Prevalence of Tooth Decay amongst Children Enrolled in the Centro de Desarollo Infantile Preschool Program of Estelí, Nicaragua

Nicholas Capezio and Michelle Henshaw, Global and Population Health

The purpose of this project was to assess the oral health status of children in Esteli, Nicaragua and to create a preventative program in order to best address the community needs. An oral health needs assessment of elementary and preschool children was conducted. Dental decay (caries experience) was found in 51% of children aged 1-5 years and 83% of second and third grade students. Despite the existent school-based fluoride rinse program in Esteli's elementary schools, by second grade the children had significant dental decay. This suggests that implementation of a fluoride varnish program in the government sponsored, Centro de Desarollo Infantile (CDI) preschool program of Estelí, Nicaragua should be the next step towards improving the oral health of Esteli's children.

Salivary Biomarkets and Cardivascular Disease: A Systematic Review

Vishal Gohel, Carolyn Wehler and Judith Jones, Center for Clinical Research

Objective: The purpose of this systematic review is to understand the associations between salivary biomarkers and cardiovascular disease status. Methods: An advanced search was conducted using MeSH terms related to salivary biomarkers and cardiovascular diseases ((("Saliva"[Mesh]) AND "Biomarkers"[Mesh]) OR salivary biomarkers) AND ("Cardiovascular Diseases"[Mesh] OR cardiovascular disease). These terms were entered into the PubMed, Web of Science, and Google Scholar search databases. After duplicates were removed, the searches yielded a combined total of 372 titles. Our inclusion criteria for the search were articles that directly tested the association between specific salivary biomarkers and cardiovascular disease. Exclusion criteria were articles published before 1980, articles that were not in English, reviews, and any articles that did not distinctly separate study populations by periodontal health (confounding biomarker levels). With these criteria, 372 titles were narrowed to a final 140. The abstracts of the final titles were then reviewed for relevance, and a total of 38 abstracts were chosen for full text review. Based on thorough review of three separate reviewers, 37 papers were selected to be included in this systematic review. Results: Cardiovascular disease populations included participants with acute myocardial infarction, acute ischemic stroke, and coronary artery disease. Several salivary biomarkers are different between the healthy population compared to a cardiovascular disease population. The top biomarkers studied include salivary C-reactive protein, troponin I, lysozyme, matrix metalloproteinases 8 and 9, cortisol, and creatine kinase MB. Although there is conflicting evidence between studies, most showed statistically significant increases in salivary levels of the above biomarkers in relation to healthy control levels. Several other biomarkers were studied, but data were too limited to gather any meaningful conclusions. Summary and future research: Current studies are insufficient to make definitive conclusions. While current research has shown that there is indeed an association between some salivary biomarkers and cardiovascular disease, the details of existing studies are conflicting. There may be several reasons for discrepancies among different research groups, and our review continues. Next steps in our review process will be to use the Newcastle-Ottawa Quality Assessment scale to score the quality of our accepted articles. This scale will allow us to evaluate bias in the methods and participant selection of these studies, which will give us a better idea of how to weigh the collection of results and draw better conclusions. Our review can then be used as a baseline reference for future salivary biomarker studies. Despite the aforementioned limitations, the diagnostic potential of saliva shows promise as a non-invasive means of cardiovascular risk assessment.

Introducing the Next Level in Ethics: Understanding Cultural Diversity

Jasmine Khedkar and Larry Dunham, Department of General Dentistry

United States is a nation of immigrants. Most Americans can trace their ancestry to some part of the world. With the growing number of first generation immigrants, to address the issue of access to oral health it is a moral obligation for healthcare professionals to understand cultural diversity and cater to the needs of the underserved thus decreasing healthcare disparities. Understanding cultural diversity will not only help in increasing the access to care for patients, but will in turn help the dentists to follow the four principles of ethics and increase patient satisfaction thereby getting more referrals and build a profitable practice. Most immigrants prefer to go to healthcare professionals who have a similar background as they feel that they will get better care as the provider will be more sensitive and caring. With 200 countries migrating to USA, it's not possible to know every culture, but the essence or bottom line is to generate a higher level of care, sensitivity and awareness among healthcare professionals.

Assessment of Mirror Image Facial Asymmetries in Monozygotic and Dizygotic Twins

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Background: Mirror imaging in identical twins has long been noted and suggests that left-right asymmetry may become established early in embryogenesis. However, it is not known whether the clinical reports of “mirroring” in twins are annulled by an equal number of cases lacking mirroring. We aimed to determine whether significant mirror imaging occurs in transverse facial asymmetries in monozygotic and dizygotic twins. Material and Methods: The sample included PA cephalograms from 54 pairs of monozygotic twins and 57 pairs of dizygotic twins from the Forysth/Moorrees Twin Study. The films were digitized and anatomical landmarks identified. Using TPSDig2, linear measurements were traced between lateral and midline landmarks. Linear measurements of one twin were reflected and superimposed to compare to the second twin. A 2-tailed t-test was performed on reflected and superimposed data to determine the differences between monozygotic and dizygotic twins. Results: No significant difference in mirror imaging is present in transverse facial asymmetries between monozygotic and dizygotic twins. 8 of 14 facial regions exhibited mirroring in monozygotic twins compared to 6 of 14 in dizygotic twins. Therefore, mirroring is not a statistically significant phenomenon occurring in monozygotic twins. All 14 facial regions fit superimposed in monozygotic twins versus 7 or half in dizygotic twins. Transverse facial asymmetries were better fit non-reflected as opposed to reflected in monozygotic twins. Conclusion: From this study we can conclude that mirroring in craniofacial asymmetries does not occur any more frequently in monozygotic twins than dizygotic, and therefore does not appear to be a unique pattern to add to the previous evidence. However, it was revealed that monozygotic twins did exhibit significantly more facial regions that were identical in their asymmetries compared to dizygotic twins.

Grain Size of YTZP Zirconia with Various Sintering Schedules

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Grain size of YTZP Zirconia with Various Sintering Schedules Objectives: The flexural strength of zirconia with various sintering schedules has been determined from previous study. In order to understand the origin of such different physical properties, grain size has been observed and measured. Materials and Methods: VITA YZ zirconia was sectioned into bend bars and sintered to produce specimens approximately 4 x 3 x 25 mm. These were randomly sorted into various groups for sintering according to Table 1. After three point bending test, grain size has been observed and measured using Hitachi SU6600 SEM. Statistical analysis was performed using Tukey HSD test at $p < 0.05$. Conclusion: Rapid sintering cycles produce significantly smaller grain size than standard sintering cycle. Although smaller grain size should increase physical properties in theory, many physical defects (voids in zirconia samples from rapid sintering groups) significantly lower its flexural strength compared to standard sintering cycle.

The Photodynamic Therapy (PDT): A New Face of Periodontal Treatment

Jignesh Rudani, Advanced Standing Program

The PhotodynamicTherapy (PDT): A New Face of Periodontal Treatment J Rudani, Boston University, Boston Objectives: Photodynamic therapy (PDT) is a light induced non-thermal inactivation of cells, microorganisms which can be used in adjunct with mechanical therapy for treatment of periodontitis. Photodynamic action describes a process in which light, after being absorbed by dyes, sensitizes organisms for visible light induced cell damage called photo lethal sensitization. The goal of this study is to investigate the possible role of PDT in the treatment of periodontitis as an adjunctive therapy. Methods: A systematic review of the literature was performed. Also, the references from all the selected full-data studies were searched for relevant articles. Conclusion: Within the limitations of this review, different clinical studies indicate that PDT has the potential to kill pathogenic bacteria and inhibit destructive host responses and this may contribute to its clinical usefulness as an adjunct to mechanical therapy in treatment of periodontitis. In the future, long-term randomized clinical trials are needed to be conducted.

HbA1c Levels in Non-diabetic Patients: A Comparative Study in Participants with and without Periodontits

Mansi Shah and Hesham Nouh, General Dentistry

Objectives: Several studies have observed the relationship between glycosylated hemoglobin (HbA1c) and periodontitis in diabetics but very few studies have showed the relation between HbA1c level in non-diabetics and periodontitis. This study was aimed to assess the association between periodontitis and HbA1c levels in non-diabetics. To achieve this aim, the study compared participants with and without periodontitis, before and after periodontal therapy (scaling and root planning). Methods: This comparative study consisted of 20 non-diabetic participants, aged 35 to 65 years old. Participants were divided into two groups based on the periodontitis status: Group I had10 participants without periodontitis (healthy), and Group II (diseased) consisted of 10 participants with periodontitis. Different clinical parameters like gingival index, probing pocket depth, body mass index, clinical attachment level and HbA1c levels were measured for all the participants. Each participant received non-surgical periodontal therapy and they were examined after three months for all the clinical parameters. Results: Statistically significant differences were observed between the healthy and diseased groups for gingival index, probing pocket depth and HbA1c levels after therapy. In healthy group, there was no significant difference in clinical attachment loss was noted after three months. However, the participants in the diseased group showed improvement in the clinical parameters at the end of therapy. Though their HbA1c level also decreased significantly, the values were not the same as those of the healthy participants. Conclusions: HbA1c levels of non-diabetic participants with periodontitis reduced significantly after three months of non-surgical periodontal therapy, although levels never reached that of the non-diabetic participants without periodontitis.

The Top Five Salivary Biomarkers in Periodontal Diseases: A Systematic Review in Progress

Kushal Zinzuvadia, Heidar Zohrehei, Kasra Dabeshim, Eleni Kanasi, Simran Grover and Judith Jones, Center for Clinical Research

Objective: The purpose of this systematic review is to examine the association of salivary biomarkers and periodontal disease. Methods: An advanced search was performed by using PubMed as MeSH database. Preliminary search with use of two key words (Salivary biomarkers and periodontal disease) yielded combined total of 764 studies. Articles were excluded if they were not available in English, studies in children, studies in animals and abstracts that were limited or irrelevant. After applying our inclusion-exclusion criteria, the search result narrowed down to 72 studies. All selected articles were reviewed and abstracted. Result: More than one hundred different biomarkers have been used in these articles; however, this study focuses on the most common salivary biomarkers. IL1-β is the most common biomarker (14 studies) and generally increased IL1-β in periodontitis except 3 studies that showed either a decrease or no change in levels. Matrix Metalloproteinase 8 (MMP-8) was the second most common biomarker and generally showed increase in periodontal disease except two studies that showed either decreased or no change in levels. Tumor Necrosis Factor Alpha (TNF-α) increased in periodontitis except 2 studies that showed either a decrease and 6 studies that showed no change in evels.IL 6 increased in 4 studies; however, it did not show any changes in remaining 4 studies. 8-OHdG increased in all studies. Next Step: The top five biomarkers in saliva associated with periodontal disease will be assessed for quality using the Newcastle-Ottawa Quality Assessment Scale

The Second Five Salivary Biomarkers in Periodontal Diseases: A Systematic Review in Progress

Heidar Zohrehei, Kushal Zinzuvadia, Kasra Dabeshim, Eleni Kanasi, Simran Grover and Judith Jones, Center for Clinical Research

Objective: The purpose of this systematic review is to examine the association of salivary biomarkers and periodontal disease. Methods: An advanced search was performed by using PubMed as MeSH database. Preliminary search with use of two key words (Salivary biomarkers and periodontal disease) yielded combined total of 764 studies. Articles were excluded if they were not available in English, studies in children, studies in animals and abstracts that were limited or irrelevant. After applying our inclusion-exclusion criteria, the search result narrowed down to 72 studies. All selected articles were reviewed and abstracted. Result: More than one hundred different biomarkers have been used in these articles; however, this study focuses on the most common salivary biomarkers. LDH generally increased in 3 studies, however, one study showed decrease and one study reported a significant lower concentration among smokers. AST is the next common biomarker being used and generally showed increase with exception of one study that reported decrease in its level. MMP9 Showed increased in all studies without any exception. Albumin Generally Increased in periodontitis , however one study showed decrease post treatment. Next biomarker ALT, showed increase in two studies and decrease in one study; however one study reported no significant changes. Next Step: The top five biomarkers in saliva associated with periodontal disease will be assessed for quality using the Newcastle-Ottawa Quality Assessment Scale.

Effect of Different Piezotome Settings on 2D Cell Culture Systems and Impact of Surgery with Piezoelectric Knife on Bone Biology

Fahad Aljamal, Serge Dibart, Erdjan Salih, Department of Periodontology

Objective: To determine the effects of Peizotome with different puissance (power) and vibrational frequencies on 2D confluent and non-confluent cell culture models and impact of clinically used surgical piezoelectric knives/devices on bone biology. Materials and Method: Prostate PC3 cancer cells were cultured in 12 well plates and two experimental approaches used were: (a) sets of confluent cells, and (b) sets of cells in suspension at seeding, to be subjected to different piezotome settings. A set of cells in each group (a) & (b) were treated with either 255 or 50 VA fixed puissance (power) but different modulations (vibrational frequencies) 10, 30 and 100 Hz, respectively, for fixed time of 5 seconds. The controls were corresponding sets of cell wells with no piezotome treatment. For live bone surgical defects neonatal mouse calvarial bone organ cultures were used with the same settings of piezotome puissance and modulations as those for the cell cultures above. The impacts of piezotome on cell cultures were evaluated by quantitative counts of cell survival and on the bone surgical defects by bone repair/healing and bone resorption. Result: Counting of media cells and those attached to plate for cell survival showed major differences in the impact generated by puissance 255 *versus* 50 with the same modulations of 10, 30,100 Hz. The degree of sensitivity of confluent cells *versus* cells in suspension for the same puissance and modulations used was also very diffreent. Furthermore, repeat treatments of 5 seconds led to progressive decrease in cell survival. In live bone organ culture surgical defects the repair/healing was significantly enhanced when piezoelectric was used with puissance 255 and modulation 30 Hz as compared with using traditional diamond bur for defect generation. Conclusion: This study revealed that the effect of piezotome with puissance 250 VA is much stronger on both cells in suspension and those attached to the plate than 50 VA. The effect of multiple 5 second piezotome treatment was also more influential on unattached cells than attached cells with only marginal effects of the different vibrational frequencies. In live bone microenvironment the piezoelectric device may be used to enhance bone formation/healing or resorption with appropriate selection of the specific puissance and vibrational frequency combinations.

Lysing Specific Demethylase 1 is a Potential Target of Epigenetic Oral Cancer Therapy

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Oral cancer statistics are dismal: half of oral cancers are not diagnosed until cancer has spread to nearby tissues, at which stage the 5-year survival rate is ~ 50 percent. Oral squamous cell carcinoma (OSCC) often evades traditional treatment strategies due, in part, to aberrant epigenetic modifications and to the generation and expansion of a subpopulation of immature cells with “stem cell-like” properties that have the ability to self-renew and expand, referred to as cancer-initiating cells (CICs). Aberrant epigenetic modifications by lysine-specific demethylase 1 (LSD1) have been shown to regulate active chromatin states leading to cancer growth and metastasis in lung, liver, and esophagus. Recently, we showed that LSD1 expression was increased in non-metastatic and metastatic OSCC orthotopic mouse models compared to vehicle injected mice. The goal of this study was to evaluate if inhibition of epigenetic modifications induced by LSD1 overexpression could block oral cancer proliferation, growth, and metastasis. Methods: Immunostaining analyses were performed with the anti-LSD1 antibody. The effect of LSD1 loss on function was evaluated by LSD1 shRNA compared to non-target shRNA, and a small molecule inhibitor of LSD1 in oral cancer cell line- and patient-derived primary cells by RT-qPCR and microarray analysis. Finally, loss and gain of function studies were performed in orthrotopic oral cancer mouse models. Results: Examination of the association of LSD1 gene expression and histological tumor grades from TCGA oral cancer mRNASeq datasets of 41 morphologically normal and 89 OSCC specimens revealed that LSD1 was upregulated in OSCC. Immunohistochemistry analysis performed on 3 clinical OSCC specimens showed that LSD1 protein expression was increased in tumor tissues compared to hyperplasia and dysplasia. The knockdown of LSD1 with shRNA in metastatic HSC3 and nonmetastatic CAL27 cells showed inhibition of cell proliferation compared to non-target shRNA. LSD1 specific inhibitors LSD-C76 (Xcessbio) and LSD-GSK1 (GlaxoSmithKline) significantly reduce tonsillar epithelial and osteosarcoma primary tumor cells proliferation. Microarray analysis of patient-derived tonsilar epithelial, myoepithelial, osteoacarcinoma cells treated with LSD1-GSK inhibitor showed unique gene signature each cell type and globally inhibits oncogenes such as CTGF, FGF5, HAS2, ETS1, SERPINE1, LMO7, DDAH1, IL7R, NPR3, KIT, AKT1, RAB11B, MYC, CBL and others. Orthotpic injection of HSC3 cells overexpressing LSD1 promoted tumor growth and metastasis in nude mice, whereas, orthotopic injection of HSC3 cells expressing LSD1 shRNA showed a significant reduction in tumor growth and metastsis compared to nontarget shRNA expressing cells. We are evaluating the mechanism of small molecule LSD1 inhibitor in patient-derived orthotopic oral cancer mouse models. Conclusion: LSD1 is a key regulator of OSCC in vitro and in vivo. A small molecule inhibitor of LSD1 regulates unique gene signatures in different patient derived primary cells. The gain of LSD1 function promotes, and loss of LSD1 function inhibits growth and metastis of HSC3 cells in vivo. Thus, LSD1 could be a potential target for epigenetic OSCC therapy. Support: CTSI and EPOC ARC funding AU 5303015 8000000.

Association between Alcohol Consumption and Periodontal Disease among the US Population

Mishali AlSharief and Elizabeth Kaye, Department of Health Policy and Health Services Research

Introduction: In this report, we examined the association between alcohol consumption and periodontal disease in the US adult population using combined data from the 2009 to 2010 and 2011 to 2012 cycles of the National Health and Nutrition Examination Survey NHANES. Methods: From the NHANES database, we included dentate adults, aged 30 years and above who also completed the two-day dietary recall. Our exposure of interest was alcohol consumption while our outcome was periodontal disease using the CDC/AAP recommended case definitions described by Eke et al, 2012. Logistic regression was used to estimate odds of periodontal disease in categories of 1-13g, 14-27g and ¡Ý 28g of alcohol vs. nondrinkers. Results: After controlling for ethnicity, age, poverty income ratio, smoking and diabetes results showed no consistent significant associations between the level of alcohol consumption and periodontal disease. Conclusions: Although a positive association between alcohol consumption and periodontal disease has been reported in a number of studies, our results do not confirm those associations. Longitudinal studies are needed to better assess this association.

Degradation of Gliadins in the Gastro-intestinal Tract of Mice

Ghassan Darwish, Chin-Hua Yang, Na Tian, Guoxian Wei, Eva J. Helmerhorst, Department of Molecular and Cell Biology

Introduction. Celiac disease is a chronic immune-mediated inflammation of the duodenum, triggered by gluten contained in wheat, barley and rye. Our previous studies have shown that Rothia, Gram-positive oral bacteria, have the ability to degrade and detoxify gluten in vitro. The objective of this study was to test if R. aeria bacteria can degrade gluten that is naturally contained in mice food in vitro and in vivo. Methods. Gluten digestion in vitro was monitored in mice chow with and without added R. aeria bacteria (OD620 200 per 1 g of chow) after 0, 2 and 4h incubation. For the in vivo experiment, two balb/c mice were fed with chow with and without added R. aeria bacteria and sacrificed after 2 h. The stomach, duodenum, jejunum and ileum contents were harvested and gluten degradation was assessed by SDS PAGE and immunoblotting, and with the G12 ELISA assay detecting immunogenic gluten epitopes. Results. In vitro, gliadins were stable in food incubated in the absence of R. aeria, but degraded within 2h in the presence of R. aeria. In vivo, fasted mice readily consumed the 1 g food without or with the added bacteria. After 2h, the distribution of the chyme in the stomach and the small intestines was determined to be approximately 64%/36%. Gliadins were detected by immunoblotting in the stomach, but not in the small intestinal samples, of both the control and the R. aeria-fed mice. G12 ELISA results provided preliminary evidence that gluten epitopes in the duodenum, jejunum and ileum of R. aeria-fed mice were reduced as compared to the control mice. Conclusion. These pilot study results provide the basis for further investigation to fully establish the extent to which R. aeria can digest and abolish immunogenic gluten epitopes in vivo. Supported by NIH/NIAID grants AI087803 and AI101067.

Measuring Divergency in the Frontal Plane

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Objectives: The aim of this study was to determine if there is any correlation between divergency and the transverse width of the maxilla and mandible at the first molar level, and angulation of 1st molar teeth. Methods: CBCT images of 83 patients were selected from Boston University¡¯s repository for this retrospective study. Patients with: Previous orthodontic treatment, Angle Class II or Class III malocclusion, missing any teeth excluding third molars, and with visible molar angulations outside normal values were excluded. The images were analyzed by Dolphin imaging software and grouped into hypo-, normo- and hyperdivergent groups with Frankfort horizontal to Mandibular plane angle (FMA) <22, ¡Ý22-¡Ü28, >28 degrees respectively. Angulations of the right and left maxillary and mandibular molars to occlusal plane, palatal width and lingual width at first molar level were measured. The differences between maxillary and mandibular widths and their ratios to the actual widths were calculated as well. One-way ANOVA and post-hoc Tukey HSD tests were used for statistical analysis to determine if there is any significant difference between the divergence groups in the above-mentioned measurements. Results: Preliminary results show that only 2 of the 9 initial measurements were statistically significant. The mean left maxillary molar angle was significantly low ($p<0.05$) and the mean palatal width was significantly high ($p<0.05$) in hypodivergent patients when compared to the hyperdivergent group. Conclusions: Clinicians need to embrace that the patients that fall on either end of the spectrum i.e. hypodivergent and hyperdivergent will have to be treatment planned differently.

Determine Optimal Culture and Dosage Conditions for Rothia Bacteria Degrading Gluten in the TIM-1 human Digestion Model

Chin-Hua Yang, Ghassan Darwish, Na Tian, Guoxian Wei and Eva J. Helmerhorst, Department of Molecular and Cell Biology

Introduction. Celiac disease is a T-cell mediated-inflammatory disorder of the small intestine precipitated by gluten ingestion. Gluten can be effectively degraded by Rothia aeria, a natural resident oral microbe. We hypothesized that Rothia bacteria can degrade and detoxify gluten in vivo and can be developed as a first probiotic for celiac disease. Aims. To select the optimal culture conditions for R. aeria enzyme expression in vitro and determine the required R. aeria to gluten ratio to achieve digestion within 2h, the residence time of foods in the stomach/upper intestines. Methods. R. aeria culture variables evaluated were dilutions of Brain Heart Infusion (BHI; 4%, 20% and 100%), temperature (28oC and 37oC), carbon sources (glucose, succinate, glycerol, or casein) added to M9 minimal salts, and cultivation time (16-96h). Enzyme activities in suspensions normalized for optical density (OD) were measured with a paranitroanilide-derivatized enzyme substrate. Gliadin degradation was investigated with a fixed gliadin concentration (250 µg/ml) and various R. aeria cell densities (OD620 1.0, 0.5, 0.25), and was monitored by SDS-PAGE. Results. Enzyme activity was minimally affected by BHI broth strength. Cells grown at 37oC showed on average a higher enzyme activity than cells grown at 28oC. No bacterial growth was observed in M9 broth +2% glucose, succinate, or glycerol. Enzyme activities in M9+2% casein were lower than in BHI. As the incubation time in BHI progressed, cell-associated enzyme activities decreased. Rapid gliadin degradation was observed in R. aeria suspension with an OD620 of 1.0. Conclusions. Full strength BHI broth and 48h cultivation at 37°C were ultimately chosen as the cultivation condition to obtain high R. aeria cell numbers. Based on the dosage experiments a 1:1 ratio of R. aeria OD620 1.0mg gluten was chosen for future studies in an in vitro human digestion model. Supported by NIH/NIAID grants AI087803 and AI101067.

The Role of BMP Signaling in Deriving Endothelial Cells From Human Pluripotent Stem Cells

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RATIONALE Mutation of the BMPRII receptor is believed to contribute to heritable pulmonary arterial hypertension (hPAH) in part by affecting pulmonary endothelial cell (ECs) phenotype and function. It is still unknown if this mutation is affecting the early embryonic processes involved in EC development or solely affects adult ECs after being exposed to environmental stresses. Recently a protocol has been published for differentiation of human induced pluripotent stem cells (hiPSCs) into ECs using 4 factors; Activin, FGF2, VEGF and BMP4 (ligand to BMPRII). The aim of this project is to study the role of BMP4 in EC development and derivation using hiPSCs generated from normal controls as well as from patients with hPAH. Methods: hiPSCs were differentiated in vitro using a media containing 10ng/ml of Activin, FGF2, VEGF and BMP4 to induce mesoderm over 4 days, followed by VEGF and FGF2 to subsequently specify ECs. By day 12, cells that expressed CD31, CD144 and KDR were sorted and replated in endothelial maintenance media. Time series characterization was performed by FACS and RTqPCR to quantify mesodermal and endothelial marker genes. Results: FACS and RTqPCR results suggest hiPS cells in this protocol differentiate first into a posterior primitive streak like stage (indicated by high T and low FOXA2 expression), then into a heterogeneous population of mesodermal subsets (day 4). Subsequently, lateral plate mesoderm cells predominate over intermediate or paraxial mesoderm, and by day 12 cells expressing mesodermal (KDR) and endothelial markers (CD31 and CD144) can be sorted to purity and maintained for several passages in culture. To understand the role of BMP4 in this process, each factor was withheld from various stages of the protocol. Results indicate that before day 4, BMP4 alone is necessary and sufficient to direct cells through posterior primitive streak into mesodermal progenitors that upon subsequent VEGF/FGF2 exposure can give rise to CD31+CD144+KDR+ endotheliallike cells by the end of the protocol. BMP4 is dispensable after day4, whereas VEGF and FGF2 are dispensable in first 4 days of the protocol. hiPSCs from patients with BMPR2 mutations form endothelial cells in this protocol, but whether their efficiency of differentiation or phenotype is perturbed remains in question. Conclusion This protocol replicates the developmental pathways of EC derivation in embryos, and suggests that BMP4 is necessary and sufficient to derive mesodermal subsets with EC competence but is dispensable for subsequent endothelial lineage specification.

Oncogenic Roles of N-glycosylation-Wnt/β-catenin Axis in Oral Cancer

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Introduction: Oral squamous cell carcinoma (OSCC) accounts for the majority of head and neck cancers, for which five-year overall survival rates are currently ~65 with few therapeutic options available. Evidence suggests that aggressive cancers arise from populations of cancer initiating cells (CICs) that exhibit the properties of stem cells and may drive tumor development and resistance to therapy. The Wnt/β-catenin signaling cascade and protein N-glycosylation have been shown to play important roles in stem cell phenotypes. Our studies have shown that increased N-glycosylation activates Wnt/β-catenin signaling and tumor spheroid growth in OSCC cells. We hypothesized that aberrant activation of N-glycosylation-Wnt/β-catenin axis in oral tissues drives the CIC state and promotes OSCC progression to aggressive disease.
Methods: We have applied advanced 12-color flow cytometry with cell sorting (FACS) for simultaneous single cell phenotypic characterization and purification of oral CICs from OSCC cell lines and fresh human tumor specimens. The roles of N-glycosylation and β-catenin signaling were determined by genetic and pharmacological knockdown and examination of mRNA and protein levels using qPCR, immunoblots and immunofluorescence imaging, and orthotopic tumor growth in nude mice. Results: FACS sorting of metastatic OSCC SCC2 and non-metastatic CAL27 cells using lectins PHA+ (high N-glycosylation) and ConA+ (low N-glycosylation), revealed a much greater fraction of PHA+ SCC2 cells than CAL27 cells, suggesting that increased N-glycosylation was associated with OSCC metastatic phenotypes. These PHA+ cells were characterized by CD44+ CD166+ primitive cell surface markers, also found in fresh primary human OSCC specimens. Inhibition of β-catenin-CBP interaction with ICG-001 reduced DPAGT1/N-glycosylation and led to growth arrest, suggesting that N-glycosylation functioned along the Wnt/β-catenin-CBP axis in promoting oral CICs. Further, treatment of human SCC2-cell line-derived orthotopic tongue tumors with ICG-001 in nude mice reduced tumor growth and metastases. Conclusion: We have applied high resolution 12-color FACS to phenotype oral CICs and evaluate functional significance of N-glycosylation-Wnt/β-catenin axis. Results suggest that N-glycans can serve as powerful detection tools for more aggressive OSCC. In addition, N-glycosylation likely collaborates with β-catenin/CBP signaling to maintain oral CICs, and ICG-001 may represent a novel therapeutic for the treatment of OSCC in humans.
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Loss of Fam20a Leads to Dwarfism Due to Proliferation and Maturation Defects in Chondrocytes

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Background: Family with sequence similarity 20, member A (Fam20A) has been recently identified as a Golgi kinase and reported to play important roles in the biomineralization process and chondrocyte function. Previously the Fam20a global knockout (KO) mouse model was generated and homozygous KO mice showed growth retardation, however, how FAM20A is involved in the developmental process of long bone remains unknown. Objective: Our objective was to investigate the morphological and molecular changes in the endochondral ossification process by using Fam20a KO mice. Methods: Homozygous Fam20a KO mice were used in this study and compared to wild type (WT) littermates. Skeletal tissues of WT and KO mice were stained with alcian blue and alizarin red and morphologically compared at post-natal day (P) 0 (i.e. newborn) and 4 weeks. Femur bones were analyzed by micro-computed tomography (μCT) at 4 weeks. Total RNA was extracted from the growth plates of WT and KO, and the gene expression levels of chondrogenic markers were analyzed by quantitative real time PCR (qRT-PCR) at P7. Histological and immunohistochemical analyses were performed to investigate the extent of chondrocyte proliferation, maturation and apoptosis using femur bones containing growth plates at P0. Statistical analysis was performed using student's t-test. Results: Our data showed that the body size and bone length of Fam20a KO mice were significantly smaller/shorter than those of WT at P0 and 4 weeks. Morphological analysis by skeletal staining showed that Fam20a KO femur bones were smaller and exhibited more delayed ossification than those of WT at P0 and 4 weeks. The μCT analyses of trabecular bone in femur distal metaphysis demonstrated that Fam20a KO mice had lower total volume, lower bone volume and thinner trabeculae as compared to WT at 4 weeks. Cortical bone assessment by μCT in mid diaphysis showed that Fam20a KO had thinner bone cortex with lower bone volume as compared to WT mice. The qRT-PCR data showed the downrgulation of parathyroid hormone-related peptide (PTHrP) gene expression by 8 folds and upregulation of collagen type X by 5.7 folds in Fam20a KO mice as compared to WT, indicating that chondrocyte proliferation activity in KO was decreased, but chondrocyte maturation was accelerated. Histological examination of femur bone revealed that there were disorganization of chondrocyte zones and enlarged chondrocytes in the growth plate of Fam20a KO mice compared to WT. Moreover, immunohistochemical analysis showed that lesser numbers of chondrocytes were positive with anti-Phospho-Histone H3 (PH3) staining as well as cleaved anti-Caspase 3 staining, indicating that chondrocytes in Fam20a KO were less proliferative and apoptotic. The maturation marker, Collagen type X protein was more expressed in the hypertrophic zone of Fam20a KO mice than in WT, indicating the accelerated chondrocyte maturation. Conclusion: Our data demonstrate that deficiency in Fam20a causes disruption in endochondral ossification of long bone development. The data in this study indicate that FAM20A regulates the balance of chondrocyte proliferation and maturation.

Exposure to Tobacco Smoke and Dental Caries among US Adolescents

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Objectives: Exposure to tobacco smoke has been related to many adverse health outcomes. The objective of this study is to investigate the association between dental caries in adolescents' permanent teeth and their exposure to tobacco smoke. Methods: Data from the National Health and Nutrition Examination Survey (NHANES 2001-2004) were analyzed. Subjects were 4399 adolescents aged 11-19 years old who completed dental examination and had serum cotinine results. Participants were classified into unexposed, passive smokers and active smokers based on serum cotinine level. The main outcome variable was caries experience defined as decayed, missing, and filled teeth (DMFT) score of 0, 1-4 or 5 and more. Covariates were demographics, poverty income ratio, household reference-person's education level, total sugar in diet, and usage of dental services. Using SAS 9.4, Chi-square tests and multinomial logistic regression models accounting for the complex survey design of NHANES were used to estimate the prevalence and odds ratios for caries among adolescents with different tobacco smoke exposures. Results: The prevalence of DMFT was significantly different among participants with different tobacco smoke exposures. About 48.6% of non-exposed participants had no caries compared to 44.3% passive smokers and 29.3% active smokers. After adjusting for confounders, exposure to tobacco smoke remained as significant determinant of DMFT (P-value= 0.04). When compared to non-exposed peers, both passive and active smokers were more likely to have a higher DMFT score OR= 1.3 (95%CI: 1.0-1.7) and OR= 1.8 (95%CI: 1.1-2.7) in passive and active smokers, respectively. Conclusions: The findings suggest that exposure to tobacco smoke is associated with higher DMFT among US adolescents. Caries experience seems to increase as the level of cotinine in serum increases. Though longitudinal studies are needed to confirm the association, the findings highlight the importance of attention to the potential dental harms of tobacco exposure among adolescents.

Retrospective Review of Early Bone Loss: Comparison of Three Different Implant Systems

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Aim: To evaluate bone loss at the time of uncovering between three different implant systems in a retrospective study. Material And Methods: 644 implants (390 Nobel Biocare, 190 Straumann, and 64 Biomet 3I implants) placed at one educational institution during the period of January thru December of the year of 2013.The records were reviewed by one independent examiner (not involved in the implant placement or uncovering), using periapical radiographs and clinician notes taken on the day of the uncovering procedure. The Uncovering was performed by the same resident who placed the implant. Results: The percentage of implants with early bone loss was found to be 10%, 5% and 4% for Nobel Biocare, Straumann and Biomet 3I implant systems, respectively. No significant differences for early bone loss at time of uncovering (P value >0.05) were detected between the three systems. Conclusion: Within the limitations of this retrospective study, the three implant systems showed similar incidence of bone loss at time of uncovering.

Inhibition of Lysyl Oxidase like 2 Controls Oral Cancer Progression

Faranak Mahjour, Mahzad Koochaki, Manish V. Bais and Philip C. Trackman, Department of Molecular and Cell Biology

Background: Lysyl oxidase catalyzes the oxidation of lysine residues which is required for elastin and collagen crosslinking and extracellular matrix maturation. This family is made up of five members: lysyl oxidase (LOX) and lysyl oxidase like-1-4 (LOXL1 – LOXL4). Lysyl oxidase family members have been reported to be involved in cancer progression recently due to their excess modification of the extracellular matrix to create microenvironments which become metastatic niches. Lysyl oxidase like-2 (LOXL2) is elevated in oral cancer and promotes metastasis. Cancer associated fibroblasts have a profound influence on the development and progression of carcinomas. The tumor-stroma crosstalk has been shown to play an important role in tumor progression. Here we determine the effects and mechanism of LOXL2 inhibitor on the progression and invasiveness of Oral Squamous Cell carcinoma in vitro and in vivo. Methods: LOXL2 inhibitor was added to human gingival fibroblasts (HGF) which were influenced by four different oral cancer cell conditioned media. The effectiveness of LOXL2 inhibitor on cell proliferation was determined by Proliferation Assay (CYQUANT). Signaling Array Kit and western blot were used to evaluate the mechanism of effect of LOXL2 inhibitor on fibroblasts to attenuate fibroblast proliferation triggered by conditioned media. In addition, human SCC2 cells expressing red fluorescent protein (DsRed) were injected into the tongues of nude mice. LOXL2 inhibitor was injected into mice by Intraperitoneal injection three times a week. Tumor growth and tongue volumes were monitored by caliper measurements, and by in vivo imaging system (IVIS). Proliferation marker levels were assessed in the tumors using immunohistochemistry. Result: Proliferation of gingival fibroblasts significantly increased by cancer cell conditioned media. In the presence of LOXL2 inhibitor in conditioned media, the proliferation decreased significantly. LOXL2 inhibitor decreased the level of phosphorylation of PDGFR at βY771 and βY857/ αY849 sites but not at βY751 site. LOXL2 inhibitor also decreased the activation of Erk 1/2. Tumor growth and metastasis significantly decreased in the cancer mice model by treatment with LOXL2 inhibitor. LOXL2 and proliferation marker (Ki-67) levels in tongue tissue sections decreased by the effect of LOXL2 inhibitor. Conclusion: Phosphorylation of Y771 in PDGFRβ provides the binding site for GTPase activating protein of Ras (Ras GAP). Thereby inhibition of PDGFRβ (Y771) activation inhibits Ras/MAP kinase signaling , ERK 1/2 activation and cell proliferation. LOXL2 inhibitor in conditioned media does not inhibit the activation of PDGFRβ at Y751 phosphorylation site which drive AKT/PI3K pathway. Inhibition of PDGFRβ phosphorylation at Y751 site by LOXL2 inhibitor occurs in the presence of PDGF-BB ligand. It suggests that activation of PDGFR by conditioned media and its inhibition by LOXL2 inhibitor is not caused by PDGF-BB, but occurs by a different ligand. LOXL2 enzyme is critical for oral cancer progression and metastasis. Inhibition of LOXL2 could provide strategies to develop therapeutic drug for the treatment of oral cancer.

A Survey on Opinion and Practice of Regenerative Endodontics among Endodontists and Dental Practitioners in North America

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Objectives: to assess the opinion of endodontists and other dental professionals toward regenerative endodontic procedures (REPs) and to evaluate their preferred technique to perform REPs. Materials and Methods: After institutional review board approval, 85 clinicians practicing in North America participated in a web-based survey (Google Forms). The participants were asked 10 or 17 questions based on their responses during the survey. Participants were asked about their educational level, professional status, opinion toward REPs and their preferred technique to perform REPs Results: Although 20% of participants didn't believe that there is enough evidence to support REPs and 45% were not sure, 76% of them still believe that REPs is a better treatment option than apexification and 69% believe that it could be a future alternative to osseointegaratged implant. The results also showed different preferred techniques to perform REPs, among the results; about one third of the participant (38%) preferred the use of mixture of antibiotics to disinfect the root canal space, while 20% preferred calcium hydroxide. Almost half of the participants (53%) were using 1 mg/ml or above of each antibiotic in the mixture, while 47% are using 0.1 mg/ml. Interestingly, about 41% of the participants believed that they could perform REPs in one visit. Conclusions: While most participants are optimistic about REPs, but they still believe that more evidences are needed to support it. Participants are using different materials and techniques to perform REPs, and most of them are using protocols that differ from the "AAE's clinical consideration for REPs"

Lysyl Oxidase Dysregulation in Diabetic Bone Disease

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Bone is a dynamic tissue which requires intricate regulatory processes that must work in synchrony to maintain a healthy skeleton. Alterations in this regulation can lead to bone disease. Diabetes is characterized by hyperglycemia resulting from a lack of insulin or reduced insulin sensitivity and affects over 20 million people in the United States. Individuals with diabetes experience decreased bone quality and an increased susceptibility to fracture, leading to significant disability and morbidity. Diabetic bone disease primarily results from an anabolic defect in which there is deficient osteoblast activity, in contrast to the mainly catabolic dysregulation seen in osteoporosis. Osteoblasts produce fibrillar collagens during bone formation, which require lysyl oxidase (LOX) enzyme-dependent modification of specific lysine residues and subsequent cross-linking in order to form a functional fibrillar network and confer strength to bone. The number of lysyl oxidase-dependent cross-links in collagen is decreased in diabetes, leading to compromised bone microarchitecture. LOX is also required for the proliferation and lineage-commitment of osteoblast progenitor cells. Incretins are gastric hormones released by intestinal K-cells into the circulation which regulate insulin release from pancreatic beta cells. One of these hormones, glucose-dependent insulinotropic peptide or GIP is anabolic in bone independent of its effects action on the pancreas to stimulate insulin release. Deletion of the receptor for this hormone (GIPR), which is expressed on osteoblasts, results in a reduction in bone strength and material properties in mice similar to the low bone formation osteopenia seen in diabetes. GIP also augments LOX activity in osteoblasts, resulting in an increase in mature, stable collagen cross-links. In diabetes the cellular response to GIP is abnormally low, while serum GIP levels remain the same or are elevated. The working model for the proposed research is that diabetes interferes with GIP-stimulated LOX expression in osteoblasts, leading to the osteopenic phenotype seen in mouse and human diabetic bone. Preliminary data indicate that LOX gene expression is directly up-regulated by GIP in calvarial osteoblasts and that diabetes induction by streptozotocin in LOX +/- mice leads to an exacerbation in trabecular defects beyond those seen in non-diabetic LOX +/- mice. Based on previous studies, we expect that GIP mediated up-regulation of LOX in normal osteoblasts occurs through activation of the PKA/Canonical Wnt pathway by the GIP receptor, a type II G protein coupled receptor. We further propose that the decreased cellular response to GIP in osteoblasts in diabetes is due to dysregulation of the mechanism that normally controls GPCR signaling, the restoration of which could treat diabetic osteopenia.

Vacuolar/Cytoplasmic pH Gradient by Inhibitors of ATP and Ionophores Increase Cell Uptake and Endosomal Escape of the Tumor Suppressor Lysyl Oxidase Propeptide (rLOX-PP)

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The lysyl oxidase propeptide (LOX-PP) is derived from pro-lysyl oxidase (Pro-LOX) by extracellular biosynthetic proteolysis. LOX-PP inhibits breast and prostate cancer xenograft tumor growth and has tumor suppressor activity. Although, several intracellular targets, molecular mechanisms of action and cellular uptake pathway of rLOX-PP (rat LOX-PP) have been identified, the enhancement of its intracellular delivery and endosomal escape were not defined as potential tumor suppressor applications. Here we demonstrate that the pH gradient across endosomes to cytoplasm is the driving force for rLOX-PP delivery to its cytoplasmic targets in PWR-1E, DU145, PC3, SCC9, MDA-MB-231 cell lines. In addition, the ionic properties of cationic rLOX-PP generates pH gradient for its endosomal escape. We defined that increasing cytoplasmic pH (pHc), increases both uptake and endosomal release of rLOX-PP into the cytoplasm.

Inhibition of Oral Squamous Cell Carcinoma with beta-catenin Antagonist ICG-001

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BACKGROUND: Oral Squamous Cell Carcinoma (OSCC) accounts for the majority of head and neck cancers, for which the five-year overall survival rates are currently about 65% with few therapeutic options available. Increasing evidence suggests that aggressive cancers arise from tumor propagating cells (TPCs) that exhibit the properties of stem cells and may drive tumor development, recurrence and resistance to therapy. The Wnt/β-catenin signaling cascade and protein N-glycosylation have been shown to play important roles in stem cell phenotypes and to be deregulated in OSCC. Specifically, our studies have shown that increased N-glycosylation activates Wnt/β-catenin signaling and tumor growth in OSCC cells. We hypothesized that targeting the β-catenin/CBP branch of the Wnt/β-catenin signaling pathway, shown to function in proliferation, survival and stemness, will inhibit OSCC growth and TPC maintenance/ expansion and promote cellular differentiation. METHODS: Using immunoblots and immunofluorescence staining coupled with confocal imaging, we examined the effects of ICG-001, a small molecule inhibitor of β-catenin-CBP interaction, in OSCC cell lines and in xenograft mouse models of tongue cancer. RESULTS: Our data showed that inhibition of β-catenin/CBP interaction with ICG-001 reduced the expression of the DPAGT1 gene. This gene encodes the first and rate-limiting step in the assembly of the lipid linked oligosaccharide, an N-glycan donor during glycoprotein biosynthesis. Reduced expression of DPAGT1, in turn, resulted in decreased cellular N-glycosylation, enhanced cell-cell adhesion, loss of mesenchymal phenotype, and inhibition of tumor growth in vivo. CONCLUSION: These results suggest that N-glycosylation functions in the β-catenin/CBP axis of Wnt/β-catenin signaling in promoting a mesenchymal phenotype, and that ICG-001 represents a potential therapeutic for OSCC.

Comparison of Two Clinical Evaluation Methods for Diagnosis of Temporomandibular Disorders (TMD) in Orthognathic Surgery Patients

Poornima Kadagad, Pushkar Mehra & Radhika Chigurupati, Department of Oral and Maxillofacial Surgery

Statement of the problem: The presence of concomitant Temporomandibular Disorders (TMD) is considered a risk factor that can affect the outcomes of orthognathic surgery. Therefore, it is important for the surgeon to determine the severity and diagnosis of the TMD by clinical examination prior to surgery. Accurate diagnosis of TMD is a challenge as the diagnosis of TMD in patients with dentofacial deformities (DFD) can range from myalgia, arthralgia, articular disc disorders to degenerative joint disease. The objective of the study was to compare two clinical evaluation methods to accurately diagnose Temporomandibular Disorders in orthognathic surgery patients. Materials and methods**:** In this observational study, sixty nine patients scheduled to undergo orthognathic surgery at an academic medical center by a single surgeon were clinically evaluated for temporomandibular disorders. The study subjects consisted of orthognathic surgery patients undergoing preoperative evaluation in an outpatient clinic 2-4 weeks prior to surgery**.** Inclusion criteria were: a) Patients above 14 years of age with DFD undergoing orthognathic surgery, b) Standardized preoperative clinical examination for TMD, and, c) Availability of complete records. Clinical TMJ examination was performed in each patient by two independent examiners, using distinct evaluation methods. Method 1) Examination by an independent oral and maxillofacial surgeon who is not part of the clinical care of the patient. This method used validated symptom questionnaire and clinical examination form with an algorithm to arrive at a diagnosis as described by the Diagnostic Criteria for Temporomandibular disorders (DC/TMD). Method 2) Examination by an experienced oral and maxillofacial surgeon who is the treating the patient, using a standard evaluation form. Both examiners assigned the patients to one of two groups: TMD group or Non-TMD group. Data obtained from both types of clinical examination was further compared for differences in each individual diagnosis categorizing of TMDs- myalgia, arthralgia, articular disc disorders to degenerative joint disease. Results: Of the total 69 patients enrolled in the study, 46 were females and 23 were males. Average age of patient was 24 years with a range of 14-45 years. There is overlap of different categories of TMD diagnosis as patients can have more than one diagnosis of the TMD. A summary of the differences observed between the two evaluations is shown below:

	Method 1 DC/TMD protocol exam (n=69)	**Method 2 Routine TMJ exam (n=69)**
Myalgia, Arthralgia, TMD-related Pain and Headache	22	42
Intra-articular disorder	18	36
Degenerative Joint disorder	10	0
No. patients assigned to TMD group	33	41
No. patients assigned to Non-TMD group	36	28

Conclusions: Based on the results of comparison between two methods, we observed that an experienced surgeon categorized the orthognathic surgery patients in the TMD group more often than an independent surgeon using DC/TMD method. Both methods arrive at the diagnosis however, the DC/TMD method allowed the independent examiner to distinguish the different types of temporomandibular disorders myalgia, arthralgia, intra-articular and degenerative disorders accurately.

Kava Treatment Reduced Porphyromonas Gingivalis-induced Alveolar Bone Loss

Jayesh Patel, Abdul Alshammari, Jacob Al-Hashemi, Salomon Amar, Department of Molecular and Cell Biology

BACKGROUND: Porphyromonas gingivalis (P. gingivalis) is a well-documented pathogen in chronic periodontitis, an inflammatory disease that results in destruction of periodontal tissues. Kava, a compound extracted from the Kavain plant, has been shown to have an anti-inflammatory effect in various systemic inflammatory diseases. The aim of this study was to assess the effect of this Kavain-derived compound on periodontal destruction in a periodontitis-induced mouse model. METHODS: The study involved 49 mice divided into control, diseased, and treatment groups. Diseased mice were infected with P. gingivalis via oral gavage and a type 2 collagen antibody over the course of 15 days. Treated mice received treatment with Kava after disease induction over the same period of 15 days. The positive controls included mice infected P. gingivalis only and mice exposed to the type 2 collagen antibody only. Bone loss was assessed by morphometric analysis of left mouse maxilla and by histomorphometric analysis of TRAP-stained and H&E stained tissue sections of right mouse maxilla. RESULTS: The P. gingivalis and collagen antibody infected group showed significantly increased alveolar bone loss and osteoclastic activity throughout the experimental period in comparison to the control groups. Mice that received Kava treatment after infection showed a significant decrease in alveolar bone loss and osteoclastic activity than mice that did not receive any treatment. Mice that received Kava treatment after infection also showed a significant decrease in inflammatory cell activity than mice that did not receive any treatment. CONCLUSION: Our results showed evidence that P. gingivalis induced periodontitis can be treated by a modified Kava compound in a murine model. Mice pre-treated with Kava showed similar levels of bone loss and inflammatory cell activity compared to those treated post-infection. In addition, the experimental model demonstrated the use of morphometry and histomorphometry to accurately quantify alveolar bone loss. Further modification of Kava could yield a more effective mediator of inflammation in periodontitis.

Comparative Study of Breast Cancer Cells and Effects of Clinically Used Bisphosphonates, Zolendronic Acid and Alendronate, in 2D Cell Culture and 3D Live Bone Microenvironment

Alaa AlQutub, Abeer Alasmari, Serge Dibart, and Erdjan Salih, Department of Periodontology

Objectives: Over 80% of women who reached an advanced stage of breast cancer develop bone metastases. Bisphosphonates (BPs) have been used clinically as anti-cancer/metastatic agents in cancer patients with a wide range of tumor types including breast cancers. This study was designed to compare the effects of zolendronic acid (ZOL) and alendronate (ALN) on human breast cancer cells under 2D cell culture conditions and in a 3-D live bone organ microenvironment. MATERIAL AND METHODS:We have evaluated a range of concentrations (30 nM, 0.5 μM, 2 μM, 5 μM, 10 μM and 20 μM) of ZOL and ALN on breast cancer cells MDA-MB-231 and its bone-seeking variant MDA-BO in cell culture model using two different approaches with drug treatments: (a) at confluence, and (b) at the seeding time non-confluent state. Cell count was performed at days 3 and 8 to compare the drug's effect over time. Next, the effect of BPs on the cancer bone metastasis/interactions and bone biology was investigated utilizing 3-D live mouse calvarial bone organ co-cultures with breast cancer cells in a roller tube model system over 8 days. These model systems under bone resorption and formation conditions were evaluated by chemical, biochemical, TRAP and ALP enzyme activities, neutral red/silver nitrate staining, histological/quantitative histomorphometric analyses of the used media and calvarial bones. RESULTS: The effect of ZOL and ALN on MDA-231 and MDA-BO in 2-D cell culture conditions when cells were confluent showed: (a) susceptibility of these cells to BP treatment at clinically relevant doses, and (b) there was a significant differences in the sensitivity of MDA-231 (70% dead) versus MDA-BO (40% dead) at 20 μM dose over 8 days. However, quite distinct from 2D cell culture results, in a 3D live bone microenvironment the cancer cells colonized bone, proliferated and survived without being affected. CONCLUSIONS: These studies revealed that the effect of BPs on breast cancer cells in 2D cell culture conditions are very different as compared to 3D live bone microenvironment due to PBs being rapidly removed from the media and trapped within the mineralized bone matrix. Furthermore, in 2D cell cultures the two breast cancer cell types showed very different sensitivity towards the BP exposure with high resistance of the bone seeking/specific MDA-BO as compared to MDA-231.

Novel Role of LOXL2 in Osteoarthritis of TMJ and Knee

Weam Alshenibr, Yazeed Alkheriji, Amelia Wise, Sadanand Fulzele, Pushkar Mehra, Mary Goldring, Louis Gerstenfeld and Manish Bais, Department of Molecular and Cell Biology

Novel role of LOXL2 in Osteoarthritis of TMJ and Knee Weam Alshenibr1, Yazeed Alkheriji1, Amelia Wise2, Sadanand Fulzele3, Pushkar Mehra4, Mary B. Goldring5, Louis C. Gerstenfeld2 and Manish V. Bais1,* 1Department of Molecular and Cell Biology, Boston University Henry M. Goldman School of Dental Medicine, Boston, MA 02118; 2Department of Orthopaedic Surgery, School of Medicine, Boston University, Boston, MA, USA;3Department of Orthopaedic Surgery and Institute of Regenerative and Reparative Medicine, Georgia Regents University, Augusta, GA 30912, USA; 4Department of Oral and Maxillofacial Surgery, Boston University Henry M. Goldman School of Dental Medicine, 100 East Newton Street, Boston, MA 02118, USA and 5Hospital for Special Surgery, Tissue Engineering, Regeneration and Repair Program; and Department of Cell and Developmental Biology, Weill Cornell Medical College, New York, New York, USA Introduction: Osteoarthritis (OA) is the most common degenerative joint disease which affects the joint structures leading to disability. The key changes that lead to progressive, irreversible destruction in OA joints include defective extracellular matrix (ECM) remodeling and the loss of chondrocytes due to apoptosis. Studies in the last 20 years have documented the increased prevalence of knee pain and symptomatic knee OA. Similarly, of temporomandibular joint (TMJ) disorders OA is the most common. The prevalence in the United States among adults having at least one symptom of TMJ disorders is 33%, and the female-to-male ratio ranges from 3:1 to 9:1. Our previous studies showed that lysyl oxidase like-2 (LOXL2) is elevated in the regenerative response during mouse fracture healing and promotes chondrocyte differentiation (1,2). The goal of the study was to evaluate the role of LOXL2 in the pathophysiology of OA and its potential to act as an anabolic agent in OA cartilage. Methods: Tissues from osteoarthtic human temporomandibular joints (TMJ), hip and knee joints were evaluated for LOXL2 protein expression by immunostaining and LOXL2 mRNA expression by quantitative real-time PCR (RT-qPCR). The anti-catabolic/pro-anabolic effects of LOXL2 overexpression on both global and specific gene expression in cultured OA chondrocytes treated with IL-1β- and TNF-α was examined by microarray and RT-PCR. Results: Analyses of human TMJ, hip and knee joints showed that LOXL2 is expressed specifically in damaged regions of cartilage as a potential regenerative response to cartilage injury. LOXL2 mRNA expression was up regulated by TGF-β1 and down-regulated by IL-1β- and TNF-α in primary chondrocytes. LOXL2 overexpression in OA chondrocytes increased the levels of CSPG4, ACAN, SOX9, and COL2A1 mRNA and reduced the levels of MMP1, MMP3 and MMP13 mRNA. LOXL2 also reversed the adverse effects of IL-1β and TNF-α by increasing COL2A1 mRNA and decreasing apoptosis measured as caspase 3/7 activity. Conclusion: This is the first study to show that LOXL2 is expressed specifically in the damaged OA cartilage, whereas overall expression is not increased. These studies suggest that LOXL2 plays a protective feedback role in the pathophysiology of OA by inhibiting specific catabolic responses while promoting anabolic and regenerative responses. We are evaluating the anabolic role and mechanism of LOXL2 in osteoarthritis mouse models. Acknowledgements: NIH/NIDCR R03DE025274- (Manish V. Bais) and Trackman lab.

Mandible Dental Arch Form Determination from CBCT at 3 Levels: A Pilot Study

Berokh Bavar and Leslie Will, Department of Orthodontics and Dentofacial Orthopedics

Objective: Mandibular dental arch form was divided to 3 different hights to assess surrounding basal structure and the underlying bone width around the apical base. The objective of this research is to evaluate variation of mandibular arch forms at different heights and to determine possibility of developing a standardized clinical arch form. Method: 86 CBCTs of patients of a private clinic were screened and 11 fitted in the inclusion criteria of a Class I occlusion and having all their natural teeth. The selected CBCTs then were imported to Mimics software (Materialise NV, Belgium) and they were traced. Each tooth was sliced midsection at 3 different heights: CEJ, Apex and 5mm apical to the apex. Midpoints between the buccal and lingual plates were located for every tooth between and including the first molars. The points were connected forming 3 splines, which then were exported to Geomorph software (cran.r-project.org, Geomorph package, Dean Adams author, Iowa State2015) for shape analysis and variation detection. Results: All 3 arch forms can be visualized in 3 dimensions and a different shape of dental arch form can be identified at each level. The variation in the arch form among subjects is significantly smaller in the CEJ level. The variation at apical level is 10 times higher than the variation at CEJ level. This shows that variation in dental arch forms in the subjects increases apically. Conclusion: Mandibular dental arch form and surrounding bone structure demonstrate more variation apically. It may be concluded that variation should be considered when using standardized arch forms for different patients.

Oral Health Quality Of Life in Adult Orthodontic Patients

Martha Neely, Raffi Miller, Sharron Rich, Leslie Will, Wanda Wright, Judith Jones, Department of Orthodontics and Dentofacial Orthopedics

Objective: Examine Teen Oral Health-related Quality of Life (TOQL) for use in adults receiving orthodontic treatment and assess the validity and reliability by age-group. Methods: Teens ages 10-17 and adults 18 and over were asked to complete surveys at the Orthodontic Clinic at Boston University School of Dental Medicine. The survey consisted of sociodemographic information, dental behavior questions, and the TOQL instrument (Wright et al., under review). Malocclusion severity was assessed using the Index of Orthodontic Treatment Need (IOTN). Results: 161 teens and 146 adults participated; teens had a mean age of 13 years and 52% were male; adults had a mean age of 32 and 37% were male. Subjects in both groups represented diverse racial and ethnic backgrounds. In general, scores overall and by domains were higher for adults than for teens, signifying a greater effect on the quality of life. Mean TOQL scores (17 items) were worse (17.5) in adults than in teens (11.86, $p<0.01$); emotional domain scores were 16.49 in adults compared with 9.31 in teens ($p<0.01$) and the social domain score was 32.29 for adults compared with 21.35 for teens ($p<0.01$). The oral, physical and role domains were not statistically significant. Construct validity was supported by strong association of TOQL scores with global self-reported oral health. Cronbach's alpha (estimation of reliability of a psychometric test) was higher in adults for all the domains (TOQL 17 items for adults 0.75 compared to 0.68 in teens). Conclusion: Adults who come for orthodontic treatment are more affected by their malocclusion as compared to teens. Total score and the emotional and social domains are significantly higher for adults than teens. The project suggests that TOQL is a valid and reliable way to measure impact of malocclusion in quality of life in both adults and teens.

Influence of Surface Coatings on Cutting Efficiency of Nickel-Titanium Rotary Endodontic Files in Artificial Canals

Yousef Alnowailaty, Sami Chogle, Richard Pober and Russell Giordano, Department of Restorative Sciences/Biomaterials

Since the Nickel-Titanium endodontic files were introduced, NiTi showed enhanced performance in root canal instrumentation when compared to stainless steel files. NiTi files had a higher tendency to break inside root canals compared to stainless steel files, which happened unexpectedly and without visible deformation. Eliminating this problem may improve patient care. Previous research showed better fatigue life when files were coated with low surface tension silanes This research aimed to evaluate the cutting efficiency and cyclic fatigue of Protaper® files and EndoSequence® files when their surface character was modified through coating them with two types of silanes, as “dry” lubricants. Octadecylsilane and (3-Hepta- fluoroisopropoxy) propylsilane. Each file type was devided into 3 groups (as received, 3-HEPT coated, and ODS coated). A specially designed platform was made to test the cutting efficiency of 120 edodontic files in custom made epoxy resin blocks using a universal testing machine (Instron®) and an endodontic motor. The files rotated at 300rpm and were introduced into the blocks at 10mm/minute. Weight of the blocks was measured before and after testing and weight difference was recorded, maximum and minimum load that the files were subjected to during testing were measured and load difference was recorded. Results did not show significant difference in the amount of material removed between the silane-coated and uncoated files $p< 0.0001$, this is acceptable since the coating was considered a monomolecular layer limited to the surface of the files, with no changes to the core properties or the cross section of the files

Directed Differentiation of Human Induced Pluripotent Stem Cells into Endothelial and Neural Crest-Like Cells

Mohmaed Ahmed and Darrell Cotton, Department of Regenerative Medicine

Rationale and Objectives: Different cell types composing the pulp are of multiple embryonic germ layer origins (odontoblast is of neural crest origin, while endothelial cells are of mesodermal origin). Hence, to regenerate pulp cells there is a need for stem cell populations with bona fide capacity to differentiate into the cell types originating from these embryonic germ layers. The aim of this project is to establish a directed differentiation protocols of human induced pluripotent stem cells (hiPSCs) into endothelial (ECs) and neural crest (NCCs) cells using tooth development as a template. The overall aim is to develop an alternative, inexhaustible source of cells that can be used to regenerate pulp-dentin complex. Materials and methods; hiPSCs were cultured as a monolayer in a sequence of differentiation medias supplemented with growth factors to induce either EC and NCC differentiation. The resulting cells were characterized by immunostainning, flow cytometry, and RT-qPCR to assess for expression levels of known endothelial and neural crest markers. In addition, ECs were further characterized using functional in vitro capillary network formation (CNF) and acetylated low density lipoprotein (Ac-LDL) assays. NCCs were further differentiated into mesenchymal stromal cells (MSCs) and the resulting cells were characterized by flow cytometry to assess expression of MSCs markers Results Approximately 90% of the generated cells expressed endothelial markers (KDR, CD31, CD144 and NP-1) and neural crest markers (HNK-1 and p75) by the end of their respective differentiation protocol. In addition, the generated ECs were able to make tube like structures similar to control (HUVECs) in CNF assays and took up Ac-LDL. The generated ECs and NCCs continued to proliferate while maintaining their phenotype for several passages. By day 12 of NCC to MSC differentiation, cells lost their NCC markers (HNK-1 and p75) and had expression pattern similar to MSCs (positive expression of CD146, CD13, CD73 and CD90 while negative for CD34, CD45 and CD14). Conclusions: These results indicate the ability to generate cells with phenotypic characteristics similar to ECs, NCCs and MSCs from hiPSCs using directed differentiation protocols. Further experiments are planned to investigate their regenerative potential in-vivo.

Fabrication of Esthetic Orthodontic Brackets in Different Shades

Najla Sulaiman A Alrejaye and Russell Giordano, Department of Restorative Sciences/Biomaterials

Available commercial ceramic brackets are made of Alumina, either monocrystalline or polycrystalline; each comes in one shade, transparent or translucent, respectively. Objective: To obtain esthetic brackets in different shades equivalent or comparable to natural tooth shades. Methods: Yttria stabilized Zirconia (In-Ceram® YZ, Vita Zahnfabrik) and Alumina (In-Ceram® AL, Vita Zahnfabrik) Cerec blocks were prepared as slices which were immersed in specific concentrations of various salt solutions: Praseodymium, Iron, Cerium, Cobalt, Chromium, Nickel, Zinc, Neodymium, Erbium and Manganese. After sintering, the shade of each specimen was determined using VITA Easyshade Compact (VITA Zahnfabrik) and verified visually under color–corrected full spectrum light. Subsequently, Zirconia and Alumina brackets were fabricated using CAD/CAM system (Cerec inLab, Sirona) and then colored. Brackets were also fabricated from composite (ParadigmTM MZ100, 3M ESPE). Fabricated brackets were compared to commercial Inspire monocrystalline (Ormco) and Mystique polycrystalline (GAC) alumina brackets. Results: Fabricated zirconia, alumina, and composite brackets had shades that closely matched VITA tooth shades, whereas commercial alumina brackets did not match tooth shades. Specimens that were closely matched to VITA shades are shown in the following table: Colored Material Coloring Element Solution Concentration VITA 3D Master Shade (g/ dl) Zirconia Pr III 0.25 2M3 Zirconia Pr III 0.1 2M3 Zirconia Fe II 1 2M3 Zirconia Fe II 2 4L2 Zirconia Ce III 30 1M2 Zirconia Ni II 0.05 0M3 Zirconia Co II 0.0055 0M1 Zirconia Cr III 0.0265 1M1 Alumina Co II 0.0055 2M1 Alumina Fe II 2 3M2 Conclusion: Zirconia and Alumina brackets can be produced in natural tooth shades using different cations at specific concentrations, which would enhance their esthetic appearance.