BOSTON UNIVERSITY SCHOOL OF DENTAL MEDICINE

SCIENCE DAY 2005

Thursday, March 24

9 AM – 5 PM
100 East Newton Street
Room 309 and third-floor hallway

Early Embryonic Mouse Salivary Gland Immunostained for Extracellular Matrix and Adhesive Protein.

Sponsored by the American Student Dental Association, the Student Research Group, and the Predoctoral Research Program

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

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Science Day at Boston University School of Dental Medicine

Dr. Maria Kukuruzinska

Welcome to Boston University School of Dental Medicine Science Day 2005. This event honors our predoctoral and postdoctoral students' and postdoctoral fellows' research efforts through poster exhibits and oral presentations. Fourteen students and fellows present poster exhibits and ten students and fellows give platform presentations.

Established 24 years ago, this event was formerly called Clinic Day. Six years ago, to reflect an increased emphasis on the research mission of the school, Clinic Day was renamed Clinic-Research Day. Three years ago this event was once again renamed, this time as Science Day. The new name reflects the increasingly important role science plays in dental medicine, and emphasizes Dean Spencer N. Frankl's and the administration's vision for the future direction of the school's educational, clinical, community, and research programs.

Our selection of Dr. Matthew Hoffman as the keynote speaker emphasizes how biomedical science can drive clinical applications in health care.

Dean Frankl and the administration have been very supportive of the school´s research activities. Three years ago the school established the Office of Science Information, whose goal is to increase the flow of information about research discoveries to the school. The office´s display cases in the lobby of the school showcase student and faculty research and highlight innovations in science and technology that are either relevant or may become important to dental medicine in the future. At these display cases students, faculty, staff, and patients read about the advances in the Human Genome Project, the molecular genetics underlying oral organ development, and the latest discoveries in stem cell research.

The success of Science Day derives from cumulative changes brought about by the vision of the school's administration and its strong support of research activities at the school.

Maria Kukuruzinska, PH.D
Director, Predoctoral Research Program
Professor, Molecular and Cell Biology

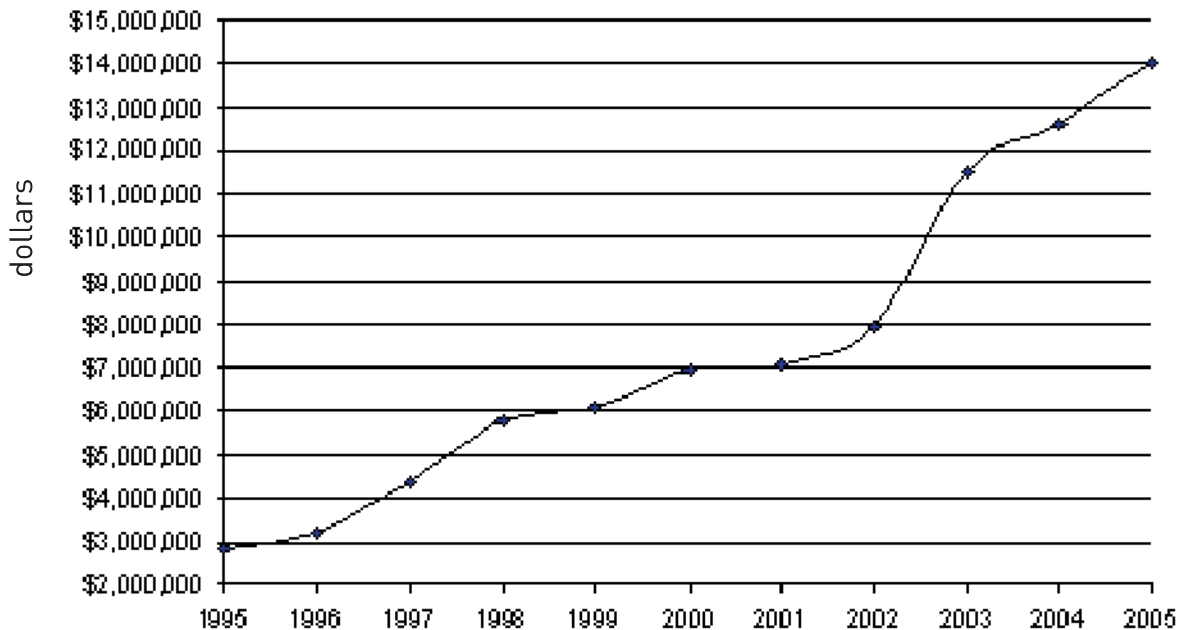
Welcome from Dean Frankl

Dean Spencer Frankl

Our annual BUSDM Science Day gives us an opportunity to showcase our predoctoral and postdoctoral students' exceptional research. Since the first Science Day in 1982, the projects have grown in both number and scope. With several new research-focused departments created during the 1990s (the Department of Health Policy and Health Services Research, the Department of Molecular and Cell Biology, and the Clinical Research Center), we continue to expand our commitment to scientific excellence and advancement.

During the past ten years we have more than quadrupled our research funding:

Growth in Research Funding at BUSDM 1995-2005



This steady growth would not be possible without our outstanding faculty and the students they mentor.

Spencer N. Frankl, DDS, MSD
Professor and Dean

Research Mentors

Diagnostic Sciences and Patient Services

- Paula Friedman, DDS, MSD, MPH, professor. Research Area: Geriatric Dentistry

General Dentistry

- Judith Jones, DDS, MPH, associate professor and chair. Research Area: Geriatric Dentistry

Health Policy and Health Services Research

- Raul Garcia, DMD, MMS, professor and chair. Research Area: Epidemiology
- Michelle Henshaw, DDS, MPH, assistant professor. Research Area: Public Health
- Elizabeth Krall, MPH, PH.D, associate professor. Research Area: Public Health
- Ana Karina Mascarenhas, BDS, MPH, DRPH, associate professor. Research area: Epidemiology and Health Services Research

Molecular and Cell Biology

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

Oral and Maxillofacial Surgery

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

Orthodontics and Dentofacial Orthopedics

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

Orthopedic Surgery

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

Pediatric Dentistry

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

Periodontology and Oral Biology

- Salomon Amar, DMD, PH.D, professor. Research Area: Cell Biology
- Dana Graves, DDS, DMSC, professor. Research Area: Cell Biology
- Robert Gyurko, DDS, PH.D, assistant professor
- Hatice Hasturk, DDS, PH.D, assistant professor.
- Eva Helmerhorst, MS, PH.D, assistant professor. Research Area: Biochemistry
- Alpdogan Kantarci, DDS, PH.D, assistant professor.
- Cataldo Leone, DMD, DMSC, associate professor. Research Area: Biochemistry/ Periodontology
- Frank Oppenheim, DMD, PH.D, professor and chair. Research Area: Biochemistry
- Philip Trackman, PH.D, associate professor. Research Area: Cell Biology
- Thomas Van Dyke, DDS, PH.D, professor. Research Area: Periodontology/ Immunology

Restorative Sciences/Biomaterials

- Laisheng Chou, DMD, PH.D, professor. Research Area: Cell Biology/Oral Medicine
- Russell Giordano, DMD, DMSC, associate professor. Research Area: Biomaterials
- Zhimon Jacobson, DMD, MSD, clinical professor. Research Area: Implantology
- Dan Nathanso, DMD, MSD, professor. Research Area: Biomaterials

Predoctoral Research Committee Members

- Maria Kukuruzinska, PH.D, professor, director, and chairperson
- Cataldo Leone, DMD, MD, associate professor and co-chair
- Donald Ferguson, DDS, MSD, chair, Orthodontics and Dentofacial Orthopedics
- Debrorah Fournier, PH.D, director, Educational Research and Evaluation
- Paula Friedman, DDS, MSD, MPH, professor
- Raul Garcia, professor and chair, Health Policy and Health Services Research
- Russell Giordano, DMD, DMSC, associate professor, Biomaterials
- Kathy Held, M.ED, assistant professor, General Dentistry; associate director, Extramural Programs
- Afaf Hourani, MPH, MS, manager, Science Information
- Jeffrey Hutter, DMD, M.ED, associate dean for academic affairs; chair, Endodontics; director, Postdoctoral Program in Endodontics
- Judith Jones, DDS, MPH, chair, General Dentistry
- Mari Megias, MS, associate director of communicationss
- Elisa Sin DMD 07, president, Student Research Group
- Thomas Van Dyke, DDS, PH.D, professor and director, Clinical Research Center

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- Dr. Cataldo Leone, DMD, DMSC
- Dr. Elisabeth Krall, MPH, PH.D
- Dr. Judith Jones, DDS, MPH
- Dr. Miklos Sahin-Toth, MD, PH.D

- Dr. Eva Helmerhorst, MS, PH.D
- Dr. Alpdogan Kantarci, DDS, PH.D

Student Research Group (SRG) 2005 Officers

- Elisa Sin DMD 07, president
- Levi Maltby DMD 08, vice president
- Brian Schmid DMD 08, secretary
- Bernardo Bianco DMD 08, treasurer

American Student Dental Association (ASDA)

Officers

- David Blackburn DMD 06, first delegate
- Leon Yu DMD 07, second delegate
- Chrys Constantinou DMD 08, alternate delegate
- Riza Santos DMD 07, secretary
- Joanna Ayala DMD 07, treasurer
- Monika Srivastava DMD 07, legislative liaison
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- Arash Molayem,DMD 07, social co-chairperson
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- Khurrum Sheikh DMD 07, DMD 2 representative
- Ale Salehpour DMD 06, DMD 3 representative
- Rohini Badlani DMD 05, DMD 4 representative
- Vilas Balakrishna, AS 05, AS representative
- Vikas Goel DMD 06, information technology manager

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- Bita Binesh DMD 07

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- Chris Lingard DMD 08
- Erick Lukman DMD 08

Program

Vendor Exhibition (9 am-4 pm), First-Floor Hallway and Cafeteria

Poster Presentations (10 am-12 pm, 1 pm-4 pm), Third-Floor Hallway

Keynote Presentation (12 pm-1 pm), Room 309

"Salivary gland development: implications for therapeutic gland regeneration" by Matthew Hoffman, BDS, PH.D

Dr. Matthew Hoffman

Dr. Hoffman is chief of the Matrix and Morphogenesis Unit at the National Institute for Dental and Craniofacial Research (NIDCR). The overall goal of this NIDCR unit is to understand how extracellular matrix and growth factors regulate salivary gland branching morphogenesis during development. Dr. Hoffman received his BDS from the University of Otago School of Dentistry in New Zealand and his PhD at the University of Rochester School of Medicine and Dentistry. His research interests include the developmental mechanisms of salivary gland development with emphasis on genetics and growth factor-matrix interactions.

Oral Presentations (1:30 pm-4 pm), Room 309

Vendors

Aspen Dental
AXIS Dental Corp
Brasseler
Calumet Photographic
Carl Zeiss
The Colonnade Hotel
Dentalworkers, Inc.
Dentsply Professional
Designs for Vision
Eastern Dentists Insurance
General Scientific Corp.
Gentle Communications
Gillette Company (Oral B Labs)
Hu-Friedy
Ivoclar/Vivadent
Lexi-Comp
Mass Dental Society
Menta-Dent
Patterson Dental
Procter & Gamble
Sonicare/Philips Oral Health
Sullivan-Schein Dental
3M / ESPE
Tom's of Maine
Ultradent Products
United States Airforce
United States Army
Water Pik

Winning Presentations

Predoctoral Poster winner Sunanda Bhushan with Dr. Maria Kukurzunska

Predoctoral Students Poster Presentation Award

- Sunanda Bhushan, Richard Pober and Russell Giordano. Depart-ment of Biomaterials: "Coloration of partially stabilized zirconia"

Predoctoral Oral winner Joanna Ayala with Dr. Maria Kukuruzinska

Predoctoral Students Oral Presentation Award

- Joanna Ayala, Juan Dong and Mary MacDougall. University of Texas:" Alterations of dentin sialophosphoprotein gene in dentino-genesis imperfecta and dentin dysplasia"

Postdoctoral Poster winner Ronit Antebi-Hadar with Dr. Tom Van Dyke

Postdoctoral Students Poster Presentation Award

- Ronit Antebi-Hadar, Dan Nathanson and Christopher Hughes. Department or Restorative Sciences/Biomaterials: "Bonding self-etching adhesives to enamel and dentin in-vitro"

Postdoctoral Oral winner Eraldo Batista Jr. with Dr. Tom Van Dyke

Postdoctoral Students Oral Presentation Award

- Eraldo Batista Jr, Alpdogan Kantarci, Hatice Hasturk and Thomas Van Dyke. Department of Periodontology and Oral Biology: "Cloning of a novel diacylglycerol kinase α (DGK α) transcript generated by alternative splicing and expressed in neutrophils of localized aggressive periodontitis patients"

Postdoctoral Fellows Poster winner Adel Alagl with Dr. Frank Oppenheim

Postdoctoral Fellows Poster Award

- Adel Alagl, Eva Helmerhorst, Robert Troxler and Frank Oppenheim. Department of Periodontology and Oral Biology: "Histatin degradation and complex formation in the oral environment"

Postdoctoral Fellows Oral winner Aneta Liwosz with Dr. Frank Oppenheim

Postdoctoral Fellows Oral Presentation Award

- Aneta Liwosz and Maria Kukuruzinska, Department of Molecular and Cell Biology.:"The Role of N- glycans in E-cadherin adhesion Dynamics"

ADA/Densply winner Nathaniel Caldon with Dr. Maria Kukuruzinska

American Dental Association/Dentsply Award

- Nathaniel Caldon, Robert Gyurko, Alpdogan Kantarci and Thomas Van Dyke. Department of Periodontology and Oral Biology: "Diabetes alters superoxide release in murine polymorphonuclear lymphocytes (PMN)"

Student Presentations

Poster Presentations

Predoctoral Students

- Sunanda Bhushan, Richard Pober and Russell Giordano. Department of Biomaterials: "Coloration of partially stabilized zirconia"

- David Buczak, J. Renz, Louis Gerstenfeld and George Barnes. Department of Orthopaedic Surgery: "Runx2 regulates TGF β responses associated with metastasis and osteolytic disease"

- Nathaniel Caldon, Robert Gyurko, Alpdogan Kantarci and Thomas Van Dyke. Department of Periodontology and Oral Biology: "Diabetes alters superoxide release in murine polymorphonuclear lymphocytes (PMN)"

- Tin-Minh Le and Michelle Henshaw. Department of Health Policy and Health Services Research: "Increasing access to one of the ten greatest public health achievements of the 20th century"

- Elisa Sin, P. Kang, K. Vogt, SE Gruninger and Chakwan Siew. ADA: "Tissue factor expression in Porphyromonas gingivalis-infected human monocytes"

- Malvika Tickoo, Hina Ali, Zori Rabinovits, Judith Jones and Takanari Miyamoto. Department of General Dentistry: "Clinical risk assessment program for dental caries; case series"

Postdoctoral Students

- Ronit Antebi-Hadar, Dan Nathanson and Christopher Hughes. Department or Restorative Sciences/Biomaterials: "Bonding self-etching adhesives to enamel and dentin in-vitro"

- Satheesh Elangovan, Marek Kloczewiak, and Frank G Oppenheim. Department of Periodontology and Oral Biology: "Binding of tritiated human serum albumin to calcium hydroxyapatite and to carbonated calcium hydroxyapatite"

- Anas Halaweh, Hsin-tzu Wong, Xiaoren Tang, Deborah Levy Marciano and Salomon Amar. Department of Periodontology and Oral Biology: "Develop a LITAF knowckout mouse by targeted mutation and homologous recombination"

- Edit Szepessy and Miklos Sahin-Toth. Department of Molecular and Cell Biology: "N-terminal processing of α1-antitrypsin by human mesotrypsin"

Postdoctoral Fellows

- Adel Alagl, Eva Helmerhorst, Robert Troxler and Frank Oppenheim. Department of Periodontology and Oral Biology: "Histatin degradation and complex formation in the oral environment"

- Luis Bredeston, John Samuelson and Carlos Hirschberg. Department of Molecular and Cell Biology: " Golgi and endoplasmic reticulum functions in Entamoeba histolytica"

- Walter Siqueira, Maria Santos, Elisabeth Oliveira, Eva Helmerhorst and Frank Oppenheim. Department of Periodontology and Oral Biology: "Electrolyte concentrations in whole saliva from individuals with cerebral palsy"

Oral Presentations (1:30-4:30)

Predoctoral Students

- Joanna Ayala, Juan Dong and Mary MacDougall. University of Texas:" Alterations of dentin sialophosphoprotein gene in dentinogenesis imperfecta and dentin dysplasia"

- Monica Desanjh and Donald Ferguson. Department of Orthodontics: "Effect of alveolar corticotomy and augmentation grafting on long term orthodontic outcome stability"

Postdoctoral Students

- Eraldo Batista Jr., Alpdogan Kantarci, Hatice Hasturk and Thomas Van Dyke. Department of Periodontology and Oral Biology: "Cloning of a novel diacylglycerol kinase α (DGK α) transcript generated by alternative splicing and expressed in neutrophils of localized aggressive periodontitis patients"

- Tesfahun Desta, Rongkun Liu, Solomon Amar and Dana Graves. Department of Periodontology and Oral Biology: "P. Gingivalis induces apoptosis in gingival fibroblasts independent of cysteine proteases"

- Rayyan Kayal, Megan Bauer, Brian Allen, Louis Gerstenfeld and Dana Graves. Department of Periodontology and Oral Biology: "Diabetes may impair fracture healing by excess removal of cartilage"

- Ming-Dih Jeng and Laisheng Chou. Department of Restorative Sciences/Biomaterials: "The ion exchange of sol-gel scaffolds in vivo"

- Krystyn Ross, Tianlei Lei, Cathrine Costello and Maria Kukuruzinska. Department of Molecular and Cell Biology: "E-cadherin protein complexes as predictors of cellular adhesive stability"

- Caterina Venuleo, Eva Helmerhorst, Anuradha Beri and Frank Oppenheim. Department of Periodontology and Oral Biology: "Candida glabrata: a unique species with a marked resistance to cationic antifungal proteins"

Postdoctoral Fellows

- Rongkun Liu, Hesham A-Mashat, Harbinder Bal, Tesfahun Desta, Suneel Kandru and Dana Graves. Department of Periodontology and Oral Biology: "Diabetes enhances fibroblast cell death through TNF-α mediated caspase activation"

- Aneta Liwosz and Maria Kukuruzinska. Department of Molecular and Cell Biology: "The Role of N-glycans in E-cadherin Adhesion Dynamics"

Abstracts

Coloration of Partially Stabilized Zirconia

Sunanda Bhushan, R. Pober and Russell Giordano, Boston University School of Dental Medicine

Objectives: Various dental porcelains and glass ceramics exhibit excellent esthetic properties but low/moderate strengths (50-150MPa). Yttria-stabilized-zirconia ceramics are amongst ceramics with high strength and fracture toughness (900-1200MPa). These are used for abutments, posts, implants and all-ceramic restorations, both single crowns and FPD's. However, their bright white color often necessitates opaquing and veneering. The purpose of this study was to produce yttria-zirconia (YZ) ceramics in various shades equivalent to the color spectrum of natural teeth using different cation solutions.

Methods: YZ bisque-fired blocks (VitaZahnfabrik) were sliced to 2.5mm, cleaned in dilute alcohol and dried (100°C). Porosity was measured in 'mercury-porosimeter'. Samples were immersed in specific concentration of various cation salt solutions: cobalt, chromium, erbium, neodymium, praseodymium, iron, nickel, barium, manganese, bismuth, cerium, zinc & tin at -26mmHg for 65min. Wet, dry and final weight after sintering in 'Zyrcomat' furnace was recorded. Color was checked under color-corrected full-spectrum light and compared to a control. Amount of absorbed cation in the ceramic sample was measured using EDS.

Results: Colors obtained were: blue(Co, Ne), green(Cr,Ni), pink(Er), yellow(Ce, Pr), yellow-brown(Fe), black(Mn). The minute quantities of cation required to obtain shades in the tooth color range were undetectable by EDS. Samples with high saturation of hue showed a uniform reduction in concentration of cation from surface to center, e.g.,0.28wt% vs. 0.14wt%-Ni sample, 0.36wt% vs. 0.20wt%-Co sample respectively, undetectable by naked eye.

Conclusion: YZ samples readily absorb salt solution which is enhanced under vacuum. Very minute quantities of cations impart color to ceramic throughout the entire material. Depth of perception and translucency are both maintained. Surface can be polished and adjustments can be made without change in color. Particular color shades in the tooth color range may be obtained by use of different cations at specific concentrations. Supported by a private grant.

Runx2 Regulates TGFβ1 Responses Associated with Metastasis and Osteolytic Disease

David Buczak, Jennifer Renz, Louis Gerstenfeld and George Barnes, Department of Orthopaedic Surgery, Boston University School of Medicine and Boston University School of Dental Medicine

Metastatic breast cancer cells preferentially localize to skeletal sites and predominantly produce osteolytic disease. Metastases associated lytic bone disease is the result of cancer cell mediated disruptions of the normal homeostatic interactions between osteoblasts and osteoclasts. We recently demonstrated that metastatic breast cancer cells express Runx2, a central regulator of bone formation and homeostasis. Furthermore, we have demonstrated that Runx2 activity is required for breast cancer cells to generate osteolytic lesions in bone both in vivo and in vitro. Our current objective is to elucidate the functional role of Runx2 in regulating breast cancer cell responses to the local bone environment. We propose that Runx2 regulates the ability to respond normally to TGFβ1 in metastatic cancer cells, and that these Runx2 mediated TGFβ1 responses lead to preferential establishment in bone. Our method for testing this hypothesis includes testing the TGFβ1 responses of control versus dominant negative Runx2 expressing breast cancer cells at the functional and gene expression levels. The effects of TGFβ1 on parental and Runx2 dominant negative cancer cell lines were assessed through growth, proliferation, adhesion, and invasiveness assays. We also analyzed the expression of specific TGFβ1 responsive genes associated with metastasis including urokinase plasminogen activator (UPA), its receptor (UPAR) and the related plasminogen activator inhibitor (PAI-1). Our results demonstrate that disruption of Runx2 in breast cancer cells alters both the TGFβ1 Smad-mediated and Smad-independent p38 MAPK signaling pathways, the expression of specific TGFβ1 responsive genes and cellular functions associated with metastasis and bone resorption. In conclusion, we have demonstrated that Runx2 activity is required for a breast cancer cells to respond normally to TGFβ1 signaling associated with the establishment of skeletal metastases. Supported by a Susan G. Komen Breast Cancer Foundation grant BCTR-0503818 to G.L.B.

Diabetes Alters Superoxide Release in Murine Polymorphonuclear Lymphocytes (PMN)

Nathaniel Caldon, Robert Gyurko, Alpdogan Kantarci, and Thomas E. Van Dyke, Department of Periodontology and Oral Biology, Boston University School of Dental Medicine

Objective: To determine the effect of hyperglycemia on the capacity of PMN superoxide release

Material and Methods: Mice carrying a point mutation in one of the insulin genes (Ins2Akita mice) and normal wild type mice were injected with casein or zymosan intraperitoneally to elicit an inflammatory effect and PMN production. Mice were sacrificed and peritoneal fluid was collected by abdominal lavage. PMN were isolated by centrifugation through a Ficoll-Hypaque density gradient. Extracted cells were counted with a hemocytometer and separated into 5x105 cells per microplate well. Superoxide production was measured immediately after stimula-

tion with the bacterial chemoattractant N-formyl-Methyl-Leucine-Phenylalanine (fMLP) using a cytochrome C reduction assay.

Results: Superoxide production in diabetic mice having blood glucose levels higher than 250mg/dl was elevated by 45% compared to wild type mice when challenged.

Conclusion: Diabetic mice produce more superoxide when challenged by fMLP compared to non-diabetic wild type mice, suggesting that hyperglycemia pre-activates or primes PMN. Supported by NIH/USPH grants DE1319 and DE014568.

Increasing Access to One of the Ten Greatest Public Health Achievements of the 20th Century

Tin-Minh Le and Michelle Henshaw, Department of Health Policy and Health Services Research, Boston University School of Dental Medicine

Introduction: Community water fluoridation (CWF) is the single most effective method of preventing dental decay and improving oral health over a lifetime, for both children and adults. Since fluoridated toothpaste is readily available for purchase over the counter, individuals, especially policymakers, may discount the benefits of CWF. The purpose of this project is to explore more fully the relationship between community water fluoridation and the prevalence of dental decay in Massachusetts children with the ultimate goal of informing the state's public oral health policy.

Methods: In 2003, a statewide oral health survey of 3,439 3rd grade children was conducted in Massachusetts. The data collected included history of decay, presence of untreated decay and insurance status. 3078 subjects had complete data and were therefore included in the data analysis.

Results: The mean age of subjects was 8.6 years, 49% were male, 1,724 lived in fluoridated communities, and 1,354 resided in non-fluoridated communities. 61% had private dental insurance, 26% had public dental insurance, and 13% were dentally uninsured. 52% were caries free, 48% had caries experience and 26% had untreated decay. Of children living in non-fluoridated communities, 49% have a history of decay compared to 40% of children who live in fluoridated communities ($p<0.05$). These results were consistent when the subjects were stratified by dental insurance status. 37% of subjects with private dental insurance living in non-fluoridated communities had caries experience compared with 19% of those living in fluoridated communities. For those with public insurance, 68% in non-fluoridated communities had caries experience compared with 59% living in non-fluoridated areas.

Conclusion: Disparities still exist between children living in fluoridated and non-fluoridated communities even when adjusting for insurance status. There are 154 cities and towns in the state, or 38% of the Massachusetts population, which have public water systems that could be fluoridated but currently are not. Data such as this, underscores the importance of CWF for all of Massachusetts children. Additional work in this area includes using GIS to map the oral health and fluoridation census data and to determine the relationship between city fluoridation status and city demographic characteristics. Supported by Blue Cross Blue Shield of Massachusetts Foundation and NIH K23 DE00454 grants.

Tissue Factor Expression in Porphyromonas Gingivalis-Infected Human Monocytes

Elisa Sin, Peter Kang, K. Vogt, S. E. Gruninger, and Chakwan Siew, American Dental Association, Chicago, and Boston University School of Dental Medicine

In periodontal disease, oral microorganisms can penetrate the surrounding tissues and blood stream, where they can interact with endothelial cells and monocytes. It is known that bacteria-infected monocytes induce tissue factor (TF, 47 Kd transmembrane glycoprotein) expression and play a central role in the initiation of the coagulation cascades. However, the mechanism by which infection of monocytes by periodontal pathogens activating TF expression which in turn leads to coagulation disorders remains unclear. Objective: This study aims to identify whether oral microorganisms activate TF expression and induce procoagulant activity in human monocytes. Methods: Human monocytes (THP-1) were incubated with Porphyromonas gingivalis ATCC 33277 (P. gingivalis) at 37oC in 5% CO2 in a time- and dose-dependent manner. The TF expression and procoagulant activity were determined with an ELISA assay for TF antigen and TF activity kit, respectively. Results: TF expression and procoagulant activity in P. gingivalis-infected THP-1 were demonstrated in a time- and dose-dependent manner relative to the concentration of P. gingivalis. Incubation for 1 hour expressed 10, 218, and 1387 ng TF/105 THP-1 infected with 102, 104, and 106 P. gingivalis/ml, respectively. Incubation with P. gingivalis for 1 hour exhibited slightly enhanced procoagulant activity. However, incubation with 1 x 102 and 1 x 106/ml of P. gingivalis for 4 hours demonstrated 129% and 146% of procoagulant activity of control group, respectively. Conclusion: This study demonstrates that P. gingivalis can activate TF expression and procoagulant activity in human monocytes, supporting that an oral microorganism may be a factor in blood coagulation disorders, which can progress to cardiovascular disease. Supported by an ADA Foundation grant.

Clinical Risk Assessment Program for Dental Caries: Case Series

Malvika Tickoo, Hina Ali, Zori Rabinovitz, Judith Jones, and Takanari Miyamoto, Department of General Dentistry, Boston University School of Dental Medicine, Department of Health Policy and Health Services Research, Boston University School of Dental Medicine[2]

Introduction: Research has shown that the appropriate amount of therapeutic, restorative and preventative care is dependant to a considerable extent on patient's caries history and current caries status. The acceptance of an evidence based management model of dental caries that has been considered by many dental schools, can be possible when the schools consent to a common approach to teaching the various components of caries risk assessment and treatment. The goal of this case series is to introduce the clinical risk test (CRT) as a caries assessment tool to identify patients who are at high-risk for developing dental caries. A radar chart system was also developed to visualize additional clinical parameters and as an additional possible tool of clinical education.

Methods: CRT kit includes tests for Streptococcus mutans, Lactobacillus, saliva buffer capacity and quantity of saliva. Other known clinical factors such as fluoride usage, plaque levels, dietary habit, smoking status, and full mouth radiographs were also collected.

Results: The study revealed that CRT along with the radar chart system can prove to be effective tools in identifying caries prone patients and in understanding associated risk factors.

Conclusion: The Caries Risk Test (CRT) can prove to be a useful diagnostic tool in identifying patients who may be at high-risk for dental caries. This can also help dental students in understanding the factors involved in dental caries and therefore in diagnosis and management of the disease. Furthermore, the information gap between the didactic and clinical courses of preventive dentistry may also have been narrowed and possibly even filled by this system.

Bonding Self-etching Adhesives to Enamel and Dentin in vitro

Ronit-Antebi-Hadar, Dan Nathanson and Christopher Hughes
Boston University School of Dental Medicine

Purpose: To evaluate the shear bond strength of new self-etching dental adhesive systems to enamel and dentin in-vitro.

Methods: Five adhesive systems were tested: A. Adper-Prompt (3M ESPE); B. AdheSE (Ivoclar); C. G-bond (GC); D. Xeno III (Dentsply); and E. Adper - Single Bond Plus (3M ESPE) as control. Extracted human teeth (n=180) were mounted in plastic rings using acrylic resin. Dentin test samples ("Dentin") were prepared by sectioning the mounted teeth through the dentin layer. Enamel samples were either abraded and polished to a flat surface ("Cut–Enamel") using wet diamond grinding discs (70, 45, and15 microns) or cleaned with pumice ("Uncut-Enamel"). Composite resins (from corresponding manufacturers) were bonded to the various surfaces using a special Teflon mold with a 2mm cylindrical opening. Bonded samples were stored in water for 24 hours. Bond strengths were measured in shear mode in an Instron machine, and data was analyzed by ANOVA

Results: Mean shear bond strengths (MPa) are shown below:

Adhesive System Substrate	Adper-Prompt (3M)	AdheSE (Ivoclar)	G-bond (GC)	Xeno (Dentsply)	Adper-Single Bond Plus (3M)
Dentin	29.34 (11)	25.35 (12)	28.77 (6)	21.22 (9)	27.22 (12)
Uncut Enamel	24.73 (11)	22.87 (7)	27.35 (6)	23.61 (7)	30.87 (7)
Cut Enamel	22.71 (7)	21.09 (6)	26.84 (7)	21.68 (8)	28.88 (11)

Conclusions: For the five adhesive systems tested there was no significant difference in bond strengths to dentin vs. enamel (both cut and uncut). Except for a difference in bond strengths between Xeno III and Adper Single Bond Plus (P=0.02) there was no significant difference among the adhesive systems tested. This in-vitro test concludes that most new self-etching adhesives tested are as reliable for bonding to dentin and enamel as the conventional control group. Increased phosphorylation of pleckstrin in diabetic mononuclear phagocytes and the role of pleckstrin in RAGE induced proinflammatory cytokine secretion. Supported by a private grant.

Binding of Tritiated Human Serum Albumin to Calcium Binding of Tritiated Human Serum Albumin to Calcium Hydroxyapatite and to Carbonated Calcium Hydroxyapatite

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

Dental enamel is a highly mineralized tissue containing 96 % of inorganic substances. The main component of enamel is calcium hydroxyapatite (HA), with small amount (up to 5.8%) of its carbonated form (CHA) and many trace elements such as F, Pb, Zn, Fe, Cu, Al, Na, K, Mg and Sr. Numerous saliva and acquired enamel pellicle protein binding studies conducted earlier used pure calcium hydroxyapatite devoid of any carbonate as an enamel model.

1. to develop a micro-method for salivary proteins binding studies;
2. to synthesize HA and CHA form under comparable conditions;
3. to compare the binding pattern of human serum albumin, a tooth enamel pellicle component, to HA and CHA.

HA/CHA sediments were generated on the bottom of 96 well microtiter plate by centrifugation of 1 mg of hydroxyapatite suspensions in water. Human serum albumin (HSA) was labeled with tritium (3H) using tritiated acetic acid anhydride. Tritiated HSA has been incubated in the wells with HA/CHA for 30 minutes at room temperature and then centrifuged. The supernatants were removed for radioactivity counting. The sediments were washed with phosphate buffer saline and dissolved in 0.05 ml of 1 M citric acid. The radioactivity of collected supernatants and dissolved sediments was measured in the liquid scintillation counter.

HA and CHA have been synthesized under identical experimental conditions with exception of constant amount (4% by weight) of sodium bicarbonate instead of ammonium dihydrogen phosphate added to the reaction mixture. Visual inspection of Ha/CHA sediments showed that CHA sediments are more compact and opaque than those consisting of pure HA

The binding studies show that HA binds up to two times more of HSA as compared to CHA. The differential content of carbonate in human enamel may have significant consequences in formation of the acquired enamel pellicle, therefore the pathogenesis of caries and/or periodontal disease. Supported by NIH/NIDCR grant DE14950.

Develop a LITAF Knowckout Mouse by Targeted Mutation and Homologous Recombination

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The long-term objective of our work is to develop new approaches to control the harmful effects of inflammatory processes such as those observed in periodontitis),while maintaining the beneficial effects (e.g. tissue repair, resolution of infections). Accurate discrimination between normal and dysregulated inflammatory processes requires a deeper understanding of the regulatory mechanisms af-

fecting gene transcription of pro-inflammatory cytokines such as TNF. The studies we propose here build upon our recent results, in which we identified, cloned, and partially characterized the novel transcription factor LITAF. We have defined a role for LITAF as a positive regulator of TNF expression, and have found that binding of the LITAF regulatory element to the TNF promoter occurs within a region from nucleotides -550 to -487. Our laboratory hypotheses are: (1) LITAF regulates TNF gene expression in vivo and (2) that LITAF plays an important role in inflammatory disease. To further our understanding of the role of LITAF gene product, through its capacity to regulate TNF activity, in the development of inflammatory diseases, including periodontitis, we have generated a LITAF knockout mice. The present mouse model offers opportunities to further investigate LPS induced inflammatory disease at the in vivo level. Supported by NIH/NIDCR grant DE14079.

N-Terminal Processing of α1-Antitrypsin by Human Mesotrypsin

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Introduction. Human mesotrypsin does not form tight inhibitory complexes with natural polypeptide inhibitors and generally digests polypeptide substrates poorly. This unusual property is caused by Arg198, which interferes with substrate and inhibitor binding. Recently, we have shown that mesotrypsin rapidly cleaves the reactive-site peptide bond of the Kunitz-type soybean trypsin inhibitor and degrades the Kazal-type pancreatic secretory trypsin inhibitor. These observations established a physiological role for mesotrypsin in digestive degradation of dietary trypsin inhibitors and raised the possibility that it has a pathological role in the development of human pancreatitis. To extend these studies, we have characterized the interaction of mesotrypsin with the serum protease inhibitor α1-antitrypsin (α1AT), the prototype of the "suicide-mechanism" serpin inhibitors.

Methods. Recombinant human α1AT and human trypsinogens were expressed in Escherichia coli and affinity purified. Native human α1AT was purchased from Sigma. Inhibitory activity and proteolytic cleavage of α1AT was followed by spectrophotometric trypsin assays, gel electrophoresis, Western blotting and N-terminal sequencing.

Results. Mesotrypsin did not form a covalent complex with α1AT, while anionic and cationic trypsins were readily complexed and inhibited. No competitive inhibition of mesotrypsin was observed with α1AT up to 20 μM concentration and mesotrypsin did not cleave the reactive-site peptide bond of α1AT. Unexpectedly, however, mesotrypsin rapidly proteolyzed the Lys10-Thr11 peptide bond of α1AT, which resulted in the release of the N-terminal decapeptide. The N-terminally processed α1AT retained its inhibitory activity toward cationic and anionic trypsins or neutrophil elastase.

Conclusions. The interaction between human mesotrypsin and α1AT follows a different paradigm than the previously described interactions with canonical trypsin inhibitors. Mesotrypsin does not recognize the Met358 reactive-site of α1AT, and thus exhibits essentially complete resistance to its inhibitory action. In turn, this allows specific and rapid N-terminal processing of α1AT, which formally categorizes α1AT as a novel natural substrate for mesotrypsin. Supported by NIH/NIDDK grant DK058088.

Histatin Degradation and Complex Formation in the Oral Environment

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Histatins are human salivary antifungal proteins which are enriched in histidines. In a previous study we have quantitated histatins in salivary secretions and whole saliva (WS) using a zinc precipitation method (Flora et al., 2001). High histatin concentrations, comprising histatins 1, 3 and 5, were found in human parotid secretions (PS) and submandibular/sublingual secretions (SM/SL) with levels ranging between 4.5-5.5 mg% and 14.0-18.4 mg% in PS and SM/SL, respectively. WS histatin levels were significantly lower ranging between 0.22-0.54 mg%. Objective: To investigate the basis for this histatin concentration difference between pure glandular secretions and WS. Methods: Pure histatin 1, 3 or 5 were added to boiled or unboiled WS samples from one individual to a final concentration of 50 μg/ml. At various time intervals (0, 1, 5, 10, 30 and 60 min) 1-ml samples were removed, boiled and subjected to zinc precipitation. The precipitate containing the histatins was analyzed by HPLC for quantification using peak integration and comparison to histatin standards. Results: Histatin 1, 3 or 5 added to unboiled WS rapidly and completely disappeared within 5 min of incubation. Histatins added to boiled WS were relatively stable over the 60 min time interval but the amount of histatin recovered from 1-ml at t = 0 min was always lower than the 50 μg added. Typically, the reduction of histatins amounted to 26% for histatin 1, 37% for histatin 3 and 61% for histatin 5. Conclusion: The rapid disappearance of histatins from unboiled WS and the protection of histatins by boiling suggests that these proteins, once in the oral cavity, undergo proteolysis. The 26-61% loss of measurable histatins from boiled WS points towards the association of histatins with other proteins forming complexes that are not accounted for by the quantitation method. Supported by NIH/NIDCR Grants DE05672, DE07652 and DE14950

Golgi and Endoplasmic Reticulum Functions in Entamoeba Histolytica

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Entamoeba histolytica (Eh) is a protozoan parasite which causes dysentery and liver abscess in developing countries of Africa, Asia and Latin America. Infection occurs by ingestion of the cyst form which is then transformed to trophozoites that colonize the intestine and other organs such as liver.

The lack of a morphologically defined Golgi apparatus (GA) led to the hypothesis that these protists had evolved prior to the acquisition of such an organelle even though Eh has glycoproteins and lectin binding sites.

Recent ultrastructural evidence as well as immunolocalization studies with trophozoites suggest that GA like elements do occur in this organism. Thus cryofixation and cryosubstitution studies showed flattened cisternae in the cytoplasm which

resemble the GA of higher eukaryotes. In addition antibodies against mammalian GA proteins such as €COP and ARF were found to label vesicles in trophozoites with the latter being disrupted by Brefeldin A and okadoic acid, both known effectors of disruption of the GA in higher eukaryotes. Previously a combination of cytochemical approaches as well as transmission electron microscopy had identified in Eh vesicles with glucose-6-phosphatase and thiaminepyrophosphatase activities as well as vesicles fluorescently labeled with C6-NBD-ceramide; both of these approaches have been used to identify GA elements of higher eukaryotes .

Very recently the complete genome of Eh has been published and we found three sequences for putative nucleotide sugar transporters (EhNST).

We here provide the first direct evidence for GA like functions in Entamoeba histolytica as well as for components of endoplasmic reticulum protein folding quality control. Using a combination of bioinformatics, cell biological and biochemical approaches we have: 1) Cloned and expressed EhNST1 in S. cerevisiae. This is a transporter for UDP-Gal with a Km=2.9 μM 2) Detected apyrase activities in a 100K-Eh fraction. These are latent, suggesting they are in the lumen of vesicles. 3) Used bioinformatics to find several apyrases but no nucleoside diphosphatase in the Eh genome. 4) Characterized 100K-Eh vesicles which transport UDP-Gal and transfer Gal to endogenous acceptors with a Km=2.7 μM. 5) Detected galactosyltransferase activity/ies (transfer of Gal from UDP-Gal to endogenous acceptors) in disrupted Eh-100K vesicles with Km=93 μM. 6) Additionally, Eh100K vesicles showed UDP-Glu transport/transfer activity which is saturable with a Km =2.1μM. 7) Detected two kinds of glucosyltransferase activities in 5K and 100K fractions. The "heavy" vesicle fraction contains the UDP-Glu:glycoprotein glucosyltransferase activity (the so called Parodi's enzyme) with a Km for UDP-Glu of 19 μM while the "light" vesicle fraction contains another glucosyltransferase activity which does not discriminate between denatured and native thyroglobulin with Km a for UDP-Glu of 81μM and 8) The "heavy" vesicle fraction also showed UDP-Glu transport/transfer activity with Km=3.7μM.

Together, these studies demonstrate that Entamoeba histolytica contains different vesicles involved in glycosylation and protein folding quality control, analogous to Golgi apparatus and endoplasmic reticulum functions of higher eukaryotes. Supported by NIH/NIGMS grant 5R01GM30365.

Electrolyte Concentrations in Whole Saliva from Individuals with Cerebral Palsy

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Cerebral palsy is a heterogeneous group of non-progressive motor disorders caused by chronic brain injuries that originate in the prenatal period, perinatal period, or the first few years of life. The contribution of this disease to impaired oral health is clearly indicated by the high incidence of dental caries and gingivitis. To what extent salivary parameters contribute to these oral conditions in these patients has been poorly studied. Objective: To measure ion concentrations such as sodium, potassium, calcium, phosphorus and magnesium in whole saliva of adolescents with

cerebral palsy. Methods: The inorganic composition of saliva of 36 adolescents aged 12 ± 1.8 years with cerebral palsy were compared with those of 36 age-matched healthy subjects. Saliva was collected with a suction device. Electrolyte concentrations were determined by inductively coupled argon plasma with atomic emission spectrometry. Results: The flow rate was lower in individuals with cerebral palsy (0.69 ± 0.28 ml/min) than in the control group (1.16 ± 0.28 ml/min)($p<0.05$). No statistically significant differences were observed for phosphorus, magnesium and calcium concentrations between adolescents with cerebral palsy and the control group. The sodium concentration was lower in the cerebral palsy group (4.45 ± 0.97 mEq/l) than in the control group (5.89 ± 0.85 mEq/l)($p<0.05$). On the other hand, the potassium concentration in whole saliva from adolescents with cerebral palsy (10.36 ± 2.10 mEq/l) was higher than the matched control adolescents (8.30 ± 1.07 mEq/l)($p<0.05$). Conclusion: Our results suggest that cerebral palsy is associated with altered resorption/secretion patterns of sodium and potassium, possibly through modification of Na^+/K^+ pump activity. Supported by CAPES and NIH/NIDCR grants DE05672, DE07652 and DE14950.

Alterations of Dentin Sialophosphoprotein Gene in Dentinogenesis Imperfecta and Dentin Dysplasia

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The dentin sialophosphoprotein (DSPP) gene encodes two major tooth matrix proteins, dentin sialoprotein (DSP) and dentin phosphoprotein (DPP). Mutations in the DSPP gene have been reported as autosomal dominant disorders to cause Dentinogenesis Type II (DGI-II), Dentinogenesis Type III (DGI-III) and Dentin Dysplasia Type II (DD-II). DGI and DD have many similarities; however, the major distinction is DD individuals do not show discoloration in permanent teeth. DD have structural changes in secondary dentition, such as partial obliteration of the pulp chambers while DGI is more of the classic inheritance disease presenting with abnormal dentin tubules, bulbous crowns, and obliteration of the pulp chamber. Objective: The objective of our study was to determine if a mutation within the DSP region of the DSPP gene is associated with the pathogenesis of DGI and DD. Methods: Collected ten affected families of DGI and DD. Retrieved buccal or blood samples of affected and unaffected family members and then isolated DNA. Using samples of all families of affected and unaffected with DGI or DD, PCR was performed using specific primer sets of DSP region of the DSPP gene. PCR products were purified and DNA was sequenced. Results: We have identified four different alterations in the DSPP gene in 7 of 10 families affected with either DGI-II or DD-II. One alteration was found in intron 2 in the GT repeat region and another alteration was at the donor site of intron 3. Two alterations were found in exon 4. Conclusion: With many reports stating inherited dentin diseases show alterations in exon 2 and exon 3, our data only showed alterations in introns and exon 4. The GT repeat region can be used as Sequenced Tagged Sites (STS). The alteration in intron 3 can possibly affect splicing of the gene. Our results indicate that multiple genes could cause DGI and DD. Further studies are needed to determine if these

alterations are a mutation or polymorphism. Supported by DE07268, DE09875, and UTHSCSA-ERC (JD & MM).

Effect of Alveolar Corticotomy and Augmentation Grafting on Long-Term Orthodontic Outcome Stability

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Alveolar Corticotomy forms the vanguard of new treatment in the ever-expanding field of orthodontics. This technique, which is also known as Accelerated Osteogenic Orthodontics, has been shown to be more clinically effective in eliminating malocclusion, while reducing treatment time by two-thirds when compared to conventional non-extraction orthodontics. Oral corticotomy is a surgical procedure where specific incisions are made into the alveolar bone, buccal and lingually. This transient decrease in mineral content is the underlying reason for the rapid tooth movement. A specific matrix of 2 parts by vol DFDBA (demineralized freeze dried bone allograft) and 1 part by vol osteograft (bovine bone xenograft), wet with cleocin phosphate is placed in the surgical site.

Alveolar augmentation grafting has been shown to elicit a sustained increase in the volume of the periodontium. However, no attempt has been made to assess the effect of augmentation grafting on stability of orthodontic treatment. The purpose of this study is to assess long-term alveolar corticotomy outcome stability relative to alveolar augmentation grafting. Supported by the Department of Orthodontics and Dentofacial Orthopedics general research funds.

Cloning of a Novel Diacylglycerol Kinase α (DGKα) Transcript Generated by Alternative Splicing and Expressed in Neutrophils of Localized Aggressive Periodontitis Patients

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Background: Localized Aggressive Periodontits (LAP) is characterized by early and severe attachment loss, often in young individuals. The lipid second messenger DAG is involved in the production of superoxide, a reactive oxygen species that is upregulated in LAP neutrophils and induces tissue destruction. We have previously shown that neutrophils from LAP subjects present increased levels of DAG, suggesting an abnormality in its natural regulator, Dialcylglycerol kinase α (DGKα).

Aim: To characterize the pool of DGKα transcripts expressed in LAP neutrophils.

Methods: RNA was isolated from LAP neutrophils and reverse transcribed. After second-strand synthesis, full-length inserts were cloned into vectors and used to transform E. coli cells and generate a cDNA library. Colonies were screened with DGKα isotopic probes and positive clones were identified, isolated and sequenced. Confirmation of the findings was assessed through long-distance PCR, 5' and 3' RACE and sequencing. The cloned DGKα open reading frames were reamplifed by

nested PCR and used as templates in the coupled reverse transcription/translation assay to assess protein synthesis. Custom primers and TaqMan probes were designed and Real-time PCR used to quantitatively assess the expression of the different transcripts in neutrophils of LAP and control individuals.

Results: Analysis of the cDNA library revealed the expression of a novel, shorter DGKα transcript lacking 89 bp but with no changes in their 3' and 5' UTRs. Coupled in vitro transcription/translation showed a frameshift in the open reading frame and creation of a premature stop codon. Real-time PCR showed that the truncated form was particularly upregulated in LAP individuals as opposed to control subjects.

Conclusion: LAP neutrophils overexpress a novel, previously uncharacterized shorter DGKα transcript generated by alternative splicing, which is translated into a truncated protein. These findings provide evidence for a DAG/DGKα–mediated signal transduction abnormality in LAP neutrophils and provides a molecular basis for the reduced DGK activity observed in LAP. Supported by NIH/USPHS grants DE13499 and GCRC grant RR00533.

P. Gingivalis Induces Apoptosis in Gingival Fibroblasts Independent of Cysteine Proteases

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P. gingivalis is an oral bacterium that causes pathology in a number of dental infections. A common sequelae of infection is death of fibroblasts. Because previous investigators had reported that gingipains produced by P. gingivalis induced apoptosis we tested the hypothesis that protease activity was responsible for P. gingivalis induced fibroblast apoptosis. Method: Human gingival fibroblasts (HGF) were incubated with wild type P. gingivalis to establish the mechanism of cell death. Apoptosis was measured by the amount of cytoplasmic histone-associated DNA as determined by ELISA and necrosis was measured by release of lactate dehydrogenase. In some cases a protease inhibitor, leupeptin was used to suppress gingipain activity. Results: P. gingivalis induced apoptosis within 90 minutes but did not affect cell attachment until six hours ($P<0.05$). At high doses P.g. induced both cell rounding and apoptosis, while at low doses only apoptosis was induced. P.g. did not induce fibroblast necrosis ($P>0.05$). Application of the protease inhibitor leupeptin prevented cell rounding. However, it had no effect on P. gingivalis stimulated apoptosis ($P>0.05$). Conclusion: The principal mechanism by which P. gingivalis induces human gingival fibroblast apoptosis is not dependent of gingipain activity. Supported by NIH/NIDCR grant DE07559.

Diabetes May Impair Fracture Healing by Excess Removal of Cartilage

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Introduction: Fracture repair involves the formation of a callus that stabilizes the limb during the healing process. The callus is formed from mesenchymal tissue which then matures to cartilage. The cartilage is resorbed and replaced by bone. Diabetes negatively affects repair of many tissues including bone. To gain insight into how diabetes affects fracture healing studies were carried out focusing on the potential impact of diabetes on cartilage.

Objective: The aim of this study is to identify how diabetes affects cartilage.

Materials and Methods: The experimental mice were treated with low dose injection of streptozotocin which induces auto-immune mediated type 1 diabetes that has many common features as human type I diabetes. Control mice were injected with vehicle alone, citrate buffer. After three weeks of being diabetic, fracture of the tibia was induced and the mice were sacrificed after 12, 16 and 22 day. Tibias were collected and sections were stained with Van Gieson, Saffranin-O/fast green and tartrate resistant acid phosphatase. Histological analysis of the center of fracture, 0.5 and 1.0 mm proximal and distal of the fracture center was done using Image Pro plus software.

Results: Callus size was larger in the control group during 12 day but the difference increased during 16 day and 22 day. The Saffranin-O/fast green stain showed that cartilage size was larger in the control group in day 12 and day 16, but in day 16 the cartilage was severely reduced in the diabetic group. In 22 day the diabetic group has more cartilage than control group. New bone formation was analyzed through Van Gisson stain which showed a higher amount in the control groups during 16 day and the diabetic groups catch up during 22 day. The results also show that the difference between control and diabetic becomes less as we move further from the center of fracture.

Conclusion: Cartilage formation is an important part of bone healing. In diabetes this cartilage is lost prematurely, so there is no scaffold for new bone to form on. This leads to delayed new bone formation. Supported by NIH/NIAMS grant AR49920.

The Ion Exchange of Sol-Gel Scaffolds in Vivo

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Objectives: The primary purpose of this study was to examine the ion release of sol-gel glass (SG) and its effects on the mineralization of bone tissues in vivo. Methods: Fifty-six rabbits were included in this random blind study to provide a total of 112 surgical sites for four different groups: two groups of SG with variant compositions (SG1, SG2), Bioglass (BG), and the control. The materials were placed in 7 mm diameter defects createdat the mesial ends of the tibia. No material was placed in the empty defects of the controls. Each material group was divided into four time periods, three, six, twelve and twenty-four weeks. Seven surgical sites per time period were used for each group. After the animals were sacrificed, each sample

was embedded in methyl methacrylate and examined by with an energy dispersive spectrum (EDS) to verify the ions released and the mineralization of surrounding bone tissues. Results: Silicon significantly released from the SG particles with time, but the amount of silicon released from BG was not significant throughout the 24-week period (t-test, $p<0.01$). Silicon levels in the surrounding tissues were higher in the SG1 and SG2 groups than in the BG group at 3 and 6 weeks (ANOVA $p<0.01$). The SG1 groups yielded a higher mineralization of newly formed bone tissue in the periphery than the other groups at 3 weeks (ANOVA $p<0.01$).

Conclusion: The sol-gel based glass had a stronger interaction with living tissue than the Biogalss did, as demonstrated by the increased silicon content around the implant sites and the resulting high mineralization in surrounding tissues at the early stage of the bone healing. Supported by USBiomaterials Co. grant.

E-cadherin Protein Complexes as Predictors of Cellular Adhesive Stability

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E-cadherin functions as the primary epithelial intercellular adhesion receptor with key roles in development and cancer. A transmembrane protein, E-cadherin makes calcium-dependent homotypic cell-cell contacts known as adherens junctions (AJs). The stability of AJs depends on the coupling of E-cadherin cytoplasmic domain to catenins and various crosslinking and scaffold proteins that link the complex to the actin cytoskeleton. In normal epithelial cells, the stability of E-cadherin cell-cell contacts is proliferation-dependent: in sparse cultures E-cadherin is found in unstable AJs, while in dense cultures E-cadherin localizes predominantly to stable AJs. However, cancer cells that express E-cadherin, such as oral squamous cell carcinoma A253 cells, form mostly weak AJs. To learn more about E-cadherin association with various cytoskeletal and adhesive molecules in normal and cancer cells, we have developed a method for separating distinct E-cadherin protein complexes. Using MonoQ anion exchange chromatography, we have isolated different fractions of E-cadherins from A253 cells. Immunoprecipitation and Western blotting showed that in these fractions, E-cadherin was in complexes that differed in the abundance and/or nature of associated proteins. Within the same cell, low ionic strength fractions typically contained E-cadherin associated with stabilizing catenins, such as α-catenin, while high ionic strength fractions had E-cadherin associated with destabilizing proteins, such as IQGAP. Moreover, the relative ratio of E-cadherin in unstable complexes was increased in cancer cells compared to normal cells. Collectively, our studies show that E-cadherin forms different types of protein complexes even within the same cell, and that the balance among such complexes defines the overall stability of E-cadherin mediated cell-cell contacts. This method of characterization of E-cadherin protein complexes will be useful for predicting the overall stability of AJs in normal and tumor cells. Supported by the PHS grant RO1 DE10183-11 and DE14437-3 to MAK.

Candida Glabrata: A Unique Species with a Marked Resistance to Cationic Antifungal Proteins.

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Various Candida species have been shown to be susceptible to killing by histatin 5. In contrast, C. glabrata has shown an unexpected resistance to histatin 5. Objective: To assess which cellular properties are associated with the insensitivity of C. glabrata to histatin 5. Methods: Histatin 5 fungistatic activity was determined in a growth inhibition assay in diluted Sabouraud dextrose broth supplemented with yeast extract. Results: While three C. glabrata strains were insensitive to histatin 5 (IC50>225ug/ml), three genetically very similar strains were susceptible. These strains are Saccharomyces cerevisiae, Kluyveromyces delphensis and Kluyveromyces bacillisporus, exhibiting IC50 values of 6±2ug/ml, 15±1ug/ml, and 11±3ug/ml, respectively. Since C. glabrata is lacking complex I in its respiratory chain, the sensitivity of a homozygous complex I null mutant of an otherwise sensitive C. albicans strain was investigated. IC50 values of this null mutant were similar to those of the wild-type strain, rangeing between 10 and 18 ug/ml, indicating that complex I is not involved in the histatin 5 killing mechanism. To assess whether C. glabrata was uniquely insensitive to histatin 5, the fungistatic effects of two other, very different cationic antimicrobial proteins, PGLa and Magainin 2, were investigated. Data showed that C. glabrata is also resistant to these two proteins (IC50>85ug/ml). Conclusion: The insensitivity of C. glabrata to histatin 5 is a) apparently species specific, b) not related to the absence of complex I, and c) not an exclusive property of histatin 5. Supported by NIH/NIDCR grants DE05672, DE07652 and DE14950.

Diabetes Enhances Fibroblast Cell Death Through TNF-a Mediated Caspase Activation

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Objective: To test the hypothesis that diabetes enhances the destructive effect of an oral pathogen by enhancing fibroblast apoptosis. Methods: P. gingivalis was inoculated into the scalp of control or type 2 diabetic mice. Mice were sacrificed at the repair phase (8 days). The scalp tissue was collected and analyzed by TUNEL, RPA and fluorimetric assay to detect the apoptosis, collagen gene expression and caspase activity, respectively. Results: Diabetes increased P.gingivalis induced apoptosis for both time points by approximately 60%. The increased apoptosis could be explained by a 25% increase in caspase-3 activity. In order to detect whether this caspase-3 activated apoptosis pathway is functional significance a caspase inhibitor was injected together with P. gingivalis. The results indicated that the inhibition has significantly reduced the rate of fibroblast apoptosis in the diabetic group, which brought a 50% increase of procollagen gene expression. To determine whether the host response was responsible for the increased caspase-3 activity, a specific TNF inhibitor was tested.

The results demonstrated that when TNF was inhibited caspase-3 activity was reduced approximately 30%. Fibroblast apoptosis reduced by 50%, which leads to 25% increase of fibroblast density and 40% increase of new matrix formation. Conclusion: The host response to P. gingivalis leads to TNF-a stimulated caspase-3 activation in fibroblasts, which in turn leads to greater fibroblast cell death. The premature fibroblast cell death reduced the capacity of diabetics to repair the bacteria-induced wound. Supported by NIH/NIDCR grant DE07559.

The Role of N-glycans in E-cadherin Adhesion Dynamics

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E-cadherin is the main calcium-dependent epithelial cell-cell adhesion receptor that plays pivotal roles in morphogenetic processes, polarity and differentiation. A transmembrane glycoprotein, E-cadherin mediates cell-cell adhesion through the assembly of adherens junctions (AJs), or multiprotein complexes linked to the actin cytoskeleton. The formation of AJs is initiated by the binding of E-cadherin extracellular domains on adjacent cells; the association of E-cadherin cytoplasmic tails with the actin cytoskeleton via catenins and other interacting proteins stabilizes AJs and provides strength to adhesion. Although the extracellular domains of E-cadherin are modified with N-glycans, their function in the stability of AJs remains unknown. Previous studies showed that E-cadherin underwent changes in its N-glycosylation status in vivo, with extensively N-glycosylated glycoforms found in unstable AJs and scarcely N-glycosylated species in stable AJs. To investigate how N-glycans affected E-cadherin-mediated cell-cell adhesion, we generated its N-glycosylation variants lacking selected N-glycan addition sites. We then investigated their adhesive properties in CHO cells that lack endogenous E-cadherin and in mdCK cells, which form E-cadherin mediated cell-cell contacts. In both cells types, E-cadherin N-glycosylation variants were present in complexes containing high levels of the AJ stabilizing proteins and this correlated with their stable association with the actin cytoskeleton. This indicates that E-cadherin N-glycosylation variants function as dominant positives. Since weak adhesion is required for tissue remodeling during morphogenesis, while strong adhesion drives cytodifferentiation and maintenance of adult tissue architecture, these results indicate that N-glycosylation is a modulator of E-cadherin function in vivo. Moreover, since inappropriate weakening of E-cadherin cell-cell contacts is associated with human cancers, our studies suggest dysregulation of E-cadherin N-glycosylation is a contributing factor in tumorgenesis. Supported by NIH grants DE10183 and DE14437 to MAK.