There were no significant differences between the groups with respect to PCNA, MMP-2, MMP-14, and TUNEL positive cells. However, the expression of MMP-9 was significantly higher in group 1 than group 2 ($p < 0.05$), while the expression of caspase-3 was significantly lower in group 1 than group 2 ($p < 0.05$). These results indicate that exposure to high doses of ZA resulted in higher levels of apoptosis and lower levels of MMP-9 expression in the oral epithelial cells and this supports the idea of soft tissue toxicity with bisphosphonate treatment.

P36 Verrucous Carcinoma of the Oral Cavity – A Unique Presentation.

A. GUPTA, A. MADAWI, Z. KOSSAK, J. OJHA

University of Detroit Mercy School of Dentistry

Oral verrucous carcinoma (VC) is a rare, low-grade variant of squamous cell cancer (SCC) most commonly seen in patients who chronically use smokeless tobacco. VC is most often seen in males over 55 years of age as a white, exophytic lesion with papillary projections. The disease is commonly extensive by the time of diagnosis and many cases have embedded SCC within the VC lesion. We present a case of VC which does not show typical VC clinical characteristics. This lesion presents on the dorsum of the tongue as a thick, white plaque with no papillary or verruciform projections. The clinical findings and histopathology can be mistaken for a much more benign lesion and adequate histopathologic sampling and analysis is key to making a diagnosis. Since VC is considered to be an extremely well differentiated variant of squamous cell carcinoma, the surgery is not as extensive as required for routine SCC. The dental practitioner is advised to be aware of the clinical variance of this lesion and to be vigilant in conducting examination of the patient.

GENERAL HEALTH

P37 Pre-hypertension in the Child Population: Pilot Study of Three Races.

J. CARSON[1],* M. MEADOWS[1], G. J. ECKERT[2], J. KOWOLIK[1]

[1] Indiana University School of Dentistry; [2] Indiana University School of Medicine

Objective: The object of this study is to evaluate whether pre-hypertension and hypertension are apparent in the child population. **Methods**: Blood pressure, pulse, weight, and height were recorded in 6 to12 year old children, 25 in each group of Black or African American, Hispanic or Latino, and Caucasian or White. The children were attending the IUSD Pediatric dental clinic for routine dental care. Parents were given a short questionnaire which asked the child's age, gender, race/ethnicity, physical activity, after-school sports, family hypertension history, and general health. **Results**: Data was compared to growth charts to obtain height percentiles and then to National Institute of Health blood pressure tables, based on height percentile. Weight, height, and age were also used to calculate Basal Metabolic Index (BMI). Preliminary results of 38 subjects show 4% of Caucasian children, 28.5% of Hispanic children, and 33.3% of African American children are pre-hypertensive or hypertensive. This is consistent with adult studies which have shown that African American adults are at a higher risk of having hypertension. Family hypertension history had some correlation, for 18.75% of those who stated family history of hypertension had children with hypertension; however, 100% of hypertensive children had a family history of hypertension. All children with hypertension or pre-hypertension are stated to be physically active in the parental questionnaire. Gender played no role in blood pressure, but was significant for high percentiles in BMI among females. There were statistically significant differences in BMI among ethnic groups. **Conclusions**: Pre-hypertension and hypertension in children is a serious problem. If blood pressure were taken in all child and adult dental patients as a screening mechanism, those with pre-hypertension and hypertension could be identified. Those affected patients could then be educated on the lifestyle changes required to enable them live a healthier, active life.

HEALTH SCIENCE

P38 Oral Health Literacy of the Patient Population in a Dental Hygiene Clinic at Indiana University School of Dentistry.

L. L. COAN[1],* R. JACKSON, E. HUGHES, G. J. ECKERT[2]

[1] Indiana University School of Dentistry; [2] Indiana University School of Medicine

Purpose: The purpose of this investigation was to gather data concerning the level of health literacy in adults who frequent the dental hygiene clinic at Indiana University School of Dentistry. Further it explores the attitudes and willingness of students to screen for health literacy in patients seen in clinic. It was hypothesized that the patient pool in dental hygiene would be reflective of all patients in the IUSD system. Knowledge of patient health literacy in

the population pool would assist faculty in development of informed consent materials at the appropriate health literacy levels for all IUSD patients. **Abstract**: As part of a new segment of the curriculum, second year dental hygiene students received a lecture concerning the prevalence of poor literacy in America and the possible consequences of poor literacy on their patients' ability to maintain oral health. The dental hygiene students were given experience with administrating a validated medical health literacy tool; the Simple Test of Functional Health Literacy in Adults (S-TOFHLA). This investigation had two goals; 1) to continue to gather data concerning the level of health literacy of adult patients at Indiana University School of Dentistry (IUSD) and 2) to tabulate data from the dental hygiene students as to their willingness to approach their patients about being screened for literacy. Adults were recruited from the Dental Hygiene Clinic during the individual's normal clinical appointment. Potential panelists were asked to read and sign an informational letter of consent and then the S-TOFHLA was administered by the student using the instructions and narrative provided by the authors of the S-TOFHLA. Ninety-one individuals agreed to participate although the demographic questionnaire was completed by only 67 participants. The mean S-TOFHLA for all 91 subjects was 31 with a range of 4-36 correct responses. The results indicated that 13% of the cohort of individuals scored in the "inadequate" or "marginal" categories. Because of the relatively small sample size, no significant differences were seen by educational attainment although interestingly, of those claiming to have achieved a college degree or trade school certificate or higher, the S-TOFHLA scores ranged from 21-36 correct responses indicating that educational attainment and literacy are not well correlated. Lower S-TOFHLA scores were also associated with the presence of periodontitis, and perceived symptoms of dry mouth. No association was found to having dental insurance, caries risk status or to self-reported frequency of tooth brushing or flossing. In regards to the hygiene students, their comments indicated that it was fairly evenly divided between having no apprehension about requesting their patients take a literacy assessment and being uncomfortable with the request. It would be of benefit in the future to inquire of our students as to whether they would consider assessing patient literacy as part of their practice routines upon graduation. **Conclusions**: A significant proportion of the IUSD patient population was found to have less than adequate health literacy skills. Because of this finding, the authors will propose a series of measures to increase health literacy awareness among our students, staff and faculty and to improve the written materials provided to our patients as a means of improving the level of healthcare. It is hoped this increased awareness will play a role in decision-making policies related to health literacy in the future practitioners.

HEALTHCARE SYSTEM

P40 Influence of In-school Experience on Decision to Treat Special Needs Patients in Private Dental Practice.

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Indiana University School of Dentistry

Objective: The aim of this study was to determine if dental students who obtain hands-on experience, in an elective class, with treating special needs patients are more or less likely to

treat patients with special needs upon graduation. **Method**: This study used a postal questionnaire to compare the attitudes of an equal number of IUSD graduate dentists, from each class graduating year, who did and did not participate in the service learning elective program. To eliminate variables, the gender ratio, the town size and location were kept identical between the groups. All responses were de-identified for confidentiality. **Results**: Survey results were tabulated using counts and percentages for the two groups of dentists. Comparisons between the groups for survey items that had either 2 possible responses or multiple responses with unordered categories were performed using Pearson chi-square tests. Comparisons for survey items that had ordered categories, such as length of time in practice, were performed using Mantel-Haenszel chi-square tests. Of those who took the elective class 40% responded with 70% being female while those who did not take the class only 21% responded with 52% female. For those who took the elective class, 70% specialized on graduation compared to 11% of the others. Nearly 50% of those who had not taken the class or specialized reported inadequate training as one of the barriers and now wished they had so that they would be better prepared. Time required and comfort levels were also more important to this group. It was interesting that all respondents agreed reimbursement level was not a major factor when deciding to treat special needs patients. Limitations: The response rate was low and could question the validity of the results. More responses were received from those who took the class (40%) than did not (21%) and this may affect the conclusions drawn. Additionally, 47% of those who took the elective specialized in pediatric dentistry, which will also impact their decision to treat special needs patients. **Conclusion**: It is well recognized that people with special needs have great problems accessing dental care. It is also thought that when the dental student experiences the care of patients with special needs while in dental school they will be more likely to care for these patients in their dental practice. The results of this small survey have supported this hypothesis.

IMMUNOGENETICS

P41 Genetically Distinct Oral Immunity to Pathogenic Adhesin Epitopes.

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The mucosal immune system revolves around secretory immunoglobulin A (SIgA) and is very important in the pathogenesis of dental caries (caries). This system is tied to human leukocyte antigen (HLA) genes that have been implicated in health and disease. It is not known whether *HLA-DRB1*04* is a caries susceptibility allele or how it affects salivary IgA response to caries pathogens. We hypothesize that there is an association between *HLA-DRB1*04* and immunodeficient antigen presentation of pathogenic adhesin epitopes, diminishing salivary IgA response to cariogenic bacteria. Our objective is to determine salivary IgA reactivity in distinct HLA groups. The study population was divided into two groups: *HLA-DRB1*04* NEGATIVE (n=7) and *HLA-DRB1*04* POSITIVE (n=8). We selected 10 biologically relevant epitopes of an adhesin

(I/II) on *Streptococcus mutans*, which were synthesized (Peptide 2.0, Chantilly, Virginia). Per IUPUI/Clarian IRB approval, we collected human saliva for antibody (1 mL stored at -20^{o}C) and genetic analyses (3 mL stored at room temperature). The saliva for DNA analysis was stored in Oragene® DNA sample collection kits (DNA Genotek, Kanata, Ontario, Canada), then extracted per manufacturer's instructions. HLA typing was performed using the LightCycler ® 480 Real-Time PCR System (Roche Applied Science, Indianapolis, Indiana). Antibody-antigen (i.e., salivary IgA-epitope) binding levels were analyzed using an enzyme-linked immunosorbent assay (ELISA). Differences in salivary IgA reactivity were determined using a chi square test. The mean salivary IgA reactivity (values reflect ELISA absorbance at 490 nm) of *HLA-DRB1*04* subjects was 0.58 (range 0.45-0.68) whereas the NEGATIVE group was 1.25 (range 1.02-1.46). Although we did not observe a statistically significant difference between the groups ($p = 0.36$), we did see a trend in favor of our hypothesis. This preliminary study suggests that *HLA-DRB1*04* subjects showed a trend toward lower levels of salivary IgA to *S. mutans* adhesin I/II. Supported by CTSI, NIH/NCCR PHS TL1RR025759.

IMPLANTOLOGY

P42 In-vivo Evaluation of Novel Custom-Made Press-Fit Implants.

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Computer-tomography (CT)-based custom-made dental implants through electron beam melting (EBM) allows the root portion and the abutment be made as one piece and have the same shape of the patients tooth. This may reduce the potential bone loss and the need for grafting in certain clinical cases. It may also reduce surgery and recovery times and be more cost effective. The early in vitro studies showed great biocompatibility, good mechanical properties, and a good potential for bone ingrowth. The objective of this study is to evaluate the in vivo performance of the novel press-fit dental implant fabricated via (EBM) and compare it to a commercially-available porous-coated press-fit dental implant (Endopore). Twelve cylindrical shaped implants 3x5 mm long were made by EBM using Ti6Al4V ELI alloy. Twelve commercial implants (Endopore™ Innova Corp., Toronto, Canada) of the same geometry were used as controls. Samples were implanted in rabbit tibia and retrieved after six weeks postoperatively. Six specimens from each implant type were embedded undecalcified, sectioned, and stained with toluidine blue for histomorphometry analysis. Bone to implant contact (BIC) was measured. On the six remaining samples from each implant type, the mechanical properties were evaluated by push-out test on a material testing machine. The samples were loaded at a loading rate of 1 mm/min. The push out strength was measured and the apparent shear stiffness was calculated. The results were analyzed with a paired-t test. The histology shows osteointegration of surrounding bone with both implant types. Bone was found to grow into the porous space between the beads. Both the Endopore and the EBM showed similar BIC. The mean BIC for the Endopore and EBM implant were 35±6% and 32±9%, respectively. It failed to reach statistical significance ($p>0.05$). The peak push-out force for

Endopore and EBM implants were 198.80±61.29N and 243.21±69.75N, respectively. The apparent shear stiffness between bone and implant for the Endopore and EBM implants were 577.36 ±129.99N; and 584.48±146.63N, respectively. Neither the peak push-out force nor the apparent shear stiffness of the implants was statistically different between the two groups ($p>0.05$). The results suggest that the implants manufactured by EBM perform equally well as the commercial implant Endopore in this current animal model. Funded by Baylor College of Dentistry.

Presentations

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

INFECTION CONTROL

P43 Bacterial Contamination of Dental Impression Guns Used in IUSD Clinics.

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Bacteria, including MRSA often contaminate dental instruments and equipment. Dental impression guns are frequently used within the operatory without barriers or disinfection between patients. IUSD infection prevention guidelines do not specifically cover impression guns. In this study, we attempted to detect and quantify bacteria on impression guns used in IUSD clinics. Used were 2 impression guns used in Clinic A as well as 2 impression guns donated by a supplier for the project. Sampling included 4 sites on each gun and involved 2 sterile cotton swabs moistened with sterile physiologic buffered saline (PBS, 0.85 M, pH 7.2). Both swabs went into a single tube containing 2.0 mL of sterile PBS, vortexed and spread plated onto selective media. Incubation was both anaerobic and aerobic at 37^{o}C for 48 hours. All 4 guns produced MRSA along with numerous other colonies of oral and non-oral bacteria. MRSA was present on 5 of the 8 sites on the 2 IUSD guns and on 6 of the 8 sites on the donated guns. The guns then underwent disinfection using 2 CaviWipes™ per sampling site. Repeated sampling resulted in only a slight decrease in the number of bacterial colonies. After autoclaving and sampling, there were no colonies detectable. After being autoclaved again, the 4 autoclaved guns went back to Clinic A for 3 weeks. During each use plastic covers went over the guns. After removal of the plastic covers, clinic staff disinfected the guns using ProSpray™ disinfectant. After 3 weeks, the guns underwent sampling as previously described with growth detected on 3 of the 16 surfaces. No MRSA was present. Our work indicates that use of common infection prevention methods appears to reduce the presence of MRSA and other bacteria on impression guns. Autoclaving, as expected, produced sterile guns. Autoclaving followed by the use of plastic gun barriers followed by disinfection appeared to reduce bacterial accumulation. Supported by IUSD Infection Control Research & Services and the IUSD Dental Student Research Program.

P44 Methicillin Resistant *Staphylococcus aureus* Present on Dental Student Laptops.

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Computers have become ubiquitous in healthcare. Studies suggest that keyboards and mice could contribute to cross-transmission of potentially pathogenic microorganisms. The objective of this study was to determine the presence of methicillin resistant *Staphylococcus aureus* (MRSA) on three different groups of dental student laptop computers before and after either daily or weekly disinfection procedures. These groups included Fourth Year Dental Student (4YDS) laptops after three weekly disinfection procedures, and Second Year Dental Student laptops after three weekly disinfection procedures (2YDS1) and after five daily disinfection procedures (2YDS2). The first study included 42 4YDS and 52 2YDS1 laptop computers which underwent a series of three weekly disinfections. The second study included a third group of 33 2YDS2 laptops that received daily disinfection for one week. Sampling of the top surface of each laptop was performed using physiological buffered saline (PBS)- moistened cotton swabs, which occurred prior to disinfection. Swabs went into tubes containing 2.0 mL of PBS and were vortexed for 15 seconds. Using a spiral plater, the solutions were transferred onto enriched trypticase soy agar (ETSA) and mannitol salt agar (MSA) plates. Incubation was aerobic at 37oC for 48 hours. Sub-culturing of colony types using trypticase soy broth then occurred. Tubes with growth underwent spread plating onto MSA, ETSA with cefotaxime discs, oxacillin resistance screening agar and MRSA selective agars - CHROMagar and MRSASelect. Counting colonies occurred after incubation. The first two groups (4YDS and 2YDS1) received three weekly disinfection procedures with CaviWipes (Metrex, Romulus, MI), while disinfection of the third group (2YDS2) received daily disinfection procedures for one week using the same disinfectant. Sampling and plating procedures after disinfection were the same as prior to disinfection. Pre-disinfection specimens from 4YDS laptops produced 148 isolates of which 41 were *S. aureus* and 7 laptops yielded 11 MRSA isolates of which 8 were also cefotaxime resistant, while 2YDS1 weekly (2YDS2 daily in parenthesis) laptops produced 109 (127) isolates with 74 (71) being *Staphylococcus aureus* and 23 (13) laptops yielded 23 (62) MRSA isolates with 6 (6) being cefotaxime resistant. Sampling after disinfection from 4YDS laptops produced 114 isolates of which 49 were *S. aureus* and 6 laptops produced 8 MRSA isolates of which 5 were also cefotaxime resistant while 2YDS1 weekly (2YDS2 daily in parenthesis) laptops produced 90 (90) isolates of which 59 (35) were *S. aureus* and 35 (8) laptops produced 36 (17) MRSA isolates of which 2 (0) were cefotaxime resistant. Overall, the presence of MRSA isolates was demonstrated on laptops in 4YDS, 2YDS1, and 2YDS2 groups. A higher percentage of 2YDS1 and 2YDS2 laptops produced *S. aureus* and MRSA isolates than did 4YDS laptops. Results also indicated that five daily disinfection procedures were more effective than three weekly disinfection procedures. However, more frequent disinfection procedures in addition to other measures would appear necessary to achieve laptops completely free of MRSA.

P45 Microbial Contamination of Stainless Steel Crowns Prior to Intraoral Placement.
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Stainless steel crowns are placed on a daily basis in pediatric dental offices and clinical settings. Proper infection prevention techniques are critical aspects of dentistry at all levels. There are multiple procedures used in SSC placement. The objective of this study was to determine the number and types of bacteria present on clinically stored stainless steel crowns (SSC), and determine potential contamination sources. Sampling included two sizes of E2 and size E4 stainless steel crowns (SSC) from each quadrant and from SSC storage compartments present in six different clinical sites. Stainless steel crown sampling was by immersion in a non-specific broth media followed by incubation and finally plating onto selective agar media. Compartment sampling included use of moistened swabs and processing as described for SSC. Statistical analysis included Chi-square tests and analysis of variance (ANOVA) and compared differences in SSC type, clinic location, and amount of contamination. All SSC's and storage containers had some level of contamination, including MRSA in some cases. Private practice settings had significantly (P=0.0001) more contamination than university settings. Temperature (P=0.27) and crown size (P=0.65) did not have a significant effect on the presence of bacteria (P=0.27). Contamination levels appeared related to sterilization methods and storage methods used. Recommendations to minimize SSC contamination prior to intraoral placement may include heat sterilization and minimizing the transfer of crowns chairside.

MICROBIOLOGY/IMMUNOLOGY

P46 Effect of Nicotine on the Growth and Biofilm Formation of *Streptococcus mutans*.
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Streptococcus mutans plays a major role in the formation of dental caries. Oral bacteria are exposed to a variety of factors that affect growth and biofilm formation. However, there are different environmental conditions under which the growth and biofilm formation of the bacteria are affected, including exposure to nicotine. There are several proteins that play a role in the biofilm formation of the *S. mutans*. Previous work had indicated that increased concentrations of nicotine upregulated expression of *S. mutans* antigen I/II, a surface adhesin responsible for binding to salivary agglutinin. The objective of this project was to determine the effect of nicotine on the growth and biofilm formation of *S. mutans* on salivary-coated microtiter plates. In order to examine whether the growth and biofilm formation of *S. mutans* UA159 on saliva-coated microtiter plates are affected by nicotine, microtiter plates were coated with diluted saliva. The plates were incubated with *S. mutans* cells that had grown in varying concentrations of nicotine, ranging from 0.125 to 4.0 mg/ml for 16 h. The planktonic cells were transferred to a second microtiter plate, and their growth was measured at 600nm. The first microtiter plate containing biofilm cells was incubated with a formaldehyde solution overnight

to fix the biofilm cells to the plate. The formaldehyde was removed by washing with saline, and the plate was stained with crystal violet and read at 600nm to measure the amount of biofilm formation. The experiments indicated a significant decrease ($p<0.05$) in the amount of planktonic cells in all concentrations of nicotine, which correlated to an increase in the amount of adhered cells. The results also established a significant increase in the amount of biofilm formation of the cells grown in 2.0 mg/ml of nicotine when compared to the 0 mg/ml control. This data establishes that nicotine has an effect on the growth and selective biofilm formation of *S. mutans* to saliva-coated microtiter plates, possibly related to the increased expression of antigen I/II previously reported by our laboratory. The increased biofilm formation by *S. mutans* after exposure to nicotine provides one explanation for increased dental caries in smokers. This work was funded by the MURI Program of the Center for Research and Learning and the Indiana University- Purdue University Tobacco Cessation and Biobehavioral Signature Center.

P47 Effects of Dental Materials With Nicotine on *Streptococcus mutans* Growth.

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Objectives: Triethylene glycol dimethacrylate (TEGDMA) is a methacrylate monomer compound which produces toxic triethylene glycol (TEG) and methacrylic acid (MA) upon hydrolysis resulting in approximately 1.25 mM concentrations in crevicular fluid. Research has shown that microleakage from dental restorations made from these materials contribute to secondary caries. Nicotine, from smoking, weakens salivary function and encourages the growth of *Streptococcus mutans,* which produces enzymes and organic acids and eventually causes dental caries. **Methods:** In this study, we examined the effects of TEGDMA, TEG or MA with and without nicotine on *S. mutans* growth (absorbance and viable bacterial numbers) at different concentrations. The same procedure was repeated for both *Streptococcus gordonii* and *Streptococcus sanguis*. **Results:** We observed that TEGDMA, TEG and MA significantly inhibit ($p<0.05$) the growth of *S. mutans* to various degrees, TEGDMA up to 85%, TEG up to 81% and MA up to 91%. However, by adding TEGDMA, TEG or MA together with nicotine, *S. mutans*, but not *S. gordonii* and *S. sanguis,* growth is enhanced compared to the samples incubated without TEGDMA, TEG and MA. **Conclusion:** Our data indicate that nicotine protects *S. mutans* from the growth inhibitory effect of TEGDMA, TEG and MA. Hence, we propose that smoking influences secondary caries by affecting dental materials specifically TEGDMA and *S. mutans*.

P48 Effects of Mecamylamine and Epibatidine on Activation of *Streptococcus mutans*.

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Nicotine exposure upregulates *Streptococcus mutans* growth and surface proteins, leading to increased adherence to tooth structure, and increased caries incidence. At this time, specific nicotinic receptors on *S. mutans* have not been found; however preliminary evidence indicates *S. mutans* has β2 nicotinic receptors which would provide a mechanism of nicotine entrance into *S. mutans* cell. Epibatidine is a nicotine agonist at nicotinic acetylcholine receptors,

especially α4β2 and the α3β4 sub-types. Mecamylamine is a competitive antagonist at α3β4 sub-type nicotinic receptors. **Objective**: Examine the effects of pretreatment with mecamylamine and epibatidine on nicotine-induced activation of *S. mutans*. **Methods**: Cultures of *S. mutans* UA159 cells were routinely incubated overnight for 16 h in sucrose-free tryptic soy broth (TSB) media. Bacterial cells were grown to late-exponential phase (OD=0.8). A 96 well plate with airtight tape was used to grow UA159 cultures plus experimental treatments anaerobically. Controls included a blank, TSB (no bacteria), and TSB + 10 uL of UA159. Experimental conditions include 10 uL of UA159 plus the following: nicotine in concentrations of 0.25, 0.5, 1.0, and 4.0 mg/ml, mecamylamine (1.0 mg/ml), epibatidine (1.0 mg/ml), mecamylamine with the same nicotine concentrations, and epibatidine with the same nicotine concentrations. Mecamylamine and epibatidine were added to test wells 30 minutes prior to adding nicotine. Bacterial growth was kinetically measured every 20 min by optical density (OD) at 600 nm. Each experimental condition was run in triplicate, and each experiment was conducted three times. **Results**: Nicotine, mecamylamine, and epibatidine demonstrated a significantly shorter ($p<0.05$) time to maximum absorbance and significantly greater maximum absorbance compared to UA159 alone. Mecamylamine and epibatidine plus nicotine samples indicated similar maximum absorbances to mecamylamine and epibatidine samples alone. Conclusion: Mecamylamine and epibatidine have an agonist effect on *S. mutans* leading to upregulated growth, similar to that of nicotine, supporting the evidence of the presence of β2 nicotinic receptors on the surface of *S. mutans*.

P49 Effects of Nicotine on Nicotinic Acetylcholine Receptor (nAChRs) Expression in *Streptococcus mutans* Biofilm.

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Streptococcus mutans is a cariogenic bacterium that metabolizes a wide variety of carbohydrates which results in production of lactic, acetic, formic and propionic acids. The presence of the above acids is associated with a rapid increase in the mineral dissolution rates of tooth enamel and dentine. Previous studies have suggested a trend of increased caries risk and an up-regulation of oral bacterial virulence in tobacco product users; however, the exact process is not yet apparent. The complete genome sequence of *S. mutans* UA159 demonstrated a significant homology with the β2 subunit gene of the known sequences of human nAChRs. In this study, the effects of nicotine on expression of the β2 subunit gene of AChRs in *S. mutans* biofilm were investigated. The *S. mutans* biofilm was grown in tryptic soy broth which contained sucrose and nicotine. The nicotine concentrations varied from 0 to 1.0 mg/ml which are of physiological significance. Enzyme-linked immunosorbent assays (ELISA) were performed to detect the expression and relative levels of the nicotinic receptors on the wall of *S. mutans* bacterial cells in the biofilm. The results confirmed the presence of the nicotinic receptors on the *S. mutans* biofilm surface with or without nicotine content. Moreover, the data indicates a significant up-regulation ($p<0.05$) of the nicotinic receptors expression in *S. mutans* biofilm with increasing nicotine concentration. These results may contribute to understanding of nicotine entrance and metabolism processes by the bacteria and their future inhibition. Further experiments are being done to examine several nAChR agonists on the upregulation of the

receptors by nicotine. This work was funded by the MURI Program of the Center for Research and Learning and the Indiana University- Purdue University Tobacco Cessation and Biobehavioral Signature Center.

P50 Increased Virulence Properties of a Nicotine-resistant *Streptococcus mutans* Mutant.

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Objectives: Smokers have increased caries, while tobacco and nicotine at physiological concentrations (0.25-1 mg/ml) enhance *Streptococcus mutans* growth and acid production. We hypothesize that nicotine accumulates in dental biofilm and therefore *S. mutans* in the dental plaque of smokers may be able to tolerate higher nicotine concentrations with increased virulence properties in terms of glucan binding, glucan production and final pH. **Methods**: A nicotine-resistant *S. mutans* strain was created by passage of UA159 wild-type (WT) in increasing concentrations up to 10 mg/ml of nicotine. The mutant and WT grown to late log-phase were examined in a glucan-binding assay by measuring the amount of biotinylated-dextran bound to the cells. In addition, the final pH was measured and glucan in the culture supernatant of 16 h sucrose-grown cells was determined by the phenol-sulfuric assay. **Results**: *S. mutans* growth is significantly delayed above 4 mg/ml of nicotine. We noticed that the 10 mg/ml mutant colonies were significantly stickier than WT colonies grown on sucrose-containing plates and the mutant cells had a larger glucan pool upon Gram staining. The mutation was stable because after 10 passages in nicotine-deficient media nicotine-resistance at 10 mg/ml was maintained. The glucan-binding assay indicated that the mutant significantly bound 2.5-3 fold more dextran than the WT strain. However, the final pH and the amount of soluble glucan produced from the two strains were similar. Conclusion: The results indicate that the mutant was able to bind significantly more glucan than the WT and maintained the ability to produce sufficient acid to cause caries. Previous data indicated that tobacco/nicotine upregulate glucan-binding proteins and glucosyltransferase in the WT strain. Together, this data suggests that the mutant produces more insoluble glucan and/or cell-associated glucan than the WT. Tobacco/nicotine plays a pivotal role in the caries process increasing the virulent properties of *S. mutans*.

P51 Influence of Cigarette Smoke Extract on Neutrophil-Epithelial–Bacterial Biofilm Interactions.

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Objectives: We set out to determine the influence of cigarette smoke extract (CSE) on cytokine and protease networks produced in a novel model of mucosal inflammation representing the mucosal surface (primary human gingival epithelial cells; HGECs); the major overlying sulcular immune infiltrate (primary human neutrophils; PMNs) and a relevant inflammatory stimulus (the major periodontal pathogen, Porphyromonas gingivalis). **Methods**: HGEC monolayers (1 x 106 cells) were overlaid with PMNs (1 x 107 cells) then P. gingivalis biofilms (10 bacteria : 1

PMN) in KSFM or KSFM supplemented with CSE (100 and 1000 ng/ml nicotine equivalents). Cytokine (IL-1b and IL-8) and protease (MMP-3, -8, -9; NGAL; TIMP-1) release into cell-free culture supernatants were measured by ELISA. **Results**: P. gingivalis alone induced low levels of Il-1b and IL-8 from epithelial cells, but high levels of both cytokines in the presence of PMNs. CSE-exposure (100 and 1000 ng/ml nicotine equivalency) significantly compromised P. gingivalis-induced cytokine secretion (both $p < 0.05$). P. gingivalis induced impressive (> 30 mg/ml) secretion of neutrophil-gelatinase (MMP-9)- associated lipocalin (NGAL) which was not influenced by CSE ($p < 0.05$). Conclusion: Tobacco smoking increases susceptibility to periodontitis; promotes more rapid disease progression; increases susceptibility to P. gingivalis infection; but reduces clinically overt inflammation. Here, we show that P. gingivalis induces large amounts of NGAL, which would be expected to abet collagen degradation and promote disease progression. CSE-exposure reduces the pro-inflammatory cytokine burden, which is likely to promote P. gingivalis survival. Therefore, these results are in keeping with established clinical dogma and establish mechanisms that help explain increased susceptibility to periodontitis in smokers. Further mechanistic elucidation may identify therapeutic targets for the treatment of tobacco-induced periodontitis and other smoke-induced chronic inflammatory diseases.

P52 Molecular Detection of Cytomegalovirus and Epstein-Barr Virus in Periapical Lesions.

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Objective: It has been reported that the herpesviruses, Epstein Barr Virus (EBV) and Human Cytomegalovirus (CMV), may be present in periapical lesions and may contribute to periapical pathosis in lesions of endodontic origin. Cytomegalovirus (CMV) and Epstein-Barr Virus (EBV) have both been indentified in periapical tissues using polymerase chain reaction (PCR) but these results have not been widely replicated. **Methods**: After approval of the research by Human Studies, extracted teeth with attached intact periapical lesions were collected from the Department of Oral Surgery. DNA from the lesions was extracted using QiaAMP DNA Mini Kit, and total DNA was measured using Nanodrop. Polymerase chain reaction was used to detect for the gB protein of CMV and the gp350 gene of EBV using specfic primers. Primers specfic for human glyceraldehyde-3-phosphate dehydrogenase (GapDH) was used as a positive control. PCR reaction was analyzed for products using a 2% agarose gel. Results were then visualized under UV light. **Results**: Eight out of ten samples collected were reported to be symptomatic periapical lesions. Agarose gel analysis resulted in four out of ten samples with a positive band for EBV. Two of these samples were reported to be symptomatic. Agarose gel analysis of CMV resulted in two out of ten samples with a positive band, with both of these samples being reported as symptomatic. Conclusion: Our results show that there may be a correlation between symptomatic periapical lesions and presence of CMV. However, the results for EBV are less conclusive. Currently, I am trying to replicate these results, and increase my patient database.

P53 Nicotine Effect on Hydrophobicity and Biofilm Formation of *Streptococcus mutans*.

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Objectives: Smokers have increased caries while *Streptococcus mutans* plays a major role in tooth decay. Antigen I/II is a surface protein adhesin of *S. mutans*. The relative abundance of antigen I/II on the surface of *S. mutans* can be measured by assessing the hydrophobicity. Previous studies have shown that antigen I/II mutation decreases the hydrophobicity of *S. mutans*, and nicotine up-regulates antigen I/II protein expression. **Methods**: The effect of nicotine on the hydrophobicity of *S. mutans* was measured. Nicotine dilutions from 0.16-5 mg/ml were made in Tryptic Soy Broth without sucrose. Bacteria were grown in each of these dilutions for 16 h at 37^{o}C in 5% CO_2. The cells were washed three times in saline and hydrophobicity was measured using a hexadecane assay. Biofilm formation was measured using the same nicotine dilutions placed into the wells of 96 well sterile microtiter plates, and incubated for 16 h. The wells were rinsed, stained with crystal violet for 10 min, rinsed again with saline and the absorbance of the biofilm cells was measured at 490 nm. **Results**: The results demonstrated that the hydrophobicity of *S. mutans* significantly increased ($p<0.05$) up to 2 fold as the nicotine concentration increased up to 1.25 mg/ml then hydrophobicity leveled off. The increase in hydrophobicity suggests that nicotine users will have increased *S. mutans* dental biofilm and caries. The biofilm data indicates that as the nicotine concentration increased, biofilm formation of the bacteria significantly increased between 2 and 4 fold. Conclusion: This data suggests that the increase in hydrophobicity and biofilm formation of *S. mutans* to the tooth surface observed with nicotine is directly related. Nicotine plays a pivotal role in the caries process of smokers by increasing the ability of *S. mutans* to adhere to tooth surfaces.

P54 The Accumulation of Nicotine in Nicotine-treated *Streptococcus mutans* Biofilm. I.

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Streptococcus mutans is a known causal agent of plaque and dental caries. An accumulation of biofilm subjects the oral cavity to dental disease. Previous studies indicate that smokers have an increased rate of biofilm formation and caries than nonsmokers. Earlier work demonstrated that nicotine and tobacco upregulated antigen I/II expression in *S. mutans,* which correlates with increased attachment to smooth surfaces. Therefore, it is important to determine if sufficient levels of nicotine accumulates in dental biofilm in order to affect antigen I/II expression. The aim of this study was to ascertain the amount of nicotine accumulation in in vitro created biofilm by enzyme-linked immunosorbent assay (ELISA). *S. mutans* UA159 biofilm containing nicotine and tobacco condensate from 0-2 mg/mL was grown for 20 hours in 5% CO2 at 37oC, probed with anti-nicotine primary and HRP-labeled secondary antibodies, and the absorbance measured at 490nm for in situ accumulation of nicotine. Nicotine accretion was significantly elevated ($p<0.05$) when biofilm was incubated with higher concentrations of both nicotine and tobacco condensate compared to the negative control. The highest amount of

nicotine in biofilm was observed at the highest nicotine concentration used when treating the biofilm. Additional studies of nicotine-treated biofilm which were collected and coated uniformly onto ELISA plates provided similar results. The increase of biofilm buildup in the oral environment could lead to a greater amount of demineralization of the enamel due to acid released from biofilm cells. Moreover, saliva would be unable to penetrate biofilm and thus could not remineralize the surface. This data supports the observation that smokers have an increased rate of biofilm formation due to exposure of tobacco components. This work was funded by the MURI Program of the Center for Research and Learning and the Indiana University- Purdue University Tobacco Cessation and Biobehavioral Signature Center.

ORTHODONTICS

P55 A Survey of the Perceived Importance of Tasks Delegated to Orthodontic Assistants.

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No current and/or comprehensive data exist reporting which tasks are most important for an orthodontic assistant to perform in a contemporary orthodontic office. Such information would be useful in designing educational curricula and continuing education courses in orthodontic assisting. The purpose of this study was to establish if various tasks commonly delegated to orthodontic assistants are perceived by orthodontists as being more important than others and to identify the relative differences between each. A total of 161 typed surveys were mailed to practicing orthodontists in Indiana, and 107 responses were received. The surveys listed forty-five concepts or techniques that orthodontic assistants are commonly expected to understand or perform. Orthodontists were asked to evaluate the importance of each in their practice according to a 5-point Likert scale. The results were reported using means, standard deviations, and medians. Relationships between orthodontists' responses and years in practice were also evaluated. The highest three means were associated with Post-Op/Hygiene Instructions, Infection Control/Sterilization, and Powerchain Placement. The lowest three means were associated with J-Hook Headgear Fitting/Adjustment, Chin Cup Fitting/Adjustment, and Cephalometric Tracing. The data suggest that various concepts and techniques that orthodontic assistants are commonly expected to understand and perform are perceived at different levels of importance by orthodontists. Supported by IUSD Research Fellowship.

P56 Histomorphometric and Biomechanical Analyses of Osseointegration of Four Different Implant Surfaces.

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Introduction: Anchorage control plays a pivotal role in the effective management of orthodontic cases for attaining both structural and facial esthetics. Attaining maximum or absolute anchorage has always been an arduous goal for the practicing orthodontist often

resulting in a condition, dreaded by most, called 'anchor loss'. In recent years titanium mini-screws have gained enormous popularity in the orthodontic community and are being considered as 'absolute' sources of orthodontic anchorage. The topography of the dental implant/ Mini implant surface has been widely studied, and various surfaces have been proposed for titanium dental implants/ Mini implants. However, the results of these studies are inconclusive concerning the best implant surface for obtaining clinical success. **Methods**: A total of 128 mini implants, differing in surface treatment, will be placed into tibia and femurs of 8 (4 to 5 months of age) male New Zealand white rabbits. On the basis of surface treatment, the mini implants will be divided into four types: 1) Machined, 2) Acid Etched, 3) Grit Blasted, and 4) Grit Blasted and Acid Etched. Each leg of rabbit will receive a total of sixteen implants, four each in the mid diaphyseal regions of the tibia and the femur. Eight weeks after the surgical procedure, all the animals would be euthanized with an overdose of anesthesia and the femur and tibia would be dissected free. From femur and tibia equal number of specimens will be used for mechanical testing (Nanoindentation+ Torque) and histomorphometric analysis. **Results**: After 8 weeks of healing, higher bone to implant contact and higher removal torque was observed in Grit Blasted and Acid Etched implant surfaces when compared to rest of implant surfaces. Scanning electron microscopy revealed that less interfacial gap between the Grit Blasted and Acid Etched implant and bone in comparison to other implant surfaces. **Conclusions**: Analysis of the data suggests that the implant surface roughness affects the biomechanical quality of osseointegrated bone.

P57 Orthodontic Needs and Esthetic Self-Perception in Honduran Adults.

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The decision to undergo orthodontic treatment is determined by a number of factors including social, economic or cultural issues. In some populations, treatment is not available to everyone and a measurement instrument may be used to identify those who would most benefit from it. The Index of Treatment Need (IOTN) has proven to be valid, reliable and quick to assess orthodontic treatment need. Orthodontic need has not been reported in Honduras and the aims of this study are to: 1) determine the normative and perceived orthodontic treatment needs of an adult population using the dental health component (DHC) and aesthetic component (AC) of the IOTN 2) determine the esthetic self-perceptions of this population using a visual analogue scale (VAS) and 3) compare the esthetic self-perceptions (VAS) with the perceived treatment need (AC). 91 adults (mean age 28.2) utilizing an outreach clinic in Pimienta Cortés, Honduras, were given a questionnaire addressing age, gender, socio-economic status, ethnicity and distance traveled for treatment. Perceived orthodontic need was indicated using a photographic scale. A visual analog scale was used to assess the self-perceived attractiveness of an individual's teeth by marking a response on a 100 mm line (0 being the least attractive). The questionnaire was followed by a clinical examination, by a calibrated examiner, to assess normative need by measuring multiple factors of malocclusion including overjet, overbite, molar relation, and crowding. **Results** showed that 66% of the sample has a normative "great" to "severe" need for treatment while only 3% of the evaluated population indicated a "great" self-perceived need for treatment. The mean VAS score was 60.7, which

only weakly to moderately correlated with the scores for perceived need. The normative need for orthodontic treatment in this specific Honduran population falls within the range of previously reported need in other countries. The weak to moderate correlation between the populations' esthetic perception and their perceived need for treatment suggests the necessity to use a modified instrument in this population. Supported by an EEG grant and the Kishibay and Eteson Craniofacial and Orthodontic Research Fund.

P58 Rescue of Coronal Suture Fusion With Relaxin in Craniosynostotic Rabbits: A Preliminary Analysis.

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Objectives: Craniosynostosis affects 300-500 per 1,000,000 births and may result in craniofacial and neural growth disturbances. Histological data has shown that thick collagenous bundles are present in the sutural ligaments, which may tether the osteogenic fronts, resulting in premature fusion. The hormone Relaxin has been shown to disrupt collagen fiber organization. It may prevent craniosynostosis by relaxing the sutural ligaments and allowing the osteogenic fronts to separate normally and stay patent. The preliminary study was designed to test this hypothesis in a rabbit model of delayed onset coronal suture synostosis . **Methods**: To date, 8 New Zealand White rabbits with craniosynostosis were randomly assigned to three groups: 1) Sham control (n=3); 2) BSA treated protein control (10ug/suture) (n=2), and; 3)Relaxin treated rabbits (10ug/suture) (n=3). At 10 days of age the BSA and Relaxin were mixed in a slow release (56 day) collagen vehicle and injected subperiosteally above the coronal suture. Longitudinal radiographs and body weights were collected at 10, 25, 42, and 84 days of age and the sutures harvest for histological examination. **Results**: Preliminary analysis of the radiographs revealed that rabbits treated with Relaxin had patent coronal sutures, thin osteogenic fronts, and wide sutural ligaments at 42 days compared to controls. However, no significant differences ($p>0.05$) were noted in any of the cephalometric measurements between Relaxin treated rabbits and controls rabbits at any age. **Conclusions**: These preliminary data support our initial hypothesis that the use of Relaxin may transiently disrupt collagen organization and rescue sutures destined to undergo premature suture fusion. However, further cephalometric and histological analyses from a larger sample size is needed to assess whether this protein therapy may have potential clinical utility in infants with insidious or progressive craniosynostosis.

PEDIATRIC DENTISTRY

P59 Induced Root Apexification in Young Permanent Incisors Using Calcium Hydroxide.

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Purposes: This pilot study was conducted in order to determine the speed of apical barrier formation and other variables related to it in traumatized young permanent incisors treated with apexification. **Methods:** Three symptomatic young permanent incisors with non-vital and open apices were treated using calcium hydroxide apexification according to a specific clinical protocol. The cases were reviewed clinically and radiographically after 6 months, then every 3 months, until apexification occurred. **Results:** The apexification procedure was successful in the 3 cases. During the initial 6 month period, apical barrier was formed in 2 cases. An additional 3 months waiting period was required for the apical barrier formation of the third case. Two of the apical barriers were located ≤1 mm from the apex. The third apical barrier was located 2-3 mm below the apex. No intra-appointment symptoms were reported for the 3 cases. Clinical symptoms resolved in all of the 3 teeth. **Conclusions:** Single application of calcium hydroxide may be enough for apical barrier formation and resultant apexification. Current recommendations suggest a 2 or 3 month follow-up interval. This study suggests that an initial 6 month follow-up interval after the first application of calcium hydroxide may be used instead of 2 or 3 months. This would assist in reducing appointments, cost and radiation exposure.

PERIODONTICS

P60 A Bayesian Approach to Assessing Factors With Bone Loss Around Endosseous Implants.

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Purpose. To examine factors that influence the rate of bucco-lingual bone-loss occurs around endosseous immediate implants. **Methods**: Mesial and distal vertical bone heights, bucco-lingual bone width, and plaque index were measured and calculated at implant placement (0 months), 4 months, and 12 months. Implant site bone density was given a categorical value of 0 to 3 from tooth replacement number, corresponding to posterior maxilla to anterior mandible. Data was standardized into standard normal variables. Terminal events were defined as occurring when >50% of bucco-lingual bone loss occurred (from time of implant placement). Statistically, the time data was stochastically perturbed, and all data was embedded into a proportional hazard's model with a two parameter Weibull baseline in the Bayesian paradigm in the software WINBUGS. A Markov Chain Monte Carlo (MCMC) algorithm procedure was used

for parameter inference. The chain was run for 150,000 iterations, and the first 50,000 were discarded as burn-in. Predictive distributions were calculated for a variety of experimental conditions to generate predictive probability distributions of bucco-lingual bone loss of >50% occurring. **Results**: Clinical covariates influence baseline bucco-lingual bone-loss rate in the rank order (comparing magnitude of expectation of coefficient): distal bone-loss rate (2.645), initial bucco-lingual bone width (1.108), oral hygiene (0.9596), mesial bone-loss rate (0.8308), and bone density (0.0569). Conclusion: Mesial and distal bone-loss rates are strongly predictive of bucco-lingual bone loss rate. This is likely due to shared inflammatory etiology between covariates (dependency). Initial bucco-lingual bone width and plaque index are also strongly predictive. Bone density did not have a significant predictive effect.

P61 Effect of Non-surgical Periodontal Therapy on sTLR2 Levels in Saliva.

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The diagnosis of periodontitis, monitoring of disease activity & efficacy of periodontal treatment is challenging and highly subjective. The American Association of Periodontology (AAP) in its consensus statement states that the current system of diagnosis is a “measure of accumulated past disease at a site rather than current activity.” In addition response to periodontal therapy is measured by subjective clinical parameters, and there are no objective biomarkers to elucidate the effect of therapy. Thus, there exists a need for an objective measure to diagnose active periodontitis, and it’s response to therapy. Although considered as chronic bacterial infection, the periodontal disease pathology is mediated by host response to the local microflora. Toll like receptors area family of germ line encoded receptors that recognize and respond to the local flora. A soluble form of toll like receptor 2 (sTLR2) was recently identified in human plasma, breast milk and saliva. It has been shown that TLR-2 suppresses excessive host response against putative periodontal pathogens such as Porphyromonas Gingivalis, T. Forsythus, or their products. Most molecules and cells from the periodontium and GCF end up in saliva. The purpose of this study is to test the hypothesis that the salivary levels of sTLR-2 may correlate with health/disease status of individuals and treatment would shift sTLR2 levels towards health in diseased individuals. Unstimulated whole saliva (UWS) was collected before and after non surgical periodontal therapy from 20 subjects based on the AAP classification {10 subjects from healthy/gingivitis (HG) subjects and 10 from patients with generalized severe chronic periodontitis- (CP)}. sTLR-2 level in clarified UWS was assessed qualitatively and quantitatively by Western blot and ELISA respectively. STLR2 levels were found at significantly reduced levels in CP as compared to HG before non surgical periodontal therapy. After periodontal therapy levels of sTLR2 significantly increased in CP correlating with improvement in clinical parameters. These results suggest that sTLR2 levels maybe reflective of periodontal health Vs disease state. The results also suggest sTLR2 may serve as an adjunctive tool in the diagnosis of periodontal disease and in the monitoring of disease activity. Supported by Dr. Mythily Srinivasan, IUSD and IUSD research committee.

P62 The In-vitro Response of Human Neutrophils to *Fusobacterium nucleatum*.

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Fusobacterium nucleatum is one of the most numerically prevalent bacteria associated with gingivitis and is associated with bacteremia of oral origin. The interaction between oral bacteria and host defense is a central mechanism linking oral and systemic diseases. We hypothesized that the metabolic oxidative activity of peripheral blood neutrophils is stimulated by exposure to *F. nucleatum*. **Objective**: To evaluate the oxidative response of human peripheral blood neutrophils, in terms of priming and activation, to *F. nucleatum*. **Materials & Methods**: The oxidative activity of peripheral blood neutrophils was evaluated by lumiol-enhanced chemiluminescence. Assays were conducted using *F. nucleatum* ATCC 10953. In addition, *Staphylococcus aureus* ATCC 9144 and *Aggregatibacter actinomycetemcomitans* ATCC 33384 were used as bacterial controls. All bacteria were killed by exposure to UV light prior to use in the assays. Comparisons among the groups were performed using ANOVA. **Results**: Activation of neutrophils with *F. nucleatum* resulted in significantly higher total chemiluminescence ($P<0.05$) as compared to activation with *A. actinomycetemcomitans* and *S. aureus*. In turn, the total chemiluminescence induced by activation with *A. actinomycetemcomitans* was higher than that produced by activation with *S. aureus* ($P<0.05$). Not priming the neutrophils or priming the neutrophils with N-Formyl-L-methionyl-L-leucyl-L-phenylalanine or *F. nucleatum* prior to activation with *F. nucleatum*, did not significantly affect the levels of total chemiluminescence. **Conclusions**: Activation of human peripheral blood neutrophils with *F. nucleatum* induced the highest levels of total chemiluminescence whether neutrophils were primed or unprimed. The findings of this study may shed light on the mechanistic pathways by which oral infection may impose risk for systemic diseases. Supported by NIH # R01 DEO15145-01.

P63 The Response of Human Neutrophils to Nicotine and *Porphyromonas gingivalis*.

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Introduction: Tobacco smoking is considered a major modifiable risk factor for periodontal disease. It affects the oral environment including the gingival tissue and vasculature, as well as the host immune response. Nicotine is the addictive ingredient in tobacco and has been shown to affect multiple cellular processes. Neutrophils are the first line of defense and are critical cells in the maintenance of periodontal health through their role in the control of bacteria, but they can also contribute to the progression of periodontal disease by the production of reactive oxygen species (ROS). ROS are released to kill the bacteria, but the extracellular release of ROS results in collateral damage of the surrounding tissues. The role of specific gram-negative bacteria in the etiology and pathogenesis of periodontal disease has been well established. Lipopolysaccharides from periodontal pathogens such as Porphyromonas gingivalis (P. gingivalis) stimulate the respiratory burst of neutrophils. The objective of this study was to explore the oxidative activity of neutrophils when stimulated with P. gingivalis, nicotine, or

both. Materials and methods: Seven buffy coats were purchased from the Central Indiana Blood Center with IRB approval and the neutrophils were separated by the Double Dextran Gradient Method. Subsequently, the generation of ROS was determined using luminol-dependent chemiluminescence assays. For each run of the experiments, 500 µl of neutrophil suspension of 1,000,000 cell/ml, 300 µl of phosphate buffer solution, and 100 µl luminol was dispensed at baseline. Then 80 µg/ml of nicotine, 80µg/ml of nicotine + 10% of P. gingivalis, and 10% of P. gingivalis alone were added. The reaction was followed for 90 minutes and neutrophil activation was recorded as total integrated energy output. **Results**: The P. gingivalis and P. gingivalis + nicotine groups had significantly higher active, mean, and peak chemiluminescence than the nicotine group ($p<0.0001$). The P. gingivalis and P. gingivalis + nicotine groups were not significantly different ($p>0.90$). Conclusion: Nicotine, in the presence of P. gingivalis, did not alter or decrease the ROS release when compared with the stimulation with P. gingivalis alone. Nicotine alone caused ROS generation that was statistically significantly lower than the P. gingivalis group and the P. gingivalis + nicotine group. This study was supported by an internal grant by Dr. Kowolik's lab.

P64 Tβ4 Levels in Gingival Crevicular Fluid Correlate to Pocket Depth.

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Thymosin beta 4 (Tb4) is a small actin-binding peptide with anti-inflammatory, cytoprotective, anti-apoptotic, and wound healing characteristics. Tb4 is present in tears, saliva, gingival crevicular fluid (GCF) and wound fluids. **Objective**: The objective of this study was to determine if Tb4 levels in GCF varied in healthy human patients and in patients with periodontal disease. **Methods**: Adult patients of the University of Detroit Mercy School of Dentistry clinic who had given informed consent were used in this study. GCF was obtained by placing a PerioPaper collection strip (Oraflow, Inc.) in the gingival sulcus for 30 seconds. 43 GCF samples were obtained from regions of the gingiva that showed no clinical signs of disease, and from regions with periodontal disease as determined by probing depth (PD) ranging from 5 to 10 mm. The volume of GCF collected on each strip was measured using the Periotron 8000. GCF proteins were eluted and samples were analyzed by ELISA to determine Tb4 concentration (Alpco Diagnostics). **Results**: The mean concentration of Tb4 in GCF taken from all regions with a PD less than 4 mm was 5.24 $\pm$ 0.19 mg/ml; from all regions with a PD greater than 4 mm the concentration was 6.15 $\pm$ 0.25 ($p = 0.0133$). Tb4 levels inversely correlated to PD; for PD of 5 mm the concentration was 6.33 $\pm$ 0.30 mg/ml, for PD of 6 mm the concentration was 5.19 $\pm$ 0.50 mg/ml, for PD of 7mm the concentration was 4.55 $\pm$ 0.30 mg/ml, and for PD of 10 mm the concentration was 3.99 $\pm$ 0.30 mg/ml. **Conclusions**: Periodontal disease results in Tb4 protein concentrations in GCF that are different from gingival health. GCF Tb4 levels appear to vary in periodontal disease depending on probing depth, suggesting a possible link between PD and levels of inflammation.

PULP BIOLOGY

P65 Cigarette Smoke Condensate Affects Repairing Ability of Human Pulp Fibroblasts.

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Tobacco usage has been known for its adverse effects in the oral cavity and on other tissues. However the mechanism of tobacco on oral tissues has not been understood well, since at least 400 out of 4,000 chemicals in tobacco are toxic, which complicates the research studies. **Objective**: This study was designed to observe the effects of cigarette smoke condensate (CSC), a particulate matter of cigarette smoke, on the dentin repairing ability of human pulp fibroblasts (HPFs). The mRNA expression of dentin specific proteins, including dentin matrix acidic protein-1 (DMP), dentin phosphoprotein (DPP) and dentin sialoprotein (DSP) was examined utilizing Reverse Transcription Polymerase Chain Reaction (RT-PCR). **Methods**: HPFs were maintained as cell lines in the laboratory. 200,000 cells were seeded per petri dish and allowed to grow until 90% confluent. Then 200μg/mL of CSC in serum (-) media were used to treat HPFs for 24 and 72 hours. The mRNA was extracted utilizing Qiagen RNeasy mini kit (Qiagen Sciences Inc., Germantown, MD) following the manufacturer's instruction. The mRNA concentration was measured using NanoDrop spectrophotometer (ThermoScientific, Wilmington, DE). RT-PCR was performed using Qiagen one-step RT-PCR kit. The RT-PCR products were resolved on a 0.9% (w/v) agarose gel, and band density was measured to identify the changes in mRNA expression of DMP, DPP and DSP. Each experiment was repeated three times. One-way ANOVA was used to analyze the difference of mRNA expression between groups. **Results**: The concentration of mRNA after 72 hours of CSC treatment was decreased significantly compared to that of 24 hr treatment. The effects of CSC on the mRNA expression of DMP, DSP and DPP varied. Conclusion: CSC affected the dentin repairing ability of HPFs.

SALIVARY RESEARCH

P66 Diagnostic Use of Epithelial Cells in Saliva.

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Introduction: The use of saliva as a biological specimen for identifying diagnostic and prognostic markers for oral and systemic diseases is progressing rapidly. Most investigations have concentrated on evaluating clarified saliva for clinical diagnostics. In addition to the soluble components, saliva is a rich source of cellular components including epithelial cells and blood corpuscles. Here we report the potential use of epithelial cells in saliva as an efficient biological specimen for studying host microbial relationship in chronic generalized periodontitis.

Method: Unstimulated Whole Saliva was collected from healthy individuals. Salivary epithelial cells were separated and cultured in medium supplemented with keratinocyte growth factor, antibiotics for 24 hrs and then stimulated with 10mg/ml of *E.coli* lipopolysaccharide(LPS). Supernatants collected at different time points were assessed for pro-inflammatory cytokines (IL-6, IL-8, and IFN-gamma) by Enzyme Linked Immuno Sorbant Assay(ELISA). Real-time PCR was performed for TLR2 and 4 genes. **Results:** The proinflammatory cytokines IL-6 and IFN-gamma significantly increased at 24hrs in epithelial cell cultures stimulated with LPS. Real-time PCR analysis showed upregualtion of TLR-4 mRNA in epithelial cells isolated from the saliva of normal individuals. The up regulation of TLR 4 gene in LPS induced cells, correlated well with the significant increase in proinflammatory cytokines suggesting that the salivary epithelial cells recognizes the oral bacteria by means of TLR's expressed on their cell surface. **Conclusion:** salivary epithelial cells can be used as potential diagnostic marker for diagnosing and also measuring the disease progression of oral diseases like periodontitis and oral lichen planus.

P67 Safety and Performance Evaluation of Saliwell GenNarino.

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Objective: The objective of this clinical trial was to test the safety and efficacy of electrostimulation using the Saliwell GenNarino (GN) device. This was a part of a world-wide study, with about ten subjects enrolled per site, with seventeen sites enrolled, and one hundred forty subjects expected. **Methods:** Dental impressions of both arches were taken with alginate impression material and stock trays, and poured immediately with yellow stone. The casts were sent to Israel for fabrication of the GN, and the device was received about one month later. The use of the device was compared between active vs. sham mode for one month each in a double-blind design (Phase 1). In phase 2, the xerostomia relieving effect of the active device was assessed in an open label design for an additional nine months, divided in three trimesters differentiated by the length of time of device wearing (1,5, or 10 minutes). **Results:** Eleven subjects were enrolled. Eight subjects completed phase one of the study, and five subjects completed the entire study. One subject was extremely pleased with the GN, and nearly doubled both unstimulated and stimulated salivary flow. Six of eleven subjects dropped from the study. Two subjects dropped because they could not function without their sialogogue medication (both were taking Evoxac® (cevimeline HCl)). One subject dropped because the device was not tolerated. One subject dropped due to distance traveled and did not think the device was helping. Two subjects experienced a dead battery during the study. **Conclusions:** The GN offers patients a non-drug, non-invasive option for the treatment of xerostomia. It is a custom-made removable device which patients can wear up to ten minutes per hour. The GN appears safe and patients seem to tolerate it well.

SPECIAL NEEDS

P68 Communication and Desensitization Aides for Dental Patients with Developmental Disabilities.

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Communication and interaction with verbal and nonverbal individuals with autism and intellectual disabilities can be challenging for a dental practice. Our team is developing and planning to test social communication tools for dental offices, similar to those used by therapists and caregivers within home, school and medical settings and also a desensitization video containing still pictures of an adult with developmental disabilities going through the steps that are common to dental appointments. The specific objective of the social communication tools is to allow individuals of many ability levels to better comprehend the expectations in their environment. The objective of the desensitization video is to reassuringly explain the experience of visiting a dentist to encourage a reduction in pre-visit anxiety and alleviate additional stress that can be a result of overstimulation. The social communication cover the following topics tools are pain, tooth, water, drill, and air and are being created through consultation and collaboration with the Christian Sarkine Autism Treatment Center. The video has been developed by our team using a consenting individual and is designed to be used either prior to a dental visit or in the dental office waiting area. These aides are currently being utilized with patients at the Indiana University Hospital Dental Clinic.

TISSUE REGENERATION

P69 Homology of Tβ-4 and VEGF in Xenopus, Axolotl, and Short-toes.

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Thymosin beta 4 (Tβ-4) and vascular endothelial growth factor (VEGF) are important growth factors in angiogenesis, a process critical to regeneration. Axolotl (*Ambystoma mexicanum*) is a unique animal model capable of regenerating their tissue parts after amputation, while short-toes (a mutant axolotl) and African clawed frog *(Xenopus laevis*) are considered as regeneration-deficient since they cannot replace their lost parts. However, we lack sequence information for VEGF from axolotl and short-toes, and that of Tβ-4 from all three animals, which has impaired our study of their roles in regeneration. In this study we partially sequenced and compared the mRNA of VEGF and Tβ-4 in Axolotl, short-toes, and African clawed frog. VEGF primers were designed based on *Xenopus* cDNA sequence while the Tβ-4 primers were designed based on human cDNA sequences. The total RNA was extracted utilizing RNeasy kit (Qiagen Sciences Inc., Germantown, MD) following the manufacture's instruction. One-step

reverse transcriptase polymerase chain reaction (RT-PCR, Qiagen) was performed following manufacture's instruction. The RT-PCR products were run on 0.9% agarose gel and then sent for two direction sequencing analysis (ACGT, Inc., Wheeling, IL). After comparing the RT-PCR products sequences, the result suggested that VEGF and Tβ-4 are highly conserved(>90%) among *Xenopus laevis*, axolotl and short-toes.

P70 Matrix Metalloproteinases Expression During Limb Regeneration.

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Axolotl (regeneration-competent) is one of the unique vertebrates which can regenerate missing organs such as limbs, jaws, spinal cord, tail etc, anytime during their life cycle. There also exists a recessive mutant phenotype of axolotl called short toes (s/s, regeneration-deficient). s/s can regenerate its tail and spinal cord but cannot maintain the growth of blastema, which results in the failure of limb regeneration. Remodeling of extracellular matrix (ECM) during early blastema formation, also known as histolysis, leads to the release of stem cells and activation of various growth factors. Therefore, histolysis is considered as a crucial step in regenerating the exact replica of missing limbs in axolotls. Matrix metalloproteinases (MMPs) are zinc dependent endopeptidase, that have been suggested to play roles in histolysis. However, it still remains unclear if histolysis is different in limb regeneration between regeneration competent and deficient animals. In this study we analyzed the expression patterns of MMPs and the tissue inhibitors of the MMPs (TIMPs) in axolotl and s/s utilizing MMP array (RayBiotech,Inc., Norcross, GA), zymography and western blot. The cut-off limbs of axolotls and s/s were used as control. The animals were allowed to regenerate and the blastema was collected at three stages: epidermis closure (EC), dedifferentiation (DD), and early bud (EB). The total proteins were extracted from all the samples. 20 µg of protein was used to perform MMP array according to manufacturer's protocol. It detected MMP-1, -2, -3, -8, -9, -10, and -13, as well as TIMP-1, -2 and -4 in the control, EC, DD and EB samples from axolotl and s/s. Gelatin zymograghy with 20 µg of protein confirmed that MMP-2 and -9 were expressed in all the time points of axolotl and s/s samples. The expression patterns of MMP-9 was similar in the axolotl and s/s till DD stage, while later in EB stage axolotl showed a decrease in MMP-9 expression and s/s had increased expression. Western blots performed with 40 µg of protein against MMP-2 and -9 antibodies confirmed the zymography results. These results suggested that the expression patterns of the MMPs especially MMP-9 is different in regeneration competent and deficient animals. One of the keys for a healthy blastema formation which can multiply and later repattern into the missing limb might be the release of the right amount of MMP at the right time. This study was supported by an IUSD start-up grant to F. SONG and grant from W. M. Keck foundation to D. L. Stocum.

TOBACCO CESSATION

P71 Effect of Nicotine on *Streptococcus mutans* and *Streptococcus sanguis* Growth.

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It is well documented that smoking leads to increased caries rates in humans. *S. mutans* has been shown to be the primary causative agent of human dental caries, and it is thought that the increased caries rates observed in smokers could in part be attributed to the favorable response of *S. mutans* to nicotine exposure as compared to other noncariogenic oral microflora. Previous studies have shown an inverse relationship between numbers of *S. mutans* (late colonizer) and *S. sanguis* (early colonizer) in saliva and dental biofilm. Other studies have shown that the growth of *S.mutans* and antigen I/II expression is increased by nicotine exposure, however the effect of nicotine on the growth of the noncariogenic bacterium *S. sanguis* has not yet been investigated. **Objective:** To determine the effect of various nicotine concentrations on *S. mutans* and *S. sanguis* growth in sucrose-free media. **Methods:** Serial dilutions of nicotine ranging from 0.25 to 4 mg/mL were made in sucrose-free TSB media. Using a 96-well microtiter plate, 195 uL of each TSB solution were pipetted into individual wells. To these wells, 5 uL of overnight cultures of either *S. mutans* or *S. sanguis* were added. For controls, 195uL of TSB containing no nicotine were pipetted and 5 uL of either *S. mutans* or *S. sanguis* were added. 200 uL of plain TSB served as a blank. The plate was then sealed, placed in a microplate reader at 37^{o}C, and the absorbance at 600 nm was read every five min for twenty h with mixing between readings. From this data, the maximum growth rate during the log phase of growth of the planktonic cells was calculated, along with lag time, maximum absorbance, and time to reach maximum absorbance. Analysis was completed in triplicate. **Results:** Compared to the control samples which demonstrated an average maximum log phase growth rate of 0.465 for *S. mutans* and 0.596 for *S. sanguis,* nicotine treatment increased the growth rate of both bacteria with *S. mutans* reaching 0.747 (a 1.6-fold increase in growth rate) at 0.25mg/mL nicotine, and *S. sanguis* reaching 1.027 (a 1.72-fold increase in growth rate) at 1.0mg/mL nicotine. Additionally, at 2mg/mL nicotine concentrations, the growth rate of *S. mutans* was significantly decreased ($p<0.05$) as compared to the control, whereas the growth rate of *S. sanguis* at this concentration remained increased. Finally, at low nicotine concentrations (0.25 and 0.5 mg/mL), the growth rate of *S. mutans* was increased 1.6 and 1.54 fold respectively, and *S. sanguis* had a growth rate only 0.79 times that of the control at 0.25 mg/mL nicotine and 1.40 times the control at 0.50 mg/mL nicotine. **Conclusion:** This data indicates that the growth rate of both *S. mutans* and *S. sanguis* is increased in the presence of nicotine. Additionally, at a nicotine concentration of 2mg/mL, growth of *S. mutans* is almost completely inhibited, whereas the growth of *S. sanguis* at this same concentration shows a 1.22 fold increase compared to the control. Conversely, at low nicotine concentrations (0.25 mg/mL and 0.50 mg/mL), *S. mutans* growth is increased to a significantly greater extent than *S. sanguis,* which supports the existing data reporting elevated caries rates in smokers and the inverse relationship between the bacteria. From this data, it is observed that at low nicotine concentrations, *S. mutans* is more sensitive to nicotine and shows a higher growth rate than *S.*

sanguis. Similar results have been observed in additional assays. This work was partially funded by the Indiana University-Purdue University Tobacco Cessation and Behavioral Signature Center.

P72 Effects of Camel Snus® on Keratinocytes.
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As a result of the bans on smoking in certain areas and public places, tobacco companies are marketing alternative smokeless and spitless tobacco products such as Camel Snus (R. J. Reynolds, Winston Salem, NC). Snus consists of the same chemicals as other tobacco products. Nicotine has been shown to affect the proliferation of gingival fibroblasts, as well as to induce cytokines/growth factors that play a role in periodontal disease. The aim of this study was to investigate the effects that Camel Snus has on keratinocytes in regards to proliferation, toxicity, and expression of cytokines/growth factors. The Snus was extracted by incubating the pouches in distilled water at 37 C^0 for 1 hour. Keratinocytes (CCL-4, American Type Culture Collection in Manassas, VA) were seeded in 6 well plates at 750,000 cells per well. They were then exposed to a range of extract concentrations. Cell proliferation and cytotoxicity were determined by WST-1 (Roche Applied Science, Indianapolis, IN) and Cytotoxicity Detection Kit Plus (Roche Applied Science, Indianapolis, IN) assays, respectively. Cytokine/growth factor expression was determined by using human cytokine antibody array I detection kits (RayBiotech, Norcross, GA)**.** 41% Snus extract resulted in 25±2.26% cell viability and killed 43±17.58% of the cells when compared to the control group. 27% SNUS extract resulted in 95.5±7.78% cell viability and no cell death occurred. 27% Snus extract was utilized to assess alterations in cytokine/growth factor expression. Cytokine-Induced Neutrophil Chemoattractant-2 (CINC-2) increased 2.98 fold, Lipopolysaccharide-induced 2 N-terminal cysteines separated by one amino acid chemokine (LIX) increased 4.68 fold and tissue inhibitor of metalloproteinases (TIMP-1) increased 6.75 fold. We concluded that keratinocytes incubated with 27% Snus extract revealed a significant increase in the CINC-2, LIX, and TIMP-1 compared to the control group.

P73 Effects of Tobacco on Collagen Expression from Human Gingival Fibroblasts.
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Effects of Tobacco on Collagen Expression from Human Gingival Fibroblasts. L. VOILES, J. SUN, L. J. WINDSOR. Department of Oral Biology, Indiana University School of Dentistry. Collagen is a major connective tissue protein and also constantly being remodeled, degraded, and synthesized in the basal membranes and extracellular spaces, as well as in other tissues. Nicotine, the addictive chemical found in tobacco, along with polycyclic aromatic hydrocarbons (PAH), affects the expression of collagen in the oral cavity. Over 6000 chemicals are found in tobacco. Chemicals from tobacco are found in their highest concentrations in the oral cavity where intake occurs. This can lead to multiple diseases including peridontitis, loss of teeth, fibrosis, and cancer. This study examined the effects that nicotine and cigarette smoke

condensate (CSC) has on type I collagen expression. Human Gingival Fibroblasts (HGFs) were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% bovine growth serum. Collagen expression was examined in untreated control cells, cells treated nicotine (250 µg/ml), and cells treated with CSC (100 µg/ml). The cells were treated for three days and the mRNA extracted. Polymerase Chain Reaction (PCR) was then used to analyze the collagen mRNA expression of HGFs treated/untreated with nicotine and CSC. Collagen expression was altered in the nicotine and the CSC exposed HGFs in comparison to the untreated control. This study was sponsored by the Indiana University-Purdue University Indianapolis Multidisciplinary Undergraduate Research Institute and the Tobacco Cessation and Biobehavioral Signature Center.

P74 Effects of Tobacco on Growth-Regulated Oncogene Alpha Expression.

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Periodontal disease is a condition of the oral cavity that develops from untreated and worsening gingivitis in which toxins from bacteria cause inflammation of periodontal tissues and eventually the breakdown of tissue. Nicotine and cigarette smoke contribute to the development of periodontal disease. Growth-regulated oncogene alpha (GRO-α) is a chemoattractant that significantly contributes to inflammatory responses. It has been shown that untreated human gingival fibroblasts (HGFs) express low levels of GRO-α. However, nicotine and *Porphyromonas gingivalis (P. gingivalis)* lipopolysaccharide increased the expression of growth-regulated oncogene alpha (GRO-α). This project examined the effects of cigarette smoke condensate (CSC), nicotine, and *P. gingivalis* on GRO- α expression. HGFs were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% bovine growth serum. When the cells reached confluence, they were sub-cultured by trypsinization and seeded at a concentration of 700,000 cells per 10 cm culture dish. After two days, the cell culture dishes were then exposed separately to nicotine (250 µg/mL), CSC (100 µg/mL of total particular matter), *P. gingivalis* supernatant (10%), *P. gingivalis* supernatant with nicotine, or *P. gingivalis* supernatant with CSC. Cell supernatants were collected after three days and analyzed by ELISAs to determine the levels of GRO- α. Initial results have shown that GRO-α concentrations increased with *P. gingivalis*, but not with nicotine or CSC. This study was supported by The Indiana University-Purdue University Indianapolis Multidisciplinary Undergraduate Research Institute and The Tobacco Cessation and Biobehavioral Signature Center.

P75 Heterogeneity of Human Gingival Fibroblasts in Tobacco-stimulated Collagen Degradation.

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Matrix metalloproteinases (MMPs) are a large family of zinc-dependent endopeptidases and their activity is modulated by tissue inhibitors of metalloproteinases (TIMPs). Smoking is a risk

factor for periodontal disease. Cigarette smoke condensate (CSC) is the particulate matter of cigarette smoke. Human gingival fibroblasts (HGFs) are one of major cellular components in periodontal tissue. CSC can increase collagen degradation of HGFs by enhancing and altering the localization of MMPs. Previous clinical studies also showed that some smoking people even with very high dental plaque index still had good periodontal status and did not develop periodontal disease. **Objectives**: The aim of this study was to investigate the heterogeneity of HGFs to CSC-stimulated collagen degradation and to start examining its mechanisms. **Methods**: Eleven HGF cell lines were established from healthy gingival tissue from patients undergoing crown-lengthening surgery. HGFs were seeded as single colony (75,000 cells/well) in 6-well Type I collagen coated plates and exposed to 100 μg/ml CSC (Murty Pharmaceuticals, Lexington, KY) diluted in serum-free media with/without a MMPs inhibitor (GM6001, 100 nM, Chemicon, Temecla, CA) for 3 days. HGFs were seeded with serum free media alone as controls. The mRNA levels of multiple MMPs/TIMPs were measured by reverse transcription-polymerase chain reaction. **Results**: CSC increased collagen degradation in 7 HGF cell lines (CSC-susceptible HGFs), but not in 4 HGF cell lines (CSC-unsusceptible HGFs). GM6001 inhibited CSC-stimulated collagen degradation in all of CSC-susceptible HGFs. The mRNA levels of MMP-1, MMP-2, MMP-3, MMP-14, TIMP-1, and TIMP-2 increased 2.5, 1.3, 3.9, 2.0, 1.6, and 1.3 fold, respectively, in the CSC-susceptible HGFs. However, expression of MMPs/TIMPs basically didn't change in the CSC-unsusceptible HGFs, except for MMP-3 which increased 1.4 fold. **Conclusions**: Heterogeneity of HGFs existed in regard to the CSC-stimulated collagen degradation and the altered expression of the MMPs/TIMPs may be responsible for this heterogeneity. This project was supported by the IUPUI Tobacco Cessation and Biobehavioral Center.

P76 Piloting a Tobacco Intervention in the Regenstrief Emergency Dental Clinic.

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Tobacco use is the single most preventable cause of death and disease in the U.S. and Indiana has the 2nd highest smoking rate in the country. Tobacco use is also detrimental to the post-operative healing process. The objective of this project was to develop, conduct, and evaluate a brief tobacco cessation intervention with patients of the Regenstrief emergency dental clinic. After a short orientation, 4th year dental students on clinic rotation conducted an "Ask-Advise, Refer" intervention for a 4 week period. Upon clinic check-in, patients were asked to complete a short tobacco history questionnaire. During their appointment, the student reviewed this form, advised the patient to quit, and assessed their interest in quitting. Interested patients were offered a postcard listing cessation resources. At the end of each 1 week rotation, students completed a survey evaluating the intervention. Of the 327 patients who completed the intervention, 50% were tobacco users and 46% of them were interested in quitting. When offered, 35% indicated interest in cessation referral. Thirty students (86%) completed the evaluation survey; perceptions of the intervention ranged from neutral to favorable. The results suggest that tobacco cessation interventions can be a quick and feasible option to be used in the emergency dental setting. Supported by Tobacco Cessation and Biobehavioral Center.

P77 Promotions and Public Perceptions of New Dissolvable Tobacco Products.
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Indiana University School of Dentistry; Indiana University Kelley School of Business
The R.J. Reynolds Company recently test marketed the new dissolvable tobacco products Camel Orbs, Sticks, and Strips, in Columbus, OH; Portland, OR; and Indianapolis, IN. The purpose of this study is to document and evaluate the promotion of these new tobacco products in central Indiana. Researchers conducted a field audit of point-of purchase displays and other promotions such as contests, coupons, and giveaways to assess key marketing messages and locational distribution within a 20-mile radius of Indianapolis including Hendricks, Boone, Hamilton, Morgan, Johnson, Shelby and Hancock counties. Following IRB approval, researchers also measured public perceptions of these products via survey. After auditing the greater Indianapolis area, trends in advertisement emerged: Only 48% of audited retail locations carried these new products. They were predominantly found in gas stations (28% of total locations). In addition, they were found in drug (18.8%), convenience (17.4%), grocery (15.7%), liquor (11.4%), and tobacco stores (8.6%). Of the stores documented, 52.9% carried Orbs, 86.5% carried Sticks, and 86.5% of the stores carried Strips. Prices ranged from $3.59 to $4.19 with a majority (64.9%) priced at $3.99. Advertisements were found in store windows, hanging ceiling signs, and with the products. Public perception survey results are pending. From this data, these new products are being selectively distributed. In addition, we believe that ads such as “Style?” and “Dissolvable Tobacco” are targeting current, adult tobacco users visiting gas stations, drug, convenience, and grocery stores who are willing to spend approximately $4.00 per package. This study was sponsored by the Indiana University-Purdue University Indianapolis Multidisciplinary Undergraduate Research Institute.

P78 The Effects of Nicotine on Human Endothelial Cells.
K. DELACRUZ,* J. SUN, L. J. WINDSOR

Indiana University School of Dentistry

Past research has indicated that nicotine, the major addictive constituent in cigarette smoke, is directly related to the effects of smoking on the cardiovascular system such as the development of arterial diseases. The lining of vascular blood vessels is composed of endothelial cells, which are directly involved in the central functions of the cardiovascular system through regulation of blood flow and blood pressure. Research studies have demonstrated that nicotine causes various changes in the cellular behavior of human endothelial cells, including morphological changes and an increase in cell death. This study was performed to assess the effect of nicotine on the cytotoxicity and cell proliferation of human endothelial cells. Human umbilical vein endothelial cells (HUVECs) were obtained from American Type Culture Collection (ATCC) and cultured in Endothelial Basal Medium-2 (EBM-2) supplemented with 10% fetal bovine serum at

37ºC in 5% CO_2. HUVECs were seeded in 6-well plates with 100,000 cells per well and were allowed to attach overnight. Each well was then exposed to a different concentration of nicotine (0, 50, 100, 200, 400, and 800 μg/mL) in serum-free EBM-2. Endothelial cell cytotoxicity and cell proliferation were measured by the lactate dehydrogenase (LDH) assay (Roche Diagnostics) and water-soluble tetrazolium-1 (WST-1) assay (Roche Diagnostics), respectively. LDH assay results indicated increasingly higher percentages of cytotoxicity from nicotine concentrations of 200, 400, and 800 μg/mL in comparison to the control. WST-1 assay results exhibited decreased cell proliferation at 400 and 800 μg/mL nicotine in comparison to the control. Morphological changes and endothelial cell death were observed at the 800 μg/mL nicotine concentration. According to data, nontoxic levels of nicotine that can then be utilized to analyze other cellular mechanisms include concentrations at 50 and 100 μg/mL. Results of this study indicate the relation between nicotine and human endothelial cells and the contribution of nicotine to various effects on the cardiovascular system.

P79 The Effects of Zoledronic Acid on Osteoblasts.

B. SEXTON,* and L. J. WINDSOR

Indiana University School of Dentistry

Background: Bisphosphonates are a unique class of medications used to treat an increasing array of pathological conditions including Paget's disease, cancer, and osteoporosis. These inorganic pyrophosphates localize to bone and are thought to inhibit osteoclast activity, differentiation and maturation, as well as increase osteoclast apoptosis. Recent data suggest that these compounds are responsible for serious adverse effects including, but not limited to, osteonecrosis of the jaw (ONJ). Zoledronic acid is a potent nitrogen containing bisphosphonate indicated for use in the treatment of hypercalcemia of malignancy, multiple myeloma, and bone metastasis of solid tumors. **Objective**: The purpose of this study was to determine the effects of Zoledronic acid on the human osteoblast-like osteosarcoma cell line MG63 proliferation and viability, as well as on MG63-mediated collagen degradation and MMP activity. **Methods**: A water soluble tetrazolium (WST-1) assay kit and a lactate dehydrogenase (LDH) assay kit were used to determine the effects of Zoledronic acid on cell proliferation and viability, respectively. This allowed for the identification of appropriate non-toxic concentrations of Zoledronic acid for further analyses. Six-well plates coated with collagen were used to determine the effects of Zoledronic acid on collagen degradation. Cells ($1.0x10^5$) were seeded in collagen coated plates and grown for 1, 3, 5 and 7 days in 2 mL Dubelco's Modified Eagle's Media devoid of growth serum with Zoledronic acid. The Zoledronic acid concentrations of 10^{-6} up to 10^{-8} M were utilized in the cell-mediated collagen assays based on the WST-1 and LDH assays. Zymography was performed to examine the MMP-2 level in the media from the MG63 cells and to also determine the ability of Zoledronic acid to inhibit MMP-2. The MMP-2 in MG63 conditioned media was separated by zymography and the gels were incubated for 22 hours at 37º C with or without 10^{-5} to 10^{-2} M zoledronic acid. **Results**: The WST-1 assays showed significant ($p<0.05$) changes in cell proliferation at 10^{-5} and 10^{-4} M (74.9 and 15.0%, respectively). LDH assays showed significant ($p<0.05$) cytotoxicity at a concentration of 10^{-4} M (16.6%). The MG63-mediated collagen degradation assays showed non-toxic levels of zoledronic acid caused little to no change in collagen degradation. Zoledronic acid was found to

be toxic to human osteosarcoma cells at concentrations of 10^{-4} M and higher. Zoledronic acid did not inhibit collagen degradation at non-toxic levels.

P80 Tobacco Influences *Streptococcus mutans* Sucrose-dependent and Sucrose-independent Adherence.

R. HUANG,* C. ZHENG, R. L. GREGORY

Indiana University School of Dentistry

Streptococcus mutans is the major cause of dental caries and utilizes both sucrose-dependent and sucrose-independent mechanisms including antigen I/II binding to salivary agglutinin in the salivary pellicle. Previous research demonstrated that there was some relationship between smoking and *S. mutans* adherence, but the mechanism is still unknown. The purpose of this study was to investigate the effect of cigarette smoking condensate (CSC) on the attachment of *S. mutans* to a solid surface, focusing on its sucrose-dependent and sucrose-independent attachment and related Ag I/II expression. *S. mutans* strain UA 159 was used for both sucrose-dependent and sucrose-independent attachment studies. Strain NG-8 and PC3370 (Ag I/II-defective strain) were used only for the sucrose-independent attachment study. For sucrose-dependent adherence, UA 159 was treated with different concentrations of CSC (0, 0.0625, 0.125, 0.25, and 0.5 ug/ml). Biofilm was cultivated with 1% sucrose for 24 h, and media and unattached or loosely attached bacterial cells were gently washed away. Biofilm was stained with 0.1% crystal violet, washed twice, and the bound dye was extracted by ethanol-acetone. Biofilm formation was measured by absorbance at 575 nm. For sucrose-independent adherence, UA 159, NG-8, and PC 3370 were treated with 0.25 ug/ml CSC. Pooled human whole saliva was collected from three healthy persons, diluted 1:10, and filtered. 96-well-plates were coated with filter-sterilized saliva overnight, washed three times with PBS, and blocked with 3% bovine serum albumin. Bacteria (OD=1.2) labeled with biotin were incubated in the wells for 1 h. After washing three times, the wells were treated with streptavidin for 30 mins. Finally, OPD was added to the wells and absorbance was measured at 450 nm. The whole cellular proteins from UA 159, which were treated with 0.25 ug/ml CSC, were extracted, separated by electrophoresis, and probed for Ag I/II using Western blots. For sucrose-dependent analysis, adherence of *S. mutans* treated with CSC was enhanced in a dose dependent manner. The percent enhancement of adherence was 34 and 76% in the 0.125 and 0.25ug/ml CSC groups, respectively. For sucrose-independent analysis, adherence of *S. mutans* to saliva-coated plates was enhanced in the UA 159 and NG-8 groups, but not the PC 3370 (Ag I/II-defective) group. Western blot analysis also indicated higher Ag I/II expression of TSC-treated *S. mutans* than control cells. CSC enhances *S. mutans* sucrose-dependent adherence, which confirmed previous studies. CSC also enhances *S. mutans* sucrose-independent adherence, which relies on Ag I/II upregulation expression. 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 Signature Center.

CLINICAL CASE REPORTS

ENDODONTICS

CC1 Nonsurgical Root Canal Therapy of a Three Rooted Maxillary Second Premolar.

B. RICKETTS,* K. SPOLNIK, M. VAIL

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The main objective of endodontic therapy is to treat pulpal and periradicular tissues in order to retain the natural dentition so that normal form, function, and esthetics will be maintained. In order to achieve this goal, it becomes imperative to remove canal contents, specifically living, infectious, microorganisms by a system of chemomechanical debridement. The advent of newer technology in instrumentation, irrigation, and magnification has provided an enhanced ability to achieve maximum canal disinfection. However, the practitioner must have a detailed and extensive knowledge of the most common patterns in root canal morphology and anatomy for each tooth. This case report presents a case of a three rooted maxillary second premolar that was treated with nonsurgical root canal therapy. The clinician must understand the rare anomalies that exist that can complicate root canal treatment so that treatment modalities can be modified to promote successful healing.

ORAL/MAXILLOFACIAL SURGERY

CC2 Surgical Treatment of a Keratocyst Odontogenic Tumor: A Case Report.

J. BARNEY,* M. VAIL, K. SPOLNIK

Indiana University School of Dentistry

Keratocystic odontogenic tumor (KCOT), formerly referred to as odontogenic keratocyst (OKC), is important to consider when treating periapical lesions, because of its high recurrence rate, its often aggressive nature, and its association with nevoid basal cell carcinoma syndrome. This case report details an 82-year-old male initially who presented with buccal swelling around tooth number twelve. Endodontic therapy proved ineffective against reducing the swelling, thus a surgical approach was used to enucleate and curette the lesion for a definitive diagnosis. Following apical surgery and biopsy, a diagnosis was established as a Keratocystic Odontogenic Tumor. The majority of periapical radiolucencies are associated with necrotic teeth. But when vitality testing is not conclusive and endodontic therapy has not provided proper healing, it is important for the treating practitioner reassess the situation and to expect another possible pathology. A definitive diagnosis can only be made with surgical curettage and histological examination, as with this case report.

ORAL-FACIAL PAIN

CC3 Differentiating Odontogenic and Non-odontogenic Pain Occurring Simultaneously. A Case Report.

S. BINKLEY* M. VAIL, K. SPOLNIK

Indiana University School of Dentistry

Determining the exact etiology is a continuous challenge in diagnosing oral-facial pain for the dental practitioner. Differentiating between odontogenic and non-odontogenic causes can prove difficult. Disease entities from either one or both of these categories can occur separately, sequentially, or even coincidentally. This case report details a 58-year-old female initially diagnosed with Trigeminal Neuralgia that was later discovered to be Herpes Zoster. She then experienced several months of post herpetic neuralgia resulting in paresthesia to her lower left quadrant. During episodic breaks in the paresthesia, she would complain of symptoms consistent with those of a cracked tooth. The patient's symptoms resolved when a diagnosis of cracked tooth syndrome was eventually made and treated with root canal therapy and a full coverage restoration on the tooth. This case report reinforces why a systematic approach to diagnosis is paramount in determining the cause of oral facial pain, especially in cases that are multi-factorial, and how to utilize all information gathered to render appropriate treatment.

PROSTHODONTICS

CC4 Lateral Rotational Path RPD to Replace Missing Hard and Soft Tissues.

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Rotational path removable partial denture incorporates a curved path of placement, permits the rigid portion of the framework to gain entry to undercut areas of the abutment teeth which otherwise would not be accessible. The prosthesis is then rotated to its final position. The primary advantages of such design are improved esthetics and reduced tooth coverage. The objective of this clinical report is to describe the fabrication technique of lateral rotational path single clasped removable partial denture to replace missing hard and soft tissues. A 43 year old female patient presented to graduate prosthodontic clinic requesting replacement of her old RPD with a fixed prosthesis. Patient presented with missing teeth # 9-11, Seibert class III ridge deficiency (deficient height and width) as a result of a cyst removal from anterior maxilla in late 1970's. After two attempts of bone augmentation, the edentulous ridge was still inadequate to accept dental implants. The patient elected to have a rotational path RPD. A surveyed PFM crown with mesial rest seat preparation and distobuccal undercut was fabricated on tooth #3. A single clasped rotational path RPD was constructed. The patient was very comfortable and satisfied with the esthetic and functional outcomes of the treatment. The use of lateral

rotational path removable partial denture might be considered a viable treatment option for severe ridge deficiency to restore esthetics and function.

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