Accessory Mental Foramen, an Anatomical Variant of Mental Foramen, Detection Using Cone Beam Computed Tomography

Shilpa Admala, Lakshmi Garladinne and Sami Chogle
Department of Endodontics

Introduction: Numerous anatomical variations are reported in the literature in the mandibular region along the course of the mandibular canal and at the mental foramen region. Literature review showed presence of accessory mental foramen (AMF) in some populations, and its importance and prevalence in our population due to its clinical implications. On two dimensional images it may be confused for periapical or periodontal pathology, or being unaware of its presence may pose complications during periapical surgeries, implant placements or orthognathic surgeries in the surgical access line of the mandible. Literature shows incidence of AMF to be around 1.5 to 10 % in various populations, often missed in conventional radiography. Imaging by cone beam computer tomography (CBCT) is far more accurate and superior than conventional radiography. **Aims & objectives:** Retrospective analysis of CBCT volumes done to 1. Check for presence of accessory mental foramen 2. Location of AMF in relation to mental foramen 3. Unilateral/ bilateral occurrence of AMF 4. Distance of AMF from 1st and 2nd premolar root tips **Materials & methods:** Cone beam CT volumes acquired using i-CAT™ between January.01.2011 and Dec.31.2016 were selected to be viewed according to the selection criteria. All HIPAA identifiers were removed and the DICOM volumes were evaluated using Anatomage®, Invivo 6 a third party software. IRB approval was obtained prior to the start of the study. Initially, the course of mandibular canal was outlined by tracing the canal. Mental foramen identified and its distance from base of mandible were measured. The axial sections were measured at an interval of 0.5mm, and the parameters for cross section were with a width of 40mm, interval of 1.5mm and thickness of 2.0mm The Axial slice indicator was placed on the top of the foramen and was used as a reference point for all measurements to prevent inter examiner variability. **Conclusion:** Presence of accessory mental foramen were looked for and if present, the distance between mental foramen and AMF were measured and its position in relation to mental foramen are described as superior/ inferior/ buccal/ lingual to mental foramen.

Lower Rates of OMS Resident Personal Achievements May be Related to Increased Levels of Anxiety during Training

Hamza Al Atassi, Michael C. Shapiro, Sowmya R. Rao, Jason Dean and Andrew R. Salama

Department of Oral & Maxillofacial Surgery

Purpose: Anxiety is remarkably prevalent among medical residents in training and is often associated with fatigue, psychological dysfunction, and burnout. Personal achievement is a characteristic of burnout, which, if unaddressed, may lead to medical error, and decrease in the overall quality of patient care. The purpose of this study is to determine the prevalence of anxiety in association with the level of perceived personal achievement in Oral and Maxillofacial Surgery (OMS) residents. **Materials and Methods:** An anonymous 20-question cross-sectional online survey was developed using the anxiety component of the Hospital Anxiety and Depression Scale (HADS-A) and the personal achievement component of the Maslach Burnout Index (MBI). The survey was sent to all the OMS residents enrolled in programs affiliated with the American Association of Oral and Maxillofacial Surgery in 2017. Univariate and bivariate analyses were conducted to obtain summary measures of the predictor (anxiety) and the outcome (personal achievement) stratified by age, gender, marital status, program type (4 year/ 6 year), and year of residency. Multinomial logistic regression models were obtained to evaluate the association of anxiety to personal achievement. A 2-sided P value less than .05 was considered statistically significant. **Results:** Two hundred thirty eight responses were received (20% response rate); 80% of respondents were men (n= 189), 60% were above 30 years of age (n=138), 46% were unmarried (n=109), and 56% were enrolled in a 4-year program (n= 134). 58% of respondents had moderate to severe levels of anxiety. 49% had low to moderate levels of personal achievement. Women were more likely to have severe anxiety compared to men (60 vs. 37%; P < 0.01). Residents with severe anxiety were 91% more likely to report low levels of personal achievement compared to residents with low anxiety (OR= 0.09; 95% CI= 0.03-0.22; P < 0.0001). Significant inverse relationship between personal achievement and anxiety has been detected where the odds of achieving high personal achievement scores decreases significantly as the severity of anxiety increases. **Conclusion:** More than half of OMS residents in the US report moderate to severe anxiety. Higher levels of anxiety are associated with lower personal achievement levels. It is important to understand the negative impact that anxiety has on residents, including the unintended consequences.

The Relationship Between Early Childhood Caries and Food Insecurity, Using NHANES 2013-2014

Christine Chiao and Elizabeth Kaye
Department of Health Policy and Health Services Research

Objective: Access to affordable and nutritious foods is a significant public health concern, and research has found food insecurity to be related to a poorer health outcomes in both adults and children. Early Childhood Caries (ECC) is a highly prevalent disease in children under six years of age, and disproportionately affects minorities and low SES groups- groups which exhibit increased utilization of social services and programs such as food assistance programs. This study seeks to examine the relationship between Early Childhood Caries and food insecurity using data from the 2013-2014 National Health and Nutrition Examination Survey (NHANES). **Methods:** Cross sectional data analysis utilized demographic, oral health exam and food security questionnaire data from NHANES 2013-2014. The following items from the NHANES 2013-2014 survey were used as measures of food security: "Worried run out of food", "Food didn't last", "Household food security category", "Emergency food received", "Ever received food security benefits", and "WIC benefit received in the last 12 months". **Results:** In the sampled population, 19.7% of participants had ECC as defined by the AAPD. After adjusting for age, gender, ethnicity, and family income, "Emergency food received" and "Ever received food security benefits" were found to be significantly associated with ECC. Participants who indicated have had "Emergency food received" had 2.9 times the odds ECC than their counterparts who had not received emergency food (95% CI: 1.3-6.6,P=0.010). Additionally, those who indicated having "Ever received food security benefits" had 1.9 times the odds of those who indicated they had never received benefits (95% CI: 1.3-2.8, p=0.0009). **Conclusion:** Taken together with existing research, the results of this study indicate that future research and intervention should focus on targeting populations that utilize food assistance programs to also address oral health concerns.

Understanding Pathologies Associated with Deficient LATS1/2 Kinase in Salivary Glands

Ariana Dela Cruz, Weihao Wang, Andrew Tilston-Lunel, Kathrine Skarstein, Maria Kukuruzinska and Xaralabos Varelas
Departments of Biochemistry and Molecular & Cell Biology

Objectives: The salivary gland epithelium lines a complex network of branched ductal tubes that end in acini, which together produce, transport and secrete saliva. The development and homeostasis of the salivary epithelium requires the precise coordination of a multitude of signals, many of which are poorly understood. Our prior studies have indicated that the nuclear activity of the transcriptional regulator Yap, a key effector of the Hippo pathway, is critical for the patterning of Krt5/Krt14-positive progenitors in the developing submandibular gland (SMG). Further, exclusion of Yap from the nucleus, which is mediated by the Lats1 and Lats2 (Lats1/2) kinases, is essential for the developmental maturation of the ductal epithelium. We therefore hypothesized that the Lats1/2 kinases play a critical role in the homeostasis of the adult salivary gland. To test this, we conditionally deleted Lats1/2 in mature luminal epithelial cells in mouse SMG ducts. **Methods:** Conditional deletion of the Lats1/2 genes was achieved by crossing Krt8-CreERT2 mice with Lats1/2 loxP/loxP mice. Lats1/2 knockout was achieved in 12-week-old female mice following tamoxifen treatment. Paraffin-embedded sections of Lats1/2 deleted (Lats1/2-cnull) SMGs were compared to age- and sex-matched tamoxifen-treated controls (CON) using immunohistochemistry analyses. **Results:** Lats1/2-deleted SMGs exhibited prominent morphological alterations with an apparent loss of acinar morphology, disorganized ductal structures, and stromal disorganization. Increases in proliferative Ki67+ ductal epithelial cells were also observed, along with an interstitial accumulation of CD45+ lymphocytes. **Conclusions:** SMGs of Lats1/2-cnull mice exhibited abnormal morphology resembling diseased salivary gland epithelium, including features similar to those found in Sjogren's Syndrome. Further analysis of the Lats1/2-deleted mouse model will guide a better understanding of salivary gland pathologies, which will hopefully offer new opportunities for development of early diagnosis or targeted treatment interventions.

Volumetric Changes in the Nasal Cavity after Minimally Invasive Sinus Technique

Mohit Jethani, Peter Catalano, Michael Schlewet and Melih Motro

Department of Orthodontics and Orthofacial Orthopedics

Background: Before the advent of endoscope assisted/guided surgery, correcting abnormal sinus anatomy historically involved invasive procedures like the Lothrop, Caldwell-Luc, etc. Over the last decade, Minimally Invasive Sinus Technique (MIST) has been suggested as a targeted and non-invasive anatomically based reproducible approach towards restoring/removing sinus obstructions. **Aim:** To evaluate the efficacy of minimally invasive sinus technique in improving airway volumes and thus improving the airflow for young children and adolescents suffering from compromised airway. **Method:** In this prospective cohort study, 17 patients with compromised airway (9 males and 8 females) underwent MIST surgery to open and connect the nasal cavity with the agger nasi, anterior and middle ethmoidal sinuses. CBCT scans were taken pre and post-surgery. Using Mimics software for 3D reconstruction and integrated cephalometric analysis, the scans were analyzed to measure the difference in airway volumes. **Results**: Significant increase in the nasal cavity volumes was found after surgery in 15 of the 17 patients. 2 patients showed evidence of synechiae (inflammation of the sinus and nasal soft tissues) in the post surgical scan, thereby showing a contrasting decrease at post surgical evaluation. The t-test values for the pre and post surgical comparison groups were as follows: Significance (P) – 0.0008 Pre Surgical group: Mean – 9517.47, Standard Deviation (S.D.) – 3108.74 Post Surgical group: Mean – 12648.76, Standard Deviation (S.D.) – 4297.55. **Conclusion:** Minimally Invasive Sinus Technique as used for this surgery has immense potential in its application as a novel approach for improving the airway flow and can be used in conjunction with orthodontics when necessary to improve overall quality of life.

Ductal Tree Patterning during Branching Morphogenesis of the Submandibular Gland

Edith Lin, Weihao Wang, Janice Walker, Meghan Bouchie, Alison Romisher, A.Sue Menko and Maria Kukuruzinska

Department of Molecular & Cell Biology

Background: The development of ductal structures during branching morphogenesis relies on signals that specify ductal progenitors to set up a pattern for the ductal network. **Methods:** Here, we identify cellular asymmetries defined by the F-actin cytoskeleton and the cell adhesion protein ZO-1 as the earliest determinants of duct specification in the embryonic submandibular gland (SMG). Apical polarity protein aPKCz is then recruited to the sites of asymmetry in a ZO-1-dependent manner and collaborates with ROCK signaling to set up apical-basal polarity of ductal progenitors and further define the path of duct specification. **Results:** Moreover, the motor protein myosin IIB, a mediator of mechanical force transmission along actin filaments, becomes localized to vertices linking the apical domains of multiple ductal epithelial cells during the formation of ductal lumens and drives duct maturation. **Conclusion:** These studies identify cytoskeletal, junctional and polarity proteins as the early determinants of duct specification and the patterning of a ductal tree during branching morphogenesis of the SMG

Cell Free Circulating Tumor DNA Mutation Profiling of a Colorectal Carcinoma Patient by Next Generation Sequencing for Guidance in Identifying Drug Options, Novel Mutations and Personalized Treatment-Case Study

Gokhan Ozdener and Melih Motro

Department of Orthodontics and Orthofacial Orthopedics

Background: Traditional cancer treatment follows standard drug regimens for all patients with similar types of cancer and metastases. However, recent development of the DNA sequencing technologies and databases reveals that there are more complex mutations in similar cancer types. Therefore, patient specific, personalized cancer treatments have gained importance. However, identifying mutations is not easy in clinical settings because a needle biopsy is often not optimal and applicable to all cancer patients. Collection of tumor samples by needle biopsy is also challenging; tumor cells mixed with normal cells can result in misinterpretation of the frequency of mutations. **Method:** To obtain high-quality samples of tumor DNA and to generate a less invasive sampling, I implemented a blood-based technique to collect cell free circulating tumor DNA (cfct-DNA) for further mutation analysis and personalized treatment options. For example, I performed the Ion AmpliSeq-Next Generation Sequencing based diagnostic test (IA-NGS) with a customized gene panel of 409 tumor suppressor/oncogene related genes on ctcf-DNA samples of a cancer patient. The overall sensitivity of the test is high with respect to SNP, MNP, DEL, INS mutations. **Results:** In particular, high frequency (100-20 %) 3049 mutations in 369 genes were identified for a colorectal carcinoma patient. 880 mutations in 278 genes are cancer driving mutations and 850 mutations on 260 genes are novel mutations that were reported for the first time. Further, driver DNA mutations were utilized to predict affected-downstream protein sequences and their dysfunctions by matching DNA mutations with their protein localizations. To identify appropriate treatment options, more than 500,000 potential compounds were scanned from databases to overlap with matching known cancer pathways and treatments that include hormone therapies, signal transduction inhibitors, gene expression modulators, apoptosis inducers, angiogenesis inhibitors, immune enhancers and heat therapy options. Primary and secondary treatment options were identified for targeted therapy for the colorectal cancer patient. For initial study, the treatment efficacy was monitored by PET-Scan after 12 weeks of recommended therapy. **Conclusion:** Overall, the high sensitivity and less-invasive NGS based cfct-DNA mutation profiling demonstrated the feasibility of detection of actionable alterations and potential new drug targets in a patient's mutation profile. This approach is also applicable to maximize efficiency of targeted therapy options for improving quality of care for cancer patients.

Public Housing Community Member Perspectives on Biomedical and Behavioral Research

Erika Ramos, Michelle Henshaw

Department of Health Policy and Health Services Research

Objectives: Understanding participant perspectives about research, particularly those of racial minority populations, is essential to the success of community-based studies conducted in public housing or other low-income communities. There is little to no research pertaining to participant understanding of biomedical versus behavioral research in these vulnerable and historically exploited communities. Thus, the goals of this study are to gain insight into (1) participant understanding of biomedical research versus behavioral research, (2) facilitators and barriers to participation in biomedical versus behavioral research, and (3) whether the kind of research provider affects the decision to participate in a study. Knowledge gained from this study may affect the design and application of community-based interventions that promote health among economically disadvantaged communities. The hypotheses of this study are that (1) public housing community members (PHCM) have a better understanding of biomedical research when compared to behavioral research, (2) the facilitators and barriers to participation will be the same for biomedical and behavioral research, and (3) PHCM are more likely to participate in studies if the research provider is familiar to the subject. **Methods:** PHCM perspectives about research were assessed via a 90-question quantitative anonymous survey administered to public housing residents 18 years or older. 266 surveys were collected at Boston Public Housing events throughout the city. **Results**: Results show that PHCM believe they have a better understanding of behavioral research compared to biomedical research. Subjects who had participated in a behavioral clinical trial (TSHS) were better able to categorize the study as behavioral or biomedical when compared to subjects who were not enrolled in TSHS. Nevertheless, 52% of the TSHS-enrolled subjects were not able to correctly categorize TSHS clinical trial as behavioral. Results also show no variance between the factors that facilitate and interfere with participation in biomedical research when compared to behavioral research studies. In addition, the first and second most likely research provider to convince PHCM to participate in both biomedical and behavioral research studies respectively is their own doctor and their own dentist. While the first and second least likely research provider to convince PHCM to participate in both biomedical and behavioral research studies respectively is a drug company and an insurance company. **Conclusions:** It is unclear whether PHCM have a better understanding of biomedical research than behavioral research. Additionally, it has been shown that the factors that affect PHCM participation are the same regardless of the type of research study—biomedical or behavioral. Finally, PHCM are more likely to participate in community-based research studies if the study is managed by someone familiar, such as their doctor or dentist.

Early Detection of Oral Cancer: A Dental Responsibility

Hirwa Shah and Larry Dunham

Department of General Dentistry

Introduction: Oral cancer being the sixth most common cancer worldwide is making it a global concern. Delay in diagnosis of Oral Cancer has claimed thousands of lives and continues being a rising threat. Indian subcontinent accounts for one-third of the world's oral cancer burden with the highest incidence of smokeless tobacco use. United States inspite of being in better state of health shows a rising mortality attributed to oral cancer. Therefore, the need for the prevention and implementation of effective treatment measures are of utmost importance. **Objectives:** Our goal is directed towards developing a better teaching model that targets an interdisciplinary approach as developed in other branches to give a greater exposure before stepping in the real world outside. It entails on refining our diagnostic skills and identifying better ones which ensure a thorough evaluation and identification of early signs and symptoms of oral cancer. **Methods:** This is a literature review based on descriptive and statistical analysis. A broad collection of 18,285 articles was narrowed down to 108 articles through comprehensive screening and using accurate MeSH terms. A detailed evaluation of 22 relevant articles that were pertinent to our research topic were narrowed down. These articles focused on knowledge of dental students and dentist on early signs and symptoms of oral cancer, public awareness about their oral health as well as the referral and treatment protocols taken. **Results:** Studies have shown that most general dentists are quite adequate in identifying only smoking as well as alcohol as a risk factor for oral cancer. Most dentists find themselves comfortable in identifying only ulcers followed by leukoplakia as a form of oral cancer. Moreover ulcerations were the most common and recognizable form of disease. **Conclusion**: In India, there are additional unique risk factors that are strongly associated with oral cancer. They include: betel quid and areca nut chewing. Betel quid and areca nut chewing are of unique importance because they cause oral submucous fibrosis (OSMF). Other limitations to identifying OC signs were lack of knowledge and awareness amongst patients who presented quite late for a dental checkup.

Assess the Correlation among International Dentists with a Specialty Dental Degree from Outside North America with Performance in a Course Related to that Specialty in the Advanced Standing Program

Anchita Venkatesh, Matthew Mara and Janet Peters

Department of General Dentistry

Introduction: In the academic year 2015-16, sixty percent of US dental schools admitted a total of 533 internationally trained dentists into a two year DMD or DDS advanced training program. Boston University Henry M. Goldman School of Dental Medicine (BUGSDM) two-year training program for foreign trained dentists is called the Advanced Standing (AS) program which admitted 83 students in the 2015-2016 academic year. It is common for first year AS students to enroll in the program after completing a specialty degree outside of North America or additional training, such as a master's degree in the US. Regardless of their previous experience or specialty training outside of North America, few exemptions are made for courses relating to previous specialty training outside of the US. The variability in teaching protocols and grading systems from around the world make it extremely hard for dental schools to allow exemption of courses for foreign trained specialists. Currently, there is no information on whether AS students with a specialty degree perform better in a course pertaining to that specialty compared to their peers. **Aims:** The overall objective of our study was to understand if there is a correlation of performance by an international dentist with a specialty degree from outside North America to a final grade in a course pertaining to that specialty in the AS program. Our secondary objective was to understand if the performance in a course pertaining to that specialty is specific to a certain country or region. **Methods:** A Fourteen question survey with both open and close ended questions with branching logic was developed and administered to Advanced Standing class of 2018 at BUGSDM electronically via Qualtrics. Data was entered and imported into Microsoft Excel from Qualtrics. Descriptive statistics were calculated using Microsoft Excel. **Results**: Students with a specialty degree,33.3 % found the pre-clinical course neither hard nor easy and 20% found it to be slightly difficult and 20 %found it moderately easy. 60 % of the students with specialty training spent 0-4 hours practicing for the pre-clinical course relating to their specialty which was similar to students with 2-5 yrs experience (58.3%) and students with >5 years' experience (50%) while students with <2 years' experience, 37.03 % spent 0-4 hours and 29.6% spent 5-8 hours. **Conclusions**: Students with a specialty degree self-reported a grade of A/A- in the course relating to that specialty. Regardless of previous education, specialty or work experience, students in the AS program suggest that pre-clinical courses require practice and effort to excel. Data collected from this survey suggests that the Advanced Standing curriculum at BUGSDM should be standard for all students regardless of prior experience outside of the US. Data collected from this survey suggests there is no co-relation to performance in a course pertaining to the specialty is specific to a certain country or region.

Transforming E. coli and Bacillus with Plasmid Encoding Gluten-degrading Enzymes

Jessica Wang, Eva Helmerhorst, Guoxian Wei
Department of Molecular and Cell Biology

Background: Celiac disease, one of the most common diseases worldwide, is characterized by a life-long gluten sensitivity accompanied by an auto-immune reaction. Gluten-degrading enzyme Rmep have been isolated from Rothia mucilaginosa, a natural colonizer of the oral cavity. Like nattokinase, Rmep degrades an immunogenic α-gliadin peptide and has important implications as enzyme therapy for celiac disease patients. **Objective**: To initiate transformation of E. coli and B. subtilis with plasmids encoding gluten-degrading enzymes: Rmep isolated from R. mucilaginosa, and nattokinase from B. subtilis. **Materials and Methods:** Plasmids pHT43-Rm encoding Rmep and pHT01-Bs encoding nattokinase were used for the transformations, with pUC19 as a control. E. coli DE3 and B. subtilis WB800N were used as competent cells. E. coli DE3 cells were first transformed with pUC19 and pHT01-Bs by heat shock. B. subtilis WB800N were transformed with pHT43-Rm, pHT01-Bs and pUC19 by heat shock and by following conventional electroporation protocol. IPTG induction and AAPF cleavage test were conducted to examine endogenous and secreted nattokinase activity in transformants. **Results**: Following heat shock using pUC19 and pHT01-Bs, transformation of E. coli was achieved and the transformed cells grew abundantly on LB-Amp plates, although expression was not induced by IPTG and AAPF cleavage activity was not detected. This may be attributed to E. coli's lack of cellular machinery for protein processing. B. subtilis WB800N subject to both heat shock and electroporation grew on LB plates but did not grow on LB-Amp plates, demonstrating unsuccessful transformation using conventional methods. **Conclusion**: E. coli DE3 was successfully transformed with pUC19 and pHT01-Bs by heat shock. Yet, conventional electroporation methods for B. subtilis WB800N require further optimization. Potential modifications include adding trehalose in the electroporation media while applying a stronger pulse.

Osseointegration in Piezoimplants Versus Cylindrical Implant

Trevor Fujinaka, Lorena Corzo, Wanpeng Xu, Taisuke Ohira, and Serge Dibart

Department of Periodontology

Introduction: Dental implants can successfully restore edentulous areas but current surgical techniques are inadequate in areas lacking sufficient bone volume. Piezoelectric surgery has shown encouraging effects on hard and soft tissue healing and when splitting narrow alveolar ridges to place cylindrical implants. A new wedge-shaped titanium "Piezo-implant" requires piezoelectric osteotomy. This study measured osseointegration and alveolar ridge changes comparing "Piezo-implants" and conventional threaded cylindrical implants. **Methods:** After 3 months post-extraction, 18 conventional cylindrical implants (Nobel Active, Nobel Biocare®) and 18 wedge-shaped "Piezo-implants" were placed using a split-mouth design in 3 minipigs (n = 36). The cylindrical implant sites were prepared with rotary instrumentation while the "Piezo-implant" sites were prepared using piezoelectric surgical inserts and the corresponding implants were placed. Quantitative microcomputed tomography analysis (Scanco Medical®) assessed peri-implant alveolar bone volume and osseointegration. Histologic and histomorphometric measurements evaluated peri-implant healing and bone-to-implant contact. Submicron x-ray imaging determined bone structure along the implant surfaces. **Results:** A multiple linear regression model with R-square of 0.69 demonstrated a significantly higher BV/TV in the mandible (t ratio = 6.21, p < 0.01), significant increase of BV/TV over time (t ratio = 5.21, p < 0.01), and significantly higher average BV/TV in "Piezo-implants" than the cylindrical implants (t ratio = -2.38, p < 0.01). Histological sections demonstrated that "Piezo-implants" had a greater % BIC at 4 and 12 weeks, a greater percent new bone growth within the ROI at all three time points, and a slight increase in bone maturation at 12 weeks when compared to the cylindrical implants. High-resolution x-ray microscopy exhibited a tighter interface between implants and alveolar bone formation in the "Piezo-implants". **Conclusion:** Bone formation increased significantly over longer healing periods, was greater in the mandible compared to the maxilla, and higher in the "Piezo-implant" group. "Piezo-implants" had a more desirable healing and may be suited for narrow ridge spaces without additional ridge augmentation. Thus, the novel wedge-shaped "Piezo-implant" system should be considered a viable option in cases with narrow alveolar bone. Further research is needed to establish clinical validity.

Lay People's Preference for Smile Esthetics Following Orthodontic Treatment

Johnny Gonzales, Ahmed Alsulaiman, Melih Motro and David Briss

Department of Orthodontics and Orthofacial Orthopedics

Objectives: To evaluate how certain visual extra- and intraoral smiling variables influence laypeople's preference for a step or no step between the incisal edges of the maxillary central and lateral incisors in orthodontically treated patients, and how subject demographics may modify such an association. Results may yield clinical implications that could assist in orthodontic treatment planning. **Methods:** Sixteen cropped, smiling photographs with differing esthetic variable combinations for skin pigmentation, lip thickness, amount of gingival exposure, and tooth length were duplicated and placed in a presentation, with one duplicated pairing per slide. One of the duplicates was modified utilizing a photograph editing software (Photoshop CS6) to create a 1mm step between central and lateral incisors, and the other had no step. Photos were shown to 200 laypeople, and they were asked to choose which smile they prefer for each pairing. Chi-square test and logistic regression analysis were performed using SAS. **Results:** More college-educated people (63%) were surveyed than people who did not attend college. For ten out of sixteen esthetic categories, people preferred no step as compared to step. Age and smile esthetic preference are not independent for categories eight, nine, eleven and sixteen. Gender and smile esthetic preference are not independent for category five. Race and smile esthetic preference are not independent for category seven. Education and smile esthetic preference are not independent for categories four and sixteen. **Conclusions:** The results suggest that more people prefer no step between maxillary central and lateral incisors as compared to a step, and that patient background and demographics have little-to-no influence on this preference.

Defining Hard and Soft Tissue Asymetry Using 3-Dimensional CBCT Analysis

Marisa Reason, Melih Motro and David Briss

Department of Orthodontics and Orthofacial Orthopedics

Objectives: The aim of the study is 1) establish appropriate, reproducible soft tissue landmarks and a bony counterpart in CBCT images 2) evaluate the correlation between the skeletal and soft-tissue landmarks 3) use the measurement correlations to objectively define asymmetry. By determining these measurement correlations between hard and soft tissue landmarks, we can discover where asymmetries originate. This will provide a set of data that can be used as reference for future studies. **Materials and Methods:** Pre-treatment Cone Beam Computed Tomography (CBCT) films of 160 esthetic human subjects previously used by another investigator were screened for use in our current research. These DICOM files were imported into InVivoDental5.3 software (AnatomageTM; San Jose, Calif.) for screening and 60 CBCT scans were selected for analysis. A soft tissue mask was created on all scanned images, establishing hard and soft tissue reconstructions. 17 anatomic bony and soft tissue landmarks were identified on each scan in reference to coronal, sagittal and horizontal planes. The reference planes were defined as Nasion, the most posteriorly identifiable point on the fronto-nasal suture line (x), Midpoint of Clinoid Processes (y), and the most inferior point of the inter-maxillary suture, as located between the central incisors (z). Each landmark was given an x-, y- and z- coordinate representing its three dimensional position and bilateral linear measurements to the reference planes were recorded. Ratios were then established between the soft tissue point and bony tissue counterpart in all three planes. InVivoDental5.3 software was used to calculate linear distances between each landmark and the reference planes. Descriptive statistics of our sample and correlation analyses were performed. The mean average and standard deviation of each hard and soft tissue landmark was calculated. Also, the correlations between the respective hard and soft tissue landmarks were calculated. **Results**: A comparison between the hard and soft tissue landmark means was accomplished with the use of paired t-tests with a 5% significance level, or p value of 0.05. It was founded that hard and soft tissue landmarks do have positive correlations, but four landmarks existed which showed statistical difference in location relative to the reference planes ($p < .05$). **Conclusion:** The trend of the data indicates a significant relationship between the soft and hard tissue points in three-dimensional images. The skeletal and corresponding soft tissue landmarks, which were statistically significant, could be due to tracing error, given the indiscrete location of some of the landmarks. However, these results could lead further researchers to use this information to discover the reasons behind the location differences, and start to define where asymmetries initiate in the skeleton and overlying facial soft tissue.

Retention Load of Various Ceramics and Cement Combinations to Titanium

Marwa Shembesh, Richard Pober, Yuwei Fan and Russell Giordano

Department of Restorative Sciences & Biomaterials

Objectives: The purpose of this in vitro study was to assess the retention load of composite resin cements between titanium and various ceramic materials. **Methods:** A titanium pin (Grade V Ti alloy, 6.4 mm high, 8.2° taper) was scanned using the Sirona Inlab scanner software version SW4.2.5. A crown (tooth #19) was designed to fit the pin and machined using four crown materials (Ivoclar e.max, Vita Enamic, Vitablocs Mark II, and Sirona inCoris zirconia). Pins were held in a CNC machine to reproducibly sandblast each pin at the same speed and pressure (100 psi, Prepstar) using 125 micron alumina and then cleaned in an ultrasonic bath. The milled crowns were cemented to the pins using three different cements (3M/ESPE RelyX Ultimate, Ivoclar Multilink Hybrid, and Kuraray Panavia VF). A load of 1190 grams was placed on the crowns to achieve uniform cementation. Two techniques were used for each cement, one with no treatment (no primer/no etch) and the other with treatment as recommended by the manufacturer (primer/etch). DOE was used to determine the testing sequence and the specimen number. A pull out test was applied on all specimens using a universal mechanical testing machine (Instron 5566A) at a crosshead speed of 1mm/min. Load at failure was recorded for each specimen. Data were analyzed by factorial regression model using JMP Pro 13. **Results and Conclusions:** Overall retention load values were lowest for zirconia and highest for e.max and Enamic. In general, primer/etch technique produced significantly higher values than no primer/etch. For RelyX cementation, no primer/etch produced significantly higher retention. Maximized desirability of retention load is for Enamic cemented with Multilink hybrid with primer/etch, although no statistically significant difference between the values for Enamic and e.max with Multilink hybrid cement primer/etch.

MicroRNA Differential Expression of External Cervical Root Resorption Compare to Normal Gingival Tissue in Human

Jacob Al-Hashemi; Sami Chogle

Department of Endodontics

Background: External Cervical Root Resorption (ECRR) presents challenges for endodontists regarding both diagnosis and treatment. Molecular pathogenesis of ECRR is still largely unknown or is poorly understood. MicroRNAs (miRNA) control many cell functions especially in the context of cancer and inflammation. **Objectives:** This proposal seeks to detect miRNA expression patterns in tissues found in ECRR lesions in humans compared to adjacent gingival tissue. Characterizing and identifying altered miRNAs regulation compared to controls can provide a better understanding of the underlying molecular changes during the ECRR process and reveal possible therapeutic targets. **Material and methods:** MiRNA from non-restorable teeth with ECRR referred for extraction will be collected and a miRNA-array will help to screen for more than 1,000 known human miRNAs. In addition, PCR confirmation for all differentially expressed miRNAs will follow. **Conclusion:** Once miRNAs are confirmed, a bioinformatics analysis of the possible targeted proteins and molecular pathways will help to elucidate possible pathogenicity of the disease.

Cytotoxic Effect of Chitosan Nanoparticles on Normal Human Dental Pulp Cells

Rami Alhomrany, Chang Zhang, Laisheng Chou

Department of Restorative Sciences & Biomaterials

Introduction: The chitosan nanoparticles have caught much attention recently as they possess an inherent antibacterial property. Many studies have shown that these nanoparticles can improve the antibacterial activity of several dental materials. This study aims to assess its biocompatibility on the normal human dental pulp cells. **Methods:** Normal human dental pulp cells were cultured with chitosan nanoparticles in concentrations 0.5 mg/mL, 1 mg/mL, 2 mg/mL, 4 mg/mL, and without the chitosan nanoparticles as a control for 7 and 14 days. The size of the nanoparticles was confirmed by the Dynamic Light Scattering and Transmission Electron Microscope to be below 200 nm. On each predetermined point of time, the proliferation and the viability of cells were assessed separately by two different methods. The crystal violet staining was used to measure the cell proliferation while the viability of cells was assessed by evaluating the activity of the mitochondrial dehydrogenase enzyme. **Results:** At day 7, groups of 2 mg/mL and 4 mg/mL significantly reduced the cell proliferation and cell viability compared to other groups ($P < 0.05$). The cell proliferation and cell viability were decreased significantly with all nanoparticles concentrations by the end of day 14 in comparison with the control ($P < 0.05$). **Conclusion:** Chitosan nanoparticles exhibit a cytotoxic effect on the normal human dental pulp cells.

Comparison of CAD/CAM Screw Retained Implant Crowns Sealed by Composite and Enamic Inlay

Rasha Ali, Richard Pober, Yuwei Fan and Russell Giordano

Restorative Sciences & Biomaterials

Objective: The aim of this in vitro study is to compare the effect of cyclic loading fatigue on failure load of CAD/CAM screw-retained implant crowns sealed by composite and Enamic inlay. **Materials and Methods:** Ivoclar e.max and Vita Enamic CAD/CAM screw-retained implant molar crowns (n=44 for each material) and Enamic inlays (n=44) were designed using Sirona in-Lab software system (SW4 4.2.5), and milled using CEREC in-Lab MC XL. All the prepared e.max and Enamic crowns were cemented to Sirona TiBase (B O 4.1 L) using Ivoclar hybrid multilink cement. The access holes were sealed either by using composite or and Enamic inlay (n=22 for each group). After cementation, twelve specimens from each group were subjected to a static load to failure test in a universal mechanical testing machine (Instron 5566A). The mean failure load for each group was calculated. The other 10 specimens for each group were subjected to cyclic loading fatigue under 40% of static failure load for 100,000 cycles. After cyclic loading, the surviving specimens were tested for static failure load. The comparison of failure load between tested groups was analyzed by one-way ANOVA using JMP Pro 13 with α=0.05. **Results:** Failure load values among the groups showed significant difference. Highest failure load was found in fatigued e.max crowns sealed by Enamic Inlay (3273.98 346.84 N), whereas lowest failure load for Fatigued Enamic crowns sealed by Enamic inlay (2038.94 360.71 N). Failure load of e.max and Enamic crowns did not show any statistical significance difference between static and fatigue paired groups. However, there was statistical significance different failure load between Enamic crowns sealed by Enamic inlay and e.max crowns sealed by either composite or Enamic inlay. **Conclusion:** The failure load of e.max and Enamic crowns sealed by composite resin was not significantly different. e.max crowns sealed by composite and Enamic inlay showed comparable results. Using Enamic inlay to seal CAD/CAM screw-retained implant crowns is a feasible new approach.

Dental Care Utilization Among US Elderly with Chronic Medical Conditions

Eman Al-joghaiman, Thayer Scott, Elizabeth Kaye and Woosung Sohn

Department of Health Policy and Health Services Research

Objective: To investigate whether comorbidity of systemic health conditions is a barrier to dental care utilization among the elderly. **Methods:** Data on dental care status and systemic diseases from the National Health and Nutrition Examination Survey (NHANES 2013–2014) for 1004 elderly subjects (>65 years) were analyzed. The main outcome was utilization of dental care, defined as having a dental examination within the past year. The main predictor variables were the presence and number of multiple diseases including hypertension, diabetes, hepatitis, asthma, arthritis, congestive heart failure, coronary heart disease, chronic obstructive pulmonary disorder, cancer, and osteoporosis. Confounding factors included in the analysis were race, age (65–75 or ≥75 years), sex, income (<$45,000, $45,000–$74,999, ≥$75,000), and education (less than high school, high school diploma, associate or college degree). Chi-square test and multiple logistic regression analysis were conducted using SAS (version 9.4). SAS Survey Procedures with sampling weights were used to estimate correct variance accounting for the complex sampling of the NHANES study. **Results:** 91.2% had at least one comorbidity; the most prevalent was hypertension (73.4%). Patients with chronic obstructive pulmonary disorder (COPD) were significantly less likely to have utilized dental care in the past year (OR 0.42; 95% CI 0.19–0.93). Patients with three or more and four or more comorbidities (20.57% and 7,46% of the participants, respectively), but not those with two or fewer, were significantly less likely to have utilized dental care after controlling for all confounding factors (OR 0.56, 95% CI 0.35–0.89 and OR 0.38, 95% CI 0.20-0.74). **Conclusion:** Elderly people with COPD or with three or more systemic diseases are more likely to underutilize dental care. Future programs and/or policy to increase dental care utilization among the elderly should consider interventions that focus on those burdened by several systemic diseases.

Racial/Ethnic Disparities in Opioids Prescription by Dentists

Adeem Alofi, Astha Singhal, Elizabeth Kaye, Thayer Scott, Sharon Rich, Woosung Sohn

Department of Health Policy and Health Services Research

Objectives: To examine the patterns of opioid and non-opioid analgesics prescription among dental professionals and explore potential racial/ethnic disparities in analgesics prescriptions by US dentists. **Methods:** Data from Medical Expenditure Panel Survey (MEPS) were analyzed for the year 2014. We included all dental visits during that year associated with an analgesics prescription by dentists. Prescribed analgesics were identified using Multum lexicon drug classification. Chi-square test was used to assess differences between opioid and non-opioid analgesic groups by race/ethnicity, age, gender, income, and dental specialty. Logistic regression models were constructed to measure the association between patients' race and dentists' analgesic prescription controlling for age, gender, income, and dental specialty. The person-weight for all the variables were appropriately adjusted to account for the sampling design complexity. **Results:** In 2014, 62.6% of individuals had at least one dental visit, of those 2.9% received analgesics, and 76.6% of these prescriptions were opioids. In contrast, 56% of analgesics prescribed by health professionals were opioids. Dentists prescribed 4.4% of total opioids prescriptions in that year. Dental specialists were more likely to prescribe opioids than general dental practitioners (p=0.04). Of those who received opioids, 72% were Whites, 11% were Blacks, 8% were Hispanics and 2% were Asians; whereas those who received non-opioid analgesics were 50% Whites, 17% Blacks, 22% Hispanics, and 6% Asians (p<0.001). Age, gender, and income were not significantly associated with opioid/non-opioid prescription. After adjusting for confounders, Hispanics, Blacks and Asians were significantly less likely to receive opioids compared to Whites (OR= 0.27, 0.50, 0.33 respectively, p-value<0.05). **Conclusions:** The results suggest that there is a potential racial/ethnic disparity in opioid/non-opioid analgesics prescription by dentists in the U.S. However, it is unclear whether this disparity is attributed to overtreatment among white patients or under treatment of other racial minorities. Further research is needed to better understand the causes behind it.

Bone Microenvironment-Mediated Cancer Cell Dormancy, Dissemination, and Drug Resistance

Alaa AlQutub, Fahd Aljamal, Serge Dibart and Erdjan Salih

Department of Periodontology

Introduction: Bone provides an exceptional microenvironment that represents an oasis for cancer cell colonization and drug therapy resistance of which many facets are to date not well understood due to the lack of an ideal model system. Most solid tumors such as breast have a high propensity to colonize bone and once established, results in a terminal and incurable disease. The biological reasons for drug resistance, failure of treatment and reoccurrence are presently unclear. We have utilized our novel ex-vivo cancer-bone metastasis model systems involving co-cultures of free floating mouse calvarial bones with cancer cells and clinically used therapeutic agents to illuminate the above clinical observations. **Materials and Methods**: A 20-days experiment was designed for MDA-MB-231 and MDA-BO cells in roller tube system under resorption and formation models in the presence and absence of clinically used bisphosphonate, zolendronic acid (ZOL). The groups contained three subgroups; controls with calvaria alone, calvarial bone co-cultured with tumor cells, and calvaria with tumor cells treated with four repeat doses of 2 µM (1µg/2 ml DMEM media) of ZOL (total cumulative 4 µg). The formation groups were supplemented with 150 µg/ml ascorbic acid. Cell count was performed on trypsinized calvaria at 2, 8, and 14 days. Media was changed every 2 days and the changed media was re-seeded in a 24-well for 20 days. To test the impact of chemotherapy agents on cancer-bone metastasis another experiment was set to test effect of 10 µM of Docetaxol on MDA and BO cancer cells under formation and resorption conditions using the above design. Cell count was performed on trypsinized calvaria with cancer colony at 8 days. **Results**: Tumor burden: Counts from trypsinized calvaria with tumor colony showed no statistically significant difference in the tumor burden between ZOL treated and untreated groups in the resorption condition from day 2 through day 14 in both MDA and BO cells. Under Formation condition, MDA showed no significant difference up to 8 days. However, the difference was significant at days 14 with ZOL treated group having less burden. For BO cells, the tumor burden was not statistically significant different between treated and untreated groups. Comparing the treated and untreated groups with clinically used chemotherapy agent Docetaxol revealed that only ~30% of the cells were affected by chemotherapy in the bone formation model, while ~70% was affected in the resorption model with both cell types. Dissemination model: Counts from the re-seeded media of the 3rd change through the 7th change revealed that the dissemination rate for MDA and BO cells under formation condition is significantly less than for resorption condition. Fluorescent microscopy: MDA and BO tumor-calvaria were treated with Ki 67 antibody showed that under bone resorption condition cancer-bone cell colony were predominantly in proliferation stage. In contrast under bone formation conditions cancer cells were predominantly in dormancy where the cells are stretched out. **Conclusions**: 1- Both MDA and BO cancer cells showed resistance to bisphosphonate (ZOL) treatment under formation and resorption conditions. 2- Drug resistance of MDA and BO to chemotherapy (Docetaxol) was more evident under formation condition, where cells are in dormancy and not proliferating. 3- The rate of cancer cell dissemination from the bone colony is significantly higher in resorption condition, indicating that cancer cells are dormant under bone formation condition and tend to disseminate less.

Osteocytes Induce Myeloid Proliferation and Inhibit Osteoclast Differentiation

Ehab Azab, Yuhei Uda, Ningyuan Sun and Paola Divieti Pajevic

Department of Molecular and Cell Biology

Introduction: Previous studies have shown that osteocytes, the matrix-embedded cells in bone, control bone marrow cells through direct contact and via secreted factors that can reach cells in the bone marrow microenvironment (BMM). Osteocytes express several receptors including G protein-coupled receptors (GPCR) and mice lacking Gsα expression in osteocytes have abnormal myelopoiesis. This study aimed to evaluate the effect of osteocytes on myeloid proliferation and differentiation. To investigate cross talk between osteocytes and the BMM, we established an osteocytic cell line lacking Gsα expression to investigate molecular mechanisms by which osteocytes control macrophages proliferation and differentiation. **Methods:** CRISPR/Cas9 was used to knockout Gsα in the osteocytic cells line Ocy454. Conditioned medium (CM) from differentiated Ocy-GsαControl and Ocy-GsαKO cells was used to treat myeloid cells and bone marrow mononuclear cells (BMNCs) isolated from long bones of 6 weeks wild type mice. BMNCs were cultured with Macrophage Colony Stimulating Factor (M-CSF), Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL) for osteoclast differentiation. Proliferation, TRAP staining, TRAP activity, resorption pit assay, F-actin ring and mRNA expression were used to evaluate cell proliferation, differentiation and function. Proteomics analysis of CM was performed to identify osteocyte secreted factors capable of controlling myelopoiesis and osteoclastogenesis. **Results:** Myeloid cell treated with CM from Ocy-Gsαcontrol or Ocy-GsαKO showed a significant increase in cell proliferation compared to Ocy-GsαControl CM and non- treated control. BMNCs treated with CM from Ocy-Gsαcontrol or Ocy-GsαKO showed a significant increase in cell proliferation as compared to non- treated control. Osteoclast differentiation was significantly suppressed by CM from Ocy-Gsαcontrol and further suppressed by CM from Ocy-GsαKO compared to non-treated control. Osteoclasts showed a significant defect in activity and function as compared to non-treated cells. Further experiments are ongoing to identify osteocyte-secreted factors. **Conclusions:** Osteocytes secrete factor capable of inducing myeloid proliferation and inhibiting osteoclastogenesis and this factor(s) is Gsα-dependent.

Dental Care Utilization and Disparities in Access to Dental Care Among Cancer Survivors in U.S.

Lina Bahanan, Elizabeth Kaye, Thayer Scott, Sharron Rich and Woosung Sohn

Department of Health Policy and Health Services Research

Objectives: This study aims to evaluate the association between cancer history and access to dental care. **Methods:** We analyzed data of 10,109 subjects, aged 20-80 years from The National Health and Nutrition Examination Survey (NHANES) 2011-2014. The main predictor was self-reported past history of any type of cancer (yes/no). The outcome was having a dental visit in the last year (yes/no) with reasons for the visit and barriers if no visit. Covariates included gender, race, income, education, and marital status. Chi-square and multivariable logistic regression were conducted to evaluate the association of cancer history with dental care utilization. We used SAS survey procedures to account for complex sampling design. **Results:** Cancer survivors were not more likely to have dental visits within the past year compared to controls (OR=0.95; 95% CI: 0.67-1.19). Both survivors and controls were more likely to have preventive than treatment visits (64% and 65% respectively p=0.68). The most common barrier to accessing dental care was cost (79% for both groups). Cancer survivors with at least a high school degree were significantly more likely to have any dental visit within a year compared to less educated survivors (OR=2.4; 95%CI:1.5-3.9). Survivors with higher SES were significantly more likely to have any dental visit within a year compared to those with lower SES (OR=6.3; 95%CI:3.5-11.6). **Conclusion:** Cancer survivorship was not significantly associated with any dental visits (yearly, preventive/treatment). However, financial and educational disparities exist among cancer survivors. Further research is recommended to more comprehensively understand oral health disparities among cancer survivors.

Pharmaceutical Modification of a Gluten-degrading Enzyme Derived from Oral Bacteria

Ghassan Darwish, Eva J. Helmerhorst, Guoxian Wei

Department of Molecular and Cell Biology

Introduction: Detoxification of gluten immunogenic epitopes is a strategy for the treatment of Celiac disease. Previous studies have demonstrated that the epitopes can be degraded by subtilisin-derived from Rothia mucilaginosa, a natural colonizer of the oral cavity. But the enzyme loses its activity under acidic conditions, as encountered in the stomach. Objective: To protect and maintain enzyme activity under acidic conditions by exploring pharmaceutical microencapsulation techniques. **Methods:** Subtilisin-A purchased from Sigma was coated with a polymer that commonly used in pharmaceutical industrious. The activity of coated enzyme was assessed by incubation of coated Sub-A, non-coated Sub-A and coating materials only in diluted HCl buffer at pH 3.0 at 37°C for 0, 1, 3, 5 and 24 hours. Aliquots at each time point were collected and their activity were determined using Suc-AAPF-pNA substrate. To assess the abolishment of immunogenic gluten epitopes in vivo. Balb/c mice were fed with mouse chow pellets with and without coated subtilisin-A. Mice were sacrificed and the stomach content were collected. The survival of immunogenic gluten epitopes of the samples were analyzed by ELISA-R5 kit. **Results:** Subtilisin-A was modified by microencapsulation, achieved ~43% yield of coated Sub-A containing ~8% (w/w) Sub-A. The coated Sub-A showed significantly increased protection against exposure to acid conditions (pH 3.0) in vitro. An in vivo experiment (n=30) showed that gluten immunogenic epitopes decreased by ~60% in the stomach of the mice fed with chow containing coated Sub-A (0.2mg Sub-A/ g chow) compared to that of the mice fed with chow only. **Conclusion:** Pharmaceutical modification of subtilisins can protect enzyme activity under acidic conditions and such enzymes can be considered a promising modality for future applications to treat celiac disease.

Lysyl Oxidase Like-2 Mediates Tumor to Stromal Cell Communication in Oral Cancer

Faranak Mahjour; Neha Shrestha and Philip Trackman

Department of Molecular and Cell Biology

Introduction: The lysyl oxidase family consists of 5 members and oxidizes specific lysine residues in biosynthetic collagen and elastin maturation. Lysyl oxidase like-2 (LOXL2) is elevated in oral cancer and promotes metastasis and correlates with poor prognosis. The objective of this study is to determine the mechanism by which LOXL2 promotes the progression and invasiveness of oral squamous cell carcinoma. **Methods:** In vitro: To investigate functions of LOXL2 in oral cancer, the effects of LOXL2 inhibitor (PXS-S1C) on human gingival fibroblasts treated with tumor cell conditioned medium (CM) were investigated. Cell proliferation assays, signaling arrays, gene knock down and western blots were used to evaluate the effect of PXS-S1C on CM-treated fibroblasts. The effect of PXS-S1C on cancer cells expression of LOXL2 and proliferation was determined. To find potential LOXL2 substrates in gingival fibroblasts treated with CM +/- PXS-S1C, carbonyl-containing proteins were purified by affinity chromatography and identified by MS and western blot. In vivo: The effect of PXS-S1C on cancer growth and metastasis in vivo was investigated using an orthotopic oral tongue cancer mouse model in both immunodeficient and immunocompetent mice. PXS-S1C at 10 mg/kg and 30 mg/kg was injected immediately following tumor cell injections. Tumors were monitored by caliper measurements, and by in vivo imaging (IVIS). The mice were sacrificed and their organs were subjected to immunohistochemistry staining for proliferation markers. **Results:** PXS-S1C significantly inhibited gingival fibroblast proliferation triggered by tumor cell CM and attenuated phosphorylation of PDGFRβ Y771 and P-PDGFRβ Y857, but not PDGFRβ Y751 in response to CM. PXS-S1C inhibited ERK1/2-signaling in fibroblasts and not AKT in response to CM. PDGFR activation by oral tumor cells was mimicked by PDGF-AB, but not PDGF-BB. PXS-S1C decreased the expression of LOXL2 by 5-fold in HSC3 oral cancer cells in vitro, suggesting a positive autoregulatory loop. Assessing for direct LOXL2 substrates in fibroblasts with functional consequences identified both PDGFR and integrin αV. Caliper measurements, IVIS, and immunohistochemistry demonstrated that inhibition of LOXL2 significantly decreases oral cancer progression and metastasis in vivo. Mice without PXS-S1C treatment developed larger tongue volumes ($p<0.05$), and more mice developed abnormal sized lymph nodes (9 out 12) compared to the PXS-S1C-treated mice (4 out 12) ($p<0.05$). IVIS imaging revealed inhibition of metastasis by PXS-S1C treatment. The expression of proliferation marker (Ki-67) and LOXL2 was lower in tongue tumors treated with PXS-S1C ($p<0.05$). **Conclusions:** LOXL2 secreted by cancer cells stimulates fibroblasts by enhancing PDGFR signaling and proliferation, and by stimulating αV integrin function and cell adhesion. Inhibition of LOXL2 could provide therapeutic strategies to address oral cancer.

Scriptaid Induces Osteocyte Respiration through a HDAC Independent Mechanism

Ningyuan Sun, Ehab Aza, Yuhei Uda, Chao Shi and Paola Divieti Pajevic

Department of Molecular and Cell Biology

Background: It has been known that bone has strong capability of self-repairing and it undergoes continuous remodeling to maintain its structural integrity. During development, bones (and in particular osteoblasts) rely on glucose uptake for proper skeletal development whereas in adult bone, the relative role of glucose metabolism, is currently unknown. Osteoblasts take up one fifth of the glucose consumed by muscles and they sense and use glucose through a Glut1 and AMPK-dependent pathway independent of insulin signaling. Glucose uptake in cells of the osteoblast lineage is necessary for osteoblast differentiation, bone formation and glucose homeostasis. Whereas the role of glucose in osteoblasts is well established, its effect on osteocytes is still unknown Osteocytes are terminally differentiated osteoblasts deeply embedded into the mineralized matrix of bone. In vitro studies have shown that PTH promotes bone anabolism, in part, by stimulating, in osteoblasts, anaerobic glycolysis while suppressing glucose oxidation through the TCA cycle. In osteocytes, PTH suppresses Sost expression (a potent inhibitor of bone formation) by inducing HDAC4/5 nuclear translocation and Mef2C suppression. Recently, scriptaid, a HDAC complex co-repressor, has been shown to induce Mef2c expression and exercise-like adaptation in mice. In muscles, scriptaid disrupts the co-repressor complex comprising HDAC3, SMRT/N-cor and induces nuclear export of HDAC4/5 with increase in Mef2c, Glut4 and Pdk4 expression. This suggested that scriptaid might upregulate Mef2C and Sost expression in osteocytes. **Methods:** To investigate scriptaid influence on osteocyte, we used an osteocytic cell line, Ocy454 12H established in our laboratory. **Results:** Unexpectedly, the compound potently suppressed Sost, whereas induced Glut4 and Pdk4 expression. Scriptaid also stimulates osteocyte respiration and glucose consumption rate. However, this metabolic regulation is through a HDAC independent mechanism. Moreover, additional genes such as MEPE and RANKL that participate in bone modeling and remodeling are also significantly regulated by scriptaid treatment. **Conclusion:** Taken together these studies demonstrated that scriptaid induces osteocyte metabolism through a HDAC independent mechanism and regulates key genes related to bone remodeling, which may have profound effect on general glucose level and bone formation.

A Trypsinogen Activation Peptide Mutation Worsens Cerulein-induced Pancreatitis in the Mouse

Zsanett Jancso, Miklos Sahin-Toth

Department of Molecular and Cell Biology

Background: Mutations in the activation peptide of human cationic trypsinogen (PRSS1) have been found in association with hereditary pancreatitis. Biochemical experiments using recombinant proteins demonstrated that the activation peptide mutations strongly stimulate autoactivation of cationic trypsinogen. These observations supported the hypothesis that increased intra-pancreatic trypsinogen activation causes hereditary pancreatitis. **Aims:** The aim of the present study was to generate a mouse model carrying a hereditary-pancreatitis associated activation peptide mutation and characterize the impact of the mutation on experimentally induced pancreatitis. **Methods:** Our genetically modified mouse strain carries the p.K24R mutation in the activation peptide of the mouse cationic trypsinogen (isoform T7). This corresponds to the p.K23R mutation in PRSS1. Acute pancreatitis was induced by 12 hourly injections of cerulein. Pancreatitis severity was determined by histology scoring, measurement of edema, MPO and serum amylase. Pancreatic trypsin activity was measured at 30 min after a single dose of cerulein. Results. Intra-pancreatic trypsin activity and all parameters of acute pancreatitis severity were markedly elevated in mice carrying the p.K24R mutation relative to the C57BL/6N controls. **Conclusions:** A trypsinogen activation peptide mutation associated with hereditary pancreatitis in humans increases intra-pancreatic trypsin activity and worsens pancreatitis responses in this novel mouse model.

Pancreas Pathology in a Mouse Model of Rapid Trypsinogen Activation

Anna Orekhova, Andrea Geisz, Zsanett Jancso and Miklos Sahin-Toth

Department of Molecular and Cell Biology

Introduction: Hereditary pancreatitis is a genetic disorder which is caused by mutations in human cationic trypsinogen. Some of these mutations affect the activation peptide of cationic trypsinogen causing intrapancreatic trypsin activation. Earlier in vitro studies showed that such mutant variants autoactivated much faster than wild type and had propensity for trypsinogen activation as in case with well-known human mutation D22G. To investigate this process in more detail the analogous mutation D23A was introduced into mouse T7 trypsinogen. Considering the fact, that this mutant variant had an incredibly high activation rate, 50-fold faster than wild type was generated mouse model with lower expression of T7 trypsinogen and delay the disease progression. **Aim:** The aim was to generate trypsin-dependent mouse model and to characterize pathological changes which develop and arise spontaneously from a certain age. **Materials and methods:** Mouse model harboring point mutation D23A with preservation of neomycin cassette was generated by knock-in method on C57BL/6 background. Mice were sacrificed in different ages and animal tissue and blood were taken for analyses. Histological evaluation was made for the presence of characteristic features of the disease such as acinar cell loss, inflammatory cell infiltrate, dilated ducts, acinar-to-ductal metaplasia. **Results:** Mouse model D23ANeo carrying neomycin cassette exhibit the first signs of disease at the age of 2,5 months. This model develops spontaneous pancreatitis and has features of chronic pancreatitis with loss of acinar cells and preservation of islets. There is a noticeable decrease in pancreas weight with age, which is also consistent with acinar cell loss and indicates pancreatic atrophy. Although there is a small increase in amylase level in mutant mice compared with C57BL/6N. **Conclusions:** This model supports the theory that increased and uncontrol trypsinogen autoactivation is one the main pathological mechanisms which leads to the development of hereditary pancreatitis.

3D Implantology: Revolutionizing Guided Implant Placement Using 3D Printed Guides

Jayesh Patel, Neal Fleisher and Hesham Nouh

Department of General Dentistry

Background: The use of surgical guides in dental implant surgery is well-documented and is increasingly being used during surgical placement. It is estimated that 10% of all implant surgeries utilize a surgical guide (iData research, 2015). During semi-guided implanted placement, the surgical guide helps in accurately positioning the initial osteotomy, ultimately affecting the final implant location. In such cases, the implant is placed without a guide into the osteotomy site. However, the use of the guide in semi-guided systems dramatically improves the predictability of the final placement, thus improving the overall outcome. Advances in CAD/CAM dentistry have led to increased use of milled surgical guides; however, recent development of FDA approved dental resins has led to the ability to 3D print guides, using relatively low cost equipment. **Aims:** The aim of this pilot study is to determine if there is a significant difference in the relative accuracy of location and of the initial osteotomy when using a milled versus a 3D printed surgical guide. **Methods:** The study involved five CEREC milled guides, and five FormLab 3D printed guides all produced using the same CAD scanned image file of a dentoform. A dentoform mounted on a surveyor table was used to compare the placement and angulation of the osteotomy between the milled and 3D printed guides. Following a semi-guided implant placement protocol, the initial osteotomy was created in two separate dentoforms, each using a guide key, and each made with a milled guide and 3D guide respectively. Static images of the mounted dentoform in a jig were taken, with guides firmly seated to the teeth and secured tightly. The negative control group was a static image of the respective dentoforms without any surgical guide seated. Two of the four positive control groups were static images of the 3D printed guides seated on the dentoform with the osteotomy made with the 3D printed guide and milled guide respectively. The final two of four positive control groups were static images of the milled guides seated on the dentoform with the osteotomy made with the milled guide and 3D printed guide respectively. **Results:** Using image analysis software, the distance was measured from a point on the osteotomy to a pre-determined point on the dentoforms. Measurements were taken for the negative control group, and each of the four positive control groups. No significant difference in location and angulation of the initial osteotomy was noted between the five milled guides. Similarly, no significant difference in location and angulation of the initial osteotomy was noted between the five 3D printed guides. When the milled guides were compared to the 3D printed guides, no significant difference between the placement and angulation of the initial osteotomy was noted. **Conclusions:** Guides that were 3D printed demonstrated an equal level of accuracy in site location and angulation of initial dental implant osteotomy during semi-guided implant placement. Our results show evidence that 3D printed guides serve as a viable alternative to milled surgical guides when used for semi-guided implant placement. Further comparative studies between milled and 3D printed surgical guides are necessary to demonstrate the accuracy and efficacy in the use of 3D printed surgical guides for dental implant placement.

MMP-20 Expression in Human Cancer Cells Interacting with Forming/Resorbing Bone

Melissa Ferguson, Serge Dibart and Erdjan Salih

Department of Periodontology

Background: Matrix Metalloprotease (MMP)-20 is a proteolytic enzyme previously thought to be restricted to enamel formation and development. Recent studies have found that MMP20 is expressed in different body tissues and interestingly, found to be increased in cancer pathology. Aggressive cancer can metastasize to bone, creating bone-cancer biology to be an area of interesting study. **Objectives:** The objective of this study is to investigate MMP20 protein expression in tumor cell lines interacting with bone in a ex vivo bone/cancer co-culture system. **Materials and methods:** Using a calvaria bone/cancer cell 3D co-culture roller tube model, bone remodeling was separated into a formation and resorption model to identify the novel effects of bone-cancer interaction on MMP20 expression in osteoblastic and osteoclastic differentiated states. Oral Squamous Carcinoma cell line (OSCC) and two breast cancer cell lines were investigated; MDA-MB-231 and a more aggressive/bone seeking MDA-BO tumor cell line. **Results:** Using ELISA, our study found an increase in MMP20 expression secreted from all tumor cell types interacting with calvaria in an osteoclastic differentiated state compared to calvaria in an osteoblastic differentiated state. MDA-BO is found to have an increased MMP20 expression compared to the less aggressive tumor cell lines. The cell lines MDA-MB-231 and MBA-DO have MMP20 protein expressed which were previously not investigated or found to contain MMP20. The last novel finding of our study is that bone calvaria from an ex vivo model have an inherently high expression of MMP20 protein regardless of formation or resorption status. **Conclusions:** In conclusion, our study offers support to previous studies that suggest MMP20 effects cancer prognosis and aggressiveness with our studies novel finding that the more aggressive breast cancer cell line had increased MMP20 expression. Further studies are warranted to investigate MMP20's role in more aggressive cell lines in a bone/cancer co-culture system.

Analysis of Nasal Airway Symmetry and Pharyngeal Airway Following Rapid Maxillary Expansion (RME)

Charles J. DiCosimo III, Dr. Ahmed Alsulaiman, Dr. Melih Motro, & Dr. Goli Parsi

Department of Orthodontics and Orthofacial Orthopedics

Objectives: This retrospective cohort study tested the effect of Rapid Maxillary Expansion (RME) on symmetrical volumetric changes in the nasal cavity. Volumetric changes were also analyzed for nasopharyngeal and oropharyngeal cavities as well as linear minimum cross-sectional measurements for left and right overall nasal cavities. Methods: CBCT scans of before and after RME treatment for 28 subjects (17 females, 11 males, average age 9.85 ± 2.42 years) were collected from a previously de-identified database. All subjects were treated for maxillary constriction using banded hyrax expanders. Mimics software was utilized to segment nasal and pharyngeal airways and a 3-D model was created. Anatomical points and subsequent planes using these points were established to divide the 3-D model into anterior nasal cavity right and left (ANC-R, ANC-L), posterior nasal cavity right and left (PNC-R, PNC-L), nasopharyngeal cavity, and oropharyngeal cavity. Volumetric changes for each of these compartments were then collected. For minimum cross-section analysis, initial scans were inspected visually until the thinnest portion of the left and right nasal airway was confirmed in the coronal plane. The number of slices from pronasale was recorded and the thinnest portion of the left and right nasal cavity was measured linearly. The analogous slices in the post-expansion scans were identified from pronasale and the same measurements were recorded. Paired T-test was used to quantify the pre- and post-expansion volumetric/cross-sectional changes. Statistical significance was set at the 0.05 level. Results: Posterior expansion as measured between right and left greater palatine foramen (GPF) averaged 2.41 mm (SD = 1.03 mm). There were statistically significant differences in overall nasal cavity (3240.7 ± 2726 mm3, $32.63 \pm 26.47\%$), ANC-R (325.4 ± 237.0 mm3, $28.71 \pm 21.35\%$), ANC-L (420.1 ± 328.6 mm3, $40.03 \pm 30.52\%$), PNC-R (643.4 ± 923.6 mm3, $27.01 \pm 36.82\%$), PNC-L (767.0 ± 1343.8 mm3, $40.55 \pm 68.89\%$), nasopharyngeal (1000.6 ± 917.7 mm3, $43.92 \pm 42.36\%$), and oropharyngeal (2349.2 ± 2520.8 mm3, $33.76 \pm 39.16\%$) cavity volumes. For cross-sectional analysis, the right nasal cavity (0.13 ± 0.07 mm, $41.13 \pm 24.72\%$) and left nasal cavity (0.11 ± 0.06 mm, $38.53 \pm 24.33\%$) showed similar increases in thinnest cross-sectional linear measurements. Conclusions: Results suggest that RME has significant benefits to increasing nasal and pharyngeal airway cavity volumes in all segments of the airway. Taking a closer look at the segments of the nasal cavity shows that the left side, both anterior and posterior, has more improvement than the right side counterparts by approximately 10-13%. Furthermore, in analyzing the left or right side of the nasal cavity alone, results show that the anterior and posterior segments have similar percent increases. Additionally, linear minimum cross-section of the left and right nasal cavities showed highly symmetrical improvements.

3D Evaluation of Condylar Changes After Rapid Maxillary Expansion

Camille Francois, Ahmed Alsumaiman, Melih Motro and Goli Parsi

Department of Orthodontics and Orthofacial Orthopedics

Objectives: The aim of this retrospective cohort study was to determine the effect of Rapid Maxillary Expansion (RME) on condylar shape, volume and orientation as well as on mandibular rotation. **Method:** Pre- and post-expansion cone beam computerized tomography (CBCT) images of forty-four healthy subjects (mean=13.65, SD=1.93) who previously had undergone banded RME were selected from a CBCT repository. Linear, angular and volumetric changes as well as condylar surface area differences and changes in mandibular rotation were assed using Mimics software. The condyles were segmented by a plane passing through the sigmoid notch (Snp) and parallel to a Frankfort Horizontal derivative plane (FHD). All the linear, angular, volumetric and surface area assessments were performed on the isolated 3D condyles. Changes in mandibular rotation were evaluated by analyzing the alterations in the angle between mandibular plane and FHD plane before and after RME. Paired T test was performed to compare pre- and post-expansion for all variables stated above. Statistical significance was set at <0.05. **Results:** The right and left condylar widths significantly increased by 0.56mm ±0.88mm (p=0.01) and 0.61mm ±0.82mm (p=0.002), respectively. The right and left condylar volumes significantly increased by 48.06mm3 ±110.1mm3(p=0.05) and 73.62mm3 ±104.2mm3 (p=0.003), respectively. Finally, the right and left condylar surfaces significantly increased by 24.06mm2 ±36.46mm2 (p=0.006) and 37.37mm2 ±44.67mm2 (p=0.001), respectively. No significant changes were found in condylar height, condylar orientation and mandibular plane angle. **Conclusion:** Condylar width, volume and surface were significantly increased after Rapid Maxillary Expansion. However, condylar growth still remains to be a cofounding factor that affects the results. No statistically significant effect on condylar orientation and mandibular plane was found. Future studies will be required to assess the three dimensional changes of the condyles with growth.

3D Cephalometric Analysis Using Novel Landmarks

Daniel Moynihan, Andrew Pham, Ahmed Alsulaiman and Melih Motro

Department of Orthodontics and Orthofacial Orthopedics

Objectives: The aim of this project is to use new landmarks to develop a novel 3D cephalometric analysis using anatomical landmarks that cannot be easily viewed on a planar film but on a CBCT image and study the reliability of these measurements between different examiners. Our implication for completing this study is to attempt to create new cephalometric norms on CBCT scans which have not been defined yet in current literature. **Methods:** Fifty CBCT images of patients with normodivergent, Class I skeletal patterns and without any noticeable craniofacial deformities were screened and included in the study based on the lateral cephalograms generated from CBCT data using the Roth/Jarabak Analysis. Ffifteen predetermined landmarks that found to be reliable in another associated study were used to define six planes. Eleven different cephalometric measurements were performed using landmarks and planes in 3D. Descriptive statistics (mean and standard deviation), overall interclass correlation coefficient, and individual interclass correlation coefficient of all of the measurements was calculated among two operators. **Results:** The measurements showed that the means and standard deviations of all three measurers are: for Mandibular A/P: 14.49mm ±2.26; Mandibular deviation: avg: 1.63mm, ±1.19; Maxilla/Mandibular divergence: 24.51°, ±3.85; Maxillary deviation: 0.94mm ±0.5; Pitch (Mandibular Plane), 50.38° ±2.8; Pitch (Maxillary Plane): 74.66° ±3.53; Roll (Mandibular Plane): 87.66° ±2.38, 0.05; Roll (Maxillary Plane): 87.70° ±1.85; Yaw (Maxillary Plane): 4.41°± 2.11; Yaw (Mandibular): 3.61°± 2.43; Maxillary A/P: 2.96mm ±2.26. An interclass correlation was calculated at a range from 0.53 to 0.95 with an average of 0.76±0.12. **Conclusions:** Our conclusions for this pilot study indicate that we have our first results for creating Cephalometric norms on a 3D scan. Amongst the operators, we indicate between moderate and excellent reliability. The measurements and points need to be plotted again two more times to confirm intra and inter examiner reliability.

Determining the Reproducibility of Locating Anatomical Land Marks on Human Skulls on CBCT for Orthodontic Measurements

Andrew Pham, Daniel Moynihan, Ahmed Alsulaiman, Jena Lee and Melih Motro

Department of Orthodontics and Orthofacial Orthopedics

Introduction: The objective of the study was to evaluate the reproducibility of identifying various landmarks solely from a CBCT. Landmarks include points from traditional cephalometry and points which are uniquely found on CBCT. The landmarks were strategically selected in order to be markers of different parts of the skull in order to be used to measure the skull in three dimensions in further studies. **Methods:** 50 CBCTs were taken from a database belonging to patients that required orthodontic treatment. With mimics software landmarks were identified from diverse parts of the skull that ranged from bony land marks, foramina and dentition. Three examiners located the land marks three times over a year long period. Three dimensional coordinates were determined in the x, y and z plane. Interpersonal and intrapersonal reliabilities were determined as well as deviations from the average. **Results:** Bony protrusions such as the lingulae, anterior clinoids and dentition proved to be the most reliable with the highest precision between interexaminer readings. **Conclusion:** Choosing different landmarks purely from CBCTs are found to be precise and reliable for landmarks that have clear bony protrusions. These points may demonstrate a new type of standard when determining land marks for 3-d evaluation of the skull.

Heritability of Facial Growth of Children using Lateral Cephalograms

Raman Samra, Ahmed Alsulaiman, Melih Motro and Leslie Will

Department of Orthodontics and Orthofacial Orthopedics

Objective: The purpose of this study was to test the association of parental mid-face and nasal soft and hard tissue morphology with their offspring. **Methods:** This retrospective pilot study was comprised of twenty western European families from the Forysth/Moorrees Twin Study. The lateral cephalograms of each parent and post-pubertal child, who is at least 2 years past peak growth (from literature age ≥ 16 yrs for females and ≥ 17 yrs for males) were evaluated on thirteen cephalometric variables including ANB, overjet, ratio of the nose to total face height (Nasion (N)-Anterior Nasal Spine (ANS)/N-Menton), angle of nasal bone relative to Sella (S)-N, nasal bone length, distance from rhinion to ANS, angle of ANS to S-N, distance of rhinion to pronasale, distance of ANS to pronasale, nasolabial angle, projection of nose, soft tissue convexity and nasal height. A two-way ANOVA with multiple comparisons (TUKEY) was performed to test for the differences between family members controlling for the effect of the individual family. Statistical significance was set at $p < 0.05$. **Results:** Twelve male and 8 female offspring were studied. There was a significant difference between the child and father but not the mother for angle of nasal bone relative to S-N ($p = 0.04$), rhinion to ANS (mm) ($p = 0.048$), rhinion to pronasale (mm) ($p < 0.0001$), ANS to pronasale (mm) ($p = 0.005$), projection of nose ($p = 0.0006$) and nose height ($p = 0.03$). There was no difference between males and females in the extent of their similarity to their mothers or fathers except for nasal height, where females were similar to their mother but different from their fathers ($p = 0.03$). **Conclusion:** Children are morphologically more similar to their mothers than their fathers when comparing mid-face and nasal bone soft and hard tissue parameters.

Student Learning of Digital vs Plaster Orthodontic Model Analysis

Frank Schiano, Sharron Rich and Leslie Will

Department of Orthodontics and Orthofacial Orthopedics

Introduction: Cast analysis plays an essential role in orthodontic diagnosis. Intraoral scanning producing digital models is a relatively new but increasingly common practice in graduate orthodontic programs. It is unknown how incorporating digital models in post-graduate orthodontic programs will influence student learning of model analysis. The purpose of this pilot study was to investigate the possible differences in learning speed and accuracy of basic model analysis using digital models or traditional plaster casts. **Materials and methods:** Two groups of senior dental students participated, one for plaster casts and one for digital. A study moderator provided a 15-minute tutorial instructing participants on how to analyze the casts. A standardized scoring sheet was used for data collection. Each group was given five sets of orthodontic models to analyze the following measurements: Right molar occlusion, overbite, overjet, arch length, required arch space, crowding, and incisor irregularity. The accuracy of the measurements as well as the time taken to complete all measurements on each model were recorded. Learning as measured by increasing accuracy or decreased time over the group of five casts was determined. Five orthodontic faculty served as the control group. **Results:** Sixteen students analyzed plaster casts; 15 students analyzed digital casts. Molar occlusion was judged as either correct or incorrect; means of the millimetric measurements of the other parameters were compared between groups using GLM. The digital learning group had 15 measurements that were significantly different from the faculty mean; the plaster learning group had only 2($p<0.05$). Regarding molar occlusion, the plaster group was always more accurate. The time required for the measurements decreased in each group to a similar extent, with the greatest decrease between casts 1 and 2. **Conclusions:** Senior dental students learned how to analyze plaster orthodontic models more accurately. The time required for analysis decreased over 5 trials.

The Effect of the Modified Kavain Compound on Inhibition of P.Gingivalis /LPS-induced TNF-Alpha Production in Human THP-1 Cells

Mansour Alasiri, Bushra Aljahdali, Abfullah Bamashmous, Serge Dibart, Erdjan Salih and Xiaoren Tang

Department of Periodontology

Background: TNF-α is an important cytokine mediator of inflammation which suggests that inhibition of TNF activity may provide potential for clinical application. Recent data indicated that treatment of both human and mouse cells with Kavain significantly modulates P. gingivalis- and LPS-induced TNF-α expression. **Materials and methods:** In order to obtain a selective analog with optimized biological activity and structural physico-chemical properties of Kavain, Kavain analogs were designed and synthesized. **Results:** one Kavain analogue that is similar to Kavain but soluble and does not induce a significant toxicity. **Conclusion:** Both studies in vitro and in vivo treatment showed stronger biological function as compared to Kavain.

Incretin Dysregulation of Lysyl Oxidase: A Novel Mechanism for Diabetic Bone Disease

Eileen Daley and Philip Trackman

Department of Molecular & Cell Biology

Background: Incretins are gastric hormones released by intestinal K-cells into the circulation in response to food consumption and stimulate insulin secretion from pancreatic beta cells. One of these hormones, glucose-dependent insulinotropic peptide (GIP) is also anabolic in bone. Individuals with diabetes experience diminished bone quality caused by a low bone formation osteopenia, and impaired cellular response to GIP in the pancreas, however GIP regulation of bone quality in diabetes has not been extensively explored. **Objective:** The present study seeks to identify a potential mechanism for diabetic osteopenia in which diabetes interferes with GIP-stimulated increases in the collagen cross-linking enzyme lysyl oxidase (LOX) in osteoblasts, leading to decreased collagen integrity and the trabecular abnormalities seen in diabetic bone. **Materials and Methods:** Micro-CT analysis of long bones and L5 vertebrae from LOX +/- and wild type mice made diabetic by low dose streptozotocin (STZ) induction revealed a profound exacerbation of the decreased bone volume and impaired trabecular structure seen in diabetic wildtype and LOX +/- mutant mice when the mice were both diabetic and haploinsufficient for LOX. Furthermore, qPCR of RNA isolated from diabetic wild type and mutant bones revealed a more than 20 fold decrease in LOX expression in diabetic bone. Studies done in healthy MC3T3 osteoblast cultures treated with GIP show that under normal conditions GIP directly increases LOX transcript and protein levels, mediated by the cAMP/PKA pathway and TCF/LEF response elements in the LOX promoter. The specific molecular mechanisms by which diabetes inhibits GIP-stimulated LOX expression in osteoblasts were also investigated. A novel inhibitory circuit of glucose stimulated insulin secretion in the pancreas has been discovered in which dopamine produced in the foregut can act as an "anti-incretin", antagonizing and thus regulating the effects of incretins in healthy individuals. Therefore the ability of dopamine to inhibit GIP-stimulated signaling in MC3T3 osteoblasts was examined. **Results:** Data indicate a strong dose- and time-dependent inhibition of GIP-stimulated cAMP production and LOX expression when MC3T3 osteoblasts are pretreated with dopamine. ELISA assays on serum from our STZ diabetic mouse model also confirmed significantly elevated serum levels of dopamine in diabetic mice. Finally, pretreatment of primary bone explant cultures with the dopamine receptor inhibitor amisulpride restores the impaired GIP stimulated increases in LOX expression in diabetic bone cells. These results define for the first time a potential mechanism for diabetic osteopenia in which elevated serum dopamine levels arising from peripheral tissues under diabetic conditions attenuate GIP-stimulated LOX production in osteoblasts, resulting in decreased bone strength, and the increased fracture incidence typical of diabetic bone disease. **Conclusion:** Interference with dopamine signaling would likely restore bone health in diabetes.

Edge Chipping Analysis of CAD/CAM Bur Milled Block Materials

Hetaf Redwan, Richard Pober, Yuwei Fan and Russell Giordano

Department of Restorative Sciences and Biomaterials

Objectives: To evaluate the edge chipping of different CAD/CAM dental materials using a three-axis milling machine. **Methods:** Five CAD/CAM dental materials were used in this study: 1. Lithium disilicate glass ceramic, IPS e.max CAD, Ivoclar Vivadent. 2. Leucite-reinforced glass ceramic, IPS Empress CAD, Ivoclar Vivadent. 3. Interpenetrating Phase Ceramic, Enamic, VITA. 4. Feldspathic porcelain, Vitablocs MarkII, VITA. 5. Nanoceramic filled composite, Lava ultimate, 3M. Rectangular bars with dimensions of 4 mm x 2 mm x 14 mm were designed using the Sirona inLab software system (SW 4.2.5). Ten specimens from each material were milled by CEREC inLab MC XL using a new set of burs for each material. Three bars at the milling sequence number 1, 5 and 10 were selected for the edge chipping analysis. All the edges of the selected bars were imaged using a scanning electron microscope (SU6600, Hitachi High Tech, Ltd, Tokyo, Japan). The length, depth, and area of each edge chip on the specimens were measured using an image processing software Quartz PCI Version 9. The chip measurements were recorded with the corresponding milling surface (cylinder bur surface and step bur surface) and edge locations (top, right and left edge). The chipping factor (ratio of the sum of chip lengths to total edge length) and chipping area of different surface locations and different materials were analyzed by one-way ANOVA and post hoc Tukey test to detect the difference between groups using JMP13.0 with $\alpha=0.05$. **Results and Conclusions:** Chipping factor and chipping area were affected by material type. Conventional ceramic materials had significantly higher chipping factor values than composite resin and interpenetrating phase materials. Step bur surfaces had a higher chipping area value than cylinder bur surfaces.

Effect of Inorganic Phosphate on the Proliferation and Odontogenic Differentiation of Human Dental Pulp Cells

Jomana. Alsenan and Laisheng Chou

Department of Restorative Sciences & Biomaterials

Introduction: This study was designed to investigate the effect of inorganic phosphate (Pi) at different concentrations on odontogenesis of the normal human dental pulp cells (hDPCs). **Method**: hDPCs derived from extracted teeth were cultured in growth media with supplements of Pi in 0, 2, 4, 5 and 8 ppm, for the time intervals of 16 hours, 7, 14, and 21 days. Cell proliferation rates were measured by the optical density of crystal violet dye stained cells. ALP activity was measured by fluorometric assay. Expression of Dentin sialoprotein (DSP) was measured by ELISA. The data were presented as the mean of triplicates and normalized on a per million cell basis. Statistical analysis was conducted using JMP Pro 12 (ver. 12.1.0) in one-way ANOVA and Tukey HSD post-hoc tests. **Result**: Cell attachment efficiency was reduced significantly by additional Pi of 2, 4 and 5 ppm ($P<0.05$). At 21 days, cultures with 2, 4 and 5 ppm supplemental phosphorous displayed significantly higher cell proliferation rates compared to the control group at day14 ($P<0.05$) and at day 21 ($P<0.05$). At day 7, cultures with 2, 4, 5 and 8 ppm supplemental Pi yield significantly higher levels of ALP activity ($P<0.05$) compared to the control group. At day 7, cultures with 5 ppm Pi supplement showed significantly higher levels of DSP expression ($P<0.05$) compared to the control group and the rest of the other groups. **Conclusion:** Supplemental Pi in concentration of 5 ppm could significantly induce proliferation and odontogenesis of hDPCs. This is the first report to demonstrate Pi-induced odontogenesis, leading to potential development and clinical application of future Pi containing dental pulp capping or root canal filling materials.

Spontaneous Pancreatitis in a Novel Mouse Model With aTrypsinogen Mutation

Andrea Geisz and Miklos Sahin-Toth

Department of Molecular & Cell Biology

Background: Premature intrapancreatic activation of trypsinogen has been implicated in pancreatic injury and chronic pancreatitis. Heterozygous mutations in the activation peptide of human cationic trypsinogen have been identified in patients with hereditary pancreatitis. Biochemical analyses of trypsinogen activation peptide mutants revealed that the common phenotypic change is a markedly increased propensity for autoactivation. Earlier we tested whether mutations in the activation peptide of the mouse T7 trypsinogen recapitulate the human phenotype. Our in vitro data showed that T7 mutant D23A exhibited approximately 50-fold increase in trypsinogen autoactivation. Aim: Our aim was to generate and characterize a novel T7 trypsinogen knock-in mouse model of human hereditary pancreatitis, which develops pancreatitis spontaneously. **Methods:** T7 D23A knock-in animals were generated by homologous recombination. Wild-type and heterozygous animals carrying the D23A mutation (T7D23A) were sacrificed in different ages and were analyzed for spontaneous pancreatitis. Histological features of pancreatitis (acinar cell loss, duct dilations, tubular complexes and inflammatory cell infiltration) were evaluated on hematoxylin-eosin (HE) stained sections. Fibrosis was detected by trichrome staining. Plasma amylase levels and intra-pancreatic trypsin activities were measured using kinetic assays. **Results:** Animals carrying T7D23A mutation showed significant changes in their pancreas starting from 3-4 weeks of age. HE stained pancreatic sections of 3-4 weeks old mice displayed extensive pseudotubular complexes and inflammatory cells. Elevated amylase levels and increased pancreas weight measured in 5 weeks old T7D23A animals indicate the occurrence of an acute pancreatitis attack. With progressing age we observed complete disorganization of the pancreas with loss of acinar cells, dilatation of ducts, fibrosis and replacement with adipose tissue. We found significantly higher intra-pancreatic trypsin activity in T7D23A mice relative to wild type controls. **Conclusions:** The T7D23A mouse is the first animal model that exhibits early onset spontaneous pancreatitis due to a trypsinogen mutation offering convincing evidence that increased trypsinogen autoactivation is a pathologically relevant mechanism in hereditary pancreatitis.

Chymotrypsin Like Protease 1 Knockout Mouse

Dora Mosztbacher, Zsanett Jancso, Miklos Sahin-Toth

Department of Molecular & Cell Biology

Background. Chymotrypsin is a digestive enzyme, produced by the pancreas as four isoforms (CTRB1, CTRB2, CTRC, CTRL1). Chymotrypsin C (CTRC) degrades trypsinogen, thereby it protects the pancreas against premature trypsinogen activation. Loss of function mutations in the CTRC gene contribute to development of chronic pancreatitis (CP). Mutations in chymotrypsin B (CTRB) gene may also have an effect on the development of CP while the role of chymotrypsin like protease 1 (CTRL1) in CP is unknown. **Aims.** Our aim was to investigate the effect of Ctrl1 deletion on pancreatitis responses in a mouse model. **Methods.** A Ctrl1 knockout (KO) mouse model was created with CRISPR Cas9 technique. Successful deletion of Ctrl1 was verified by reverse transcription (RT) PCR and western blot. Total trypsinogen and chymotrypsinogen content were measured by enzymatic assay. To generate pancreatitis we used intraperitoneal cerulein injection 10 times hourly. We measured pancreas mass, pancreatic water content (edema), plasma amylase activity, pancreas MPO and evaluated histology sections for edema, inflammatory cell infiltration and necrosis. We measured pancreas trypsin and chymotrypsin activity 30 minutes after a single injection of cerulein. **Results.** RT-PCR and western blot demonstrated no Ctrl1 expression in the KO strain. Total trypsinogen and chymotrypsinogen content was comparable between wild-type and KO mice. In experimental pancreatitis studies, we found no significant difference in pancreas mass, edema, plasma amylase activity and histological evaluation between wild type and Ctrl1 KO strains. **Conclusion.** Ctrl1 is expressed at low levels in the pancreas and plays no role in the development of acute pancreatitis.