

16TH ANNUAL
PACIFIC
RESEARCH DAY

Wednesday, May 28, 2014

Abstracts

Faculty, Student and Staff Presentations

Second-Year Student Research Presentations

Senior Research Presentations

IDS Student Review Presentations

IDS Student Research Presentations

Invited Presentation

Microbiology Student Research Presentations

Stockton Campus Student Presentations



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FACULTY, STUDENT AND STAFF PRESENTATIONS

Histological examination of apical papilla tissue

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OBJECTIVES: A preliminary study of the histological properties of the apical papilla, a poorly understood tissue, via immunohistochemistry. Information from this study can be used to understand the tissue structure and cellular components involved with root maturation.

METHODS: Apical papilla tissue, extracted from immature 3rd molars, was embedded into paraffin wax, and sliced into 5µm thick sections. Samples were dewaxed, rendered antigen accessible and detection specific (by blocking for non-specific binding and endogenous enzyme blocking) for immunological staining: primary antibody binding for a given protein marker of interest, followed by secondary conjugated antibodies, tertiary conjugated enzymes, DAB (brown chromogen), and then counter staining with hematoxylin (purple chromogen). Qualitative assessment was determined for the following markers: vimentin, desmin, neural intermediate filament, collagen 4, collagen 2, smooth muscle actin, CD31, CD34, CD29, CD68, CD45, and BCL2.

RESULTS: The following markers were positive in the tissue sample: vimentin, desmin, neural intermediate filament, collagen 4, collagen 2, smooth muscle actin, Stro-1, CD31, CD34, CD29, and CD45; certain markers were inconsistently detected among multiple sections. The following markers were negative in our tissue sample: BCL2 (cancer marker), and CD68

CONCLUSIONS: Tissue samples contained rich neurological, noticeable epithelial & endothelial, & very minor lymphatic cellular elements. Very minimal CD29 (mesenchymal stem cell marker) cellular elements detected within the perivascular space.

Cationic lipid transfection is superior to adenoviral transduction in HeLa cervical carcinoma, but not in HSC-3 oral squamous cell carcinoma cells

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OBJECTIVES: While viral vectors have been used commonly in the past and are generally believed to be more efficient gene delivery vehicles, non-viral lipoplexes are advantageous, because compared to viral vectors, they have very large expression cassettes, the vectors are not able to self-replicate, and plasmids can be made recombination-defective. In addition, liposomal complexes are noninfectious, cost-effective, and easy to administer. The identification of highly efficient non-viral vectors would facilitate the delivery of therapeutic genes to oral cancer cells.

METHODS: HeLa cervical carcinoma cells and HSC-3 human oral squamous cell carcinoma (OSCC) cells, were seeded in 48-well culture plates one day before transduction or transfection, and used at ~85% confluence. The cells were transduced with Adenovirus CMV eGFP–RSV Luc (KeraFAST, Boston, MA) at concentrations of 100, 1,000, or 10,000 viral particles per cell, and compared to cells transfected with TransfeX (ATCC, Manassas, VA), a cationic liposomal compound, at ratios of either 2 or 4 μ l TransfeX:1 μ g DNA. Toxicity was measured with the Alamar Blue cell viability assay. Transfection efficacy was evaluated by measuring luciferase activity using the Luciferase Assay System (Promega, Madison, WI) and a Turner Designs TD-20/20 luminometer (Sunnyvale, CA). Data were expressed as relative light units (RLU) per ml of cell lysate. Fluorescence microscopy was used to measure GFP expression.

RESULTS: In HeLa cells, luciferase expression with TransfeX transfection reached $52,140,000 \pm 2,200,818$ RLU/ml compared to $14,442,000 \pm 629,708$ RLU/ml after transduction with 10,000 viral particles per cell. Conversely, in HSC-3 cells, luciferase expression with TransfeX was substantially lower, reaching $559,467 \pm 30,624$ RLU/ml compared to $1,092,200 \pm 88,611$ RLU/ml after transduction with 10,000 viral particles per cell. There was ~20% cytotoxicity at 4 μ l TransfeX:1 μ g DNA with both HeLa and HSC-3 cells. Viral transduction with HeLa cells showed no cytotoxicity, while HSC-3 cells showed ~20% cytotoxicity.

CONCLUSIONS: Adenoviral transduction was more efficient with HeLa cells than with HSC-3 cells, while transfection with TransfeX was more efficient with HSC-3 cells. Studies with flow cytometry and fluorescence microscopy are in progress to examine the percentage of cells expressing green fluorescent protein expressed after transfection or transduction. Our findings indicate that for certain OSCC cells, adenoviral transduction may be preferable for the delivery of suicide genes. Future studies will also examine the potential enhancement of adenoviral transduction by TransfeX.

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Accuracy assessment of immediate implant placements using anatomage Invivo5[®] CAD/CAM surgical guides: A pilot study

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OBJECTIVE: Although accuracy assessments of multiple CAD/CAM surgical guides have been reported in multiple occasions, investigations regarding the use of such tools for immediate implant placements have not been reported to the best knowledge of the authors. The objective of this study is to assess the accuracy of a single, immediate implant placements using Anatomage-Invivo5[®] CAD/CAM surgical guides.

METHODS: Records between 2012-2014 were gathered for patients who had immediately placed implants using Anatomage invivo 5[®] CAD/CAM surgical guides with both pre and post-op CBCT images on file.

RESULTS: Nine implants in nine patients were included in this study. The mean deviation at the crest was 0.67mm, the mean deviation at the apex was 0.88mm, and the mean deviation of the axis was 2.30 degrees. **DISCUSSION:** Errors have been reported in the literature for all systems providing such tool. A meta-regression analysis reported by Schneider et al.² revealed a mean deviation at the entry point of 1.07mm, at the apex of 1.63, and an overall mean error in angulation of 5.261.2 Although this pilot study reports lower deviations, our sample was not large enough to compare to previous reports. In addition, this study was limited to tooth supported guides which have been shown to be the most accurate of all three types of supports (mucosa, bone, and tooth supported).

CONCLUSION: Within the limitations of this study, Anatomage-Invivo5[®] CAD/CAM surgical guides can be reliable tools to accurately place implants immediately after extraction.

This work was presented at the 29th Annual Meeting of the Academy of Osseointegration, March 6-8, 2014, Seattle, WA.

Prevalence of malocclusions among dental students

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INTRODUCTION: The vast majority of studies and textbooks state that normal occlusion is observed only in 30-35% of general population. When the Angle classification of occlusion is used, the most common is the class I malocclusion.

OBJECTIVES: The purpose of our study was to ascertain the prevalence of malocclusions among dental students, obtain information about family history, and prepare background data for heritability estimate calculation, recurrence risk calculation, and a molecular genetic analysis.

METHODS: The sample consists of 284 probands (149 males, 135 females), past and present DDS & IDS dental students from the University of the Pacific Arthur A. School of Dentistry. Two forms of data collection were used: (1) A structured questionnaire for collection of descriptive epidemiologic data and (2) a family pedigree drawn by students for recording of malocclusions that occurred in the first, second, and third generation of relatives. All information was entered into Excel and descriptive statistic tool pack was used for analysis.

RESULTS: Out of 284 probands, 15 (5.4%) did not record their occlusion. Normal occlusion was observed only in 10.6% (n=30), Angle class I in 65.1% (n=185), Angle class II in 5.9% (n=17), and Angle class III in 7.4% (n=21). Combinations of Angle classification of the first molar on left and right side were observed in 15 cases (5.4%).

CONCLUSION: Our results are not based on general population data, however, the sample is large enough to provide a valuable information in respect to the prevalence of Angle type of malocclusions in a selected population. Relatively low prevalence of normal occlusion and high Angle class I group is due to inclusion of even small irregularities of line occlusion. The study continues to increase the sample size and to analyze also a family history of previous orthodontic treatments.

Presented at the CDA Scientific Session in Anaheim, May 15, 2014.

Differentiation potential of human stem cells of the apical papilla (SCAP)

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OBJECTIVES: Investigate differentiation potential and soft tissue and hard tissue deposition properties of passage variant human apical papilla derived stem cells.

METHODS: Cellular outgrowths of minced apical papilla tissue, derived from immature 3rd molars, scheduled for routine extraction, was subcultured for 10 passages with trypsin dissociation, grown in Dulbecco's PBS, and Lonza hMSC basal media supplemented with human serum, L-glutamine, and penicillin/streptomycin. Flow cytometry was performed for each passage in order to determine the degree of expression for the following markers: CD31, CD34, Stro-1, CD90, CD105, and CD29. To assess differentiation potential, cells from passages 3 (P3) and 9 (P9) were exposed to 3 differentiation growth conditions for 2 weeks each: standard (dexamethasone, ascorbate, β -glycerolphosphate) osteogenic in 2D culture, chemically defined (L-proline, ITS+1, ascorbate, dexamethasone, pyruvate, high glucose, TGF-B3, serum-free) chondrogenic in pellet culture, and a second osteogenic medium formulation involving less ascorbic acid, less L-glutamine, no β -glycerolphosphate, and incorporation of KH_2PO_4 in 2D cultivation. Real-Time PCR analysis for the expression of 84 unique osteogenic and chondrogenesis gene markers was performed on osteogenically differentiated cells and undifferentiated cells, for comparison. Osteogenic calcium deposition was visualized with Alizarin Red S (pH 4.2). Chondrogenic proteoglycan deposition was determined with Alcian Blue (pH 0.2).

RESULTS: CD29, CD90, and CD105 expression remained consistently high (>70%) with passage. Flow cytometry analysis for MSC markers was as follows: CD105 expression decrease started between passage 8-9 and continues; CD90 began to decrease between P9-10; CD29 remained consistent high (>98%) regardless of passage number. Comparison of P9 to P3 for undifferentiated cells showed increased expression of chondrogenic markers, decreased osteogenic distinctive/termination markers, and decreased extracellular matrix deposition and remodeling activity. Alizarin Red S stain intensities from KH_2PO_4 -mediated differentiated cultures were marked and similar, regardless of P3/P9; however, in comparison, intensities were dramatically decreased in deposition in traditional osteogenic differentiation, especially with greater passage number. Alcian blue staining of chondrogenic pellets were marked and of similar intensity regardless of passage number.

CONCLUSIONS: The default differentiation pathway of apical papilla cells, under the standard growth conditions utilized here, is more of chondrogenic-, than of osteogenic-like cell fate. Traditional osteogenic differentiation potential is marked in early passage cells but dramatically reduced in later ones. However, calcium deposition activity can be induced in the presence of inorganic phosphates ubiquitous of cellular passage number. Lastly, CD29+ apical papilla cells are capable of (sulfated) proteoglycan and calcium depositing activity, characteristic of in-vivo odontogenic cells.

Enhancing the detection of hidden occlusal caries lesions with OCT using high index liquids

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OBJECTIVES: In a previous study, we investigated the influence of several high refractive index fluids on the performance of polarization sensitive optical coherence tomography (PS-OCT). That study showed that these liquids can increase the effective imaging depth and lesion contrast. Other in vitro and in vivo studies have shown that OCT can be used to show whether occlusal lesions have penetrated to the dentinal-enamel junction (DEJ) and spread laterally under the enamel. The purpose of this study was to determine if high index fluids can enhance the ability of OCT to detect hidden occlusal lesions and show if these lesions have penetrated through the enamel into the underlying dentin.

METHODS: Ten extracted teeth with occlusal lesions were chosen after examination with OCT and near-IR transillumination (1310-nm). Four fluids of different index of reflectivity were added to tooth occlusal surfaces prior to OCT imaging in sufficient quantity to fill the pits and fissures. OCT images were acquired of the tooth occlusal surfaces with each of the high index fluids applied to the tooth surfaces with the time-domain OCT system.

RESULTS: This study shows that the use of high index fluids significantly increases the visibility of subsurface lesions located under sound enamel peripheral to pits and fissures in the occlusal surface.

CONCLUSION: The requisite optical penetration/imaging depth for the detection and diagnosis of occlusal lesions is to the DEJ. If the lesion is present in the underlying dentin and the enamel above is sound, OCT works quite well in resolving that lesion and the images confirm penetration to the DEJ. If extensive demineralization is present from the enamel surface all the way down to the DEJ the results are quite mixed, i.e., sometimes the entire lesion is visible from the enamel surface to the DEJ, while more typically only the outer surface of the lesion is visible or the area where the lesion has reached the DEJ (lower part) can be seen. One limitation of radiography is that ionizing radiation is required, however it is even more important to emphasize that radiography is insensitive for the detection of early occlusal lesions. By the time lesions are apparent on a radiograph they have typically spread extensively throughout the dentin and are far too late for chemical intervention, or even conservative surgical intervention.

This work was supported by NIH/NIDCR Grant R01-DE17869.

Effects of new types of calcium silicate cements on human apical papilla-derived stem cells (SCAP): biocompatibility and calcium release

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OBJECTIVES: During endodontic procedures, a cement or paste-type material may directly contact apical tissues. Therefore, the initial reaction of cells in apical tissues with an endodontic material is important for wound healing and repair. ProRoot MTA has been successfully used for apexification, apical surgery and perforation repair. Recently, new types of MTA like cements have been introduced on the market. However, these new calcium silicate cements have not been fully investigated. The purpose of the study was to evaluate aspects of biocompatibility of various calcium silicate cements on stem cells derived from human apical papilla tissue in different culture conditions.

METHODS: Human SCAP at passages 7 and 8 were used. Cells were characterized by flow cytometry using stem cell markers CD29, CD90 and CD105. The same number of cells (2×10^4 per cm^2) was used in 24-well cell culture dishes either in direct contact with mixed cements or in indirect contact with the cement samples placed in an insert with 1μ pore size. Cell culture plates without cement were used for controls. Tooth Colored ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK), Ortho MTA (bioMTA, Seoul, Korea) and Endoseal (Maruchi, Seoul, Korea) were mixed following manufacturers' instructions, placed a Teflon tube (5-mm diameter and 3-mm thick) and allowed to set in 100% humidity for 24 hours. Cell attachment and growth was examined under a phase contrast microscope after 1, 3 and 7 days of culture. At 1 and 3 days, cell viability was measured using an XTT assay kit ($n=3$). Statistical significance was assessed with Student t-tests, compared to the control ($p<0.05$). Calcium concentrations were measured for ($n=3$) with a Quantichrom™ calcium assay kit (bioAssay systems, Hayward, CA) and the increase of calcium concentration for 24 hours was calculated by subtracting the calcium concentration of cell culture media ($n=3$).

RESULTS: Flow cytometry of the SCAP population revealed 69.1% of CD29+, 64.4% of CD90+ and 74.1% of CD105+ cells. Cells grown on ProRoot MTA and OrthoMTA were well attached and appeared to be visually healthy throughout the culture period, regardless of time of culture. However, cells on Endoseal showed less confluency and a clear inhibition zone was found in the direct contact group. Cell viability in groups of cells grown in contact with ProRoot MTA was higher than that in control group ($p=0.0023$). At 3 days, cell viability of ProRoot MTA(direct), ProRoot MTA(coculture), OrthoMTA(direct), OrthoMTA(coculture), Endoseal(direct), Endoseal(coculture) was $109 \pm 6\%$, $91 \pm 12\%$, $103 \pm 5\%$, $107 \pm 11\%$, $76.1 \pm 1\%$, $78 \pm 6\%$ of control, respectively. The changes of calcium concentration in ProRoot MTA, OrthoMTA and Endoseal after 24 hours were 0.39, 0.55 and 0.79mM respectively.

CONCLUSIONS: Among three types of cements, ProRoot MTA in direct contact showed the most favorable result. Endoseal appeared more toxic than ProRoot MTA and OrthoMTA. The amount of calcium released from the cements was highest in Endoseal.

Periodontal reaction to orthodontic treatment with Invisalign

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OBJECTIVES: The periodontium is the first responder to application of force to a tooth. Mechanisms of orthodontic tooth movement (OTM) by fixed appliances have been studied clinically and experimentally. However, no data are available about reaction of periodontium to treatment by Invisalign. Gingival crevicular fluid (GCF) is a serum transudate that is released in periodontium and penetrates through the crevicular sulcus epithelium to the oral cavity. OTM triggers an acute inflammatory reaction in periodontium. One of the major signs of inflammation is increased exudation (swelling). Therefore, we collected GCF under standard conditions and measured its volume before and after application of force.

METHODS: Ten volunteers starting the Invisalign treatment were included in the study (IRB approval Nr. 14-57). Twenty teeth were followed including two premolars, canine and two incisors in two quadrants of the upper jaw and two quadrants of the lower jaw. Samples (n =1,200) were collected on both buccal and lingual sides of each tooth using Periopaper strips (Oraflow) and a volume of collected liquid was measured using Periotron (Oraflow). Collections were done before the Invisalign treatment was started (baseline) and then one day and 14 days after the start of the first aligner. The moved and non-moved teeth were identified using ClinCheck software (Align Technologies).

RESULTS: The teeth that showed positive changes of GCF volume after deduction of baseline values were included in analysis. Typically, GCF volume increased after one day of wearing the first aligner. At 14 days, the volume declined. Interestingly, this reaction was observed not only in the moved teeth, but also in some teeth that were not moved by the aligner.

CONCLUSIONS: Volume of GCF is a good indicator of early inflammatory reaction of periodontium due to application of force. The initial response was a rapid increase of GCF volume that was followed by a slower decline. Some teeth that were not designed to be moved also showed increased exudation of GCF. It may have been caused secondarily by their occlusal contact with the teeth moved by the aligner.

This work was supported by the Research Pilot Project Award from the Arthur A. Dugoni School of Dentistry.

Treatment of *Porphyromonas gingivalis* with azithromycin encapsulated in cationic liposomes

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OBJECTIVES: *Porphyromonas gingivalis* is one of the most significant periodontal pathogens. Although debridement is the first course of action in the therapy of periodontal disease, the use of antibiotics may be necessary in severe forms of periodontitis. The localized delivery of antibiotics into the periodontal pocket and to the surface of microorganisms would have several advantages over systemic administration: (i) A high concentration of the drug at the pathogen membrane; (ii) preventing antibacterial effects on normal protective flora elsewhere in the body; and (iii) minimizing the potential for the development and transfer of drug resistance in intestinal flora. Liposomes can encapsulate antibiotics either in the aqueous interior or the lipid bilayer, and may be a potential delivery vehicle to localize the antibiotics at the surface of periodontal bacteria. Previous studies in our laboratory showed that fluorescently labeled cationic liposomes bind quantitatively to *P. gingivalis*. Azithromycin is effective against *P. gingivalis* (MIC₉₀ = 0.38 µg/ml; MIC₅₀ = 0.032 µg/ml). We therefore examined the effect of azithromycin encapsulated in liposomes and as a free drug for its effect on *P. gingivalis*.

METHODS: *P. gingivalis* strain 2561 was incubated on blood agar plates and then in medium 199 under anaerobic conditions. Liposomes were composed of palmitoylcholine (POPC), dioleoyltrimethylammoniumpropane (DOTAP) (Avanti Polar Lipids, Alabaster, AL), and azithromycin (5:5:2 molar ratio). Control liposomes contained no antibiotic. The liposomes and free azithromycin dihydrate (Sigma, St. Louis, MO) (stock dissolved in dimethyl sulfoxide (DMSO)) were added to *P. gingivalis* in phosphate-buffered saline at 1 µg/ml. After incubation for 48 h at 37°C under anaerobic conditions, *P. gingivalis* were plated on blood agar plates and incubated for 48 h at 37°C under anaerobic conditions. Colonies were counted after this period.

RESULTS: DOTAP:POPC:azithromycin liposomes at 1 µg/ml azithromycin reduced *P. gingivalis* colonies by ~40%, based on two experiments. Control DOTAP:POPC liposomes had no effect on *P. gingivalis* colony formation. DMSO alone (<0.5%) added to *P. gingivalis* reduced colonies by ~40%. Although free azithromycin at 1 µg/ml reduced *P. gingivalis* colonies by ~40%, based on two experiments, this could be attributed to the carrier, DMSO.

CONCLUSIONS: The solvent, DMSO, even at the dilution used, is toxic to *P. gingivalis*. Thus, the effect of free azithromycin cannot be determined because of DMSO toxicity. However, liposome-encapsulated azithromycin may be effective in the treatment of *P. gingivalis* infections.

Supported in part by Research Pilot Project Award 03-Activity 089 from the University of the Pacific, Arthur A. Dugoni School of Dentistry.

Gene delivery to cancer cells with Metafectene and its derivatives: Nanoparticle tracking analysis of lipoplexes

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OBJECTIVES: To identify optimal non-viral vectors for suicide gene therapy of cancer, we compared the transfection activities of Metafectene (M) and its derivatives in HSC-3 oral squamous cell carcinoma, and HeLa cervical carcinoma cells. We determined the size distribution of the vectors and their complexes with DNA (lipoplexes).

METHODS: The cells were used at 80% confluence. pCMV.Luc, expressing luciferase was complexed with different volumes of M, M-Pro (MP), M-Easy (ME) (Biontex) and Eugene HD (FHD) (Roche) and incubated with the cells for 4 h. 48 h after transfection, the cells were lysed and the transfection activity was assayed using the Luciferase Assay System (Promega). The size distribution of the reagents and their lipoplexes was determined using a NanoSight.

RESULTS: The mean sizes of the reagents were 165 nm (M), 181 nm (MP), 155 nm (ME) and 230–306 nm (FHD). The mean sizes of lipoplexes prepared with 2 µl reagent and 1 µg DNA were 282±2 nm (M), 293±30 nm (MP), 264±8 nm (ME) and 311±7 nm (FHD). The transfection activity in both cell lines decreased in the order ME>MP>M>>FHD. In HeLa cells, luciferase activity achieved with ME was 680-fold higher than that with FHD, and with HSC-3 cells, it was 83-fold higher. The low activity of FHD was surprising, as previous experiments had produced much higher activities. Transfection activity of ME in HeLa cells was 40-fold higher than in HSC-3 cells. Lipoplexes prepared with 0.5, 1 and 1.5 µl ME and 1 µg DNA had multiple peaks in particle analysis, whereas lipoplexes made with 2 µl ME were homogeneous.

CONCLUSIONS: The mechanisms of the resistance of HSC-3 cells to transfection are not known. A uniform size distribution of lipoplexes may contribute to higher transfection activity.

This work was presented at The Biophysical Society 58th Annual Meeting, February 15-19, 2014, San Francisco, CA (Abst. 3162-Pos).

2ND YEAR STUDENT RESEARCH PRESENTATIONS

Setting the scene for regenerative endodontics: the biologic rationale for growth factors, stem cells, and scaffolding

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OBJECTIVES: Recent cases reports have shown success with regenerative procedures in mature permanent teeth which highlight the continuation of a paradigm shift in endodontic possibilities. The aim of the poster is to determine the biological rationale for regenerative endodontic procedures (REPs), focusing on the interplay of components - growth factors, stem cells, and scaffolding- and to improve our understanding of current best practices and protocols.

METHODS: Literature review of Index JCR journals using Pubmed, Scopus, and Web of Science with keywords: growth factors, stem cells, scaffold, regenerative endodontics, tissue engineering, pulp regeneration

RESULTS: Successful regenerative endodontics demands a delicate environment of stem cells, growth factors, and scaffolds to dynamically induce pulpal and dentin regeneration. Proper irrigation will help to preserve stem cells. The laceration of the apical papilla allows a high concentration of stem cells to fill the pulpal space and growth factors to be release from platelets and dentin. To successfully differentiate stem cells need to interact with the growth factors through a scaffold which can be a blood clot, protein-rich plasma or made from natural or synthetic materials.

CONCLUSIONS: Stem cells, growth factors, and scaffolds play an interdependent role in REPs. REPs must optimize the availability and role of each component in a technically challenging protocol.

This work was presented in Spanish at the 34th National Congress of the Spanish Association of Endodontics and the 13th Congress of the Ibero Latin American Association of Endodontics, October 31st-November 2, 2013.

A new insertion landmark and modification of the standard technique for inferior alveolar nerve block injections

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OBJECTIVES: Nerve block anesthesia is employed in mandibular dental procedures to serially block the inferior alveolar (IA), mylohyoid (MH), and lingual nerves. Success rates range broadly (13-98%). In an effort to improve success rates we test a maxillary landmark that is consistently visible in the oral cavity and that might provide a stable bony landmark for insertion of the needle. This landmark would also simplify the injection technique. Here we: 1) test the ability of the landmark to guide the needle into the proper position, 2) test different mandibular opening positions, and 3) attempt to identify anatomical variations that impact needle placement.

METHODS: We compiled a geographically diverse sample of skulls ($n=26$). Four skulls have unerupted/erupting $M^{3/3}$, while the rest are from adults. The number of teeth present varies from complete to edentulous. CT scans of each cranium and mandible were made on a GE Lightspeed VCT scanner (helical mode, 0.3mm isotropic voxels, standard convolution kernel). Mandibular models were duplicated. The condylar heads of one mandibular model were aligned on the articular eminences (maximum opening with translation), and the other was centered in the glenoid fossa (maximum opening with no translation). Mandibles were set with a maximum incisal opening of ~40-45 mm. To mimic the injection path we oriented oblique orthoslices through the interproximal surfaces of the LP_{3-4} and the posterior extent of the right alveolar ridge (maxillopterygoid junction). Oblique orthoslices were oriented separately for each mandibular model. Injection height was determined both from the occlusal plane (established via oblique slices) and from the coronoid notch.

RESULTS: With the mandible at maximum opening and positioned on the articular eminence, the needles path terminated just superior to the mandibular foramen in 73% of cases (19/26). In 7.6% of cases (2/26), the plane ran at or just posterior to the ramal border. In 19.2% of cases (5/26), the plane ran posterior to the ramus. Neither geographic variation or the presence or absence of the $M^{3/3}$'s impacted this relationship. In those cases in which the plane terminated at the posterior ramus, or just posterior to it, neither metric nor morphological differences that account for the variation have been observed or determined, respectively. Tests of the landmark with the condyle in the glenoid demonstrated that the needle path is generally too anterior. Whereas many instances of proper needle placement are achieved with this orientation, the number of individuals falling outside an acceptable range is large. While not providing resolution of the problem of consistent placement, this comparison revealed the need to control mandibular position during IA block procedures.

CONCLUSIONS: Assessment of the maxillopterygoid junction as a landmark for needle insertion shows it to be appropriate. It also demonstrates the potential to greatly simplify the block technique by eliminating insertion depth ambiguity. However, in ~20% of cases the landmark results in a slightly more posterior position of the needle than what we considered to be acceptable. The reasons for the observed variation in needle termination are currently obscure and efforts are underway to resolve this problem. Clarifying relationships between landmarks employed in block procedures and anatomy relevant to achieving anesthesia should lead to more standardized, patient-centered techniques and improved success rates.

Dental caries and taste receptor genes

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OBJECTIVES: Dental caries is one of the most prevalent diseases worldwide and the most common chronic childhood disease in the United States. Although previous studies suggest the importance of genetic factors, not too much is known about dental caries in relation to food choices that people make due to a specific genetic mutation. Our review analyzes the genetic influence of taste perception on caries susceptibility. Specifically, we focused on the bitter taste perception from TAS2R38 genes and the sweet taste perception from the TAS1R2 genes. The results of this review proved that genotypes of taste perception do have an influence on dental caries. This information can be used to create a customized diet to prevent or decrease morbidity of dental caries in primary, mixed and permanent dentition.

METHODS: The twin study that examined the genetic component of caries looked at 44 pairs of twins and three triplets over a six-year period. The study used clinical and radiographic exams, study models and dental questionnaires. Another study looked at 46 pairs of monozygotic twins and 22 pairs of dizygotic twins reared apart to analyze teeth present, teeth present excluding third molars, teeth restored, teeth restored index, surfaces restored, surfaces restored index, and surfaces restored or carious. Other studies primarily used DMFT and DMFS scores to compare to the presence of TAS2R38, TAS1R2, and GNAT3 taste genes. For the study in Udaipur, India, data and blood or saliva specimens were collected from 54 patients with non-syndromic cleft lip and/or palate (NCLP), 57 relatives of patients with NCLP, and 38 unaffected controls. The Taqman allelic discrimination assay utilizing RT-PCR was used for rapid detection of five single nucleotide polymorphisms (SNPs) of TAS2R38 and TAS1R2.

RESULTS: Both twin studies showed a statistically significant correlation for monozygotic, but not dizygotic, sets of twins for percentage of teeth and surfaces restored ($p < 0.001$) and for percentage of teeth and surfaces restored. Polymorphisms of taste genes associated with caries and NCLP in Udaipur, India, showed that the G allele can be considered as a protective allele for TAS2R38 taste receptor gene. Patients with NCLP in this study possessed less caries protective genes and more caries risk genes. DMFT and DMFS scores compared to presence of TAS2R38, TAS1R2, and GNAT3 study showed that certain alleles (G, G, and C) of the TAS2R38 gene provided protection from caries in the primary teeth alone.

CONCLUSIONS: The TAS2R38 genotype and bitter taste perception have a strong relationship to caries incidence. This knowledge can be used to target patients who are at a higher genetic risk for caries. The high-risk patients can be helped by a hygiene and diet program for caries prevention.

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Cleft lip and palate in Vietnam and folate-related genes

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INTRODUCTION: The etiology of nonsyndromic cleft lip with or without cleft palate (NCL/P) is multifactorial, including genetic and environmental factors. Folate-related genes, methylenetetrahydrofolate reductase (MTHFR) and reduced folate carrier 1 (RFC1), are among those genetic factors most intensively studied. When their function is altered due to mutations, a decreased utilization of folate slows down cell multiplication and it may contribute to orofacial clefting. RFC1 gene encodes a cell membrane protein essential for internalization of folate bound to a folate-binding protein from extracellular fluid into cells. MTHFR gene encodes an enzyme that catalyzes formation of an active form of the internalized folate in the cell.

OBJECTIVES: Purpose of our study was to determine whether MTHFR677CT and the RFC180AG polymorphisms are associated with NCLP in CanTho, Vietnam.

METHODS: A case-control study design was used. Cases (individuals affected with NCL/P; n=38) and controls (n=33) for this study were identified during Rotaplast medical mission to CanTho, Vietnam. Diagnosis of NCL/P was determined by medical geneticist (MMT) conducting physical examination of each individual. Controls (n = 33) were recruited in the same hospital. Venous blood and saliva was obtained for DNA analysis. DNA was isolated from dry blood or saliva spots. MTHFR 677CT and RFC1 80AG genotypes were established by PCR amplification and single nucleotide conformational polymorphism detection using polyacrylamide gel electrophoresis (PAGE).

RESULTS: Mutations of the MTHFR 677th nucleotide were relatively rare in CanTho samples of cases and controls that we studied. We found a different proportion of genotypes in cases and controls for MTHFR 677CT, but only one homozygote TT (in controls). There were twice as many CT heterozygotes in cases compared to controls (34.2% vs 15.2%) and thus a higher T allele frequency in cases (0.171) compared to controls (0.106) was found. However, these differences were not significant. Very interesting findings were revealed by analysis of RFC1 80AG. Mutated allele G was common in cases as well as in controls (higher in cases - 0.554 compared to controls - 0.5). Although GG genotypes were observed in the same proportions in cases and in controls (27 % vs 27.3%), lower proportion of AA homozygotes (16.2% vs 27.3%) and higher proportion of AG heterozygotes (56.8% vs 45.4%) were observed. The difference in distribution of genotypes between cases and controls was statistically significant (p=0.041).

CONCLUSION: The present study suggests association of 80AT variant of the RFC1 gene with NCL/P in CanTho Vietnam. No association was observed for 677CT variant of MTHFR. The study continues by increasing size of samples for cases and controls and by involving also mothers and fathers of cases.

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Salivary flow rate in a rat model of Type 2 diabetes

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OBJECTIVES: Type 2 diabetes (non-insulin dependent diabetes mellitus, NIDDM) is one of the most prevalent health problems in our society today, and xerostomia is usually considered a common oral complication that occurs with the onset of the disease. Saliva plays an integral role in oral health by helping maintain the integrity of hard and soft tissues. The potential consequences of reduced salivary flow include difficulties with speech, increased caries, yeast infections, bad breath and alterations in taste. Thus, the purpose of this study was to examine submandibular salivary flow rate in a rat model of type 2 diabetes (Zucker Diabetic Fatty, ZDF).

METHODS: Six-week old male and female diabetic (ZDF obese, N=32) and non-diabetic (ZDF lean, N=33) rats were obtained from Charles River Laboratories and the onset of diabetes was verified by measuring serum glucose levels. One to 2 months after the development of diabetes, animals were anesthetized and salivary flow was measured in response to either sympathetic (2 and 4 Hz continuously, or 20 and 40 Hz in bursts of 1s every 10s) or parasympathetic (1 to 20 Hz) stimulation. The right submandibular duct was cannulated and saliva was collected and weighed. At the end of each experiment the glands were weighed and flow rate was expressed as $\mu\text{l}/\text{min}/\text{g}$ tissue. Differences in flow rate were analyzed for statistical significance using student's t test.

RESULTS: Diabetes was confirmed by elevated serum glucose levels observed in both male (443 ± 96 mg/dl vs 144 ± 46 mg/dl) and female (437 ± 100 mg/dl vs 131 ± 24 mg/dl) rats. Only minor differences were observed between male and female, control and diabetic rats at all frequencies of parasympathetic stimulation. However, when sympathetic stimulation was applied, significant differences in flow rate ($p < 0.01$) were observed between males and females, irrespective of diabetes. Salivary flow rate was approximately 50% lower in non-diabetic males compared with non-diabetic females, and diabetes resulted in a reduction in salivary flow rate by approximately 40% in male and 30% in female rats.

CONCLUSIONS: Parasympathetic impulses provide the main driving force for salivary fluid secretion. However, only small effects of type 2 diabetes were observed during parasympathetic stimulation. In contrast, submandibular salivary flow rates evoked by sympathetic stimulation were significantly lower in male compared to female non-diabetic rats, and diabetes resulted in significant reductions in salivary flow rates in both sexes. Because salivary secretion normally results from simultaneous parasympathetic and sympathetic nerve activity, these data suggest that type 2 diabetes may be responsible for the xerostomia reported in diabetic patients. However, there are currently no data that explain either the sex differences or the effect of diabetes on sympathetically-stimulated flow rate in ZDF rats.

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***In vitro* comparison of the torsional performance between ProTaper Universal and ProTaper Gold SX rotary instruments during coronal enlargement of artificial s-shaped canals**

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OBJECTIVES: New alloys have been developed to enhance the properties of rotary instruments. The purpose of this study was to compare the peak force and torque induced by two instruments with the same geometry but made of two different alloys (raw NiTi and a proprietary advanced metallurgy called NiTi Gold) during the coronal enlargement of simulated S-shaped root canals.

METHODS: 12 S-shaped plastic blocks were mounted on scanning electron microscopy stubs after an initial negotiation with a #10 K file and distributed into two groups for the coronal enlargement either with ProTaper Universal or ProTaper Gold Sx instruments. A total of 6 instruments of each system were used. The tests were run using an automated torque testing platform. Peak torque (Ncm) and force (N) were registered. After assessing the normal distribution of data, Student's t-test analysis was performed.

RESULTS: There were no significant differences between the peak force and torque generated by either ProTaper Gold and ProTaper Universal SX instruments ($p>0.05$).

CONCLUSIONS: Under the conditions of this study, differences in the torsional performance between the recently developed ProTaper Gold and ProTaper Universal SX instruments could not be demonstrated. The different metallurgical properties of raw NiTi and NiTi Gold seemed to have no effect in the peak torque and force induced during the coronal enlargement of simulated S-shaped root canals.

Treatment of *Porphyromonas gingivalis* with antibiotics and photosensitizers encapsulated in cationic liposomes

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OBJECTIVES: *Porphyromonas gingivalis* is considered one of the most significant periodontal pathogens, and is responsible for the destruction of periodontal tissues. Debridement is the first action of periodontal therapy. The use of antibiotics may be necessary in severe forms of periodontal disease. Photodynamic therapy (PDT) exploits visible light and photosensitizer to inactivate cells and has been used for the treatment of several types of malignancy. The use of photosensitizer and light as an antimicrobial agent against periodontal microbial biofilm represents as attractive method of eliminating oral bacteria. Liposomes can be used as a delivery vehicle for antibiotics and photosensitizers by encapsulating these molecules either within the aqueous interior or the lipid bilayer. Previous studies in our laboratory showed that fluorescently labeled cationic liposomes bind quantitatively to *P. gingivalis*. We therefore examined the effects of azithromycin, doxycycline, and zinc phthalocyanine encapsulated in liposomes and as a free drug on *P. gingivalis*.

METHODS: *P. gingivalis* strain 2561 was incubated on blood agar plates and then in medium 199 under anaerobic conditions. Liposomes were composed of dioleoyl-rimethylammoniumpropane (DOTAP):palmitoyl-oleoylphosphatidylcholine (POPC) (Avanti Polar lipids, Alabaster, AL):and azithromycin or doxycycline or zinc phthalocyanine (5:5:1, 5:5:1, 5:5:0.1 molar ratio respectively). Control liposomes contained no antibiotic or photosensitizer. The liposomes and free antibiotics or photosensitizer (Sigma, St. Louis, MO) were added to *P. gingivalis* in phosphate-buffered saline. After incubation for 48 h at 37°C under anaerobic conditions, *P. gingivalis* were plated on blood agar plates and incubated for 48 h at 37°C under anaerobic conditions. Liposomes containing zinc phthalocyanine liposomes were incubated with *P. gingivalis* in suspension for 48 h at 37°C under anaerobic conditions, and the bacteria were irradiated with red light for 20 min and incubated on blood agar plates for 48 h at 37°C. Colonies were counted after this period.

RESULTS: DOTAP:POPC:azithromycin liposomes and free antibiotic at 10 µg/ml azithromycin did not reduce *P. gingivalis* colonies. DOTAP:POPC:doxycycline liposomes and free drug at 1 µg/ml reduced *P. gingivalis* colonies by 36% and 25% respectively. DOTAP:POPC:zinc phthalocyanine liposomes and the free drug at 5 µg/ml reduced *P. gingivalis* colonies by about 99% and 97% respectively.

CONCLUSIONS: The photosensitizer zinc phthalocyanine was more effective against *P. gingivalis* than the antibiotics. Doxycycline had more of an effect on *P. gingivalis* than azithromycin.

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Targeted photodynamic therapy of cervical carcinoma *in vitro*

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OBJECTIVES: Photodynamic therapy (PDT) is a medical treatment that uses light to activate a photosensitizer in the presence of oxygen, and leads to local damage by the generation of reactive oxygen species. The application of liposomes as delivery systems can overcome many drawbacks of conventional photosensitizers. PDT in tandem with liposomal delivery is adaptable to the treatment of cancers and more importantly, may be a safer and more effective alternative to current therapies. Cancer cells overexpress the folate receptor (FR) that can potentially be used as a target for drug delivery. Hence, we examined the efficacy of phthalocyanine photosensitizers encapsulated in liposomes, conjugated or not with folate, using HeLa cells.

METHODS: Liposomes were composed of phosphatidylglycerol (PG) and palmitoyl-oleoyl-phosphatidylcholine (POPC), and contained either aluminum phthalocyanine chloride (AlPc) or zinc phthalocyanine chloride (ZnPc) (5:5:0.1). Phthalocyanines were also encapsulated in liposomes containing 1 mole% folate-conjugated PEG-DSPE. HeLa cells were incubated with folate or non-folate conjugated liposomes containing AlPc or ZnPc, in the range of 0.01-1.0 μ M, for 24 h at 37 °C. The cells were then exposed to light (690 nm) from a High Power LED Multi Chip Emitter for 20 min. Cytotoxicity was evaluated by the Alamar Blue Assay.

RESULTS: Encapsulation of AlPc and ZnPc in liposomes significantly reduced cell viability when compared to cells treated with free AlPc and ZnPc. Both photosensitizers did not show dark cytotoxicity in HeLa cells. For 0.1, 0.5 and 1.0 μ M liposomal AlPc, cell viability was reduced to 25.8 ± 8.2 , 0 and 0%, respectively. For 0.1, 0.5 and 1.0 μ M liposomal ZnPc, the viability was reduced to 68.0 ± 8.6 , 15.1 ± 9.9 and 0%, respectively. Folate-conjugated and unconjugated liposomes containing AlPc were then incubated with HeLa cells and the results indicated that the folate-conjugated liposomes were less effective than the unconjugated AlPc-liposomes at all concentrations.

CONCLUSIONS: PDT is a very promising and novel approach to the treatment of oropharyngeal and other cancers, and the results of this experiment demonstrated that when delivered using liposomes, the photosensitizers AlPc and ZnPc were both effective at reducing cell viability. However, conjugation of folate to the photosensitizer-containing liposomes did not yield an increased reduction in cell viability, relative to control. Future experiments will attempt to utilize the increased expression of folate receptor on oropharyngeal cancer cells as a means to more effectively and efficiently target photosensitizers.

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Highly efficient gene transfer to HSC-3 and H357 oral squamous cell carcinoma and HeLa cervical carcinoma cells by two novel transfection agents

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OBJECTIVES: Oral squamous cell carcinoma (OSCC) is one of the most common neoplasms in the oral cavity. The survival rates of OSCC patients have not increased in the last two decades. Gene therapy involves the introduction of therapeutic genes into malignant cells. Suicide gene therapy aims at specific killing of cancer cells, and includes the delivery of the genes for enzymes that convert prodrugs into active drugs. The use of non-viral vectors is a promising approach to gene delivery, since it avoids potential inflammatory effects of viral vectors. However, the transfection efficiency of non-viral vectors needs to be improved to achieve gene expression in most of the cancer cells. We investigated the ability of two novel reagents to deliver the gene for firefly luciferase into cervical and oral squamous cell carcinoma cells.

METHODS: HeLa cervical carcinoma, and HSC-3 and H357 human OSCC cells were seeded in 48-well culture plates one day prior to transfection, and used at ~85% confluence. The cells were transfected with either TransfeX (ATCC, Manassas, VA) or TransIT-LT1 (Mirus, Madison, WI). Each transfection reagent was used at ratios of 1, 2, 4, or 8 μ l per 1 μ g plasmid DNA (pCMV.Luc). Toxicities of the reagents were measured with Alamar Blue cell viability assay. Transfection efficiency was evaluated by measuring luciferase activity 48 h after transfection, using the Luciferase Assay System obtained from Promega and a Turner Designs TD-20/20 luminometer. Data were expressed as relative light units (RLU) per ml of cell lysate.

RESULTS: In HeLa cells, luciferase expression with 4 μ l TransfeX/1 μ g DNA reached 50,620,000 \pm 13,195,014 RLU/ml, the highest activity ever seen in our laboratory. TransIT-LT1 at 4 μ l/1 μ g DNA achieved 10,221,333 \pm 1,615,934 RLU/ml. In HSC-3 cells, luciferase expression obtained with TransfeX was 1,034,800 \pm 37,098 RLU/ml, and 35,067 \pm 5,621 RLU/ml with TransIT-LT1. In H357 cells that are highly resistant to transfection with previously available reagents, TransfeX showed maximum transfection efficiency at 2 μ l TransfeX/1 μ g DNA, with 2,514,000 \pm 249,800 RLU/ml.

CONCLUSIONS: Transfection of HeLa cells with TransfeX exhibited the highest transfection activity obtained in our laboratory, and was almost 5 times higher than that achieved with TransIT-LT1. In HSC-3 cells, transfection efficiency of TransfeX was 29 times higher than that of TransIT-LT1. Very high levels of luciferase activity were obtained in H357 cells with TransfeX. Future studies with flow cytometry and fluorescence microscopy will evaluate the percentage of transfected cells, and apply TransfeX-mediated transfection to suicide gene therapy, utilizing the herpes simplex virus I thymidine kinase gene + ganciclovir system.

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Biomarkers of teeth moved with Invisalign

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OBJECTIVES: A number of studies analyzed a response of periodontal ligament (PDL) to force applied by fixed orthodontic appliances. However, no study has focused on a different orthodontic system, namely the Invisalign system. The Invisalign system is based on sequential application of 20-40 aligners each of them applying a small force. The force, however, is not stable as with the fixed appliances, but intermittent. The goal of our study was to find out, if force applied by aligners triggers a similar or different inflammatory response in the PDL. The gingival crevicular fluid (GCF) is serum transudate that is released from PDL space to oral cavity. We have measured the volume of GCF fluid as an indicator of inflammatory response of PDL to applied force.

METHODS: Three volunteers starting the Invisalign treatment were included in the study (IRB approval Nr. 14-57). Twenty teeth were followed including two premolars, canine and two incisors in two quadrants of the upper jaw and two quadrants of the lower jaw. Samples (n = 640) were collected on both buccal and lingual sides of each tooth using Periopaper strips (Oraflow) and a volume of collected liquid was measured using Periotron (Oraflow). Collections were done before the Invisalign treatment was started (baseline) and then one day and 14 days after the start of the first aligner, 14 days after the start of the third aligner and one day and 14 days after the start of the fifth or seventh aligner. The baseline values were deducted from the treatment values. The GCF volumes collected from buccal and lingual sites were evaluated separately, mean values were compared to the baseline values and significance of differences was calculated using t test.

RESULTS: The highest volume of GCF was collected after application of the first aligner (either 1 day after the start or after 14 days of its application). At the end of the third aligner, the GCF volume was lower than at the end of the first aligner. The lowest volume of GCF was collected after application of the fifth or seventh aligner and, at the end, the value was close to or not different from the baseline. The same results were obtained from buccal and lingual collection sites.

CONCLUSIONS: This pilot longitudinal case study is the first to analyze a PDL tissue response to force applied by orthodontic treatment with the Invisalign system. The highest GCF volume was collected after application of the first aligner, lower at the end of the third aligner and almost the resting volume of GCF was collected with the fifth or seventh aligner (after 10-14 weeks of treatment). It is suggested that the inflammatory response to the same force applied by the subsequent aligners is diminishing.

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Role of the MSX1 gene in etiology of orofacial clefting: a review

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OBJECTIVES: MSX1, a member of the muscle segment homeobox gene family, has been considered a strong candidate gene involved in orofacial clefts and dental anomalies. The aim of this review is to summarize the available literature on the role of MSX1 gene mutations in nonsyndromic cleft lip with or without cleft palate (CL/P) and to identify areas in which further research is needed.

METHODS: A PubMed electronic database search for literature on the etiological role of the MSX1 gene in orofacial clefting was carried out using Boolean language and key words '*msx1*,' '*cleft lip*,' '*cleft palate*' and '*transcription factor*.' A manual search in the Pacific Craniofacial Genetics Laboratory literature database and in relevant studies was also performed for additional references that may have been missed by the aforementioned search strategy. The inclusion criterion for accepting articles was studying the direct role of the MSX1 gene in nonsyndromic (isolated) orofacial clefting. Out of the 162 publications initially retrieved, 27 articles were selected for review.

RESULTS: The MSX1 gene is weakly expressed in the anterior mesenchyme of the developing palate. In MSX1-deficient mice embryos, the anterior region of the paired palatal shelves failed to make contact and fuse leading to CL/P. This occurrence of cleft palate in the MSX1-null mice aided the identification of MSX1 mutations cosegregating with CL/P. Mutations in the MSX1 gene have been identified in 2% of human patients with nonsyndromic orofacial clefting. Several subsequent studies of various ethnic populations support the association of MSX1 with orofacial clefting. Some studies found no associations between MSX1 and CL/P.

CONCLUSIONS: Although, no specific variant of MSX1 has been directly linked as a major polymorphism for clefting thus far, multiple lines of evidence suggest that MSX1 is a gene that promotes the development of the orofacial complex. The literature search also supports the presence of multiple genes and environmental conditions involved in the etiology of the cleft phenotype. The challenge is now to identify the genes in which variants are more likely to increase the risk for CL/P. Further research is needed to better understand these multifactorial etiologies of clefts so that an effective approach for cleft prevention can be developed.

Mutations in exons 1 and 2 of PAX9 gene and hypodontia

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INTRODUCTION: Hypodontia is one of the most common dental anomalies that result in aesthetic and functional problems. A wide range of prevalence values for missing teeth has been reported ranging from 1.6% to 25.4% in adult population. Our understanding of the genetic basis of tooth agenesis is still limited. Several genes have been explored, including, but not limited to PAX9 and MSX1. The normal function of the PAX9 gene, a transcription factor that plays an important role in signaling between epithelial and mesenchymal cells during tooth development, seems to be critical during development of dental lamina.

OBJECTIVES: The goal of our study was to identify genetic mutations in exon 1 and exon 2 of PAX9 gene, commonly associated with a lack of tooth development in probands and their close relatives.

MATERIAL AND METHODS: Our sample consisted of 66 individuals with congenitally missing teeth and 50 of their relatives. Saliva specimens were collected from all individuals. Most of saliva specimens were collected using our own protocol. Modified chelex method was used to extract DNA. A smaller number of specimens were collected using Oragene saliva kit. Following DNA isolation, PCR was done using specific primers for each single nucleotide polymorphism, agarose electrophoresis followed to confirm PCR product, which was then purified and sent to sequencing laboratory. The sequenced specimens were analyzed for PAX9 genotypes.

RESULTS: Out of 66 individuals with missing teeth and 50 their family members that were genotyped, 13 mutations (9 in probands and 4 in family members) in exons one or two of PAX9 gene were found. Among 76 controls, none had a mutation in exons one or two of PAX9 gene.

CONCLUSION: Results of this pilot study suggest a rather strong association of exon 1 and exon 2 mutations of PAX9 gene in individuals with hypodontia in our sample. Evaluation of a larger sample will enable us to draw more definitive conclusions. Our study continues and, in addition to PAX9, also MSX1 and BMP4 gene polymorphisms are studied.

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SENIOR RESEARCH PRESENTATIONS

Effects of an interactive poster on oral hygiene knowledge in a rural village in Jamaica

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OBJECTIVES: The purpose of this study is to determine the effectiveness of an interactive poster as an intervention to increase the knowledge of oral health care in the rural community in Jamaica. The objective is to learn if the prevention information presented via the poster increases the ability of the subjects to correctly answer the evaluation questions.

METHODS: The design of the study included a pre-test and an identical post-test to determine whether the intervention worked. The sample was selected at the UOP Jamaica Mission trip. Subjects were selected randomly from the pool of people who came to receive dental treatment. All subjects were over 18 years of age. Participants were asked if they would like to participate in a short survey and learn more about taking care of their teeth. Data was collected by students participating in the dental mission trip. Each student asked the question verbally in the same manner. As a pre-test, three questions were asked of each participant at the entrance to the clinic. 30 minutes to 7 hours were then given to the participants to look at and interact with the poster in the waiting room, while waiting for an available dentist to do a dental procedure for them. Before the dental procedure was done, the subject was asked the exact same questions as a post-test to test their newly acquired knowledge. The answers were recorded on paper and then transferred to a spreadsheet electronically.

RESULTS: The review of the data indicates that the poster was successful in increasing participants' knowledge of dental care. The three questions were separated out to show the differences in knowledge in each category: brushing, flossing, and diet. The first question asked the people how many times they should brush their teeth. Initially, only 25% of the population knew that they should brush their teeth twice per day. After the intervention, 86% of the population knew the correct answer. 82% of people who answered the question incorrectly, corrected their answer after looking at the poster, while 17% of them did not. The second question asked subjects when it would be the best time to floss. Before looking at the poster 30% of the participants knew that it is best to floss at night. After the intervention 90% of the participants knew the correct answer. Of those that chose the incorrect answer at the pre-test 84% chose the correct answer on the post test and 15% did not. Last question asked the participants if the popular Jamaican drink "bag juice" is good for their teeth or no. 53% of the participants knew that bag juice was not good for their teeth before looking at the poster. After the intervention 94% of the subjects knew the correct answer. 88% of the people who had an incorrect answer in the beginning changed to the correct one after the intervention, but 11% did not.

CONCLUSIONS: The results show improvement in all three questions of the survey. Over 80% of the people who did not know the proper oral hygiene regimen before looking at the poster learned the proper way of taking care of their mouth. This is extremely important because this knowledge can be applied to improve their personal and perhaps their family's oral hygiene regimen. It was interesting to see that 36% of the population did not know how to brush their teeth while 67% of the population did not know how to floss. Overall the study provided insight into the interactive oral hygiene posters, which could be used as a resource to teach proper techniques of oral hygiene. These tools are not time consuming to the practitioner but they are very valuable to the patients receiving oral care. These types of resources could be used in other mission trips to promote proper oral hygiene techniques and improve information transfer to break down language barriers.

This work was supported by the University of the Pacific Arthur A. Dugoni School of Dentistry Department of Dental Practice.

Broadly neutralizing anti-HIV antibodies PG9, PG16, PGT121, and PGT145 do not inhibit HIV-1 envelope protein (Env)-mediated cell-cell fusion

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OBJECTIVES: An effective HIV vaccine must be able to block infection by a wide range of viral isolates. PG9, PG16, PGT121, and PGT145 antibodies were identified from culture media of activated memory B-cells of an infected donor and shown to neutralize many HIV strains (Walker et al. Science 326, 285; 2009), recognizing conserved epitopes on the viral envelope protein gp120. The PG9, PG16, and PGT145 antibodies recognize V1/V2 conformational epitopes, whereas PGT121 recognizes a V3 epitope involving carbohydrates. Since HIV-1 infection occurs via both free virions and cell-cell fusion, we examined the effect of the antibodies on HIV Env-mediated cell-cell fusion.

METHODS: We used the HIV fusion assay developed previously in our laboratory (Yee et al. Open Virol J 5, 12; 2011). Clone69TRevEnv cells (NIH AIDS Reagent Program) that express Env in the absence of tetracycline (“HIV-Env cells”) were plated, and then labeled with Calcein-AM Green (Invitrogen). Highly CD4⁺ SupT1 cells were labeled with CellTrace™ Calcein Red-Orange (Invitrogen), and then were incubated with the adherent HIV-Env cells, with or without antibodies. Antibodies were obtained from the International AIDS Vaccine Initiative and Polymune Scientific. Lectins were purchased from EY Labs. Syncytia were observed under a Nikon Diaphot inverted fluorescence microscope.

RESULTS: Monoclonal antibodies PG9, PG16, 2G12, PGT121, and PGT145 (at up to 20µg/ml) had little or no inhibitory effect on fusion between HIV-Env and SupT1 cells. By contrast, *Hippeastrum hybrid* agglutinin at 1µg/ml completely inhibited fusion.

CONCLUSIONS: Antibodies PG9, PG16, PGT121, and PGT145 are ineffective against cell-cell fusion induced by the HIV ENV membrane protein, indicating that they may not be able to inhibit the transmission of the virus or viral genetic material between cells. Lectins, however, are very effective against Env-induced cell-cell fusion, even at low concentrations.

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Targeting lectin-coupled liposomes to the highly mannosylated HIV envelope protein

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OBJECTIVES: HIV-infected cells that are producing virions express the HIV envelope protein (Env) on their surface, thereby exposing their identity. Our long term goal is to exploit this fact to target cytotoxic liposomes to HIV-infected cells by attaching carbohydrate binding proteins (lectins) that recognize the highly mannosylated Env. The lectins *Hippeastrum* hybrid agglutinin (HHA) and *Galanthus nivalis* agglutinin (GNA) bind specifically to Env, and inhibit HIV infection and Env-mediated cell-cell fusion. We coupled HHA to liposomes, and evaluated whether these liposomes could bind specifically to Env-expressing cells.

METHODS: Fluorescein-labeled HHA (EY Laboratories) was combined with N-hydroxy succinamide-palmitic acid (Sigma) at a mole ratio of 5 palmitic acid:1 lectin molecule for 24 h. The palmitoylated lectin was added to 100 nm-diameter liposomes at a ratio of 40 lectins per liposome along with deoxycholate for 24 h. The lectin-labeled liposomes were dialyzed using dialysis tubing in Hepes-buffered saline (HBS) over 24 h. The lectin-labeled liposomes were passed through a Sepharose CL-4B column equilibrated with HBS and 2 ml fractions collected. The fractions were tested for protein using the Bio-Rad assay and for fluorescence using a Perkin-Elmer LS-50B fluorometer. Clone69TRevEnv cells (NIH AIDS Reagent Program) were maintained in DMEM supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), L-glutamine, geneticin, hygromycin B and tetracycline. Tetracycline was removed from the medium to induce Env expression ("HIV-Env cells"). Cells were plated at 2.0×10^5 cells/ml in 48-well plates for 24 h, incubated with 1% albumin for 1 h at 6°C, and then incubated with 1 µg/ml lectin (free or coupled to liposomes) for 3 h on ice. Cells were observed under a Nikon Diaphot inverted fluorescence microscope with a Jenoptik digital camera. Fluorescence of the cells was also quantified using a Guava flow cytometer.

RESULTS: The fractions of lectin-labeled liposomes tested in the binding assay bound to both Env-expressing and non Env-expressing cells. The control fluorescein-labeled lectin as well as the control rhodamine-labeled lectin tested in the binding assay bound lightly to both Env-expressing and non Env-expressing cells. The control liposomes tested in the binding assay did not bind to Env-expressing or non Env-expressing cells.

CONCLUSIONS: The lectins must be examined further for Env-binding specificity. Whether synthetic lectins that inhibit HIV infection at very low concentrations bind specifically to Env-expressing cells will also be investigated.

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Liposomal Zn- and Al-phthalocyanine enhance photodynamic therapy of oral cancer

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OBJECTIVES: Photodynamic therapy (PDT) has been studied as a promising method to eliminate cancer cells. We used liposomes as a method of specific drug delivery for PDT and studied the effectiveness of the liposome-encapsulated photosensitizers, zinc phthalocyanine (ZnPc) and aluminum phthalocyanine chloride (AlPc), on oral squamous cell carcinoma.

METHODS: Liposomes were composed of palmitoyllecithin (POPC): phosphatidylglycerol (PG), and contained either ZnPc or AlPc. Free or liposome-encapsulated ZnPc and AlPc were added to HSC-3 cells in the concentration range 0.1-1 μ M, and incubated for 24 h at 37°C. The cells were then exposed to light (690 nm) from a High Power LED Multi Chip Emitter. Cytotoxicity was evaluated by the Alamar Blue assay that measures metabolic activity, using a Molecular Devices Versamax microplate reader.

RESULTS: Cells treated with ZnPc and AlPc encapsulated in liposomes resulted in further decrease in cell viability when compared to cells treated with free ZnPc and AlPc. The Alamar Blue assay showed a linear reduction with increased concentrations of ZnPc and AlPc in both free and liposomal form. For 0.1, 0.5, and 1 μ M liposomal ZnPc, the viability was reduced to 89 \pm 4, 69 \pm 4, and 41 \pm 5%, respectively. With free ZnPc, the values were 104 \pm 5, 89 \pm 5, and 75 \pm 6%, respectively. For 0.1, 0.5, 1 μ M liposomal AlPc, the viability was reduced to 54 \pm 3, 20 \pm 3, and 21 \pm 2%, respectively. With free AlPc, the viabilities were 108 \pm 5, 78 \pm 8, and 51 \pm 1%, respectively.

CONCLUSIONS: HSC-3 cells are vulnerable to liposomal ZnPc and AlPc in a dose-dependent manner, following light activation. Liposomal ZnPc and AlPc both reduce cell metabolic activity more effectively than the free photosensitizers. Our studies indicate that liposomal delivery of ZnPc and AlPc results in a more efficient elimination of oral squamous cell carcinoma.

Mannose-specific lectins that inhibit HIV infection and HIV Env-induced cell-cell fusion do not bind specifically to HIV-Env expressing cells

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OBJECTIVES: HIV-infected cells that are producing virions express the HIV envelope protein (Env) on their surface, thereby exposing their identity. This Env protein is used for fusion of HIV to CD4⁺ host cells, as well as for the fusion of HIV infected cells to uninfected cells for spread of the virus. The carbohydrate binding proteins (lectins), *Hippeastrum* hybrid agglutinin (HHA) and *Galanthus nivalis* agglutinin (GNA) bind to the mannose residues on the Env protein. These lectins inhibit Env-mediated cell-cell fusion, and HIV infection. Thus, we wanted to evaluate if the lectins bound specifically to the Env protein on the surface of cultured cells.

METHODS: Clone69TRevEnv cells (NIH AIDS Reagent Program) were maintained in DMEM supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), L-glutamine, geneticin, hygromycin B and tetracycline. Tetracycline was removed from the medium to induce Env expression (“HIV-Env cells”). Cells were plated at 2.0×10^5 cells/ml in 48-well plates for 24 h, then incubated with 1 µg/ml fluorescein-labeled HHA and GNA (EY Laboratories) lectin for 3 h on ice to inhibit endocytosis. Fluorescence of the cells was quantified using a Guava flow cytometer.

RESULTS: Both the fluorescein-labeled HHA and fluorescein-labeled GNA bound to both Env-expressing and non-Env-expressing cells.

CONCLUSIONS: The lectins HHA and GNA do not bind specifically to the Env protein, and may be binding to other receptors on the surface of Clone69TRevEnv cells. Thus, they may be inhibiting Env-mediated cell-cell fusion by a mechanism other than specific binding to the Env protein. This observation may preclude the use of lectins as a ligand to target cytotoxic liposomes to HIV-infected cells.

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Comparison of two canal preparation techniques in the induction of microcracks: A pilot study with cadaver mandibles

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INTRODUCTION: The purpose of this pilot study in a cadaver model was to compare 2 different shaping techniques regarding the induction of dentinal microcracks.

METHODS: Three lower incisors from each of 6 adult human cadaver skulls were randomly distributed into 3 groups: the control group (CG, no instrumentation), the GT group (GT Profile hand files; Dentsply Tulsa Dental, Tulsa, OK), and the WO group (WaveOne; Dentsply Tulsa Dental). In the GT group, manual shaping in a crown-down sequence with GT Profile hand files was performed. In the WO group, Primary WaveOne files were used to the working length. Teeth were separated from the mandibles by careful removal of soft tissue and bone under magnification.

Roots were sectioned horizontally at 3, 6, and 9 mm from the apex using a low-speed saw. Color photographs at 2 magnifications (25x and 40x) were obtained. Three blinded examiners registered the presence of microcracks (yes/no), extension (incomplete/complete), direction (buccolingual/mesiodistal), and location. Data were analyzed with chi-square tests at $P < .05$.

RESULTS: Microcracks were found in 50% (CG and GT) and 66% (WO) of teeth at 3 mm, 16.6% (CG) and 33.3% (GT and WO) at 6 mm, and 16.6% in all 3 groups at 9 mm from the apex. There were no significant differences in the incidence of microcracks between all groups at 3 ($P = .8$), 6 ($P = .8$), or 9 mm ($P = 1$). All microcracks were incomplete, started at the pulpal wall, and had a buccolingual direction.

CONCLUSIONS: Within the limitations of this pilot study, a relationship between the shaping techniques (GT hand and WaveOne) and the incidence of microcracks could not be shown compared with uninstrumented controls.

Role of the TAS2R38 and TAS1R2 taste receptor genes in dental caries status

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OBJECTIVES: With the high incidence of dental caries worldwide it is a major public health concern. The high incidence is due to many factors influencing the disease and its progression. Past studies have established there is a genetic component influencing the rate of caries in an individual. This study focused on two genes, TAS2R38 and TAS1R2. TAS2R38 is a bitter taste perception gene, which encodes for a G-protein coupled receptor and the TAS1R2 gene is a sweet taste perception gene, which also encodes for a G protein-coupled receptor. This study examined the TAS2R38 and TAS1R2 taste genes and their influence on dental caries rate in a population of dental students at the University of the Pacific Arthur A. Dugoni School of Dentistry. Understanding the influence of these genes to dental caries is important for prevention and patient education. With this understanding, high-risk patients could be identified and prevention strategies could be implemented early on to compensate for the genetic predisposition of dental caries.

METHODS: For each participant their caries status was determined by charting their current carious lesions, restorations, and missing teeth. From this information the DMFT (decayed missing and filled teeth) and DMFS (decayed missing and filled surfaces) scores were determined. Each individual also provided a 24-hour food recall and background health and oral hygiene information. Saliva specimens for DNA analysis were collected from all of the individuals in the study and were spotted on filter paper. DNA was extracted from the dried saliva specimens via isolation kits. Real time PCR was performed to determine the genotypes. For each individual, the caries status was placed in a low or high category. Individuals with a DMFS score of less than 14 were considered low, and individuals with 14 or higher were classified into a high category. The categories were determined based on the number 14 representing 10% of the 140 surfaces in the mouth excluding third molars. We calculated genotype proportions and allele frequencies for each SNP and compared low and high category. However, as our sample is small, we compared only percentages in those two categories.

RESULTS: Wendell's results (Wendell et al, 2010) suggest that the G allele of the TAS2R38 rs713598 C/G polymorphism is associated with protection against dental caries ($p=0.007$) and the G allele of the TAS2R38 rs1726866 G/A polymorphism is associated with caries protection as well ($p=0.03$). Also for TAS2R38 rs10246939 C/T, Wendell found the C allele to be associated with caries protection ($p=0.01$). Wendell's results were not so convincing for TAS1R2. We combined "protective" alleles for TAS2R38 and compared subgroups with low and high DMFS score (Table 3 a, b). There was no statistically significant difference in occurrence of "protective" alleles between low and high DMFS groups ($p=0.5$).

CONCLUSIONS: Our study proved feasibility to analyze taste receptor genes from saliva that was collected using a simple and effective protocol. It was also not complicated nor time consuming to collect data that are relevant to dental caries status – as dietary information, family history of dental caries, and medical history. Dental exam was recorded as DMFS and provided us with a very basic, but sufficient information. Our sample was very small to draw any conclusion, whether "protective" alleles of TAS2R38 and TAS1R2 described previously are associated with a lower rate of dental caries. Understanding the influence of these genes on dental caries is important for prevention and patient education. With this understanding, high-risk patients could be identified and prevention strategies could be implemented early on to compensate for the genetic predisposition of dental caries.

Photodynamic therapy of pharyngeal cancer with liposomal aluminum phthalocyanine chloride

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OBJECTIVES: Photodynamic therapy (PDT) utilizes visible light to activate photosensitizer molecules, which then generate singlet oxygen and other reactive oxygen species that can cause cytotoxicity. Head and neck squamous cell carcinomas may be amenable to PDT. We investigated the efficacy of PDT with free or liposome-encapsulated aluminum phthalocyanine chloride (AlPc) as the photosensitizer on FaDu pharyngeal carcinoma and HPV16+ 2A3 cells.

METHODS: Liposomes comprising phosphatidylglycerol (PG), palmitoyllecithin (POPC), and AlPc (5:5:0.1) were prepared by thin film hydration and extrusion through polycarbonate membranes. FaDu and 2A3 cells were incubated with free and liposomal AlPc in the concentration range 0.1–1 μ M for 24 h at 37°C. They were then exposed to light (690 nm) from a 9.8V High Power LED Multi Chip Emitter for 20 min, at a total light dose of 3.6 J/cm². Cell survival was evaluated by the Alamar Blue assay.

RESULTS: Treatment of the cells with AlPc resulted in a dose dependent decrease in cell viability. In the case of free AlPc at 0.1, 0.5, and 1 μ M, the viabilities of FaDu cells were reduced to 87.5 \pm 14%, 24.8 \pm 15%, and 0% of untreated controls, respectively. With 0.1, 0.5, and 1 μ M liposomal AlPc, the viabilities were reduced to 1.4 \pm 0.3%, 0%, and 0%, respectively. With 2A3 cells treated with free AlPc, the viabilities were 104.0 \pm 0.4%, 66.7 \pm 14.3%, and 51.6 \pm 12.5, and with liposomal AlPc they were 45.9 \pm 10.5%, 24.4 \pm 2.9%, and 15.5 \pm 6.1%, respectively.

CONCLUSIONS: In FaDu cells, even at the lowest concentration, liposomal AlPc was 63-fold more effective than the free photosensitizer. PDT was not as effective in 2A3 cells, although liposomal AlPc could reduce the viability to 15% of untreated controls and was 3-fold more effective than the free drug. Whether the lower susceptibility of the HPV16+ cells is related to the viral proteins expressed in these cells is yet to be determined.

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IDS STUDENT REVIEW PRESENTATIONS

Endodontic therapy vs. three unit fixed partial dentures vs. single unit implant

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OBJECTIVES: Systematic processes and practical criteria are described in this review to aid in the determination of a treatment plan decision on when to proceed with tooth preservation via root canal therapy, or replacement via a FPD, or a single-tooth implant. A compilation of the best available evidence from views of field specific experts in endodontics and implants, as well as a comprehensive literature review, provided the recommendations presented.

METHODS: An extensive analysis of various systematic reviews, texts and peer-reviewed articles was conducted. A MEDLINE search ranging from the 1990s' to 2014, using PubMedSearch parameters including a combination of keywords: "endodontics vs implants", "implants vs fixed partial dentures", "success and survival rate", "factors affecting treatment planning", "complications", and "treatment planning" was conducted with a supplemental hand search of relevant article bibliographies. Following evaluation of various abstracts and titles 39 articles were analyzed in full. After reviewing many articles the most relevant articles to this topic were selected for this presentation.

RESULTS: Iqbal and Kim (2007) completed a meta-analysis on success and survival rate of single tooth implants and endodontically restored natural teeth. 143 studies were reviewed varying in design, success criteria, operator and sample size. Similar survival rates were reported, but the implant group showed greater incidence of post-operative complications, prosthetic repairs and soft tissue maintenance. Setzer and Kim (2014) in their meta-analysis found that new technologies in endodontics have changed the statistics on outcomes, especially in retreatment cases: traditional apicoectomy- 59%, modern approach 91-93% (1-yr. FU). In the meta-analysis by Pjetursson and Lang (2007), a comparison of the 5 and 10 years survival rates of conventional FPDs (93.8%, 89.2%) and a single crown implant (94.5%, 89.4%) was conducted. Kim et. al (2011), concluded that a "single implant-supported restoration, despite its high survival rate, was shown to be the least cost-effective treatment option based on current fees" amongst all the treatment modalities for a failed endodontically treated first molar. It was found that the single-implant supported restoration was 2-4 times more costly than a nonsurgical retreatment with a crown and a FPD is more cost-effective than a single implant-supported restoration though less cost-effective than non-surgical retreatment and crown.

CONCLUSIONS: Treatment alternatives have different aims; endodontic treatment is provided to treat or prevent apical periodontitis, whereas implants and fixed partial dentures are used to replace missing teeth. An overall review of each individual patient case evaluation, operator experience and training, evidence based research, patient desires and expectations, risks and cost effectiveness aids in the treatment decision and informed consent in acceptance. Further studies with improved controls, larger sample sizes, and longer follow-up will continue to enhance clinical decision making. Based on the present evidence, methodologies with improved scientific evidence are most needed in the areas of systemic and local factors influencing implant survival/success and factors in selecting endodontic versus tooth extraction and replacement. New investigations should reflect current advances in materials and techniques and be more relevant to present-day treatment options.

Veneers in dentistry

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INTRODUCTION: Aesthetic is becoming very instrumental in the modern dentistry. As the patients' demand for aesthetics is increasing; our effort to providing a better treatment has to be increased also. Therefore, our research is focusing on the aesthetic field especially on veneers. Veneers as a procedure is highly predictable, conservative and aesthetic procedure. However, patient/case selection is a very crucial step in the procedure success, longevity and survival. Considering indications and limitations of this procedure should be evaluated carefully to ensure desirable clinical outcomes. The major four sections covered are: case selection and smile design; preparation design and material; veneers shade and cement; as well as longevity and survival rate.

OBJECTIVES: Proper case selection, clinical applications, indications and limitations are very influential factors in the treatment success. Each has to be considered carefully when treatment plan for veneers. Taking thorough Medical and dental histories is a key step in that process. Partly due to, staining challenges, tooth structure and anatomy variations. Smile design is another significant facet of the treatment plan. It is very effective measure of the patient aesthetic expectations and how realistic they are. Indications of use and limitations were evaluated in the presentation as well to evaluate their impact on the preparation design, final outcome and survival of the restoration. Diving more in the aesthetic outcomes, other parameters came to play such as, the abutment natural color and cement shade were evaluated in the presentation for their effect on final aesthetics. Testing the outcomes from the aspect of success, survival and failure rate are discussed as well. Reviewing studies that consider parafunctional habits, preparation design, material been used, the skill level of operator, the type of interventions and the clinical performance of veneers in 5,10,20 years of follow up are giving us a better understanding on veneers in general.

METHODS: A literature research was conducted, using electronic databases, relevant references, citations and journal researching for studies focusing on the veneers as an aesthetic and conservative treatment options.

CONCLUSIONS: It is our duty to carefully diagnose, analyze and deliver the best to our patients. Taking into account all of the discussed health, dental and local factors. Our aim has to be less reduction of tooth structure, greater esthetics and durability. Simply, cosmetic dentistry incorporates multispecialty interventions. Veneers is a great addition to minimally invasive dentistry however it is not a reversible procedure. Case selection and substrate enamel left play important role in the treatment outcome. Choosing of the correct material is essential for desired results. Different shades and opacities of resin cements have insignificant influence on final color match of veneers. Shade of ceramic and tooth have major influence on the final color match of veneer. Dual cured resin cements have better micromechanical properties than light cured cements and hence recommended for more posterior veneers. Porcelain veneers offer a predictable, conservative, and highly successful restoration. The estimated survival probability at 10 years was 93.5%, the main reason for failure was fracture of the ceramic and increased failure rates were associated with parafunction (bruxism) and nonvital abutment teeth.

Traditional vs. digital impressions in fixed and implant dentistry

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OBJECTIVES: The purpose of this study is to evaluate the efficiency, difficulty, accuracy of internal and marginal fit, technique sensitivity and operator's preference of digital impression compared to conventional impression, giving more focus on the fixed and implants fields in dentistry.

METHODS: Articles were gathered using PubMed. A thorough reading and interpretation of these articles assisted in comparing between internal fit of restorations produced by both techniques, marginal and dimensional inaccuracy as well as level of difficulty to inexperienced operators.

RESULTS: Impress CAD restorations fabricated with Cerec Scan system showed the best margin accuracy of 30 (± 17) among seven different types of restorations produced by different digital and traditional techniques, while Lava Zirconia restorations fabricated by Lava C.O.S scan system showed the best internal fit 29 (± 7). For technique efficiency and difficulty, it was shown that digital impressions had a significantly shorter learning curve and 77% of operators preferred it over traditional. On the diagnostic accuracy aspect, it was shown that iTero system provided almost 1-to-1 diagnostic information and more accurate than CBCT measurements. Finally, frameworks fabricated from digital and traditional impressions showed clinically acceptable marginal fit, while those fabricated from digital impression demonstrated significantly better internal fit.

CONCLUSIONS: With the recent significant advancement in digital impressions techniques and devices, along with the superior results produced, digital impressions can be the new gold standard in majority of daily dental work and will help expand the opportunity of modern, fast and efficient dentistry.

Clinical applications of dental ceramic

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OBJECTIVES: There have been significant technological advances in the field of dental ceramics over the last 10 years that have made a corresponding increase in the number of materials available. Improvements in strength, clinical performance and longevity have made all ceramics restorations more popular and more predictable. Despite improvements these restorations are still time consuming, expensive and technique sensitive when compared to conventional porcelain fused to metal. The authors of this poster aim to discuss the clinical applications and the laboratory and processing systems of 4 types of dental porcelain: Feldspathic Porcelain, Leucite Reinforced Porcelain, Lithium Disilicate and Polycrystalline Ceramics. This poster will cover the types of ceramics are commercially available based on a classification of the micro-structural components of the ceramic. In addition to clinical indications, contra-indications, advantages and disadvantages of each of the materials, current cementation protocols will also be discussed. In this review, we conducted a comprehensive literature review to compile and compare clinical evidence for a full and partial coverage restorations using all ceramic systems.

METHODS: A comprehensive review of the literature was completed seeking evidence for the treatment of teeth with all-ceramic restorations. A search of peer-reviewed literature was undertaken using PubMed, MED-LINE, JADA and CDA journals with a focus on evidence-based research articles published between 1990 and 2013. A hand search of relevant dental journals was also completed. Randomized controlled trials, nonrandomized controlled studies, longitudinal experimental clinical studies, longitudinal prospective studies, and longitudinal retrospective studies were reviewed. Data supporting the clinical application of all-ceramic materials and systems was sought.

RESULTS: The literature demonstrates that multiple all-ceramic materials and systems are currently available for clinical use, and there is not a single universal material or system for all clinical situations. The successful application is dependent upon the clinician to match the materials, manufacturing techniques, and cementation or bonding procedures, with the individual clinical situation. A close understanding of the science and dynamics of different materials is necessary for the design and function of the restoration. As one can comprehend, no material is ideal in all aspects, to natural structure of teeth.

CONCLUSIONS: New generation ceramics present dentists with many restorative options in these exciting times for dental ceramics. However, mechanical and biological properties of ceramics have exhibited a significant improvement in the past few decades. Recent surge in all-ceramic crowns for esthetics and durability is a highlight of such improvements. Further scope of research in dental ceramics can be directed to stronger and more esthetic and strong crowns and veneers, to mention a few. Also with the advent of the computer technology, it is now possible to have a huge progress in impression making and crown fabrication. The new CAD/CAM technology has allowed dentists to fabricate crowns on site in much shorter and convenient way. Definitely, future is bound to witness a bigger revolution in field of dental ceramics, with introduction of newer ceramics and nanotechnology for the betterment of dental restorations on the lines of form, function and esthetics, along with improved biocompatibility.

Minimally invasive dentistry

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INTRODUCTION: Minimally Invasive Dentistry (MID) is an approach in treating dental caries, which has evolved from past of removing the cavitations to focusing on remineralizing it. Dental caries is considered a chronic multifactorial lifestyle disease in which patient compliance and professional recommendations contribute significantly. Both dentists and patients need to adapt to this change in philosophy of how the caries disease process is treated today.

OBJECTIVES: The primary objectives of this study were to learn about four principles of MID, (1) Early detection of caries, (2) Elimination of caries risk factors (3) Remineralization of infected lesion (4) Repair of areas where cavitation is present and surgical intervention is required. It also talks about the current scenario in implementing MID in private office is also covered.

METHODS: Review of the available literatures regarding MID particularly focusing on evidence-based journals, review of clinical trials and meta-analysis within the past 14 years were used in this presentation. A total of 48 articles were chosen centered on the application of Minimally Invasive Dentistry through diagnosis, products, treatments and current challenges. Articles were gathered using PubMed and Medline databases using keywords such as minimally invasive dentistry, CAMBRA, caries, etc.

CONCLUSIONS: With the evolution of new technologies caries can be detected and intervened at a very early stage. We need to start thinking in terms of demineralization and re-mineralization. Acceptance of the CAMBRA approach by practicing dentists will lead to a significant change in how caries is managed.

IDS STUDENT RESEARCH PRESENTATIONS

Effect of different root canal irrigation solutions on push out bond strength

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OBJECTIVES: The purpose of this research was to evaluate the effect of different irrigation solutions on the bond strength of the cemented post to the dentin surface of the root canal walls. The null hypothesis was that there is no effect of irrigation solution on bond strength.

METHODS: Roots of 48 human incisors were root canal treated and then divided into four groups. Post spaces were prepared for all roots with a low speed drill to approximately 4mm short of the apical constriction. The diameter of the post space preparation (parallel) was constant for all teeth (1.5mm).

Before post cementation, root canals were pretreated with one of four protocols. 1:water; 2: 6% NaOCL and chlorhexidine; 3: 6% NaOCL and EDTA and 4: NaOCL and Qmix (Dentsply Tulsa Dental). The solutions were applied in the canal space using needle irrigation and the EndoActivator (Dentsply). Afterwards, fiber posts (GT Fiber Posts, Dentsply) were bonded using Prime and Bond Elect (Dentsply) in combination with FluoroCore 2+ (Dentsply) build-up material. The roots were cut perpendicular to the long axis in 1mm thick dentin slabs using a slow speed saw (IsoMet, Buehler). Five slices per root were obtained. The bond strength of the fiber posts in the root canals was tested using the push-out test method. An Instron Universal Testing machine was used at a crosshead speed of 0.5mm/min to determine the push-out bond strength. Multivariate statistical analysis was performed.

RESULTS: The null hypothesis of no irrigation solution effect was rejected

(p-value = .003, F-statistic = 5.33, df = 3)

There was a statistical significant higher bond strength for Qmix compared to water for the middle dentin slab location. (Tukey's HSD, Alpha =0.5)

For each of the 5 dentin slab locations group 3 and 4 had the highest bond strength and group 2 and 1 had the lowest. For overall locations the means are: Group 1: 7.8; Group 2: 9.1; Group 3: 10.6 Group 4: 10.5 MPa respectively)

CONCLUSIONS: In this study group 3 and 4 yielded in significantly higher push out strength than group 1 and 2. 6% NaOCL combined with EDTA or Omix resulted in significantly higher bonding strength of the cemented posted then using water or NaOCL with chlorohexidine as irrigants.

Dose- and time-dependent cytotoxic effects of artemisinin C on oral squamous cell carcinoma cells *in vitro*

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OBJECTIVES: Oral squamous cell carcinoma (OSCC) is the most common oral and pharyngeal cancer. Despite the aggressive management such as surgery, radiation, and chemotherapy, prognosis for patients with advanced stage OSCC remains poor. The selective expression of anti-apoptotic proteins, such as survivin, in cancers has been shown to play a role, at least in part, in chemotherapy resistance. Artemisinin C (3,5-diprenyl-4-hydroxycinnamic acid), the major biologically active phenolic component found in green propolis, is known to have antitumor activity; however, little is known about its effect on OSCC cells. This study aimed to determine the cytotoxicity of artemisinin C in HSC-3 cells as a potential novel therapeutic agent for OSCC.

METHODS: HSC-3 OSCC cells were treated with either medium (unstimulated), DMSO (vehicle control), or graded doses (25 μ M ~ 200 μ M) of artemisinin C. After 24, 48, and 72 hours of treatment, cytotoxicity was measured by WST-1 assay. Apoptosis was quantified using Alexa fluor® 488-Annexin V and propidium iodide by flow cytometry. The amounts of survivin levels in the cell lysates were quantified with ELISA.

RESULTS: Artemisinin C exhibited dose- and time-dependent cytotoxic effects on HSC-3 cells. Flow cytometric analysis showed that 22% of unstimulated HSC-3 cells underwent spontaneous cell death while 77% of HSC-3 cells were killed in response to the highest dose of artemisinin C at 72 hours. This HSC-3 cell death was associated with the reduction in survivin level. We have not observed an effect of DMSO on cell viability at the concentration used in this study and even higher concentration (~2%).

CONCLUSIONS: HSC-3 cells are vulnerable to artemisinin C in dose- and time-dependent manners. Artemisinin C induced HSC-3 cell death probably because of a decrease in the levels of the anti-apoptotic protein, survivin.

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INVITED PRESENTATION

Stimulation of endogenous stem cells by a therapeutic Wnt protein to enhance dentin regeneration

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OBJECTIVES: During a normal repair response to dental injury such as crown fractures, damaged tissue is replaced with fibrous connective tissue (scar) tissue. This scar tissue is different from the original tissue in both anatomy and function. If a regenerative response could be induced in which the damaged tissue is replaced with tissue that is identical in anatomy and function, it would be more beneficial. Here, we tested whether introduction of a potent stem cell factor, WNT3A, could convert this reparative response into a regenerative one.

METHODS: We tested the consequences of amplifying endogenous Wnt signaling either genetically or biochemically. In order to determine the response stimulated by the introduction of lipid-reconstituted Wnt3a, Wnt (*Axin2*^{LacZ/+}) reporter mice were used to identify Wnt-responsive cells in the adult dental pulp cavity. The healing response of the endogenous environment following an acute dental pulp exposure will be compared between *Axin2*^{LacZ/+} controls and *Axin2*^{LacZ/LacZ} mice, which are deficient in Wnt signaling down-regulation due to *Axin2* elimination. In parallel, acute pulp exposures performed in the rat molar were treated with lipid-reconstituted Wnt3a and the healing response was assessed over time.

RESULTS: It was shown that acute pulp exposure causes extensive cell death but in an amplified Wnt environment, which was either created genetically or biochemically, apoptosis of the pulp cells was significantly reduced and the surviving pulp cells rapidly differentiated into dentin-secreting odontoblasts.

CONCLUSIONS: By transiently amplifying the body's natural Wnt response to pulp injury dentin formation is enhanced, and the resulting dentin bridge protects the remaining pulpal tissue from damage. This finding is likely to have general application in clinical procedures where increased dentin production is desired.

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MICROBIOLOGY STUDENT RESEARCH PRESENTATIONS

Contamination of dental unit water lines by *Legionella pneumophila* at University of the Pacific Arthur A. Dugoni School of Dentistry

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OBJECTIVE: To sample dental water lines, specifically the air-water syringe, in the University of Pacific Main Dental Clinic for microbial pathogens, including *Legionella pneumophila*. Contamination of dental water units via syringes, air rotors, and slow speed handpieces with *Legionella*, *Mycobacterium*, *Candida*, and *Pseudomonas* has been found in previous studies (Walker, Bradshaw, et. al. Applied and Environmental Microbiology. 2000 and Ricci et. al. The Lancet. 2012). According to these previous studies, up to 25% of the dental water unit lines were found to be contaminated with *Legionella*. This most likely occurs because of the sloughed off biofilm. These findings show that there is a potential risk of infection of patients. Further research needs to be done in this field to understand the prevalence of contamination of dental unit water lines.

METHODS: We collected 5 ml samples from 10 water lines throughout the Pacific dental clinic in the morning before the water lines were first used for the day. Five milliliter samples were collected into sterile 15 ml culture tubes from the air-water syringe at selected dental units. Samples were then collected again from previously sampled dental units after several hours of use (patients). Samples were stored at room temperature until microbial testing. The samples (500 µl-1 ml) were plated onto separate Buffered Charcoal Yeast Extract (BCYE) agar plates using a lawn streaking method with sterile cotton swabs. A total of 11 plates (5 morning samples, 5 afternoon samples, and 1 control) were streaked using a sterile swab and incubated at 37 °C for 70 hours. Gram-staining was used to determine the type of bacterial growth under a Leica light microscope equipped with an ICC50 HD digital camera.

RESULTS: Dental unit waterlines at University of the Pacific Arthur A. Dugoni School of Dentistry are contaminated with *Legionella pneumophila*. Based on qualitative analysis higher counts of bacteria were found in the dental water unit before the workday than at the end of the workday.

CONCLUSIONS: The results from the experiment would be a great concern for most dental related professionals and the patients. However, as long as we keep the bacteria under control, spread of disease can be prevented and minimized. The primary route of infection of *L. pneumophila* is via inhalation. Therefore, the presence of *L. pneumophila* in water used for dental treatments may threaten both patients and the dental team. In order to solve this problem, a disinfectant must be used to reduce the number of biofilm formation and also tubing system of the dental units need be tested against it. In addition, flushing the water line for several minutes before the first patient and for 20-30s between patients is recommended so that the number of bacteria in the water line can be decreased.

Prevalence of *Staphylococcus aureus* in a dental student population

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OBJECTIVES: The aim of the study was to determine the prevalence of *Staphylococcus aureus* in second year dental students at the Dugoni School of Dentistry and compare it to the prevalence of the general population.

METHODS: Samples were taken from the nares and antecubital fossa of 16 male and 14 female participants. The samples were streaked onto mannitol salt agar plates and incubated for one week. Positive colonies were identified and recorded.

RESULTS: The results suggest that second year dental school students have a higher prevalence of *Staphylococcus aureus* in the nares when compared to the general population, but a lower prevalence in the antecubital fossa.

CONCLUSIONS: The results of this study suggest that there may be some lapses in the Dugoni School of Dentistry's infection control methods, and that related future research should be aware of the findings.

This study was supported in part by the Microbiology Research Laboratory, Department of Biomedical Sciences, Arthur A. Dugoni School of Dentistry.

The results were presented as part of the Microbiology course in September, 2013.

STOCKTON CAMPUS STUDENT PRESENTATIONS

Sex-based alteration of relative importance of EDRFs in modulating vascular reactivity in Zucker diabetic fatty (ZDF) rats

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OBJECTIVES: Little is known about the interaction between diabetes and sex in vasculature. Our study investigates the effects of type 2 diabetes on endothelium-dependent and -independent relaxations. Also, if there are sex-based changes in relative contributions of endothelium derived relaxing factors (EDRFs) in modulating vascular reactivity of mesenteric arteries (MA) from ZDF rats.

METHODS: 16-18 weeks old male and female ZDF rats (fa/fa) and their lean controls (fa/-) were used. Relaxation responses to acetylcholine (ACh, 10^{-8} to 10^{-5} M) in MA pre-contracted with phenylephrine (PE) were obtained before and after pretreatment with indomethacin (cyclooxygenase inhibitor), L-NAME (nitric oxide synthase inhibitor) or barium chloride (Kir blocker) plus ouabain ($\text{Na}^{+}\text{-K}^{+}\text{-ATPase}$ inhibitor). Vascular responses to sodium nitroprusside (SNP, 10^{-9} to 10^{-5} M) were also measured in MA.

RESULTS: ACh-induced relaxations were significantly impaired in MA of ZDF rats compared to their lean controls, regardless of sex. In diabetic females, the relative importance of endothelium derived hyperpolarizing factor (EDHF) in relaxation to ACh was reduced, while in diabetic males, role of nitric oxide (NO) in relaxation to ACh was reduced. Interestingly, relaxation to SNP was enhanced in ZDF rats, irrespective of sex.

CONCLUSIONS: The relative importance of NO and EDHF in regulating vascular tone of rat MA is altered in type 2 diabetes with respect to sex. Furthermore, increased smooth muscle sensitivity to NO may be an attempt to compensate for impaired endothelial function in both diabetic male and female rats.

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Sexual dimorphism in aortic endothelial function of Zucker diabetic fatty rats: Possible involvement of superoxide production

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OBJECTIVES: Little is known about the interaction between diabetes and sex in vasculature. This study was designed to investigate whether there were sex differences in rat aortic endothelium-dependent vasodilation (EDV) in Zucker diabetic fatty (ZDF) rats, and the potential role of superoxide.

METHODS: EDV to acetylcholine (ACh) was measured in aortic rings pre-contracted with phenylephrine before and after pretreatment with apocynin (100 μ M), a NADPH oxidase (Nox) inhibitor. In addition, the level of Nox (a potent source of superoxide) and PKC β mRNA expression were determined using real-time RT-PCR.

RESULTS: ACh-induced relaxations were significantly greater in female lean rats compared with male lean rats. Accordingly, male lean rats had higher PKC β I expression level than female lean rats. Diabetes significantly impaired EDV in aortic rings from female ZDF rats, however, potentiated the relaxation in males. Pre-incubation of aortic rings with apocynin increased EDV only in diabetic female group, suggesting impairment of EDV in female ZDF aorta was partly due to an increase in the activity of superoxide. Accordingly, the levels of Nox1, Nox4, and PKC β mRNA expression were substantially enhanced in aorta of female ZDF rats compared to those in lean animals.

CONCLUSIONS: These data suggest that an elevation of superoxide may partially contribute to the predisposition of the female aorta to injury in type 2 diabetes.

This work was supported by NIH/NIDCR grant (LA and RR). This work will present at 2014 Experimental Biology, April 26-30, 2014, San Diego, CA and the 2014 Research Day event, April 26th, 2014, at the School of Pharmacy & Health Sciences, Stockton, CA.