Featuring a keynote lecture on *Diabetes and Sjogren’s: From Disease Reversal in Mice to Human Trials* by Denise L. Faustman, M.D., Ph.D., Director of Immunobiology, Massachusetts General Hospital and Associate Professor of Medicine, Harvard Medical School

Image: Regeneration of salivary gland tissue in a mouse with advanced Sjogren’s Syndrome treated with a therapy to selectively kill autoreactive T cells. Contributed by the laboratory of Dr. Denise Faustman at Massachusetts General Hospital.
Dr. Denise L. Faustman, M.D., Ph.D.
“Diabetes and Sjogren’s: from disease reversal in mice to human trials”

Dr. Faustman earned her M.D. and Ph.D. from Washington University School of Medicine in 1985. Dr. Faustman did her internship, residency, and fellowships in Internal Medicine and Endocrinology at Massachusetts General Hospital. She started as an independent investigator at Harvard Medical School in 1987. Dr. Faustman is currently an Associate Professor of Medicine at Harvard Medical School and Director of the Immunobiology Laboratories at the Massachusetts General Hospital.

Dr. Faustman’s research accomplishments include the first scientific description of modifying the antigens on donor tissues to change their foreignness, a scientific accomplishment that is now in human clinical trials. This invention forms the basis of producing genetically modified pigs for organ donation and in 2003 earned her the prestigious National Institutes of Health and the National Library of Medicine Award, “Changing the Face of Medicine”, one of 300 American physicians, one of 35 in research, honored for her seminal scientific achievements in the history of the United States.

Currently, Dr. Faustman works on strategies aimed at halting established autoimmune diseases such as type I diabetes and Sjogren’s Syndrome. She was the first to demonstrate the targeted removal of pathogenic T cells allowed spontaneous regeneration of the pancreas or the salivary glands in a mouse model of autoimmunity. This targeted approach is now validated in human autoimmune disease and is the basis of clinical testing in a double-blinded placebo controlled trial. She is a member of the AAAS and serves as a frequent member of the Institute of Medicine in Washington, D.C. She is now in a clinical trial in long-term diabetics attempting to reverse type 1 diabetes with a generic drug.
**Predoctoral Students**

Courtney Brady, Kathy Held, and Ana Karina Mascarenhas. Department of Health Policy and Health Services Research, Division of Dental Public Health: “Evaluating Program White Coat, a Dental Pipeline Program for Children.”


**Postdoctoral Students**


Ghadir Atout, Thomas E. Van Dyke, and Robert Gyurko. Department of Periodontology and Oral Biology: “Actin Polymerization to Chemotactic Stimulus is Defective in Diabetic PMN.”

Susan Baloul, Louis Gerstenfeld, Robert Carvalho, Thomas Van Dyke, and Alpdogan Kantarcı. Department of Periodontology and Oral Biology: “Cathepsin K and RANKL Expression in Corticotomy-Facilitated Tooth Movement.”

Vinodh Bhoopathi, Samar Mashabi, Thayer Scott, and Ana Karina Mascarenhas. Department of Health Policy and Health Services Research, Division of Dental Public Health: “Knowledge, Attitudes, and Opinions on Bioterrorism Preparedness in Dental Professionals: A Comparative Study.”


Hisham Merdad, Mohamed Bamashmous, and Ana Karina Mascarenhas. Department of Health Policy and Health Services Research: “Oral Health of Rhode Island Retirees.”

Jan Ortiz and Lee Chou. Department of Restorative Sciences/Biomaterials: “Effects of Calcium on Survivin Expression in Human Osteoblasts.”

Corneliu Sima, Khadija Rhourida, Thomas E. Van Dyke, and Robert Gyurko. Department of Periodontology and Oral Biology: “Chronic Hyperglycemia Impairs Leukocyte Recruitment to Sites of Inflammation.”

**Postdoctoral Fellows**


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### Predoctoral Students
Jeffrey Clark, Daniel Jeong, Mohammed Nadershah, and Pushkar Mehra. Department of Oral and Maxillofacial Surgery: “Odontogenic Keratocysts: An Analysis of Surgical Treatment Options and Their Long-Term Results.”

### Postdoctoral Students
Maha Bahammam and Philip Trackman. Department of Periodontology and Oral Biology: “Regulation of Transforming Growth Factor-β1 (TGFβ1) Induced Expression of Connective Tissue Growth Factor (CCN2/CTGF) by Wnt in Human Gingival Fibroblasts.”
Jike Cui and John Samuelson. Department of Molecular and Cell Biology: “Darwinian Selection for Sequons (Sites of Asn-Linked Gicosylation) in Phylogenetically Disparate Eukaryotes and Viruses.”

### Postdoctoral Fellow
Evaluating Program White Coat a Dental Pipeline Program for Children

Courtney Brady, Kathy Held, and Ana Karina Mascarenhas

Boston University, Boston, MA

Objectives: The number of minority dentists and students in dental schools is dramatically lower than the percentage of minority citizens in the U.S. population. Since data shows that minority populations are more likely to access care if the provider is from a minority group, attempts need to be made to encourage more young people from these groups to consider dentistry and other health careers. To address this issue, several programs have been introduced that expose disadvantaged and under-represented minority (URM) students to health careers; one such program at Boston University Goldman School of Dental Medicine that targets middle-school children is Program White Coat (PWC). This week-long program focuses on stimulating children's interest in health careers, particularly dentistry, at an early age. The purpose of this study was to assess PWC’s effectiveness in raising interest and knowledge in the health professions. Methods: Children and their parents’ awareness and knowledge in the healthcare professions was assessed. Children that participated in the program in July 2008 were evaluated using pre and post program surveys, journal entries, quizzes, and verbal interviews. To evaluate the parents, they were surveyed at the end of the program. Results: Children that participated were in grades 4-6. Analysis show that the children’s general dental knowledge improved after the program. In the pre-test, only 30% thought that bacteria caused cavities, but, 80% in the post-test said that bacteria caused cavities. Similarly, 80% did not think acid caused cavities in the pre-test which changed to 40% in the post-test. Seventy-one percent of parents were not aware of additional programs for their children. Conclusion: Program White Coat has been successful in stimulating young minds toward a career in the healthcare professions. It is recommended that more schools implement similar programs to expose disadvantaged and URM students to health professions.

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Role of Lysyl Oxidase Propeptide in inhibiting EGF and FGF-2 functions in Oral Cancer cells

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Objective: Oral cancer often has devastating effects on patients due to its rapid growth and fatal consequences if not caught early. Our current understanding is that oral cancer cells grow rapidly and unchecked in part to an increased response to Epidermal Growth Factor (EGF) and Fibroblast Growth Factor-2 (FGF-2). Previous research has indicated that the 18 kDa pro-peptide domain of lysyl oxidase is effective in inhibiting FGF-2-induced growth and signaling of oral cancer cells (Palamakumbura et al, unpublished). Our goal was to investigate the possible effects of the lysyl oxidase propeptide (LOX-PP) on EGF stimulated signaling, and of three separate peptide sequences that correspond to conserved regions of LOX-PP on FGF-2 stimulated signaling in the oral cancer cell line, FADU.

Methods: FADU cells were cultured and treated with different concentrations of EGF at different time intervals to determine the optimum conditions for p44/42 MAPK phosphorylation. Then these cells were treated overnight with different concentrations of LOX-PP and induced with 10 ng/ml EGF for 5 minutes. Cells were then harvested and Western blots were prepared. Membranes were blocked and then incubated with anti-phospho p44/42 MAPK antibody overnight. Then they were treated with horseradish peroxidase-coupled anti-rabbit antibody, and signals were developed with chemiluminescent peroxidase substrates. X-rays were developed to locate the bands of phospho p44/42 MAPK. The same membranes were then stripped and similarly probed with anti-total p44/42 to normalize for gel loading. Additional groups of cells were treated with three different synthetic peptides corresponding to parts of LOX-PP overnight and induced with 10 ng/ml FGF-2 for 5 minutes. Cells were harvested and subjected to Western blotting using anti-phospho FRS2α antibody, a signaling molecule specifically phosphorylated by activated FGF-receptors. Membranes were then stripped and probed with anti-β-actin antibody for normalization.

Results: Results indicate that there is no significant effect of LOX-PP on EGF-induced p44/42 MAPK phosphorylation. Out of three peptides sequences, 2 peptides corresponding to the more C-terminal conserved regions of LOX-PP inhibit FGF-2 induced phosphorylation of FRS2α.

Conclusions: These findings indicate that LOX-PP has specificity for inhibition of growth factor-stimulated signaling. In addition, data suggest that the active region of LOX-PP as an inhibitor of FGF-2 initiated signaling in oral cancer cells is located in its C-terminal region and not the N-terminal region.

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Oral Health Quality of Life in BUGSDM

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Objective: Health care of the future will be evaluated on how well it improves health from a patients’ perspective. The purpose of this project is to describe patient-reported improvements in oral health as a function of dental care received in Boston University Goldman School of Dental Medicine (BUGSDM) clinics.

Methods: This was an IRB approved secondary analysis of existing data in BUGSDM’s predoctoral clinic patients. Primary outcomes of interest were changes in the brief oral-specific health related quality of life (OQOL) instrument (Kressin et al., 2008), the self report of oral health (OH1), and general health (GH1). Predictors of change evaluated age, sex, insurance status, type and quantities of treatment received.

Results: Participants were 108 males and 88 females, with a mean age was 41.49 years, and an average of 9.1 visits per person. Mean brief OQOL scores improved from 24 (worse) to 14, while the percent with scores of fair to poor improved from 41 to 14%. Predictors of improvement included having Medicaid vs. private insurance, age, sex, number and type of treatments. Furthermore, improvement in OH1 was highly correlated ($r = 0.34$) with improvement in GH1. ($p=0.0001$)

Conclusions: Dental care in BUGSDM patients significantly improves the oral health quality of life in patients. Our findings suggest that the brief OQOL instrument and the OH1 are useful instruments in monitoring the outcomes of dental care.

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Mechanical Properties of Zirconia with Various Surface Finishes

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Objectives: Yttria partially stabilized zirconia is a dental ceramic used to produce restorations such as dental bridges and crowns. Pure zirconia can exist in three different phases: monoclinic, tetragonal and cubic. Yttria is added to zirconia to partially stabilize it in its tetragonal phase. Transformation can still take place from the tetragonal phase to the larger monoclinic phase, which aids in the prevention of crack propagation. The surface finish and heat treatment of zirconia may have an effect on the phase, which has an effect on the flexural strength. The purpose of this study was to examine the effect of surface finish on the mechanical properties of yttria partially stabilized zirconia using ISO standards (ISO 6872: 2008).

Methods: Vita YZ Zirconia and Dentsply Cercon Zirconia cylinders were cut into discs with a thickness of 1.5 mm. Thirty Vita YZ Zirconia discs and 30 Dentsply Cercon Zirconia discs were sintered in a furnace. Ten discs from each zirconia brand were left as-sintered. The other 40 discs were polished, and then 10 polished discs from each brand were heat treated. The biaxial flexural strengths of all the discs were tested using a universal testing machine. One-way ANOVA tests were used to determine any significant differences between the groups for each brand.

Results: Data showed that Vita YZ Zirconia polished discs and polished-heat treated discs were significantly stronger than the Vita YZ Zirconia as-sintered discs. There were no significant differences between the polished discs and polished-heat treated discs. Also, there were no significant differences between the Dentsply Cercon Zirconia groups.

Conclusions: It appears that the polishing of the Vita YZ Zirconia resulted in strength increase, but heat treating did not have an effect on the material's strength. The results also imply that there was a phase transformation when the Vita YZ Zirconia discs were polished but not when the Dentsply Cercon Zirconia discs were polished. To determine if this is accurate, x-ray diffraction should be used to examine the phases of the specimens in the future.
Anticarcinogenic Properties of Capsaicin on Oral Cancer

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Objective: To determine the anticarcinogenic affects of Capsaicin on oral cancer.

Method: Meta-analysis of published, English language scientific literature, focusing on past and current data with an emphasis on capsaicin and cancer.

Results: Currently, there are numerous studies supporting the utilization of capsaicin as a promising anticarcinogenic agent. Research has demonstrated that capsaicin either activates or inhibits several cellular pathways in transformed cells which cause apoptosis. Among the various pathways that have been activated or inhibited include: NF-kB, R.O.S., Caspases, p53, JNK-1, Mitochondrial respiration, N.A.D.H. Oxidase, Cytochrome P450 to mention a few. On the contrary, there is published data claiming capsaicin possesses pro-carcinogenic properties. However, some of these studies have been replicated and the results could not detect any specific carcinogenic activity of capsaicin.

Conclusion: Presently, most data supports capsaicin’s anticarcinogenic properties on cancer. However, since most of the studies have been performed in vitro, the efficacy of this compound in humans remains unknown. In addition, other factors which must be fully understood before administering capsaicin to cancer patients are: toxicity level, length of treatment, dosage level, safety and ethical issues. Hence, additional research is necessary to completely evaluate the anticarcinogenic properties of capsaicin on oral cancer.
Meta-analysis and Review on the Effectiveness of Different Xylitol Products in Caries Prevention

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Objective: Recently there is an expansion in oral xylitol products on the market. The purpose of this study is to identify effective commercially available oral xylitol products for the purpose of implementing xylitol programs in the US.

Methods: An electronic search through PubMed database was performed to identify studies on the effectiveness of xylitol products in caries prevention. The search was limited to English language full text articles of human clinical trials. Meta-analysis was performed and pooled estimates using combined fixed and random effects models were calculated.

Results: Although there are various forms of xylitol products available in the market, online or supplied through dental offices, only a few products were clinically tested. These are xylitol chewing gum, lozenges, candies, xylitol based foods, gummy bears, tooth paste, and “Fall-Asleep Pacifier” (FAP) using xylitol tablets. Products with (100%) xylitol chewed or consumed 5 times per day with a total dose of 5-10 g of xylitol/day were most effective. Meta-analysis results showed that clinically tested products containing high levels of xylitol had significantly prevented and reduced the DMFS score compared to the control. Pooled estimate using the combined fixed and random effect of standardized mean difference was -0.26 (95% CI: -0.3, -0.20) and -0.32 (95% CI: -0.5, -0.08) respectively.

Conclusion: Practitioners should consider product form, dose and frequency when prescribing xylitol as a caries preventive tool. In an era of evidenced based dentistry, further research is needed to test the effectiveness of different xylitol products in caries prevention.
Development of *In Vitro* and *In Vivo* Methods to Evaluate Wound Healing Properties of Salivary Proteins

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**Introduction:** The rapid healing of lesions in the oral cavity has traditionally been ascribed to nerve growth factor (NGF), transforming growth factors alpha and beta (TGF-α and -β), fibroblast growth factor (FGF), and epidermal growth factors (EGF). A recent study showed that salivary histatins, being an exocrine product from the major salivary glands, can stimulate the migration of transformed human buccal epithelial cells *in vitro*. **Objective:** To develop *in vitro* and *in vivo* models to evaluate the wound healing activities of histatins and other salivary proteins. **Materials and Methods:** *In vitro* studies were carried out with human gingival fibroblasts (primary cells) and TR146 buccal epithelial cells (transformed cell line). Both cell types were cultured in Dubecco’s Modified Eagle Medium supplemented with 10% fetal bovine serum till confluence followed by starvation in serum-free medium. The effects of salivary proteins on cell migration were assayed in a 24-well transwell tissue culture plate containing uncoated filters with 8 µm pores, followed by counting of migrated cells microscopically. Cell migration was also assessed by microscopic examination of the closure of a scratch applied to a confluent layer of cells. The development of an *in vivo* dermal wound closure model involved the application of a fixed silicone splint around a 4 mm puncture wound on the dorsal surface of mice. **Results:** Both fibroblasts and epithelial cell lines were successfully cultured and examined in transwell migration and scratch assay models. Conditions for stimulation of cell migration were established in both models and preliminary data on the stimulatory effects of a variety of naturally occurring and synthetic salivary protein fragments were obtained. In addition, the methodology for the formation of dermal wound, including splint fixation and dressing placement was established. **Conclusion:** The development of two *in vitro* and one *in vivo* model for wound healing will allow the systematic comparison of a variety of exocrine and non-exocrine saliva-associated components with respect to their wound closure activities.

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Actin Polymerization to Chemotactic Stimulus is Defective in Diabetic PMN

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Objective: Chemotaxis and phagocytosis are essential steps in bacterial killing by neutrophil polymorphonucleotides (PMN). Leukocyte defects have been implicated in the development of long term complications of diabetes mellitus but the effects of high blood glucose on the cytoskeleton are not clear. Upon chemoattractant signaling actin monomers are polymerized to form F-actin with corresponding changes in the cytoskeleton and cellular shape, thus enabling cell mobility during neutrophil chemotaxis.

In this study, we investigated the effect of chronic and acute hyperglycemia on actin polymerization in fMLP-stimulated PMN.

Methods: Type-1 diabetic Akita and wild-type mice were injected interaperitoneally with 1 ml of zymosan solution (0.2 mg/ml in PBS). Abdominal lavage fluid was collected 2 hours later and neutrophils were isolated by centrifugation. Chemotaxis was induced by timed fMLP (10^{-7} M) stimulation at 37°C. Actin polymerization was determined by flow cytometry analysis of cytoskeletal staining with FITC-phalloidin. In a separate set of experiments we have challenged wild-type PMN in situ by injecting zymosan intraperitoneally with either 1 g/L (normoglycemic) or 4.5 g/L (hyperglycemic) PBS for 3 h.

Results: fMLP stimulation yielded rapid F-actin polymerization that was maximal 10 seconds after fMLP stimulation in both Akita and WT neutrophils. However, the F-actin fluorescent response was significantly smaller in Akita PMN compared to WT PMN. Within 60 seconds F-actin content returned to baseline levels in both groups. Acute glucose challenge in wild-type PMN resulted in similarly diminished actin polymerization.

Conclusions: These results indicate that actin polymerization response to chemotaxis demonstrates a slow and impaired pattern in chronic and in acute hyperglycemia. This defect might contribute to diabetic tissue damage by preventing proper movement of PMN towards microbial gradients and weakening the defense of innate immune system.

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Cathepsin K and RANKL Expression in Corticotomy-Facilitated Tooth Movement

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Coupling of anabolic and catabolic processes in alveolar bone results in orthodontic tooth movement. Corticotomy-facilitated tooth movement induces osteoclastogenesis adjacent to the surgically injured cortical bone allowing rapid tooth movement. The biological mechanism underlying this enhanced tooth movement is not well understood.

Objective: To evaluate RANKL and Cathepsin K mRNA expression in alveolar bone in response to tooth movement with or without corticotomy.

Methods: Seventy-two CRL-CD male rats were included and grouped as corticotomy alone; tooth-movement alone; and “combined” therapy with right side of each animal serving as the untreated control. Tooth-movement was performed on the first molar utilizing a 25-gram Sentalloy spring secured to a micro-screw palatal to the incisors. There was a 6-week active tooth movement (active phase). Appliances were removed and stability of the movement was assessed for an additional 6 weeks (stability phase). Animals were sacrificed at Day 0, 1, 2, 3, 4, 6, 7, 8, 9 and 12 weeks. Bone was harvested as a block from the distal to the mesial of the maxillary first molar. Bone tissue was immediately frozen in liquid nitrogen. Total RNA was extracted and quantified. Gel electrophoresis utilized to verify mRNA integrity. Molecular Analysis using q-PCR was performed to evaluate RANKL and Cathepsin K mRNA expression normalized to beta-actin levels. Statistical analysis was performed using ANOVA with LSD post-hoc test.

Results: During the active phase, both RANKL and Cathepsin K expression was increased compared to the baseline during the first week in both TM and CTM groups with no significant difference [RANKL: 3.5 vs. 4.4-fold TM and CTM, respectively, p>0.05, Cathepsin K: 2.5 vs. 3.2-fold, respectively, p=0.39]. During the fourth week, CTM group showed a further increase in Cathepsin K (3.1-fold) while TM group went back to baseline levels. Similar Cathepsin K gene expression was observed in both TM and CTM groups after 4 weeks with no statistically significant differences among groups through 12 weeks. RANKL expression reached similar results by week 6 in both TM and CTM groups [RANKL: 2.4 vs. 1.5-fold respectively, p>0.05] and no differences were observed during the stability phase among groups.

Conclusion: These findings suggest that RANKL and Cathepsin K mediate bone resorption, which underlies the enhanced orthodontic tooth movement after corticotomy.

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Knowledge, Attitudes and Opinions on Bioterrorism Preparedness in Dental Professionals: A Comparative Study

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Aim: The aim of our study is to compare knowledge, attitudes, and opinions regarding bioterrorism preparedness between dental professionals from a region that has been exposed to a bioterrorism event (Oregon) with those from a region that has not been exposed (New England).

Methods: An 18 item pre-tested, self-administered questionnaire was used to collect data during the 2005 Oregon Dental Conference (n=156) and 2005 Yankee Dental Conference (n=370). Means and frequencies were calculated. Chi square and t-tests were performed.

Results: Over 90% of both the New England and Oregon Dental Professionals were willing to provide care during bioterrorist events. The mean positive opinions regarding bioterrorism preparedness and management were higher among New England professionals (11.5 ± 2.2) compared to Oregon professionals (10.5 ± 2.8) (p<0.0001). Again, New England professionals had higher overall mean self–perceived knowledge (14.7 ± 4.9) compared to Oregon professionals (12.0 ± 5.1) (p<0.0001). No significant difference between the groups in the mean number of roles they thought the dental professionals should play during an event was observed. Both groups had a very low mean actual knowledge.

Conclusions: Both the New England and Oregon dental professionals were interested in providing assistance during a bioterrorism event, but lacked knowledge regarding bioterrorism preparedness and management, suggesting a need for more education and training on bioterrorism preparedness. New England professionals, who had never been exposed to a bioterrorism event, had better opinions and higher self–perceived knowledge than the Oregon professionals, who possibly had been exposed.
Quantitative Proteome Analysis of Saliva of Healthy Versus Periodontal Patients

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Periodontal disease is a bacterial infection associated with a chronic inflammatory response, which affects teeth supporting structures. Manifestation of periodontal disease are gingival inflammation, alveolar bone loss, tooth mobility and in severe cases tooth loss. Such changes can lead to increased levels of serum related components in the oral cavity via gingival crevicular fluid and new components from the inflammatory reaction and bone resorption.

Objective: To evaluate and determine on a large-scale quantitative alterations in the whole saliva (WS) proteome of healthy versus periodontal patients by using high-throughput mass spectrometric approaches.

Method: We have utilized isotope-coded-affinity-tag (ICAT) to differentially label by stable isotopes the WS samples of healthy and periodontal patients followed by tryptic digestion, specific affinity enrichment and nano-flow LC-ESI-MS/MS analysis for protein identification and relative quantitation.

Results: Our preliminary studies indicated three categories of observations with respect to relative quantitative protein analysis of healthy versus periodontal WS samples. A group of proteins of unchanged levels, a second group who's levels increased and a third group that showed decreased levels. Within the group who's levels remain unchanged appear to be predominantly those abundant and well known salivary proteins. Those proteins who's levels increased include but are not restricted to predominantly serum origin, cellular and a few of glandular origin. Whereas the group that showed decreased levels are of mixed origin.

Conclusion: The present study provided a unique opportunity to evaluate alterations in the WS proteome of healthy versus periodontal patients at a quantitative level. Such studies have the potential to highlight important biological changes in oral cavity fluid composition that can impact significantly the integrity of both hard and soft tissues as well as provide specific biomarkers useful for diagnostic purposes.

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Oral Health of Rhode Island Retirees

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Background: Data on oral health, including root caries experience and gingival recession in the community-dwelling U.S. elderly population is limited. Most of the recent data available are from studies on institutionalized elders. For Rhode Island, oral health information is only available through BRFSS data on the percentage of people that have had permanent teeth removed due to tooth decay or gum disease.

Objective: To describe the oral health, including root caries and gingival recession experience in Rhode Island retirees.

Methods: Data was collected as part of oral cancer screenings during a health fair for The Rhode Island Alliance for Retired Americans, held in East Providence, RI in November 2008. Seventy-three retirees 60 years and older participated. Data collected included oral hygiene, oral cancer screening, number of teeth present, decayed, missing and filled teeth, the presence of root tips, decayed and filled roots, gingival recession, and the presence, status and condition of dentures.

Results: We found that 85.9% of participants had natural teeth. Of these, 19.7% had untreated decay, 95.1% had filled teeth, 100% had at least one missing tooth, 50.8% had root caries experience and 78.7% had gingival recession. The participants’ mean number of teeth present was 19.7 ±8.0. The mean untreated decay, missing and filled teeth was 0.2 ±0.4, 12.3± 8.0 and 9.4±5.5, respectively. The mean DMFT was 21.9 ±6.0. In addition, dentate participants had a mean of 1.4 ±2.1 teeth with root caries experience, and 5.8 ±5.6 teeth with gingival recession.

Conclusion: Although the prevalence of untreated decay in this population is small, the gingival recession and root caries experience in this sample is large enough to suggest that preventive measures such as regular fluoride varnish applications may be useful in reducing root caries risk in this population.
Effects of Calcium on Survivin Expression in Human Osteoblasts

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Survivin is a newly discovered anti-apoptotic molecule from the IAP family. It is involved in the cell mitosis process and has been related to cancerous tumors. Although negative connotations of survivin are usually emphasized in the majority of researches, this project directs the attention to its positive characteristics: its assistance during proliferation to human osteoblasts. **Objectives:** The purpose of this study is to verify if survivin expression can be manipulated using external factors such as calcium ions. Survivin is not involved during differentiation of cells, therefore expression of this molecule entail that the cells are in a proliferation stage. **Methods:** The 2nd passage of normal osteoblast cell cultures derived from human donors were placed in well plates at a concentration of $1.5 \times 10^5$ cells. Cells were cultured with essential medium as a control and with medium containing supplemental calcium ions at a concentration of 30 ppm as a study group. Cell proliferation, mineralization, alkaline phosphatase activity, osteocalcin and survivin expression were measured at 7 and 21 days of culture. **Results:** Compared with the control group, cell cultures with 30 ppm calcium supplement presented an increase of survivin expression at all three time intervals, 34% at 16 hours, 35% at 7 days and 62% at 21 days. A significantly higher rate of cell proliferation was observed, an 86% increase at 7 days and a 57% increase at 21 days. The study group also showed at 21 days a 163% increase of osteocalcin expression, a 37% increase of alkaline phosphatase activity, and a 229% increase of mineralization of the cultures. **Conclusion:** This study demonstrated that survivin expression could be significantly up regulated by calcium enhanced normal human osteoblast cultures, resulting in up regulated cell proliferation.
Chronic Hyperglycemia Impairs Leukocyte Recruitment to Sites of Inflammation

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Objectives: To determine the role of chronic hyperglycemia in leukocyte rolling, attachment and overall recruitment using a model of type I diabetes, the Akita mouse.

Methods: C57BL/6 wild type and Akita mice, ages 2-4 months, were used for all experiments. Gingival intravital microscopy of postcapillary venules was used for assessment of leukocyte rolling and attachment along endothelium and dorsal air pouch lavage for overall recruitment after injection of TNFα. A hemocytometer with Wright-Giemsa staining was used for quantification of total leukocytes and individual cell types. E. Coli strain MC 1061 was incubated with Akita and WT PMN at different ratios for 30 minutes at 37°C, PMN lysed in distilled water and 35μl aliquots spread on LB agar plates and incubated overnight at 37°C.

Results: Intravital microscopy of gingival postcapillary venules showed an increased number of rolling leukocytes along endothelium in the Akita mice (WT: 12.5±3.7, Akita: 21±3.6, P<0.03, t-test, n=4). Akita had more leukocytes in the air pouch without TNFα stimulation (WT: 0.88x10⁶±0.13 cells/pouch, Akita 1.56x10⁶±0.19 cells/pouch n=4, P<0.02). Following intrapouch TNFα injection (0.5 mL of 3.5 nM TNFα in saline) Akita mice recruited fewer leukocytes (WT+TNFα 3.98x10⁶±1.58 cells/pouch and Akita+TNFα 2.01x10⁶±0.9 cells/pouch, at 4 h, P<0.01, t-test, n=6). Differential cell counts revealed a significant PMN recruitment in WT in response to TNFα, but not in Akita (WT: 64.1±5.8%, WT+TNFα 80.8±7.2%, Akita: 56.2±2.8% and Akita-TNFα: 54.9±16.4%, P<0.05, t-test, n=4). This data supports our previous findings of increased adhesion molecule expression, oxygen consumption and superoxide production in by diabetic PMN. WT PMN at ratio 1:30 reduced CFU by 30% while Akita PMN did not reduce CFU. (n=2, p<0.05)

Conclusion: Chronic hyperglycemia increases leukocyte rolling on endothelium but reduces leukocyte recruitment to sites of inflammation. Furthermore bacterial killing by PMN is impaired. These findings reveal dysregulated leukocyte recruitment process in diabetes which might contribute to the development of diabetic complications, including periodontal disease.

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ANALYSIS OF THE HUMAN TYROSYLPROTÉIN SULFOTRANSFERASE-2 (TPST2) GENE IN PATIENTS WITH CHRONIC PANCREATITIS

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Objectives: Human trypsinogens undergo post-translational sulfation on Tyr154, a process catalyzed by the Golgi resident enzyme tyrosylprotein sulfotransferase 2 (TPST2). Tyrosine-sulfuration was shown to stimulate autoactivation of human cationic trypsinogen. Because increased trypsinogen autoactivation has been implicated as a pathogenic mechanism in chronic pancreatitis, we hypothesized that genetic variants of TPST2 might alter the risk for the disease. Methods: We analyzed the 4 protein-coding exons and their intron/exon junctions of TPST2 by direct sequencing of 155 subjects with chronic pancreatitis and 169 controls. Results: We detected 10 synonymous variants within the coding region and several intronic variants that occurred with similar frequencies in patients and controls. Additionally, we identified a c.458G>A transition resulting in a predicted arginine to histidine change at codon 153 (p.R153H) in one patient and in one control. Activity and expression of the p.R153H variant was comparable to those of wild-type TPST2. Conclusions: Genetic variants of human TPST2 are rare and show no association with chronic pancreatitis.
The Lysyl Oxidase Propeptide Inhibits FGF-2 Induced Signaling and Proliferation of Osteobasts

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Lysyl oxidase is secreted as a 50 kDa pro-enzyme and cleaved into a 30 kDa mature enzyme and a 18 kDa pro-peptide (LOX-PP). The importance of the mature enzyme in cross-linking collagen and elastin molecules is well established. Here we generate and purify recombinant LOX-PP in order to investigate a possible role in regulating osteoblast proliferation and differentiation. FGF-2 is an important mitogen for osteoblasts. Data show that LOX-PP inhibits FGF-2 induced [3H]thymidine incorporation into MC3T3 osteoblasts in a dose dependent manner (0-8 µg/ml) by a maximum of 60%. The Ras-Raf-MEK-Erk pathway partly mediates the FGF-2 proliferative response. ELISA and Western blot analyses show that LOX-PP (0-8 µg/ml) inhibits FGF-2 induced Erk activation by a maximum of 65%. FRS2 (Fibroblast Growth Factor Substrate) an adaptor molecule downstream of the FGF receptors, mediates signals to the Ras-Raf-Erk pathway. LOX-PP significantly inhibits FGF-2 stimulated phosphorylation of FRS2 at 5 mins, by atleast 45%, indicating an effect at, or upstream of the receptors. Competitive ligand binding assays with [125I]FGF-2, demonstrates a concentration-dependent inhibition of FGF-2 binding to MC3T3 cell layers by LOX-PP (0-8 µg/ml), similar to the LOX-PP concentration-dependence observed in DNA synthesis assays. Additionally, our study suggests that LOX-PP may inhibit the interaction of FGF-2 to its high affinity receptors. These data indicate that LOX-PP may play a role in regulating the FGF-2 induced proliferation of osteoblasts, perhaps allowing cells to exit from the cell-cycle and progress to the next stage of development. Supported by NIH DE 14406
A Pancreatitis-Associated Chymotrypsinogen C Mutant Elicits Endoplasmic Reticulum Stress and Apoptosis

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Background and Aims: We recently identified chymotrypsinogen C (CTRC) as a novel susceptibility gene for chronic pancreatitis in humans (Nat Genet 2008; 40:78-82). Pancreatitis-associated CTRC mutants exhibit diminished secretion due to intracellular retention and degradation. We hypothesized that mutation-induced CTRC retention may result in endoplasmic reticulum (ER) stress and activate the unfolded protein response (UPR). Since prolonged activation of the UPR can lead to apoptotic cell death, a direct link may be established between CTRC mutants and acinar cell death in chronic pancreatitis. The aim of the present study was to test this hypothesis using the disease-associated p.A73T CTRC mutant.

Methods: Wild type and p.A73T mutant human CTRC were expressed in the AR42J rat acinar cell line and primary mouse acinar cells using adenovirus mediated transfection. Levels of UPR markers (BiP, spliced XBP1, and CHOP) were determined in cell lysates by western blot and RT-PCR analysis. As indicators of apoptotic cell death, caspase-3/7 activity and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) were also assessed.

Results: Cells expressing the p.A73T mutant exhibited elevated BiP mRNA and protein levels and increased splicing of XBP1, compared to cells expressing wild type CTRC. Cells transfected with the p.A73T mutant became detached over time and showed significantly increased caspase-3/7 activity and TUNEL staining, consistent with apoptotic cell death. Finally, marked induction of the transcription factor CHOP, a known mediator of ER stress induced apoptosis, was also observed in cells expressing the p.A73T mutant.

Conclusions: The results demonstrate that the p.A73T pancreatitis-associated CTRC mutant causes ER stress, activates the UPR and elicits apoptosis via induction of the pro-apoptotic transcription factor CHOP in pancreatic acinar cells. The findings suggest a novel disease mechanism in chronic pancreatitis which is unrelated to trypsin activity.

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Odontogenic Keratocysts: An Analysis of Surgical Treatment Options and their Long-Term Results

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Purpose: To investigate the incidence of recurrence of odontogenic keratocysts (OKC) after enucleation with peripheral ostectomy or resection. Patients and Methods: A retrospective chart review of patients diagnosed and treated for OKCs at Boston Medical Center were evaluated. Demographics, lesion location, age, gender distribution, treatment modalities, complications and recurrence rates were evaluated. Results: 21 patients were included in this study with an average follow up time of 21.4 weeks from the date of surgery. Patients included 13 males and 8 females ranging in age from 14 to 68 years with a mean age of 37.7 years. About 2/3 of the lesions were found in the mandible and 5 patients (23.8%) had nevoid basal cell carcinoma. 15 patients (71.4%) were treated with enucleation and peripheral ostectomy. In this group 4 patients had infection and 4 had temporary parasthesia of the inferior alveolar nerve (IAN) and 1 patient (6.7%) had recurrence. The second group consisted of 6 patients (28.6%) who had resection with 1 recurrence (16.7%). One of these patients (16.7%) had a mandibular fracture and 2 (33.3%) of them had temporary IAN parasthesia. Conclusions: Enucleation with peripheral ostectomy and/or mechanical, chemical, or thermal curettage is an effective treatment option for the management of odontogenic keratocysts, with low rates of recurrence and low surgical morbidity. The recurrence is suggested to be due to ineffective surgical treatment and seems to represent persistence of the disease.
Public Health Nurses as Oral Health Advocates: A Feasibility Study

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Objective: Caries is the most common infectious disease in young children, and is more often found in minority populations and those of low socioeconomic status. Although these disparities are multifactorial, one major factor in the disproportionate disease burden is access to care. In this study, we intend to integrate an oral health screening program into an existing community home visiting program, Healthy Baby Healthy Child (HBHC). This program specifically targets areas of Boston that are disproportionately affected by health disparities, through the use of public health nurses and home visits. In this study, we hope to show that these nurses are capable of delivering oral health education to program enrollees. This has been proven beneficial in other populations, such as pediatricians, and it is hoped that this can be expanded into the public health sector. Methods: HBHC Nurses were trained on oral health education, effective patient-centered counseling, and physical screenings. After certification, nurses performed an oral-health centered home visit once every 3 months for 1 year to provide oral health education and screenings. Within 48 hours of this visit, a registered dental hygienist performed a home visit to collect information regarding the nurse visit, and perform an additional oral screening, as to achieve comparable results. Results: Only a small amount of inconclusive data has been obtained. At this point, we have confirmed that nurses are participating in oral health counseling, and oral screenings are being performed. Current literature regarding the effectiveness of non-oral health care professionals delivering these services was reviewed and found to be effective. This is due to the early intervention and preventive information provided that would not otherwise be received until the first dental appointment, typically around three years of age. In addition, studies investigating the efficacy of training these same providers to deliver oral health services was reviewed, which showed a positive attitude toward and willingness to perform these services, providing they are properly trained. Conclusion: The inclusion of oral health screening in home visiting programs like HBHC could potentially affect a large number of the neediest population. This feasibility study is the first step in discovering if the implementation of these programs is efficacious and practical. More work must be done, but the potential to increase access to care and decrease early childhood caries in this population makes the discovery worth pursuing.
Osteomyelitis of the Mandible: Analysis of Treatment Outcomes

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Objective: To assess outcomes of treatment of osteomyelitis of mandible at an academic, tertiary care hospital.

Materials & Methods: The study involved a retrospective review of patients with osteomyelitis of the mandible treated by the Department of Oral and Maxillofacial Surgery at Boston University Medical Center from 2004-2008. Criteria for inclusion included: 1) Diagnosis of osteomyelitis confirmed by histopathological examination, 2) inpatient hospitalization with debridement and decortication of the mandible via an extraoral approach, 3) placement of an indwelling catheter for drainage and irrigation, 4) Intravenous (I.V.) antibiotic therapy, and 5) appropriate follow-up. All medical records were reviewed for the following parameters: etiology, medical co-morbidities, microbiology, duration and type of antibiotic therapy, duration of inpatient hospitalization, costs related to treatment, type of surgical treatment provided, and success and failure of treatment.

Results: A total of 26 patients were identified with a diagnosis of osteomyelitis of the mandible. Of these, 13 patients were included in the study. The mean age of the patient population was 45 years (R 20-77). Etiology was related to odontogenic source of infection (30%), delayed treatment of mandibular trauma (52%), and iatrogenic/postoperative complications following surgery (18%). Majority of patient had significant medical co-morbidities which could potentially affect treatment and results. Culture specimens and bone biopsies were obtained and empiric antibiotic treatment initiated with either Penicillin or clindamycin. Duration of IV antibiotic therapy ranged from 2 - 10 weeks. Streptococcus species were found to be the most common offending organisms. All patients were prescribed short-term oral antibiotic therapy after discontinuation of IV antibiotics. The range for in-patient hospitalization ranged from 3-13 days, with the mean length of stay being 3 days. Surgical treatment options included closed reduction only, rigid internal fixation with interdental fixation, and/or external pin fixation techniques for refractory cases. Treatment was found to be successful in 11 patients (84.6%). Failed cases required additional surgical intervention which ultimately resulted in successful outcomes.

Conclusions: A protocol consisting of aggressive debridement via an extraoral approach, initial empiric followed by culture-driven antibiotic therapy, provides for predictable successful outcomes in the management of mandibular osteomyelitis.
Regulation of Transforming Growth Factor-β1 (TGFβ1) Induced Expression of Connective Tissue Growth Factor (CCN2/CTGF) by Wnt in Human Gingival Fibroblasts

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Connective tissue growth factor belongs to the CCN family of growth factors, designated CCN2 or CTGF. Transforming growth factor β (TGF-β) stimulates CCN2/CTGF expression in human fibroblastic cells. CCN2/CTGF is responsible for mediating some of the effects of TGF-β and has been implicated in the onset and progression of fibrosis in most human tissues. Studies have shown that CTGF is up-regulated by Wnt3A in mesenchymal stem cells. Moreover, an expression profiling analysis of mesenchymal stem C3H10T1/2 cells stimulated by Wnt3A revealed that CTGF is among the most significantly up-regulated genes. Interestingly, our data shows that Wnt3A does not up-regulate CTGF in gingival fibroblasts. By contrast Wnt3A inhibits TGF-β1 stimulated CTGF expression in these cells. The binding of Wnt to its receptor leads to the stabilization of β-catenin. Stabilized β-catenin translocates to the nucleus to drive the expression of Wnt target genes. To investigate whether Wnt3A inhibition of TGF-β1 stimulated CTGF depends on β-catenin we used a constitutively active (CA) mutant form of β-catenin (S33Y) and transfected it into normal gingival fibroblasts. Mutation of this residue prevents the degradation of β-catenin. Empty vector served as a control. These cells were then induced with TGF-β1 or vehicle to see the effect on CTGF protein levels. We show that cells transfected with CA β-catenin (S33Y) fails to inhibit TGF-β stimulated CTGF, which suggests that GSK-3β inhibition by Wnt signaling inhibits CTGF expression independent of β-catenin. The same experiment was carried out with IMR-90 cells (human fetal lung fibroblasts) which were used as a positive control. Results support a direct role for canonical Wnt signaling in up-regulation of CTGF, as expected. We conclude that the unexpected down-regulation of TGF-β1 stimulated CTGF expression by Wnt3A occurs independent of β-catenin activation, and therefore can be considered to occur by a non-canonical pathway. The results of these experiments are in line with previous findings from our lab that supports the unique regulation of connective tissue factor (CCN2/CTGF) expression in gingival fibroblasts that may provide therapeutic opportunities to treat oral fibrotic diseases, and is likely a mechanism of the tissue-specificity of drug-induced gingival overgrowth.

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N-Glycosylation Status of E-Cadherin Regulates Cytoskeletal Dynamics in Epithelial Cells

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Cell-to-cell adhesion is predominantly regulated by E-cadherin, a calcium-dependent cell adhesion molecule that binds to other E-cadherin molecules on adjacent cells and is located at the adherens junction (AJ) of epithelial cells. E-cadherins connect to actin filaments by way of the catenins. This linkage is mediated through α-, β-, γ-catenins, and vinculin, with β-catenin and γ-catenin (plakoglobin) binding directly to the cytoplasmic tail of classical cadherins in a mutually exclusive manner. α-catenin then binds to these catenins where it is believed to play a role in the recruitment of actin to and the organization of actin filaments at the plasma membrane.

In contrast to the well-established connection between cadherins and the actin cytoskeleton, less is known about the potential relationship between cadherins and microtubules and intermediate filaments. We have been interested in examining how the presence of, and changes in N-glycan structures on E-cadherin extracellular domains affected its adhesive function and here we will examine its effects on its interaction with the cytoskeleton.

One of the classical features of the hypoglycosylated variant of E-cadherins that we constructed is its increased recruitment of protein phosphatase 2A, which is known to stabilize intercellular adhesion. The hypoglycosylated variant V13 exhibited 4-fold increase in the amount of PP2A compared to the wild-type E-cadherin. We investigated the localization of PP2A in the E-cadherin complex by co-immunoprecipitation of β-, and γ-catenins. Blotting of β- and γ-catenin immuno precipitates revealed increased association (2.8-fold) with β-catenin in the V13 variant with a corresponding decrease in the γ-catenin V13 variant. This suggests that in stable (mature) AJs, PP2A tend to localize in the β-catenin complex.

We then focused on the microtubule-associated protein tau and the microtubule-motor protein dynein because both are active in their dephosphorylated state and both are substrates for PP2A. Our findings indicate that E-cadherin complexes lacking complex N-glycans enhance MT dynamics. A key factor in this enhanced activity is the increased recruitment of PP2A, which upon localization to the β-catenin complex can exert several roles including dephosphorylating tau, thereby enhancing its stabilizing function. More p-tau was detected in the wild-type E-cadherin (up to 2 fold). This was true for both the serine and the threonine phosphorylation sites. Tau hyperphosphorylation to p-tau modulate its ability to perform its roles and is believed to be the trigger event in the tau aggregation cascade. Clusters of tau on the MT have been reported to interfere with the function of motor proteins and the transport of cytoplasmic material along the MT.

Another proposed role for PP2A at the β-catenin complex is dephosphorylating dynein and enhancing its sliding activity. Phosphorylation of cytoplasmic dynein at its serine residues in vivo has been documented and it was suggested that changes in dynein phosphorylation regulates its functional activity and distribution. In our study we noted that, consistent with the increased recruitment of PP2A to the V13 variant, a 1.5-2 fold increase in dynein also occurred.

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Antibiotic Regimens in the Management of Facial Fractures
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Statement of Problem: Given the concern for antibiotic overuse, resistance, and the current literature across specialties stressing perioperative antibiotics to prevent postoperative surgical site infections (SSI), an evidence-based antibiotic protocol is important to oral and maxillofacial surgeons. Orthopedic literature supports pre/perioperative antibiotics for open fractures, but varies on postoperative regimens. Multiple studies confirm the benefit of perioperative antibiotics for preventing SSI in general surgery; postoperative antibiotics are not routinely recommended. While orthopedic and general surgery literature highlight this controversy, direct extrapolation to facial trauma is impossible. Based on available literature, a strict protocol for perioperative antibiotic use in management of the full scope of fractures treated by oral and maxillofacial surgeons was instituted at an urban level I trauma center and effect on infection rate was assessed.

Patients and Methods: A prospective cohort study of facial trauma patients treated by multiple Board Certified Oral and Maxillofacial Surgeons at an urban level I trauma center from August 2007 through December 2008 after institution of a strict protocol for antibiotic administration. Antibiotic use was eliminated in closed midface trauma managed conservatively and limited to one preoperative dose for all fractures operated within 72 hours of injury. Patients were stratified according to gender, age, medical and social history, nature of trauma and associated injuries, time from injury to surgery, method of treatment, antibiotic regimen, and compliance with follow up. Examination for presence of infection was performed at weeks 1, 2, 4, and 8. Outcomes were compared to those of a retrospective review of electronic medical records pertaining to facial trauma patients managed by similar surgical methods at the same institution from July 2004 through June 2007, when various durations of postoperative antibiotics were routinely prescribed.

Inclusion Criteria:
1) Minimum 1 maxillofacial fracture (isolated dentoalveolar, nasal, and skull fractures excluded)
2) All management at one institution
3) Minimum 1 month, maximum 2 months follow up after trauma
4) Complete records available

Method of Data Analysis: 162 patients met inclusion criteria for prospective cohort study and were compared to retrospective review of records from 151 patients. Clinic and radiographic records were utilized to obtain observations which were collated in a Microsoft Excel spreadsheet.

Results: Of 162 patients, there were 69 midface fracture patients (11 surgically managed) and 163 mandible fractures (151 surgically managed) - 47 (31%) underwent open reduction with internal fixation; all others were managed with closed reduction and maxillomandibular fixation.
No patients with midface fractures developed an infection during the study period. Mandibular fracture infection rates were 4/104 (3.8%) among closed reductions (CR/MMF) and 5/47 (11%) among intraoral open reductions with internal fixation (ORIF). Infection rate after institution of strict protocol of perioperative antibiotic use did not result in significant change from baseline infection rate when postoperative antibiotics were routinely given: 151 patients, 71 midface fractures (17 managed surgically), 92 mandible fractures, infection rates: closed reduction 1/51 (2%), intraoral ORIF 3/33 (9.1%).

Postoperative infections at 9 sites were managed in 8 patients. All were soft tissue infections following surgical management of compound mandibular fractures, 4 after closed reduction, 5 following intraoral ORIF. Eight resolved with local wound care and antibiotics, 1 required hardware removal.

**Conclusion:** Retrospective review of patient data showed that no specific antibiotic protocol has a direct correlation to postoperative infection rates. All infections involved compound fractures through dentoalveolar segments. Prospective cohort study did not demonstrate a statistically significant change in infection rate after standardization of antibiotic protocol. Standardization of antibiotic usage has dramatically limited the number of patients who receive antibiotics. This potentially decreases health care costs and complications associated with antibiotic resistance.
Statherin: Secretion and Degradation in the Oral Fluid Environment

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Statherin belongs to the family of phosphorylated proteins secreted by the major salivary glands. Its capacity to inhibit both primary and secondary calcium phosphate precipitation has been implicated in the maintenance of tooth enamel homeostasis. The intact statherin protein is 43 amino acid residues in length. Analysis of the whole saliva peptidome has identified multiple statherin-derived peptides, suggesting it is susceptible to proteolytic degradation by oral fluid derived enzymes. Aim: To investigate the rate and mode of statherin degradation in whole saliva. Materials and Methods: Statherin was precipitated from human parotid secretion by zinc precipitation under alkaline conditions and purified by reversed-phase high performance liquid chromatography (RP-HPLC). To study statherin degradation, 400 µg of the purified protein was added to 1 ml of pooled, 1:10 diluted whole saliva supernatant and incubated at 37º C. After 0, 6, 10, 24, 36 and 48 hours of incubation, 100 µl aliquots were removed, boiled and subjected to RP-HPLC. Proteolytic degradation fragments were collected and structurally characterized by Liquid Chromatography Electrospray-Ionization Tandem Mass Spectrometry. Results: The added amount of statherin was reduced by 90% in 53 h. The main and primary cleavage sites in statherin were found to be Arg9, Arg10, Arg13 Phe14 and Tyr18. Apparently, the C-terminus, consisting mostly of hydrophobic amino acids, is more resistant to whole saliva protease activities than the N-terminal domain comprising mostly hydrophilic amino acid residues. While Tyr18 was among to first targeted, Tyr16, Tyr21, Tyr30, Tyr34, Tyr38 and Tyr41 were untouched demonstrating the specificity of the cleavage at Tyr18. Despite cleavage in the N-terminal domain, the functional properties related to tooth remineralization are likely retained in the short N-terminal doubly phosphorylated peptides generated. Peptide domains surviving oral fluid proteolysis are of high interest with respect to function and potential clinical exploitation.

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Ameloblastoma of the Jaws: Is Recurrence Likely?

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Statement of Problem: Ameloblastoma is a benign and locally aggressive odontogenic tumor with the literature universally reporting a high recurrence rate. The purpose of this study was to evaluate the results of primary ‘aggressive’ surgical management of ameloblastoma of the jaws.

Methods and Materials: Surgical treatment was prospectively provided with a uniform protocol (resection with 1.5 cm margins) based on principles of known tumor biology, results of histopathological examination, and clinical and radiographic presentations. The study included data from patient's charts with respect to patients' age, sex, tumor size, type, location, radiographic correlation, and type of surgical treatment. The records were also assessed for success of reconstruction, implant osseointegration, results of nerve repair, and rate and timing of recurrences.

Results: A total of 33 patients were included in the study. The age of the patient sample ranged from 14-86 years (mean 36.42 years) and the follow-up ranged from 5-16 years (mean 10.4 years). No recurrences were seen in any patient at longest follow-up. Twenty-two (67%) patients were female and eleven (33%) were male. Twenty-seven (82%) were multicystic (24 mandible and 3 maxilla) and six were unicystic (4 mandible and 2 maxilla). Twenty-seven patients had an impacted tooth associated with the lesion. All five maxillary patients underwent resection and reconstruction with an obturator. Twenty-seven mandibular defects were reconstructed with non-vascularized cancellous marrow grafts harvested from the iliac crest and one patient underwent microvascular free flap reconstruction. A total of 64 endosseous implants were placed in 14 of the patients reconstructed with iliac crest bone with a success rate of 94%. All bone grafts were successful and surgical complications were minor, not requiring re-operation. Patients undergoing inferior alveolar nerve preservation and/or repair had excellent recovery of sensation at longest follow-up.

Conclusion: The results of this study show that: 1) Aggressive surgical management consistent with principles following current known concepts of tumor biology, could be considered as “curative”, and, 2) patients can undergo predictable functional and esthetic reconstruction with a very low rate of complications. Based on this experience, we propose the concept of using the term “persistence” rather than “recurrence” to describe the high recurrence rate previously reported in the literature as in our opinion, the high rate of recurrence is perhaps related to inadequate surgical management rather than the tumor recurring by itself due to its intrinsic potential.
Increased Tissue Damage of Superoxide-deficient Diabetic Mice

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Objective: Diabetes predisposes to periodontal disease and excessive superoxide production is implicated in mediating periodontal tissue damage. Here we assess the role of superoxide in the development of oral complications of diabetes.

Methods: Double mutant mice (Akita/Ncf1) were generated that are diabetic and superoxide deficient. Double mutants were generated by crossing Akita mice, carrying a point mutation in the insulin gene and Ncf1 mice, carrying a mutant p47phox, a key element of the superoxide-generating NADPH oxidase found in leukocytes. Mouse survival was monitored by genotyping and oral pathology was assessed on histological sections.

Results: The first offspring of the Akita x Ncf1 crossing (F1) are diabetic, as the Akita mutation is dominant but fully capable of superoxide production as the Ncf1 mutation is recessive. F1 mice are born at the expected Mendelian ratio (50% of offspring) and their survival is 100% during the 3-month observation period. On the other hand, second generation Akita/Ncf1 mutants (F2) that are produced in F1 x Ncf1 crosses display impaired survival as only 6% of the offspring is double mutant instead of the expected 25% at weaning (P<0.05, proportional z-test). Kaplan-Meier analysis shows impaired survival of Akita/Ncf1 mouse compared to Akita or Ncf1 single mutants. Antibiotic supplementation (Sulfatrim 100mg/kg/day) restores the Mendelian inheritance ratio observed at weaning and prolongs survival, indicating that impaired leukocyte-mediated antibacterial immune response is responsible for the death of Akita/Ncf1 mice. 75% of Akita/Ncf1 double mutant mice display unilateral mandibular swelling. Histological examination of the lesions revealed neutrophil infiltration surrounded by a fibrous capsule, consistent with acute inflammatory lesion that extended into the mandibular bone.

Conclusions: Diabetes predisposes to bacterial infections as lethality of Akita/Ncf1 is higher than Ncf1 alone. Moreover, leukocyte superoxide is paramount in the defense against oral infections in diabetes.

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Inflammation-resolving Mediator Resolvin E1 Induces Acute Changes in Osteoclast Cytoskeleton

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Objective: Resolvin E1 (RvE1) directly inhibits osteoclast (OC) formation and bone resorption in vitro via the BLT-1 receptor. Here we hypothesized that RvE1 alters the structure and function of the mature OC. In this study we have assessed the immediate effects of RvE1 on the OC actin ring.

Methods: Mouse bone marrow cells were obtained and OC differentiation was induced by M-CSF (30ng/ml) and RANKL (30ng/ml) for 4-5 days. Mature OC cultures were treated with either RvE1 or U75302 (BLT-1 receptor antagonist) or both. Control treatment consisted of ethanol the vehicle for RvE1. OC were identified as tartrate-resistant acid phosphatase (TRAP)-positive multinuclear (nuclear count>3) cells and total OC-occupied area was analyzed for time-course and dose-response studies. For cytoskeletal staining cells grown on microscope chamber slides were stained with FITC-phalloidin (for F-actin) and DAPI as a nuclear stain. The intact actin ring (as percentage of total number of intact and non-intact actin ring) and actin ring area (presented as percentage of total cell area) were analyzed.

Results: OC occupied area decreased 15 minutes after RvE1 (10nM) treatment, then returned to its original value by 60 minutes. (Repeated ANOVA, n=6, P<0.05) The BLT-1 receptor antagonist U75302 (1nM) prevented this effect. (One-way ANOVA, n=5, p<0.05) The actin ring area also significantly decreased 15min after RvE1 treatment and the proportion of OC showing an intact actin ring decreased by 18.4±1.4%, both of which were reversed by U75302. (One-way ANOVA, n=3, p<0.05).

Conclusion: RvE1 disrupts cytoskeleton arrangement acutely through binding to BLT-1 receptor, which might result in decreased bone resorption by mature OC. The inflammation-resolving mediator RvE1 may be particularly effective against inflammatory bone diseases such as rheumatoid arthritis and periodontitis as it can resolve both inflammation and bone resorption.

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Mass Spectrometric Identification of Whole Saliva Proteases Involved in Salivary Protein Degradation

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Histatins, statherins and proline-rich proteins are human salivary proteins that are rapidly degraded once they undergo the transition from the sterile salivary glandular environment to the non-sterile oral cavity. **Aim:** To identify the kinds of proteases involved in the degradation of salivary proteins in the oral environment. **Materials and methods:** To identify the whole saliva proteases with activity towards histatins, dialyzed and lyophilized proteins present in whole saliva supernatant were separated by anion-exchange chromatography. Collected fractions were desalted and screened for their proteolytic activity towards histatins. The active fractions were further analyzed by zymography using incorporated histatins as enzyme substrates. To characterize the proteases in the respective active protein bands, individual bands were excised, trypsinized in-gel and subjected to liquid chromatography electrospray ionization tandem mass spectrometry. The data obtained were searched against human, bacterial and protease databases to maximize protease identification. **Results:** Out of total 65 individual fractions collected, 28 fractions contained protease activity towards histatins. The thirteen most active fractions were further analyzed by histatin zymography. On the histatin zymogram gel, most fractions contained multiple protein bands with protease activity. Protein bands displaying proteolytic activity were particularly prominent in the 50-75 kD region. In total, 13 proteases from mammalian origin were identified. Found repeatedly were lactotransferrin, kallikrein-1, cathepsin D, and puromycin-sensitive aminopeptidase. In addition to these proteases, 10 mammalian protease inhibitors were identified which were likely closely associated with the proteases in the same fractions. The inhibitors found most frequently were alpha-2-macroglobulin-like protein 1, alpha-1-antitrypsin and leukocyte elastase inhibitor. **Conclusion:** The apparent close association of proteases and inhibitors in whole saliva suggests that salivary protein degradation in the oral cavity is a tightly regulated process.

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GSDM Science Day 2009 Award Winners

Hanny Hamoui: First prize for predoctoral student research poster presentation “Role of Lysyl Oxidase Propeptide in inhibiting EGF and FGF-2 functions in Oral Cancer cells”

Shravan Kumar Renapurkar: First Place for predoctoral student research oral presentation, “Osteomyelitis of the Mandible: Analysis of Treatment Outcomes”

Jehan AlHumaid: First Place for postdoctoral student research poster presentation, “Meta-analysis and Review on the Effectiveness of Different Xylitol Products in Caries Prevention”

Georges Traboulsi: First Place for postdoctoral student research oral presentation, “Statherin: Secretion and Degradation in the Oral Fluid Environment”

Dr. Richárd Szmola: First Place for your postdoctoral fellow research poster presentation, “A Pancreatitis-Associated Chymotrypsinogen C Mutant Elicits Endoplasmic Reticulum Stress and Apoptosis”

Dr. Xiuli Sun: First Place for your postdoctoral fellow research oral presentation, “Mass Spectrometric Identification of Whole Saliva Proteases Involved in Salivary Protein Degradation”