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11TH ANNUAL PACIFIC RESEARCH DAY

Abstracts

Faculty and Student Presentations

Orthodontics Resident Presentations

Senior Research Competition

Second-Year Student Research Competition

IDS Student Presentations

Wednesday, May 20, 2009

11th ANNUAL PACIFIC RESEARCH DAY AND STUDENT RESEARCH COMPETITIONS

ABSTRACTS

WEDNESDAY, MAY 20, 2009

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PREVALENCE OF HYPODONTIA AMONG DENTAL STUDENTS

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INTRODUCTION: Hypodontia without any systemic disorders is a common dental anomaly that occurs approximately in 25% of the population, if absence of the 3rd molars is included (Arte, 2001). Excluding 3rd molars, 1.6% to 9.6% of the population presents with one or more missing teeth (Vastardis, 2000).

OBJECTIVES: The purpose of this study is to continue in investigation of the prevalence of congenitally missing teeth among a larger pool of dental students covering also their family history of hypodontia. The gathered data and information will form a basis for evaluation of heritability and recurrence risk, and for molecular genetic study of MSX1 and PAX9 gene polymorphisms.

METHODS: The sample consists of 478 individuals (266 males, 212 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 for recording of occurrence of congenitally missing teeth and diastema in the first, second, and third degree relatives: (1) A structured questionnaire and (2) A family pedigree drawn by the student.

RESULTS: Ninety one students (19.0%) reported one or more missing teeth representing the prevalence 190.37 per 1000 (95% CI = 156.73-229.10). These students formed our sample of probands. The most common quantity of missing teeth was two (36.3%) and nearly one third of probands (30.8%) were lacking three or more. There was a slight difference in frequency between genders. The study showed that 19.9% of males and 17.9% of females were missing at least one tooth. Third molars were the most common type of missing tooth (n=70, 76.9% of probands), representing prevalence 146.44/1000 (CI= 116.62-182.09). When missing 3rd molars were excluded, the prevalence was 43.93/1000 (CI = 28.08-67.47). It was more likely to have missing teeth from both arches (35.2%) than either from the upper or the lower arch. It was more than three times as likely to be missing teeth from both the right and left (61.4%) than solely from the left (14.5%) or right side (17.5%). Nearly seventeen percent (16.3%) of probands with missing teeth had a first-degree relative also missing a tooth, 5.8% had a second-degree relative missing a tooth, 34.5% had both. No family history was indicated in 41.7% of probands.

Types of missing teeth	males		females		total	
	n	*%*	*n*	*%*	*n*	*%*
just 3rd molars	42	79.25	28	73.68	70	76.92
just laterals	5	9.43	4	10.53	9	9.89
2nd molar	0	0.00	1	2.63	1	1.10
3rd and 1st premolar	1	1.89	2	5.26	3	3.30
3rd and 2nd premolar	2	3.77	1	2.63	3	3.30
3rd and lateral	1	1.89	1	2.63	2	2.20
other	2	3.77	1	2.63	3	3.30
TOTAL	**53**	100.00	**38**	100.00	**91**	100.00

CONCLUSIONS: Prevalence of hypodontia 190.37/1000 was observed among UOP dental students. When missing 3rd molars were excluded, it equaled 43.93/1000. A positive family history for missing teeth was observed in 16.3% of our probands, suggesting that a genetic component is a likely etiological factor.

ANALYSIS OF ROOT CANAL FILLINGS USING THERMAFIL, LATERAL COMPACTION, AND A MODIFIED HYBRID OBTURATION TECHNIQUE

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OBJECTIVE: To evaluate the efficacy of three methods of obturation in terms of percentage of gutta percha filled area in the root canal.

METHODS: Thirty plastic blocks with simulated root canals were prepared using ProTaper endodontic files to a size F3 file. Each plastic block was lubricated using hand soap and irrigated using distilled water. The blocks were then divided into three groups. Each group was obturated with either Thermafil according to the manufacturer's guidelines (group 1, n=10), lateral compaction (group 2, n=10), or a modified hybrid obturation technique (group 3, n=10), consisting of a master point and added Thermafil gutta percha. All blocks were then sectioned under water cooling and using a low-speed saw at coronal, midroot, and apical levels. Each section was then prepared for microscopic analysis and photographed at 80x magnification in a stereomicroscope equipped with a high-resolution digital camera. The digital photographs were then imported into ImageJ software to determine gutta percha filled areas, total number of voids, and total void area. Statistical analysis was done using ANOVA with Scheffé post-hoc tests as well as chi square tests.

RESULTS: In coronal sections ANOVA indicated a statistically significant difference among the three groups; post-hoc tests revealed significant differences between lateral compaction and Thermafil ($p<0.05$). Midroot sections showed no statistically significant differences among the groups. In apical sections ANOVA showed statistically significant differences among groups; post hoc tests indicated differences between Thermafil and the modified hybrid technique ($p<0.05$). The total number of voids was similar at all three levels among the three experimental groups (chi square test, $p>0.05$).

CONCLUSION: Under the present experimental conditions, Thermafil produced significantly higher gutta percha filled areas compared to lateral compaction. The modified hybrid technique did not result in improved root canal fillings. More experiments and clinical studies are needed to characterize improved root canal filling techniques.

VITAMIN D-MEDIATED INHIBITION OF RAD51 EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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INTRODUCTION: Numerous studies have demonstrated the pro-apoptotic and anti-proliferative effects of the active form of vitamin D, vitamin D_3 (VD_3), in a variety of cancers, including head and neck squamous cell carcinoma. The molecular mechanisms through which VD_3 mediates these effects remain unknown. Rad51 is a key protein involved in the repair of DNA double-strand breaks induced by ionizing radiation and overexpression of Rad51 has been shown to increase cellular resistance to radiation and chemotherapy in several cancer types. High levels of endogenous Rad51 protein in head and neck squamous cell carcinoma have been correlated to poor prognosis and resistance to treatment.

METHODS: The human cell lines SCC25, SCC9, derived from squamous cell carcinomas (SCC) of the oral cavity, and HaCat (normal keratinocytes) were obtained from the American Type Culture Collection (ATCC, Manassas, VA), and cultured under recommended conditions. SCC25, SCC9 and HaCat cells were treated with 100 nm VD_3 and/or ionizing radiation and harvested at 6 hours and 24 hours post treatment. Cells were lysed in 1% Triton-X lysis buffer and subjected to routine polyacrylamide gel electrophoresis (SDS-PAGE). Following electrophoresis, the resolved polypeptides were transferred to a polyvinylidene difluoride (PVDF) membrane and incubated with an anti-Rad51 antibody (Santa Cruz Biologicals). Protein expression was visualized by enhanced chemiluminescence (ECL). Lysates were also subjected to analysis for sublethal DNA damage response using a clonagenic assay, immunohistochemical analysis for H2AX foci formation, and apoptosis.

RESULTS: Our studies have shown that VD_3 down-regulated Rad51 protein expression in squamous cell carcinoma in vitro. Furthermore, VD_3 antagonized radiation-induced induction of Rad51. Pretreatment of SCC25 cells with VD_3 twenty-four hours prior to irradiation reduced the formation of H2AX foci and decreased sublethal DNA damage repair. Combined treatment of VD_3 and radiation showed an increase in caspase-3 cleavage, a measure of increased apoptosis.

CONCLUSION: Our results suggest that application of VD_3 may decrease the ability of head and neck cancer cells to survive radiation or chemotherapy by impairing the DNA damage response ultimately leading to apoptosis. This has obvious therapeutic implications as vitamin D-mediated inhibition of Rad51 expression could modulate the response of head and neck cancers to treatment.

This work has been previously presented at the American Association of Cancer Research Annual Meeting and was supported by funds from the UC Davis School of Medicine Department of Otolaryngology/Head and Neck Surgery.

AMBISOME AND CASPOFUNGIN INHIBIT *CANDIDA ALBICANS* ADHERENCE TO HSC-3 CELLS

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OBJECTIVES: Candidal adherence to epithelial cells is significantly reduced when antifungal polyenes are present during the "adherence phase", but cell-associated *Candida* is resistant to antifungals in terms of adherence. *Candida* biofilms with reduced susceptibility to conventional antifungals, are sensitive to lipid formulations of amphotericin B (AMB) and to Caspofungin. We examined the effect of the liposomal AMB formulation, AmBisome, free AMB, and Caspofungin on the adherence of *C. albicans* to HSC-3 squamous cell carcinoma cells.

METHODS: The adherence of yeasts to HSC-3 cells was determined as described by Samaranayake et al. (1994). The cells were incubated with three oral isolates of *C. albicans* either in the presence of the drug, or pre-incubated with yeasts and subsequently exposed to the drug. The cytotoxicity was determined by an Alamar Blue assay.

RESULTS: Following a 1-h incubation in the presence of AmBisome or Caspofungin, at 1-256 μg/ml, the adherence of *C. albicans* to HSC-3 cells was reduced considerably. For example at 16 μg/ml, adherence was diminished by ~34% and 50%, in the presence of AmBisome and Caspofungin, respectively. *Candida* adherence obtained with Caspofungin in this range was significantly different from the controls ($p<0.0005$), while AmBisome-mediated inhibition was significant at 4 μg/ml and above. The susceptibility of cell-associated *Candida* to antifungals was decreased markedly. The reduction in adherence was between 3.5 and 13.1%, when compared to the drug-free controls. AmBisome was not toxic in the range 1-256 μg/ml, Caspofungin in the range 1-64 μg, and free AMB at 1 and 4 μg/ml.

CONCLUSIONS: AmBisome and Caspofungin inhibited candidal colonization when present during the "adherence phase" but did not cause detachment of cell-associated yeasts.

This work was supported by funds from the University of the Pacific, Arthur A. Dugoni School of Dentistry, and from Western Dental Services.

This work was presented at the 87th General Session of the IADR and 38th Annual Meeting of the AADR, April 1-4, 2009, Miami, FL (J. Dent. Res. Vol. 88 (Special issue A) Abstract No. 1300, Seq. #140).

AURINTRICARBOXYLIC ACID RELEASE FROM A CALCIUM ALGINATE COMPLEX: A POTENTIAL APPLICATION FOR INTRAVAGINAL HIV CHEMOPROPHYLAXIS

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OBJECTIVES: Chemoprophylaxis for effective control of HIV infection remains an elusive challenge. A drug delivery system that enhances release of an anti-HIV agent during sexual intercourse would be a desirable advance. We wanted to determine if a physiologically relevant concentration of citrate (a natural component of seminal fluid) would release a prototypical anti-HIV polymer (ATA; aurintricarboxylic acid) from a calcium alginate-ATA complex.

METHODS: At a fixed concentration of ATA in alginate, where each ml of alginate (0.5 – 2 % w/v) contained 2 mg of ATA, ATA was immobilized in calcium alginate with equal (w/v) concentrations of calcium chloride and sodium alginate. Beads of calcium alginate-ATA complex (~25 μl / bead) were produced by dropwise addition of the ATA-alginate mixture into calcium chloride (aqueous solutions). For citrate dissolution testing, 1 ml of sodium citrate (132 mM at pH 8.0) was added to 10 calcium alginate-ATA beads and incubated at 37°C in 15 ml polypropylene tubes. The beads were mixed with citrate solution by inverting the tubes at 5 min intervals. The time necessary for total dissolution of the beads was noted by visual inspection.

RESULTS: In the range of calcium alginate concentrations tested (0.5-2.0%), the dissolution time ranged from 15 min to 80 min. The dissolution time for the alginate beads varied in approximately linear fashion with the percentage of alginate ($r^2 = 0.97$).

CONCLUSIONS: These data suggest that the design of an intravaginal delivery system for anti-HIV agents could utilize citrate-mediated drug release as a physiologically rational approach for chemoprophylaxis. While ATA was used as a prototype anti-HIV drug in this system, other drugs may be more appropriate for further study. The wide range of dissolution times reveals the considerable flexibility of this approach, either as a single formulation or as an adjunctive measure.

This work was supported by intramural funding from the University of the Pacific and LeadBioTech, Inc., Seoul, Korea.

OH, BABY: INFANT SIGN TRAINING AND FUNCTIONAL ANALYSIS

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INTRODUCTION: Thompson et al. (2004, 2007) established a structured method for teaching signs to infants using delayed prompting and reinforcement. Thompson et al. suggested that signs served as functional communication; however, the experimenters did not attempt to train the signs under specific stimulus conditions or assess the function of speech. Research has recently tested the utility of functional analysis methodologies for identifying the functions of emerging language, based on Skinner's (1957) analysis of verbal behavior (Lerman et al., 2005; Kelley et al. 2007; Normand et al., 2008).

OBJECTIVES: The current study used the sign training methodology proposed by Thompson et al. (2007) to establish independent signing with infants. Functional analyses were used to identify verbal operants (e.g., mand, tact, echoic, or intraverbal) established during sign training.

METHODS: A multiple-baseline design across participants with reversal design was used to assess sign language acquisition in 6 infants. During baseline, participants received reinforcers on a time-based schedule. During sign training, infants had varied amount of time to initiate the sign that corresponded to the reinforcer. If independent signing did not occur, a model prompt was delivered, followed by a physical prompt if no signing occurred within five seconds of the model. When high levels of independent signing occur (after the reversal), a functional analysis will be conducted.

RESULTS: For one participant, frequency of independent signs increased from near 0 in baseline, to an average of 28 per session during the 20-second delay. When the reversal to baseline was reinstated, independent signing decreased to baseline levels. During the second sign-training phase, independent signs returned to the level observed in the initial intervention.

CONCLUSION: While the sign training methodology was successful in increasing independent sign language in one child, it has yet to produce independent signs in other participants. Results thus far suggest that age may be a critical factor in determining sign acquisition.

SHAPING PHYSICAL ACTIVITY IN OBESE AND OVERWEIGHT CHILDREN USING PERCENTILE SCHEDULES

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INTRODUCTION: In 2006, approximately 14% of pre-school aged children were overweight, while12% were at risk for becoming overweight (Ogden et al., 2006). Fluctuations in weight can be attributed to changes in calorie consumption (diet) or calorie expenditure (physical activity). McIver et al. (2009) published preliminary data on an observation system for coding physical activity and related environmental events. Prior to the investigation, no concurrent validity information was available for the OSRAC-P.

OBJECTIVES: The current study used pedometer-aided feedback, goal setting, and reinforcement to increase levels of physical activity in preschool-aged children. We also compared two measures of physical activity, the OSRAC-P observational system and pedometer readings.

METHODS: Participants wore pedometers to measure step totals during normal recess periods. During baseline, pedometers were masked in order to prevent feedback. During the intervention, step total goals were set based on the participants past performance. Percentile schedules were employed as a systematic method of goal setting (Galbicka, 1994). The OSRAC-P observational system was also used to code levels of physical activity and related environmental events.

RESULTS: For three out of four participants, physical activity levels increased during the intervention. The three participants displaying favorable outcomes during the intervention met their goals on at least two occasions, while the participant that did not, never met his goals. Increases in physical activity correlated with increasing OSRAC-P activity codes.

CONCLUSION: The current study provides data indicating a relation in OSCRAC-P activity codes and step total readings, as well as moderate treatment effects in increasing overall physical activity.

EFFECT OF BULK FLOW ON THE ELECTROPHORETIC DETERMINATION OF LIPOSOME CHARGE

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INTRODUCTION: Particle electrophoresis is a well-known technique for measuring the electrical charge of colloidal particles such as liposomes, lipoplexes, and isolated cells. However this method suffers from inaccuracies associated with complicated and poorly understood phenomena occurring in the electrolyte near the particle surface. Such effects arise from the fact that water molecules adjacent to the particle surface "stick" to the surface and move with the particle, while water far from the surface does not move with the particle. The water solvent is thus sheared, giving rise to a "shear surface" that resides an unknown distance out from the particle surface. The shear surface defines an effective "hydrodynamic particle." It is the charge of this particle that the electrophoretic mobility measures. The measured charge of the hydrodynamic particle may or may not be a good approximation to the actual charge of the physical particle. This approximation is particularly poor for particles with "fuzzy" surfaces such as Stealth® liposomes used for intravenous drug delivery.

OBJECTIVES: We hypothesize that elimination of the flow of nearby solvent relative to the particle surface will eliminate shear and thus eliminate the shear surface. Under such conditions the electrophoretically measured charge should be the true charge of the physical particle. This result would constitute a major advance in the technique of charge determination by electrophoretic methods. It would also provide a new tool to investigate hydrodynamic properties of the surface coats of Stealth® liposomes and similar particles of medical and biological importance.

METHODS: We control the bulk solvent flow so that the electrophoretic particle moves, not through stationary solvent, but through moving solvent. The movement of bulk solvent is provided by electroosmotic flow in the electrophoresis cell itself. Because of charge on the glass walls, the electric field that drives the particle also causes electrolyte in the cell to move. It flows with a parabolic velocity profile ranging from positive near the walls to negative at the cell center. Electrophoretic measurements in the cell normally are taken at the "stationary layers," where the solvent velocity changes from positive to negative, thus is zero. In this experiment, we measure the electrophoretic velocity also at "non-stationary" layers, which provide the bulk flow whose effects we wish to examine. The cell velocity profile is first calibrated using neutral PC liposomes. The mobilities of liposomes of known charge density (90% PC, 10% PG) are then measured at various layers in the cell, including the stationary layers. The effect of bulk flow on the inferred liposome charge is thus ascertained. Experiments are done with electrolyte solutions of various ionic strengths. Measurements will also be done with Stealth® liposomes, whose "fuzzy" surface is provided by the incorporation of PEG-PE lipids.

RESULTS: We have considerable data on the electrophoretic mobilities of charged PC/PG and Stealth® PC/PEG-PE liposomes at the stationary layers. Measurements at the non-stationary layers are currently in progress.

EFFECT OF THE FLAVONOID APIGENIN ON LUNG CANCER CELL MOTILITY

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INTRODUCTION: The formation of metastases contributes substantially to the mortality of cancer. As a constituent of the metastatic process, motility provides a potential target for the reduction of cancer mortality and morbidity. Motility involves a complex interplay between cell attachment factors (integrins, cadherins, and others) and the extracellular matrix. This interplay is controlled by the coordinated formation and breakage of focal adhesions, which alter the cytoskeletal matrix. Focal adhesion dynamics responds to a variety of inputs, including signals from integrins, growth factor signaling cascades, and the environmental sensing of hypoxia, electrical signals, and nutrients.

A variety of bioflavanoid natural compounds are thought to inhibit both cancer cell growth and motility. Apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a constituent of parsley, celery, and lettuce and has been shown to reduce cancer cell growth at high concentrations while inhibiting motility at lower concentrations.

METHODS: We have measured motility in A549 non-small cell lung cancer cells stimulated by 2.5 μg/mL Epidermal Growth Factor (EGF) and responding to a 0 – 400 ng/mL gradient of Stromal Derived Factor 1α (SDF1α, CXCL12). Cells were introduced into IBIDI chemotaxis chambers (ibidi.de) and allowed six hours to attach. Chambers were then filled with serum-free buffered growth medium containing EGF. In some experiments, apigenin (Sigma) was dissolved in DMSO and diluted into the EGF solution (DMSO final concentration <0.1%) and SDF1α was introduced into one reservoir. Chambers were then placed on the warmed stage of a Nikon TE200 inverted microscope and images were taken every two minutes at 10X magnification. Cells in the stack of images resulting from an overnight experiment were enumerated and a random sample of cells was selected for tracking and analysis using ImageJ (rsb.info.nih.gov/ij) and associated plugins (substack maker, enumeration, manual tracking, and the IBIDI chemotaxis tool).

RESULTS: During a 10 hr period, A549 cells exhibited an average path distance of 250 ± 42 μm and a Euclidian distance of 71 ± 17 μm with a velocity of 0.42 ± 0.07 μm/min. Apigenin inhibited this motility, reducing the average path distance, Euclidian distance and velocity in a dose related manner. The influence of these concentrations of apigenin on the viability of A549 cells remains to be determined.

We plan to investigate the influence of additional bioflavonoids such as epigallocatechin gallate (a major constituent of black and green tea) and kaempferol (present in brussel sprouts and apples). While a variety of mechanisms for the inhibition of cell growth and motility has been proposed for these compounds, we plan to determine the influence of inhibiting or enhancing the expression of several genes (*e.g.*, vascular endothelial growth factor, focal adhesion kinase) thought to be important in controlling or executing the motility program in these cells.

CONCLUSION: Flavanoids like apigenin have potential to inhibit motility, a requisite precursor step in the metastatic cascade.

Supported by a Holmok Cancer Research Endowment Grant (to JCL).

TARGETED APOPTOSIS OF HIV-INFECTED CELLS BY AN HIV-ACTIVATED SUICIDE GENE

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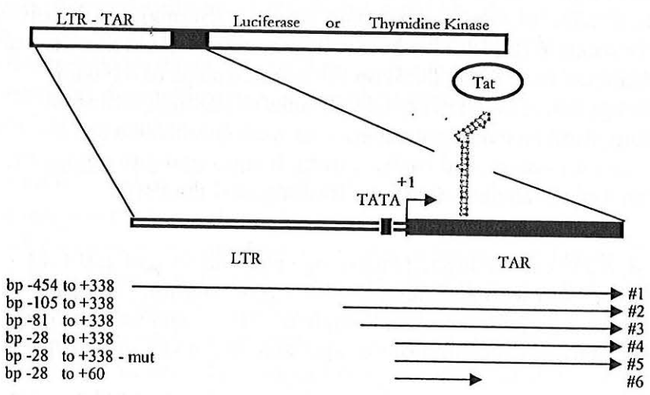
INTRODUCTION: Current anti-retroviral therapies against HIV infection are unable to eradicate the chromosomally integrated proviral genome. This creates a pool of latent viral clones that become active in the absence of anti-retroviral medication. Our goal is to target this latent pool that is unaffected by current therapies, but is responsible for the persistence of the virus and continued immune system degradation. We propose an approach that uses the HIV-specific factor TAT to activate a suicide gene, Herpes Simplex virus thymidine kinase (HSV-*tk)*. Specifically, this project will evaluate six progressively truncated versions of the HIV-LTR promoter/TAR region for their ability to drive gene expression exclusively within *tat*-expressing HIV infected cells.

OBJECTIVE: This study seeks to develop a TAT-only responsive promoter element, eliminating the non-specific activity associated with the full length HIV-LTR promoter. This HIV-specific promoter may be used to drive the expression of the HSV-*tk* gene. The enzyme produced from this gene converts the pro-drug Ganciclovir into a toxic metabolite. The specificity of the truncated promoter element will restrict the expression of HSV-*tk*, and hence the cytotoxicity, to cells expressing *Tat* that are infected with HIV.



METHODS: The full-length HIV-LTR/TAR region clone was generated using *de novo* gene synthesis. Truncated clones were generated using PCR. The clones were placed into a luciferase-expressing vector that will be co-transfected with a plasmid expressing Tat (pHXBΔbgl). Measurement of luciferase in the presence or absence of TAT will allow us to evaluate the effectiveness of the truncated HIV-LTR/TAR region in limiting transcriptional activation to *Tat*-expressing cells only. The clone or clones that demonstrate the greatest TAT-mediated specificity will be used to drive HSV-*tk* expression in cytotoxicity assays with Ganciclovir.

HYPOTHESIS: We will test the hypothesis that a truncated HIV-LTR/TAR region will drive expression of luciferase in the presence of TAT only, i.e. only in HIV infected cells.

Funded by Research Pilot Project Award 03-Activity 071 from the University of the Pacific, Arthur A. Dugoni School of Dentistry

HOMOGENEITY OF ROOT CANAL FILLINGS PERFORMED BY UNDERGRADUATE STUDENTS WITH WARM VERTICAL AND COLD LATERAL TECHNIQUES

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OBJECTIVE: To determine radiographic and microscopic appearances of root canal fillings performed by undergraduate students using vertical and lateral compaction techniques.

METHODS: Thirty dental students were instructed how to fill curved simulated canals with gutta-percha and sealer using lateral and vertical compaction. Digital radiographs were taken in bucco-lingual and mesio-distal projections; radiographs were evaluated for homogeneity and root canal wall contact. Plastic blocks with simulated canals were sectioned and cross-sections were assessed under a light microscope for voids. Probabilities were expressed as odds ratios (OR) with 95% confidence intervals (CI).

RESULTS: Radiographs showed that the chances of obtaining a homogenous root canal filling by using a vertical compaction technique were three times higher in the coronal canal third (OR 3.2; CI:1.9,5.3), the same in the middle third and two times higher in the apical third (CI:1.1,2.4) than when using lateral compaction. Microscopic evaluation of the same canals revealed that the chances of obtaining a homogenous root canal filling by vertical compaction were three times higher in the coronal canal third (CI:1.6,5.8), almost three times higher in the middle canal third (CI:1.6,4.7) and about ten times higher in the apical canal third (OR9.8; CI:2.2,43.4) than by lateral compaction. The chances of transporting filling material beyond the apex were almost five times higher (OR4.6; CI:2.8,7.6) when using vertical rather than lateral compaction.

CONCLUSIONS: Inexperienced students obtained more homogenous root canal fillings with the vertical compaction method. However, the probability of over-extruding filling material into the periapical tissue with this method was high.

This work has been submitted for publication to the International Endodontic Journal.

TEST ANXIETY AND STIMULANT USE AMONG COLLEGE STUDENTS

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INTRODUCTION: Previous research has reported a positive correlation between illicit drug use and anxiety disorders. More specifically, Sareen, Charter, Paulus and Stein, (2006) reported that participants who indicated illicit stimulant use also had a significantly higher likelihood of having an anxiety disorder. However, to the best of our knowledge no studies have reported findings concerning the possible relationship between illicit stimulant use and test anxiety among college students. Test anxiety refers to psychological and physiological responses to aversive stimuli associated with testing taking situations (e.g. increased heart rate while in the presence of a study guide). It is estimated that anywhere from 10-30% of students suffer from test anxiety. Similarly, Sharp and Rosen (2007) reported an 18% prevalence rate of illicit stimulant use among college students.

OBJECTIVES: In the current study we (1) examined the relationship between stimulant use and self reported test anxiety and (2) examined correlates of this relationship including gender, ethnicity, college major, study habits, and GPA.

METHODS: 171 undergraduate were recruited from a small private university located in Northern California. Participants were administered measures examining a variety of behaviors including test anxiety, prescribed, licit and illicit drug use within the last 30 days. Many students received extra credit and all students were entered into a drawing for a chance to win prizes.

RESULTS: Results indicated that greater caffeine use is a significant predictor of higher test anxiety and greater methylphenidate use is a significant predictor of lower test anxiety. Contrary to previous research, students with higher grade averages were more likely to report higher test anxiety.

CONCLUSION: Preliminary results indicate greater caffeine use as a significant predictor of higher test anxiety and greater methylphenidate use as a significant predictor of lower test anxiety. Students who reported higher grade averages were more likely to report higher test anxiety. Due to study limitations (i.e., relatively small sample size, low rates of substance use and the use of self-report measures) findings should be considered tentative. Future research should include a larger sample size and objective measures for substance use.

THE GENOMIC ACTION OF 17 β-ESTRADIOL ACCOUNTS FOR ALTERING CALCIUM HOMEOSTASIS IN HUMAN ENDOTHELIAL CELLS

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INTRODUCTION: Our preliminary studies showed 17 β-estradiol (E_2)-regulated Ca^{2+} homeostasis may play a role in enhancing nitric oxide production in human endothelial cells, EA.hy926. The effects of E_2 can be mediated via the genomic response or the nongenomic response. The genomic actions of E_2 require the transcription of genes, through estrogen response elements or activation of transcription factors. The nongenomic actions of E_2 are inclusive of all acute affects of E_2.

OBJECTIVE: We investigated the genomic and nongenomic actions of E_2 on intracellular calcium concentration ($[Ca^{2+}]_i$) in endothelial cells.

METHODS: Cells were treated with either a) E_2 (1 μM) in the presence or absence of the transcription inhibitor actinomycin D (1 μg/mL) for 24 h or b) E_2 (1 μM) for 5 minutes prior to the analysis of $[Ca^{2+}]_i$. Using a spectrofluorometer, $[Ca^{2+}]_i$ from cells loaded with the Ca^{2+}-sensitive dye, Fura 2-AM, was monitored in the absence of extracellular Ca^{2+}. The sarco/endoplasmic reticulum Ca^{2+} ATPase inhibitor, thapsigargin (TG, 1 μM), was used to induce Ca^{2+} release from the endoplasmic reticulum.

RESULTS: TG-induced $[Ca^{2+}]_i$ increase from cells treated with actinomycin D plus E_2 was not significantly different from cells treated with vehicle (ethanol). However, the extent of TG-induced $[Ca^{2+}]_i$ increase was significantly lower in vehicle- or actinomycin D- plus E_2-treated cells as compared with that observed in E_2-treated cells, indicating that gene transcription is required for E_2-mediated alteration in Ca^{2+} response. Five minutes of E_2 treatment did not alter TG-induced $[Ca^{2+}]_i$ increase as compared to vehicle-treated cells.

CONCLUSION: E_2 regulates Ca^{2+} homeostasis in EA.hy926 via a genomic mechanism.

This work has been supported by a grant from the National Heart, Lung and Blood Institute.

IN VITRO MODEL OF HUMAN DENTAL PULPITIS

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INTRODUCTION: A frequent cause bringing patients into the dentist's office is a tooth-related inflammation - periodontitis and pulpitis. Acute pulpitis may develop into a chronical apical periodontitis with a formation of a periodontal lesion in the bone. Recently, it has been shown that a progression of an inflammatory process is controlled by a balance between pro-inflammatory and anti-inflammatory factors, including newly discovered pro-healing lipid mediators (Serhan, 2005).

OBJECTIVE: To develop a novel co-culture model of pulpitis that would be suitable for studies of inflammatory factors and cellular reactions in a controlled environment.

METHODS: Extracted human third molars were split and the pulp was removed, minced and cultivated in plastic petri dishes in the HMSCBM medium (Lonza) supplemented with 10% human serum. After three weeks, we observed a clonal growth of dental pulp stem cells (DPSC). The Transwell system (Corning) has two compartments separated by a filter membrane. We confronted without intercellular contact pre-differentiated DPSC and human peripheral blood mononuclear cells either resting or pre-activated by phytohemagglutin (PHA) for one day. After a day of co-culture, total RNA was isolated from DPSC using RNeasy mini kit (Qiagen). Expression of inflammatory cytokines and chemokines was measured using the RT² Profiler™ PCR Array Human Chemokines & Receptors (PAHS-022A, SABioscience) on ABI 7900HT RT-PCR machine (Applied Biosystems).

RESULTS: We found a significantly increased expression of several chemokines in test DPSC confronted with PHA-stimulated PBMC, when compared with expression of the same genes in control DPSC confronted with resting PBMC: CCL26 23.5 times, CCL8 35.6 times, CXCL11 107 times, CXCL10 369.4 times, CXCL9 566.9 times. All these chemokines with elevated expression are monocyte and lymphocyte attractants.

CONCLUSIONS:
1. We have designed a novel co-culture model suitable for studies on inflammatory factors and cellular reactions of DPSC in vitro.
2. DPSC exposed to PBMC pre-stimulated with PHA responded by increased expression of five chemoattractants of lymfocytes and monocytes. This is a feature of an early inflammatory response.

ACKNOWLEDGEMENTS: We are grateful for support of the Departments of Orthodontics, Oral and Maxillofacial Surgery, and Endodontics, School of Dentistry Arthur A. Dugoni.

MAY EVERY CHILD SING AND SMILE

~ Volunteerism with Rotaplast International, Inc. ~
~ Professional dental, orthodontic, genetic, and pediatric services ~
~ Field work for research of causes of orofacial clefts ~

Marie M. Tolarová and Faculty, Residents, and Students.

University of the Pacific Arthur A Dugoni School of Dentistry, Department of Orthodontics.

During the last nine years, faculty, orthodontic residents, and students from our School professionally participated (in and pediatric fields) 120 times in 63 Rotaplast cleft medical missions. Donated value in professional services represents altogether $ 1,905,765 ($ 1,051,785 for dental /ortho services and $853,980 for genetic services). In average, in each mission our professional dental/ortho services represented $ 19,500 donated value and our professional genetic services represented $37,000 donated value.

A partnership with the Rotaplast International, Inc. – the non governmental organization that provides free reconstructive surgeries to underprivileged children affected with cleft lip and palate worldwide - allowed to our faculty, residents, and students not only to deliver professional services and acquire new experience, but also to bring back valuable data and specimens for our genetic research .
In addition to many students research projects, the data and specimens collected during Rotaplast medical mission provided fundamental source for 17 residents' research projects toward MSD degree (additional 4 are in progress). Two of them received the highest basic science research award from the American Association of Orthodontists – Harry Sicher Award (Dr. Basma Fallah in 2008, Dr. Alia Aljabeiti and Reem Salahuddin in 2009.
Rotaplast generously supports every year our field work for genetic research and thus making our craniofacial genetics research at the Arthur A. Dugoni School of Dentistry possible.

A participation of dental professionals (dentists and orthodontists) in the Rotaplast cleft medical missions became a part of our graduate program. It has brought another dimension to education and training of our graduates. With no exception, work of our residents and their contribution to the success of each mission is highly prized. They are bringing back from those two weeks - working many times more than 12 hours a day - not only what they learned professionally, but also warm feelings in their hearts remembering their patients to whom they helped to start a better future.

PRIMARY PREVENTION OF NONSYNDROMIC CLEFT LIP AND PALATE

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INTRODUCTION: Our first study in Lancet (1982, and those of others suggest that nonsyndromic cleft lip and palate (NCLP) can be prevented by periconceptional supplementation with folic acid (FA). Our studies later showed 65% decrease in recurrences with mother's daily supplementation of multivitamins with 10mg of FA (Teratology 1995) and 27-50 % decrease in occurrence when diet contained 400mcg of FA (Lancet 1995). However, several studies that followed did not show consistent results.

OBJECTIVE: To present data from our studies of genetic and environmental factors involved in etiology of NCLP and summarize data from studies by others in order to clarify realistic and effective approach for primary prevention of NCLP.

METHODS: We analyzed blood specimens for MTHFR677CT and RFC180AG from 1204 individuals affected with NCLP and 921 control individuals from 11 different locations in seven countries. For about one third of our probands and for about 25% of our controls specimens from mothers were also analyzed. For the same samples we also analyzed maternal nutrition using customized Food Frequency Questionnaire (FFQ) and additional data using General Genetic Questionnaire that we developed.

RESULTS: We found significant differences in genotype distribution for MTHFR677CT and RFC180AG among populations studied. While for example polymorphisms of both these genes were associated with NCLP in Guatemala no such association was not found in Karaikal. Analysis of FFQ revealed that four nutrients were associated with NCLP most often: low intake of folate, zinc, and B6 vitamin, and high intake of vitamin A.

CONCLUSIONS: There is enough scientific evidence that a significant proportion of NCLP can be prevented. However, different genes are creating susceptibility for NCLP and different environmental factor triggering them, exist in specific populations: ***"one size does not fit all"***. Therefore, prevention approach has to address differences in genetic and environmental factors that exist among different populations.

ACKNOWLEDGEMENT: The fieldwork for studies included in this presentation was supported by Rotaplast Intl., Inc.

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Tolarova MM, Mosby T. Primary prevention of nonsyndromic cleft lip and palate. J Dent Res 88 (Spec Iss A): 1457, 2009, www.dentalresearch.org

HIV-1-MEDIATED FUSION MONITORED BY FLUORESCENCE MICROSCOPY OF SYNCYTIUM FORMATION BETWEEN Clone69T1RevEnv AND SupT1 CELLS: INHIBITION BY LECTINS AND PEPTIDES

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OBJECTIVES: Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of the acquired immunodeficiency syndrome (AIDS). HIV-1 infection of CD4+ lymphocytes or macrophages occurs via both free virions and cell-cell transmission. The specific interaction of the HIV-1 envelope protein Env (gp120/gp41) with the CD4 surface molecule and a co-receptor (CXCR4 or CCR5) on target cells are required for both mechanisms of infection. We developed a new HIV fusion assay using fluorescently labeled Env-expressing Clone69T1RevEnv cells and highly CD4+ SupT1 cells. We examined the effect of known inhibitors of HIV-1 infection in this system.

METHODS: Clone69TRevEnv ("HIV-Env") cells were maintained at 37°C, under 5% CO_2 in Dulbecco's modified Eagle's MEM supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), L-glutamine, geneticin, and hygromycin B (DME/10*-tet). Cells were first grown in the presence of tetracycline. Env expression was induced by removing the tetracycline from the medium. Cells were plated at 2.0 x 10^5 cells/ml in 48-well plates for 24 h, and labeled with 1-2 μM Calcein-AM Green for 30 min at 37°C. SupT1 cells were maintained at 37°C in RPMI 1640 medium containing 10% FBS, penicillin, streptomycin and L-glutamine, and labeled with CellTrace™ Calcein red-orange for 30 min at 37°C. SupT1 cells (2.0 x 10^5), antibodies, lectin, and fusion inhibitor were added to wells containing adherent HIV-Env cells. Syncytia were observed under a Nikon inverted fluorescence microscope.

RESULTS: HHA lectin and T-20 fusion inhibitor at 1 μg/ml inhibited fusion between HIV-Env and SupT1 cells. Monoclonal antibodies to HIV-1 gp120 (IgG1B12, m14 IgG, and hmAb 2G12), all of which inhibit HIV-1 infection had little or no inhibitory effect on cell-cell fusion.

CONCLUSIONS: Fluorescently labeled HIV-Env and SupT1 cells can be used to monitor HIV Env-mediated fusion, and to screen membrane fusion inhibitors. Virus-cell fusion inhibitors, including monoclonal antibodies, may be ineffective against cell-cell transmission of the virus or viral genetic material.

Presented at the XIIth Bay Area Symposium on Microbial Pathogenesis, San Francisco, March 28, 2009.

PREDICTING PARATHAYROID HORMONE LEVELS IN DIABETIC HEMODIALYSIS PATIENTS USING NEURAL NETWORKS

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OBJECTIVES: Parathyroid Hormone (PTH) is an important biochemical indicator for the medical condition of osteodystrophy in patients on hemodialysis. Prior studies have been conducted to classify hemodialysis patients based on their PTH level, using Artificial Neural Networks (ANNs). This paper introduces the possibilities of predicting parathyroid hormone levels in the more specific case of diabetic patients. Our first objective was to improve prior models by predicting the patients' actual PTH level instead of classifying it to two broad categories. Additionally, we investigated the link between diabetes and PTH. Hemoglobin A1c (A1c) is a typical indicator of a patient's blood glucose level. We have compared the ability of ANNs to generalize with and without A1c as a predictor variable in order to analyze the diabetes-PTH relationship.

METHODS: Our data included 153 patients on hemodialysis for treatment of renal failure. These patients had a variety of biochemical measurements taken on regular bases that served as predictor variables. For Method A, we included all patients, and all predictor variables except A1c in the data set. For Method B, we included predictor variables, but only the diabetic patients were considered. For both methods, we used PTH for the target data. Due to the distribution of PTH levels in patients, those with a PTH of greater than 500 pg/mL were considered to be outliers and removed from the data set. For training we employed a three-layer backpropagation network. Our ANNs were implemented with the use of MATLAB (r2008a). The input data was randomly divided into multiple sets as follows: 60% training, 40% validation/testing. The network was then trained using a gradient descent algorithm with the training set for input data, and PTH as target data.

RESULTS: Performance for Method B was measured in terms of Mean Absolute Error (MAE). MAE was much smaller for Method B (53.8) than for Method A (64.3), indicating its superior performance. The greater correlation coefficient obtained in Method B (0.73 vs. 0.67) indicates that the network was able to predict PTH more accurately for diabetics than for non- diabetics.

CONCLUSIONS: This study was an exploration of the relationship between A1c and PTH in patients on hemodialysis for renal failure. We have improved upon previous use of ANNs to predict PTH in these patients. We have also compared the ability of ANNs to predict PTH with two sets of inputs. One set which excluded A1c as a predictor, and one set which was limited to diabetic patients and included A1c as a predictor. The ANNs were consistently able to predict PTH levels more accurately when A1c was included as a predictor.

This work was has been presented in the International Conference of Computing in Engineering, Science and Informatics (ICC2009), in April 2009.

TEMPERATURE CHANGES DURING ULTRASONIC IRRIGATION WITH DIFFERENT INSERTS AND MODES OF ACTIVATION

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OBJECTIVE: To evaluate temperature changes during passive ultrasonic irrigation (PUI) of intracanal solutions.

METHODS: Non-cutting Nickel Titanium inserts or stainless steel K-Files #15, #25 and #35 were used. Root canals of three extracted human maxillary canines were enlarged to apical size #45. Thermocouples were mounted inside root canals 3, 6 and 9 mm from the apical foramen. Teeth were placed in a water bath at 37°C. Distilled water (20°C) was continuously delivered through the ultrasonic unit (Group 1) or deposited into the root canal prior to ultrasonic activation (Group 2). Baseline temperatures were determined before irrigation and averaged. Ultrasonic activation was subsequently performed; fifteen measurements were done with each insert. Temperatures were recorded every second and expressed as differences to baseline values. Statistical analysis was done using ANOVA and Scheffé post-hoc tests.

RESULTS: Initially, temperature decreased by up to 7.4°C; these drops were significantly smaller in Group 1 than in Group 2 ($p<0.001$) in the middle and apical root canal third. The decreases were followed by temperature rises for all inserts in Group 2. However, in Group 1, temperatures just reached baseline values in middle and apical thirds; in the coronal root canal third lower temperatures were measured. In Group 2, mean maximum temperature rises over baseline were 7.7, 7.5 and 4.2°C in coronal, middle and canal thirds, respectively. There were significant differences between activation modes for all inserts ($p<0.001$). In Group 2, K-file type inserts size #35 generated highest and insert size #15 the lowest temperatures; the NiTi inserts was more effective than size #15 K-files and less effective than #35 K-files.

CONCLUSION: Continuous flow negated the potential of ultrasonic activation to heat irrigation solutions inside root canals. Non-cutting NiTi instruments and large K-files were more effective than small K-files in warming the irrigation solution.

This work has been submitted for publication to the Journal of Endodontics

ORTHODONTICS RESIDENT PRESENTATIONS

MTHFR 677CT IS ASSOCIATED WITH NONSYNDROMIC CLEFT LIP AND PALATE IN GUATEMALA

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INTRODUCTION: The etiology of nonsyndromic cleft lip with or without cleft palate (NCLP) is multifactorial, including genetic and environmental factors. Folate-related genes and folate intake are among those factors intensively studied recently. Methylenetetrahydrofolate reductase (MTHFR) gene encodes the enzyme involved in a control of folate metabolism. When its function is altered due to a mutation, a decreased utilization of folate slows down cell multiplication in the early embryonic development and it may lead to an orofacial cleft. However, there is no consistency of results from studies of MTHFR 677CT and NCLP.

OBJECTIVES: To find out whether C677T variant of the MTHFR gene is associated with NCLP in Guatemalan population.

METHODS: Case-control study based on 242 individuals affected with NCLP and 218 controls identified during Rotaplast medical missions at Roosevelt Hospital in Guatemala City, Guatemala was conducted. DNA was isolated from dry blood spots on filter paper. MTHFR 677CT genotypes were established by PCR amplification and single nucleotide conformational polymorphism detection using polyacrylamide gel electrophoresis.

RESULTS: Significantly different proportion of genotypes (p=0.005) with higher proportion of TT homozygotes between cases and controls, and also significantly higher T allele frequency in cases compared to controls (p=0.003) was found. In cases, 7.44% of individuals had CC genotype, 45.87% had TT genotype, and 46.69% were heterozygotes. Proportions of genotypes in controls were 16.06% CC, 35.32 % TT, and 48.62% CT. The C allele frequency was 0.308 for cases and 0.404 for controls, while the T allele frequency was 0.692 for cases and 0.596 for controls.

CONCLUSION: Results of this study suggest that the C677T variant of MTHFR gene is associated with NCLP in Guatemala.

ACKNOWLEDGEMENT: The fieldwork for this study was supported by Rotaplast Intl., Inc.

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Delurgio A, Tolar M, Gomez M, Bauter N, Komura J, Mishra S, Patil S, Mahood K, Abyu Al-Melh M, Behdad B, Berdichevsky Y, Allasuty M, Kaur A, Tolarova M.. J Dent Res 88 (Spec Iss A): 1456, 2009, www.dentalresearch.org

WHAT CAN OR CANNOT BE DONE WITH INVISALIGN®?

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OBJECTIVE: To compare orthodontic cases and to determine when Invisalign® can be used to treat such cases successfully, from a preclinical perspective. This study will be useful to the graduating students in determining the limitations of Invisalign® in their practices.

METHOD: Five cases from the Orthodontic Clinic at the University of the Pacific, the Main Clinic at Pacific, and private practices in North Carolina were analyzed in the study. All subjects had standard pre-treatment and post treatment photos. Certain parameters were used to compare each case. These focused on the success of Invisalign® in treating malocclusions, and in treating the patient's chief complaint.

CONCLUSION: Invisalign® can be used to treat complex malocclusions with the correct guidance from other practitioners in treatment planning the case initially. Other factors, namely patient compliance, play a large role in case selection.

EFFECT OF PLATELET-RICH PLASMA ON HUMAN ADULT MSC IN VITRO

Waleed Soliman[1], Manal Abu Al-Melh[1], Kimberly Mahood[1], Nicholas Bauter[2], and Mirek Tolar[2]

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INTRODUCTION: A controversy exists regarding efficiency of platelet-rich plasma (PRP) in improving an outcome of bone grafting. Conditions in vitro are well suited for a study on factors and mechanisms controlling growth and osteogenic differentiation of human adult mesenchymal stem cells (MSC).

OBJECTIVE: To determine basic bone regeneration cellular mechanisms in vitro that may or may not be affected by addition of PRP to the cultivation medium.

METHODS: Cells from human bone marrow aspirates (Lonza) are cultivated in plastic flasks or Petri dishes in the MSC Basal Medium (Lonza) supplemented with L-glutamine (2 mM), Penicillin (10 units/mL), Streptomycin (10 microg/mL) and 10% human serum with or without PRP. PRP is prepared from human blood plasma, activated by thrombin and calcium chloride and combined with human serum. Medium is exchanged twice a week. Lonza's osteogenic medium contains dexamethasone, ascorbic acid and beta glycerophosphate. Cell counts are used for measurement of growth. Osteoblasts are characterized by alkaline phosphatase stain (intracellular enzyme activity) and by Von Kossa stain showing extracellular mineral deposits.

RESULTS: Addition of PRP to the cultivation medium shortened lag phase preceding multiplication of human adult MSC. Rate of multiplication and extent of osteogenic differentiation of MSC were significantly enhanced.

CONCLUSIONS: A shortened lag phase and a steeper slope of exponential growth phase of MSC were the main effects of PRP supplied continuously in vitro. Clinically, PRP is applied when bone material is grafted. It is suggested that growth factors contained in the PRP, when supplied continuously, would shorten the lag phase and enhance exponential growth of MSC in vivo.

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Soliman W, Abu Al-Melh M, Mahood K, Bauter N, Tolar M. J Dent Res 88 (Spec Iss A): 2701, 2009, www.dentalresearch.org

SENIOR RESEARCH PRESENTATIONS

INFLAMMATORY CYTOKINE SECRETION BY THP-1 CELLS EXPOSED TO *PORPHYROMONAS GINGIVALIS*

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OBJECTIVES: Interleukin-18 (IL-18) is a pro-inflammatory cytokine that plays an essential role in the T-cell response and is elevated in inflammatory diseases such as periodontal disease. *Porphyromonas gingivalis* (*Pg*) is one of the most important bacterial pathogens that contribute to pathogenesis of chronic periodontitis. Here we examined the IL-18 levels expressed by differentiated macrophage-like THP-1 cells after exposure to live and heat-killed *Pg* and to *E.coli* LPS.

METHODS: THP-1 cells were differentiated with phorbol 12-myristate 13-acetate (PMA) for 5 days at 37°C. *Pg* grown in chopped meat medium under anaerobic conditions, was added to differentiated THP-1 cells, at ratios of 2-100 bacteria/cell, and incubated at 37°C for 24 h. IL-18 was determined by ELISA. The Multi-Analyte Profiler ELISArray kit was used to profile other pro-inflammatory cytokines and chemokines. Values were compared to that obtained with differentiated THP-1 cells treated with medium alone.

RESULTS: Exposure to *Pg* at ratios of 20, 50, and 100 bacteria/cell induced significant expression of IL-18 after 24 h. For example, treatment with live and heat-killed *Pg* at 100 *Pg*/cell produced 174.3 ± 41.3 and 118.0 ± 17.3 pg IL-18/ml, respectively. THP-1 cells treated with heated and regular conditioned *Pg* medium produced the same level of IL-18 (~124.5 pg/ml). Treatment with *E. coli* LPS at 50 and 100 ng/ml resulted in the production of 61.1 ± 3.6 pg/ml IL-18. Analysis by the Multi-Analyte Profiler ELISA indicated the stimulation of IL-1β, IL-12 and TNF-α.

CONCLUSIONS: *P. gingivalis* significantly increased IL-18 secretion by differentiated THP-1 macrophage-like cells. Our results suggest that *Pg* factors other than LPS also contribute to the activation of THP-1 cells and production of IL-18.

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This work was presented at the 12th Annual Bay Area Microbial Pathogenesis Symposium (BAMPS XII), March 28, 2009, the Mission Bay Campus of UCSF, San Francisco, and at the 87th General Session of the IADR and 38th Annual Meeting of the AADR, April 1-4, 2009, Miami, FL (J. Dent. Res. Vol. 88 (Special issue A) Abstract No. 1362, Seq. #144) by A. Kim (DDS Class 2009).

SHEAR BOND STRENGTH (SBS) BETWEEN GLASS- IONOMER CEMENT AND COMPOSITE RESIN RESTORATIVE MATERIALS

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OBJECTIVE: The purpose of this study is to test the shear bond strength between restorative Glass-Ionomer cement and composite resin.

METHODS: Glass-ionomer (Fuji IX, GC America) was placed in jigs labeled Group 1 and Group 2, with 12 samples in each group. A mylar strip with a small round opening was placed along a flat surface of the GI. The exposed area was etched (35% phosphoric acid) and bonding agent (Optibond Solo Plus [Kerr] for Group 1; Adper Scotchbond [3M ESPE] for Group 2) was placed according to manufacturer's guidelines and light cured. Composite (Premise [Kerr] for Group 1; Filtec Z250 [3M ESPE] for Group 2) was placed in the opposing jig surface and light cured. Six of the samples in each group were allowed to sit for 24 hours before the tests were conducted. The other six samples in each group were stored at 36 degrees Celsius in 100% humidity for 10 weeks to simulate oral conditions. The SBS was tested in a universal testing machine Instron 1011 at a crosshead speed of 5 mm/min.

RESULTS: The result for Group 1 (24h) was a mean SBS of 17.8 MPa with a standard deviation of 3.1 MPa. Group 2 (24h) had a mean SBS of 9.2 MPa with a standard deviation of 3.3 MPa. The t- test confirmed a greater than 95% confidence interval and a significant difference between the two material groups (p=0.0009).
The result for Group 1 (10 wks) was a mean SBS of 8.9 MPa with a standard deviation of 4.1 MPa. Group 2 (10 wks) had a mean SBS of 2.9 MPa with a standard deviation of 1.9 MPa. The t-test confirmed a greater than 95% confidence interval and a significant difference between the two material groups (p=0.007).

CONCLUSIONS: The 24 hour results show a bond strength between Fuji IX and Premise as well as between Fuji IX and Filtec Z250 composite that is below the SBS of a good dentin to composite bond (20 MPa and up) but higher than GI bond to dentin (4 to 8 MPa). However, the SBS was lower for both groups after storage of the samples in simulated oral conditions for 10 weeks. The significant difference in both sets of SBS results suggests that the SBS of GI to composite may vary between product combinations.

INTRACELLULAR FATE OF NON-VIRAL VECTORS IN ORAL CANCER CELLS

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OBJECTIVES: One problem in the use of cationic lipid-DNA complexes ("lipoplexes") for gene therapy of Oral Squamous Cell Carcinoma (OSCC) is the variability in transfection of different OSCC cells. Here we tested the hypothesis that the variability in transfection arises from the differential uptake of lipoplexes, using fluorescent transfection reagents.

METHODS: KB, HSC-3 and H-357 human OSCC cells were seeded in 48-well culture plates, and used at approx. 85% confluence. The plasmid pCMV.Luc expressing luciferase was complexed with optimal volumes (2µl/well) of Fluo-Metafectene. Transfection activity was assessed by luciferase expression assayed 48 h after transfection, using the Promega Luciferase Assay System and a luminometer. Fluorescence was quantitated 48 h after transfection using a Perkin-Elmer Luminescence Spectrometer, and observed in a Nikon-Diaphot microscope right after transfection or 48 h afterwards.

RESULTS: Representative luciferase activities in HSC-3, KB and H-357 cells were 100100, 73607 and 147 Relative Light Units/ml. Fluorescent lipoplexes were taken up by HSC-3 and KB cells to a greater extent than by H-357 cells, as determined by microscopy and fluorometry.

CONCLUSIONS: While the uptake of fluorescent lipoplexes correlates considerably with transfection activity, there appears to be a barrier to gene transfer in H-357 cells, since a significant amount of lipoplexes (albeit lower than that in HSC-3 and KB cells) are taken up by these cells. In cell lines that are difficult to transfect, the efficacy-limiting step in gene transfer to OSCC cells appears to be lipoplex processing beyond initial uptake. These additional steps may include destabilization of the endosomal membrane, escape of the DNA into the cytoplasm and transport of DNA into the nucleus. We are exploring the use of fluorescence markers for lysosomes and nuclei to better define the intracellular localization of lipoplexes.

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ENHANCING GENE DELIVERY TO CANCER CELLS BY TRANSFERRIN AND EGF

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OBJECTIVES: Despite advances in diagnosis and treatment of head and neck squamous cell carcinoma (HNSCC), including improvements in radiation therapy, surgical techniques, chemotherapy and prevention strategies, survival rates for patients with recurrent head and neck cancer are poor. Our laboratory is developing safe, non-viral vectors for the delivery of therapeutic genes to HNSCC cells. The purpose of this study was to increase transfection efficiency of the polycationic, non-viral vector Metafectene Pro (MP) via complexation of transferrin or Epidermal Growth Factor (EGF) as targeting ligands, and to determine if the resistance of some HNSCC cells to transfection could be overcome by this method.

METHODS: HSC-3 and H-413 cells were seeded in 48-well culture plates the day before transfection, and used at approx. 85% confluence. The plasmid pCMV.Luc expressing luciferase under the control of the cytomegalovirus promoter was complexed with MP and with either human rEGF or human transferrin. Luciferase activity was assayed 48 hours after transfection.

RESULTS: EGF- and transferrin-mediated enhancement of transfection was most prominent under conditions where MP alone was sub-optimal for transfection (1 µl MP/µg DNA). In HSC-3 cells, complexation of transferrin or EGF caused an approx. 7-fold increase in luciferase expression. In H-413 cells that are very resistant to transfection, complexation of EGF with MP lipoplexes resulted in a 15-fold increase in luciferase activity, while transferrin mediated only a 2-fold enhancement. Use of 1.5 and 2 µl MP in H-413 cells enabled EGF to increase transfection by 12- and 4-fold, respectively.

CONCLUSIONS: Transfection of HNSCC cell with lipoplexes can be improved with the use of transferrin and EGF as targeting ligands, even in cells that are difficult to transfect. Such lipoplexes may be used for cancer cell-specific delivery of suicide genes in the therapy of HNSCC.

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FEASIBILITY STUDY ON THE USE OF SALIVA COLLECTED DURING A DENTAL MISSION IN PERU FOR DNA ANALYSIS

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INTRODUCTION: While blood is often considered the ideal source for DNA analysis, saliva can be collected using an easier and non-invasive technique. However, it is still challenging to obtain uncontaminated saliva specimens for DNA studies, specifically during various fieldworks and dental and medical missions. The capability to collect good saliva specimens on mission trips for further DNA studies would enable us to expand genetic studies on etiology of various congenital anomalies and common dental conditions.

OBJECTIVES: The aims of this pilot study are (1) to find whether a method of collecting saliva that was previously developed in our Pacific Craniofacial Genetics Laboratory (PCFGL), can be used in the field, and (2) to develop a reliable protocol for collection of saliva specimens during dental missions to developing countries.

MATERIAL AND METHODS: Our sample consisted of 22 Peruvian volunteers from three sites during a dental mission trip to Lima and Cuzco, Peru, in March 2008. Participants were asked to rinse their mouth with 0.5 oz of Listerine® for 30 seconds followed by a rinse with water for 30 seconds in order to get rid of food particles. They were then asked to spit into a 50 mL Falcon tube until 3-5 mL of saliva was collected. The Falcon tubes were transferred to a secure location where drops of saliva were spotted on filter paper. Sterile pipettes were used to make six large spots onto filter papers that were allowed to dry. When completely dry, each filter paper was placed into a small envelope for transport back to the United States. In the PCFGL, the saliva specimens were processed using PCFGL standard protocol for SNP (single nucleotide polymorphism) testing. DNA was isolated and extracted from the saliva-spotted filter paper using two methods: the REDExtract-N-Amp PCR kit (Sigma-Aldrich) and the Chelex-100. Following DNA isolation, we analyzed MTHFR 677CT polymorphism using PCR, agarose gel electrophoresis and PAGE (polyacrylamide gel electrophoresis).

RESULTS: Both methods of DNA isolation (Sigma and Chelex) from saliva specimens yielded sufficient amounts of DNA for genotype analyses. There was 100% agreement in CC, CT, and TT genotype diagnoses when Sigma and Chelex methods were compared.

CONCLUSIONS: Our study demonstrated that saliva specimens of good quality for DNA analyses could be collected during international dental missions to developing countries. We are presenting a reliable protocol that we developed for collection of saliva in the field. This protocol offers several advantages: it is time saving and efficient, it limits exposure to blood-borne pathogens, and it is relatively inexpensive.

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EFFECTS OF GENDER ON VASCULAR RESPONSES TO SYMPATHETIC STIMULATION IN THE SUBMANDIBULAR GLAND

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INTRODUCTION: In many vascular beds estrogen increases NO production and bioavailability, and this beneficial effect of estrogen is responsible, in part, for the lower risk of cardiovascular disease in premenopausal women compared with men of the same ages. The purpose of this study was to determine whether gender affects sympathetic vascular responses in the rat submandibular gland. Specifically, we tested the following hypotheses: 1) that NO is responsible for the vasodilatory phase of the response to sympathetic stimulation and 2) that in females the balance between vasoconstriction and vasodilatation is shifted towards NO-mediated vasodilatation.

METHODS: Male and female, Sprague-Dawley rats (total n=17) were anesthetized with pentobarbitone followed by chloralose. Blood flow responses to sympathetic stimulation (2 Hz and 4 Hz continuously or 20 Hz and 40 Hz in bursts of 1s for every 10 s were measured using laser-Doppler flowmetry before and after inhibition of cyclooxygenase (indomethacin) and nitric oxide (NO) synthase (L-NAME).

RESULTS: The response to sympathetic stimulation was biphasic (vasoconstriction followed by vasodilatation), but depending on the experimental protocol employed the result was either an intense vasoconstriction (continuous impulses) or a net vasodilatation (burst stimulation). In female rats there was a large vasodilatory response to burst stimulation and there was a tendency to overcome the vasoconstriction induced by continuous stimulation. In contrast, the net vasodilatation observed in males during burst stimulation was significantly lower than that seen in females ($p<0.02$) and there was no recovery from the vasoconstriction seen under continuous stimulation. Indomethacin, either alone or in combination with L-NAME had no effect on blood flow response. However, L-NAME significantly reduced the vasodilatory responses in both males and females ($p<0.01$), and completely abolished all gender differences.

CONCLUSIONS: From these data we conclude 1) that NO is largely responsible for the vasodilatory response to sympathetic stimulation and 2) that NO-mediated responses are greater in female than in male rats.

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EFFECTS OF ANTIFUNGALS AND HISTATIN 5 ON *CANDIDA ALBICANS* BIOFILMS

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OBJECTIVES: *Candida* biofilms are more resistant to antimicrobials than planktonic cells, which may explain the high recurrence of candidal denture stomatitis. Salivary histatins, especially histatin 5 (Hst5), possess significant antifungal properties. We examined the susceptibility of *C. albicans* biofilms on denture acrylic to Hst5 and the antifungals, amphotericin B, nystatin, and fluconazole.

METHODS: Biofilms were developed on poly(methyl methacrylate) discs submerged in *C. albicans* cell suspensions. The plate was incubated for 90 min at 37°C (adherence phase). After removal of non-adherent cells, discs were submerged in YNB/100 mM glucose medium and incubated for 48 h at 37°C (biofilm formation phase). The antifungals were present either during biofilm formation, or added after biofilm formation. The metabolic activity of *Candida* biofilms was measured by the XTT assay.

RESULTS: Fluconazole showed the greatest inhibitory effect, when present during biofilm formation, reducing metabolic activity by ~30% at 0.25 μg/ml, while amphotericin B and nystatin were not inhibitory at this concentration. All three drugs were equally inhibitory (>90%) at 2 μg/ml. Biofilm-associated *Candida* was highly resistant to fluconazole in the range 1-64 μg/ml. Nystatin and amphotericin B were more inhibitory. At 1 μg/ml, amphotericin B and nystatin reduced the relative XTT activity by 56 and 85%, respectively. Hst5 inhibited the XTT activity of biofilm *Candida* by 40% at 50 μM, and 60% at 100 μM.

CONCLUSIONS: Fluconazole, an inhibitor of ergosterol synthesis, was more effective during biofilm formation, most likely because fungal proliferation is dependent on cell membrane synthesis. In contrast, the polyenes were effective after biofilm formation, because they disrupt already synthesized cell membranes. Hst5 could also affect biofilm *Candida* by internalization and ATP depletion.

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CLEFT LIP AND PALATE AND MSX1 GENE POLYMORPHISM

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INTRODUCTION: Cleft lip and palate is the second most common birth defect with birth prevalence 1 in 560 births in California. Genetic factors are thought to contribute heavily to the development of this disorder. Several genes, like TGFA, TGFB3, MTHFR, RFC1, IRF6, PVRL1, and MSX1, are considered candidate genes.

OBJECTIVES: Our case-control study was designed to evaluate a possible association between MSX1 CA-repeat polymorphism and children affected with nonsyndromic cleft lip with or without cleft palate (NCLP) in Guatemalan population.

MATERIAL AND METHODS: Case control design was used in our study. Altogether, 192 patients affected with NCL/P and 130 unaffected controls form our samples. Venous blood was drawn during Rotaplast medical missions in Guatemala City in years 2001-2003, and 2005. DNA analysis followed Pacific Craniofacial Genetics Laboratory (PCFGL) protocol including sequencing of all specimens in order to establish MSX1 CA-repeats genotypes. The genotypes consisting of alleles 1-4 were established. Allele 1 had 12 CA repeats, allele 2 had 11 CA repeats, allele 3 had 10 CA repeats, and allele 4 had 9 CA repeats.

RESULTS: The most common genotype observed in both cases and controls was A4A4. The second most common genotype was A4A2 showing a lower proportion in cases (17.19%) compared to controls (32.31%). This difference was highly statistically significant ($\chi 2 = 9.0905$, p=0.00257, CI = 0.249-0.760).The A2 allele was the second most frequent in both cases and controls, with higher - but no significantly - frequency in controls (17.69 % vs 12.76%).

CONCLUSIONS: Our pilot study complemented findings of Oh et al. (2005), Porter et al. (2006), and McDonough and Kitamura (2007) showing that the A4A4 genotype was more often found among children affected with NCL/P and also among mothers of children affected with NCL/P compared to controls and control mothers. A very interesting new finding in our study was a lower, and statistically significant, proportion of A4A2 genotypes among children affected with NCL/P compared to controls.

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2ND YEAR STUDENT RESEARCH COMPETITION

DENTAL MISSIONS AND OBTAINING SALIVA AND BLOOD SPECIMENS FOR GENETIC STUDIES. WHICH IS BETTER?

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INTRODUCTION : Many medical and dental missions providing professional services for underserved children and adults take place around the globe every year. These trips present a unique opportunity for collecting data and specimens for research studies. Venous blood, saliva, and buccal swabs are most commonly used for molecular genetic studies.
The feasibility study conducted at UOP Arthur A. Dugoni School of Dentistry in 2008 (Passamano et al, 2008) showed that good quality, uncontaminated saliva specimens can be obtained during a dental mission, safely transported to US, and used for genetic analysis. The authors developed the protocol for saliva specimen collection during dental missions to developing countries.

OBJECTIVES: The aims of this study are (1) to use the protocol for collection of saliva specimens developed by Passamano's group last year, (2) to collect blood specimens (finger sticks) from the same individuals, (3) analyze both types of specimens for MTHFR 677CT polymorphism, (4) compare MTHFR 677CT genotype distribution and allele frequencies in our Peru sample with published studies.

MATERIAL AND METHODS: Our sample consists of 59 volunteers from Cusco, Peru who agreed to provide saliva and blood specimens for our study. Demographic information and consent forms were obtained from each volunteer. Saliva specimens were collected according to the protocol developed last year. Blood specimens were obtained using capillary puncture technique (finger stick). MTHFR 677CT genotypes were analyzed using the Pacific Craniofacial Genetics Laboratory (PCFGL) standard protocol.

RESULTS: Altogether 111 specimens were obtained (51 saliva and 59 blood). The average time spent for collecting saliva and blood samples was 80 minutes for each. Creating a sterile environment and collection of volunteer data took the same amount of time. Saliva collection took 6 minutes/volunteer. Saliva transfer took 3 hours. Blood collection and transfer took 3 minutes/volunteer. The molecular genetic analysis took the same amount of time for both.
MTHFR 677CT polymorphism was analyzed for all specimens. Genotypes from saliva and blood specimens were identical for 46 out of 50 individuals. In 3 individuals, genotype was not conclusive for one of the two specimens analyzed. For 7 remaining individuals, only one type of specimen was available. Proportion of MTHFR 677CT genotypes was CC 41.3%, CT 52.2%, TT 6.5%.

CONCLUSIONS: Based on our experience, blood is easier to collect by finger stick in the field because it is less time-consuming, involves fewer steps, involves less opportunities for contamination, and contains sufficient amount of DNA for analysis.

A STUDY ON MOTHER'S NUTRITION AND NONSYNDRONIC CLEFT LIP AND PALATE IN SAN SALVADOR

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INTRODUCTION: It is generally accepted that nonsyndromic cleft lip and palate (NCLP) has a multifactorial etiology, with genetic and environmental factors involved. Among environmental factors, a mother's nutrition plays a very important role. A high prevalence of cleft lip and palate is found in the third world countries. Some studies suggest that the diet of the patient's mother may play a key role in the development of orofacial defects. Based on studies of different ethnicities and locations, Tolarova et al (2007) suggested the term "candidate nutrients" for several nutrients that seem to be critical for development of the orofacial region.

OBJECTIVE: To analyze the nutritional profile of mothers of children affected with NCLP in El Salvador, San Salvador.

MATERIALS AND METHODS: The sample consisted of 68 mothers of children with NCLP. Two interview instruments, (1) General Genetic Questionnaire - GGC and (2) Food Frequency Questionnaire – FFQ, were used for interviews conducted in Spanish. The information from these surveys was put into the DietSys nutritional software and analyzed. Results from DietSys analysis were compared to the Daily Recommended Intake (DRI) values published by the Food and Nutrition Board of the Institute of Medicine.

RESULTS: Out of 68 interviewed case mothers, 4 mothers were excluded from the study, because their daily caloric intake was either lower than 500 or higher than 3500 calories. The mean caloric intake was 1599.6 (SD=675.7). This value represents only 72.7% when compared to DRI for women and 64% when compared to DRI for pregnant women. The mean daily folate intake was 319.2 μg (SD = 188.3), 53.2% of DRI for pregnant women. Compared to DRI for pregnant women, the mothers also showed low daily intakes of zinc (82.8%), vitamin B6 (71.6%), and vitamin E (49.8%). The mean daily intake of vitamin A was 5058.8 IU (SD = 2878.6) that represents 144.8% of the DRI for women. A daily consumption higher than 10,000 IU was found in 11% of mothers.

CONCLUSIONS: Insufficient dietary folate intake appears to be the most important finding in the San Salvador study population. More data and also a comparable sample of mothers with unaffected children are necessary for any final conclusion. It has been shown that the mothers' deficiency of folate and high levels of Vitamin A (over 10,000 IU) were causing congenital anomalies.

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EFFECTS OF TYPE II DIABETES ON VASCULAR REACTIVITY IN THE RAT SUBMANDIBULAR GLAND

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INTRODUCTION: The prevalence of type 2 diabetes is increasing worldwide, and represents a major public health problem. Patients with type 2 diabetes have an increased risk for adverse cardiovascular events that result from changes in microvasculature structure and function. Previous studies have shown that the rat submandibular gland (SMG) is an excellent model for studying microvascular changes in a rat model of type 1 diabetes. Thus, the purpose of this study was to determine whether SMG vascular responses are affected in a rat model of type 2 diabetes. Specifically, we hypothesized that vascular reactivity in response to sympathetic stimulation is diminished in type 2 diabetic animals compared with age-matched controls. In addition, xerostomia is considered an oral complication of diabetes mellitus in humans. Thus, we also compared salivary flow rates between control and diabetic rats.

METHODS: Four month old male rats (Zucker Lean [ZL], n=6 and Zucker Diabetic Fatty [ZDF], n=5) were anesthetized with sodium pentobarbital (35 mg/kg i.p.) followed by chloralose (80 mg/kg i.v.). Blood flow responses in the SMG to sympathetic stimulation (2 Hz and 4 Hz continuously or 20 Hz and 40 Hz in bursts of 1s for every 10 s) were measured using laser-Doppler flowmetry and recorded on computer software program for analysis. At the end of each experiment, the salivary glands were weighed and fixed for routine histology

RESULTS: Compared with control ZL animals, ZDF rats demonstrated an increase in body weight (mean ± S.D., 380 ± 19 g vs 346 ± 20 g) and non-fasting serum glucose levels (497 ± 28 mg/dl vs 138 ± 12 mg/dl). In contrast, SMG weights were decreased (192 ± 16 mg vs 277 ± 47 mg). The response to sympathetic stimulation was biphasic (vasoconstriction followed by vasodilatation), but depending on the experimental protocol employed the result in ZL rats was either a net vasoconstriction (continuous impulses) or a net vasodilatation (burst stimulation). In contrast, the net vasoconstriction during continuous stimulation was greater in ZDF rats. In addition, burst stimulation did not result in a net vasodilatation in ZDF rats. Finally, mean SMG flow rate (ml/min/g) was significantly reduced in ZDF rats compared to that of ZL control animals under all stimulus conditions.

CONCLUSIONS: From these data we conclude 1) that there is a decrease in the vasodilatory response to sympathetic stimulation in the SMG of type 2 diabetic rats and 2) that salivary secretory responses to sympathetic stimulation are diminished in type 2 diabetes.

ENHANCEMENT OF GENE DELIVERY TO ORAL CANCER CELLS: INTRACELLULAR VISUALIZATION OF LIPOPLEXES AND USE OF CELL-PENETRATING PEPTIDES AND MICROTUBULE-DISRUPTING AGENTS

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INTRODUCTION: The survival rate for oral cancer has not changed significantly in the past few decades. Thus, finding new methods of treatment is of utmost importance. Cancer cells may be eliminated by the delivery of therapeutic "suicide" genes. Non-viral vectors are the emerging preference for gene delivery due to lower safety risks and ease of production. One problem with non-viral vectors is the variability in the transfection of different oral squamous cell carcinoma (OSCC) cells. It is important to understand the mechanisms of this variable gene delivery and find ways to overcome the cellular barriers to transfection.

OBJECTIVE: To observe the intracellular internalization of cationic liposome-DNA complexes (lipoplexes) in HSC-3 and H-413 cells, the latter highly resistant to transfection, to better understand the mechanisms of gene delivery. To test the hypothesis that cell-penetrating peptides (HIV-Tat-peptide) and microtubule disrupting agents (vinblastine) can facilitate cytoplasmic delivery and nuclear entry of DNA.

METHODS: HSC-3 and H-413 human OSCC cells were maintained in appropriate media and seeded in fibronectin-coated Lab Tek II chambered cover glasses. They were kept in 48-well culture plates the day before transfection and used at approx. 70 % confluency. The plasmid pCMV.Luc expressing luciferase under the control of the cytomegalovirus promoter was complexed with optimal volumes (2 µl/ well) of Fluo-Metafectene with or without the HIV-1 TAT peptide. Vinblastine was added to the culture medium. Lysosensor and Hoechst dye were used to stain the lysosome and the nucleus. Transgene expression was assayed 48 h after transfection using the Promega Luciferase Assay System. Fluorescence was observed 2 h, 4 h and 48 h after transfection using a Zeiss LSM 510 Confocal Fluorescence Microscope.

RESULTS: HSC-3 cells displayed a higher level of cell-associated rhodamine fluorescence compared to H-413 cells. Metafectene co-localized with lysosomes in the perinuclear region. The increase in intracellular fluorescence at 48 hours also indicates that time is a variable for increased efficacy. Pre-complexation of 1 and 5 µg HIV-Tat-peptide with 1 µg DNA enhanced gene expression by 4- and 5-fold, respectively, while the addition of the peptide+Metafectene mixture to DNA was not effective. Treatment of HSC-3 cells with vinblastine (2 µg/ml) enabled higher perinuclear localization, and enhanced gene expression, optimally in the range 2-4 µg/ml.

CONCLUSIONS: HSC-3 cells internalize Fluo-Metafectene more efficiently than H-413 cells. Cell penetrating peptides and microtubule disrupting agents may be useful for gene delivery to transfection-resistant cell lines. Optimal enhancement of gene delivery depends on the order in which cationic liposomes, cell-penetrating peptide and plasmid DNA are added together.

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LOAD AND AGE RELATED CHANGES IN HUMAN CEMENTUM

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Introduction: Functional loads are partially accommodated by primary cementum (PC), which anchors the tooth to alveolar bone and secondary cementum (SC) which is mechanistically thought to be an occlusal load absorber. Hypothesis: The inherent characteristics of PC and SC change with functional loads and with an increase in age.

Objective: The changes due to functional loads were studied by characterizing cementum from incisors (N=6) and molars (N=6) belonging to the same age group and changes due to age (younger: 19-40 and older: 55-80yrs) by characterizing cementum taken from the same tooth type. The width of cementum and hardness from CEJ to apex, type of tooth, and age were determined.

Methods: The teeth were longitudinally cut into halves, embedded and polished to evaluate cementum width and hardness.

Results: Cementum width increased from CEJ to the apex of a tooth for all groups. The cementum width of molars was twice that of incisors. A gradual increase in hardness from cementum to dentin and from CEJ to apex was observed for all groups. PC formed a steeper gradient with dentin than SC for both tooth types. Additionally, hardness gradients from cementum to dentin and from CEJ to apex for incisors were steeper than for molars. No significant changes in gradients were observed with an increase in age. Significant differences (Student's t-test, $P<0.05$) in PC and SC hardness values as a function of tooth type and age were observed:

Hardness	**INCISORS**		**MOLARS**	
	Younger	Older	Younger	Older
PC	0.55 ± 0.12	0.42 ± 0.08	0.39 ± 0.14	0.49 ± 0.10
SC	0.47 ± 0.12	0.39 ± 0.08	0.41 ± 0.10	0.46 ± 0.09

Currently the observed hardness values are being co-related to respective mineral contents.

Conclusions: The resulting properties could be an adaptation of PC and SC to accommodate the combinatorial effect of type and magnitude of functional loads and exposure to contents of oral environment.

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CARIFREE© TREATMENT RINSE EFFECTS ON S. MUTANS

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INTRODUCTION: The model of dentistry has evolved from "drill and fill" to the medical model of preventive, wellness care. Part of this model is treating dental caries as a disease. Treating the caries infection involves eliminating the current infected population of bacteria from the biofilm, and also altering the environment to ensure that they don't return. CariFree© Treatment Rinse comes in two different bottles labeled as component A and B, respectively. Its labeled use is stated to prevent "cavity-causing plaque biofilm, reduce the overpopulation of cavity-causing bacteria, and neutralize decay-causing acids with patent-pending pH+ technology."

OBJECTIVES: We tested CariFree© Treatment Rinse on S. mutans in order to study its effectiveness when the components are combined as prescribed versus the components as separate therapies.

METHODS: To test the efficacy of each component, we used S. mutans in 48 hour biofilm, single layered on a hydroxyapatite bead, and as a planktonic suspension. We measured the effectiveness, by using each component individually and also together, to see if one component had the main killing power for S. mutans. We used a live/dead fluorescence assay to measure the relative amount of S. mutans killed.

RESULTS: It was shown that component B was superior in killing the S. mutans for all cases. However, it was also shown that when mixing the two components together, the efficacy was slightly lowered compared to just component B. Thus, CariFree© Treatment Rinse does eliminate S. mutans, but at a lowered value due to the mixing of the two components.

CONCLUSION: CariFree© Treatment Rinse is indeed effective in killing S. mutans. Further study would be to test the efficacy of the rinse on other cariogenic bacteria.

GENE DELIVERY TO ORAL SQUAMOUS CELL CARCINOMA CELLS BY ADENOVIRAL VECTORS: ENHANCEMENT BY CATIONIC LIPOSOMES AND MODIFIED VIRAL FIBER PROTEINS

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INTRODUCTION: Head and neck squamous cell carcinoma is the 6th leading cause of cancer-related mortality in the United States, and affects about 50,000 people each year, 52% of whom have a mean survival time of only 5 years. Adenoviruses are being studied as vectors for the delivery of therapeutic genes to oral squamous cell carcinoma (OSCC) cells.

OBJECTIVE: To test the hypotheses that (i) adenoviral vectors will efficiently deliver the tumor-specific, survivin-driven luciferase gene to OSCC cells, (ii) cationic liposomes will enhance adenoviral transduction, and (iii) adenoviral vectors with modified fiber proteins will enhance gene delivery to OSCC cells compared to the wild-type virus.

METHODS: Cultured HSC-3 and H-413 human OSCC cells were used at 85% confluence. Two recombinant adenoviral vectors from which the E1A region had been ligated and constructed to encode luciferase were used: Ad-Sur-luc, under the control of the human survivin promoter, and Ad-CMV.luc, under the control of the cytomegalovirus promoter. The virus was incubated with the cells at different multiplicities of infection, new medium was added and luciferase expression was determined after 48 h using a Promega kit. To assess the effect of cationic liposomes, the viruses were mixed with 2 µl Metafectene or Metafectene-Pro (Biontex), and incubated with the cells as described above. A recombinant adenovirus with polylysine-modified fiber knobs (Ad-pK7-CMV.luc) was tested for its ability to express luciferase.

RESULTS: In both HSC-3 cells and H-413 cells, luciferase expression increased with the multiplicity of infection. The efficiency of transduction by Ad-Sur.Luc was much lower than than by Ad-CMV.Luc. Increasing the incubation time from 1 to 4 h increased transduction efficiency. Cationic liposomes caused a large increase in viral transduction. Gene expression in HSC-3 cells with Ad-pK7-CMV.Luc was 7-fold higher than with Ad-CMV.luc. Although H-413 cells are resistant to non-viral transfection, the use of viral vectors with cationic liposomes enhanced gene expression by >1000-fold.

CONCLUSIONS: Transduction of OSCC cells with wild-type adenoviral vectors did not result in high levels of reporter gene expression that were expected. However, complexation of cationic liposomes with the viral vectors greatly improved gene expression. The modified adenoviral vector also enhanced viral transduction, most likely by facilitating cell binding. The use of viral vectors with optimal incubation times in conjunction with cationic liposomes can be a promising tool to help achieve the delivery of suicide genes in the therapy of OSCC.

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FRACTURE STRENGTH OF TEETH RESTORED WITH DIFFERENT DESIGNED POST AND CORE SYSTEMS

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OBJECTIVE: The purpose of this study is to evaluate the fracture strength of teeth restored with varying posts and core systems cemented in dentin

BACKGROUND: The ability to restore severely broken down teeth depends largely on the successful internal reconstruction of these teeth with a strong and durable post and core system. Until recently, all-ceramic materials were not used for root post, because they could not withstand the intraoral loading conditions. A milled ceramic post and core is very strong, has a better refractive index compared to metal, can be stained to existing dentin shades, and cemented to place with auto polymerizing resin, thus providing a very strong and esthetic custom milled dowel and core.

METHODS: Thirty extracted maxillary anterior teeth were collected and root canal treated. The crown was cut off at the proximal CEJ level. Post space and retention features were created using Lightspeed and diamond instruments. Group A(n=6) were thick parallel zirconia posts, Group B(n=6) were small parallel zirconia posts, Group C(n=6) were tapered zirconia post design, Group D (n=6) were thick parallel fiber posts with composite build-ups and Group E were thick parallel cast gold post and cores. Duralay patterns were made for the samples and sent to the lab to be milled for Group A, B and C. Conventional methods of fabricating composite and gold post and core systems were used for Groups D and Group E respectively. All posts and cores were cemented within the teeth using Rely X Unicem (3 M ESPE). The teeth are then embedded into plastic containers using auto curing resin and fracture strengths tests have been performed using the universal testing machine Instron 1011 at a crosshead speed of 1 mm/min until fracture.

RESULTS: The greatest fracture strengths were found in Group A, thick parallel Zirconia posts, with average fracture strength of 578 Newtons. Group C, tapered Zirconia posts, had slightly greater fracture strength of 345 Newtons than Group B, thin parallel Zirconia posts, average of 285 Newtons. Group D, thick parallel composite Fiber Post, had the lowest average fracture strength of 214 Newtons. Group E, thick parallel Cast Gold post, had a medium fracture strength of 385 Newtons. Group A had significantly higher fracture strength than Group D($p=0.02$) while Group E had not significantly different strength than Group A($p=0.07$). The fractures most commonly occurred within the tooth structure mesio-distally rather than at the zirconium post and core.

CONCLUSION: Compared to the more commonly used cast gold and composite fiber post systems, custom milled thick parallel Zirconia posts generated the highest fracture strength as well as the highest degree of esthetics. If fracture does occur, it will most likely be within the tooth structure rendering the tooth subsequently unrestorable.

STUDY OF THE RFC1 A80G POLYMORPHISM AND NONSYNDROMIC CLEFT LIP AND PALATE IN GUATEMALA

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INTRODUCTION: Nonsyndromic cleft lip with or without cleft palate (NCL/P) is among the most common congenital anomalies. Etiology of NCL/P is multifactorial, with genetic and environmental factors contributing to the development of the abnormality. Folate, a water-soluble vitamin B9, is necessary for cell multiplication, differentiation, and maintenance. Sufficient supply of folate is especially essential during pregnancy allowing the fetal cells to undergo growth and replication (Vieira et al., 2005). For this reason, it is suspected that a significant deficiency of this vitamin, caused by either inadequate ingestion or a genetic abnormality/mutation in metabolism, can affect critical stages of embryonic development and lead to development of congenital anomalies such as NCL/P. The reduced folate carrier (RFC1) gene encodes a cell membrane transport protein that receives folate molecules from circulating blood and transports them into a cell.

OBJECTIVE: To determine if an association between the A80G variant of the RFC1 gene and NCL/P exists in our Guatemalan sample.

METHODS: Cases (n=106) and controls (n=74) for this study were identified during Rotaplast cleft medical missions at Roosevelt Hospital in Guatemala City, Guatemala. Venous blood specimens were obtained, preserved as blood spots on filter paper, and shipped to the Pacific Craniofacial Genetics Laboratory (PCGL) for molecular genetic analysis. In the PCGL, DNA was isolated from dry blood spots and RFC1 80AG genotypes were identified by PCR amplification and detection of single nucleotide conformational polymorphism using polyacrylamide gel electrophoresis (PAGE).

RESULTS: A significant difference ($p=0.0369$) was found in genotype distribution between cases and controls. In cases, 18.9% of individuals had A80/A80 genotype compared to 35.2% in controls, while 35.5% had G80/G80 genotype in cases and only 24.3% in controls. The A allele frequency was 0.415 for cases and 0.554 for controls, while the G allele frequency was 0.585 for cases and 0.446 for controls ($p=0.0127$).

CONCLUSION: Our results confirmed previous findings by Costanzo et al (2004) suggesting that the G allele in nucleotide 80 of the RFC1 gene contributes to the etiology of NCL/P in Guatemala.

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POLYMORPHISMS OF THREE "CANDIDATE GENES" FOR NONSYNDROMIC CLEFT LIP WITH OR WITHOUT CLEFT PALATE IN GUATEMALA. A PILOT STUDY

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INTRODUCTION: Nonsyndromic cleft lip with or without cleft palate (NCLP) is among the most common congenital anomalies. The birth prevalence of NCLP varies from 1 in 500 to 1 in 1000 according to ethnic background, geographic location, and socioeconomic status. The etiology of NCLP is thought to be a complex interaction between genetic and environmental factors. Several recent studies have suggested that approximately 15-20% of NCLP are determined by three susceptibility genes or their combinations: MSX1, RFC1, and MTHFR.

OBJECTIVES: The objective of our study was to combine results from our studies on polymorphisms of three candidate genes: RFC1 A80G, MTHFR C677T, TGFB3 (two mutations: rs2300607 A/T and rs2268625 C/T) in Guatemala.

MATERIALS AND METHODS: Altogether, 564 individuals were included in this study. Sample of cases consisted of 265 patients affected with NCLP and sample of controls comprised 299 unaffected individuals. All cases and controls were collected during Rotaplast cleft medical missions to Guatemala City, Guatemala from 2001 to 2005. The genotypes were ascertained on PAGE using Pacific Craniofacial Genetics Laboratory protocol (MTHFR, RFC1, TGFB3 rs2300607 A/T) and by sequencing (TGFB3 rs2268625 C/T).

RESULTS: In 45 individuals (39 cases and 6 controls), all four polymorphisms were analyzed. The most common combination of genotypes among 39 cases (n=7, 2.6%) was homozygote for mutated allele of RFC180 (GG) + homozygote for mutated allele of MTHFR 677 (TT) + heterozygotes for two mutations of TGFB3 (AT and CT). In 150 individuals (111 cases and 39 controls), only three polymorphisms were analyzed. The most common combination of genotypes in cases was MTHFR 677 CT, rs2300607 AA, and rs2268625 TT (n=17, 6.4%).

CONCLUSION: All four polymorphisms were found to be associated with NCLP in previous studies of Guatemalan population. In order to find out, whether any combination of genotypes has a stronger association with NCLP, the missing genotypes will be completed and all data will be statistically analyzed taking in consideration also types and severity of the cleft anomaly.

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CLEFT LIP AND PALATE IN PHILIPPINES AND *RFC1 A80G* POLYMORPHISM. A PILOT STUDY

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INTRODUCTION: The etiology of the nonsyndromic cleft lip with or without cleft palate (NCLP) involves interactions of environmental and genetic factors. Our previous study from the Guatemala population (Costanzo et al, 2004) suggested that A80G polymorphism of the reduced folate carrier 1 (RFC1) gene, which is involved in the transport of folate across the cell surface membrane, was associated with NCLP. Specifically, the mutated G allele was found significantly more frequently in NCLP. While some studies are in agreement with Costanzo's findings, others are not. Also studies on another gene's polymorphism related to folate metabolism – MTHFR 677CT – are showing inconclusive results.

OBJECTIVES: To analyze the A80G polymorphism of the RFC1 gene in a sample of patients NCLP from Cebu City, Philippines.

METHODS: Individuals affected with NCLP (n=76) and unaffected individuals (n=37) were identified during Rotaplast medical missions to Cebu City, Philippines in 2003, 2005, and 2007. RFC1 A80G genotypes were established by PCR amplification followed by detection of single-nucleotide conformational polymorphism using polyacrylamide gel electrophoresis.

RESULTS: In cases, 28.9% of individuals had A80/A80 genotype, 22.4% had G80/G80 genotype, and 48.7% were heterozygotes (A80/G80). Proportions of genotypes in controls were 27.0% A80/A80, 29.8% G80/G80, and 43.2% A80/G80. The allele frequency was 0.533 for cases and 0.486 for controls, while the G allele frequency was 0.467 for cases and 0.514 for controls. There was no difference found between neither genotypes distribution nor allele frequency between cases and controls.

CONCLUSION: Our pilot study suggests that polymorphism of the RFC1 A80G may not be involved in the etiology of NCLP in population of Cebu City, Philippines. This is completely opposite to the results we have found in the Guatemala population (Costanzo et al, 2004). Thus, very likely, a different spectrum of genetic factors forming a genetic susceptibility to NCLP exists in those two different populations. Additional studies are in progress.

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INHIBITION OF STREPTOCOCCUS MUTANS GROWTH WITH CARIFREE® TREATMENT RINSE

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INTRODUCTION: *Streptococcus mutans* is a common bacterial culprit of tooth decay. The dental community is trying to find ways to treat patients through chemotherapeutics in order to reduce the numbers of *S. mutans*, and therefore reduce tooth decay. CariFree® treatment rinse is a product used for this purpose.

GOAL: This study investigated the concentration and time dependencies of the rinse to kill S. mutans in a planktonic, single layer, and biofilm environment.

HYPOTHESES: Two hypotheses were created. The first hypothesis was that the biofilm created by the S. mutans would have protective characteristics compared to the planktonic preparation. Therefore, the CariFree® rinse would be slower and would require higher concentration against the biofilms on the hydroxyapetite beads, than against the planktonic bacteria. The second hypothesis was that the hypochlorite is the active ingredient causing morbidity of the bacteria.

METHODS: The fraction of live bacteria was determined for S, mutans preparations after a ten minute incubation with increasingly diluted CariFree®, and for aliquots taken at increasing times after addition of full strength CariFree®. The live fraction was calculated from measurements of SYTO9/propidium iodide fluorescence ratios.

RESULTS: The reaction rate for killing the planktonic bacteria was slightly slower than for the single layer bacteria. The reaction rate for the biofilm was much slower. The concentration dependencies were equal within experimental error for all three preparations.

CONCLUSIONS: The results for the concentration dependence probably reflect the long, ten minute, incubation period, which gave the CariFree® adequate time to diffuse into the biofilm. The results for the time dependence experiments suggest that the biofilm had protective characteristics for the bacteria. Similar results were found in literature with biofilms of MRSA on dentures killed with sodium hypochlorite (Lee et al 2009). This is also clinically significant. If a patient is to use CariFree® rinse, he or she should mechanically remove plaque for the rinse to be effective.

DENTAL STUDENT PREDICTION OF PEDIATRIC PATIENT ANXIETY

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INTRODUCTION: The vast majority of studies of psychological stress in dental practice have investigated common stressors of dentists' or dental students. Other studies have evaluated dentists' perceptions and assessments of patients. However, review of the literature reveals little information relative to dental students' ability to assess pediatric patient anxiety. Yet accurate assessment and appropriate management of patient anxiety is essential to successful pediatric patient care. Further, there is little in the literature to elucidate dental student anxiety around the pediatric patient care situation. There may be significant relationships between these two anxiety contexts which would also be beneficial in instructing students in anxiety management for themselves and their pediatric patients.

OBJECTIVES: The primary aim of this study was to evaluate dental students' ability to assess their pediatric patients' anxiety level accurately to facilitate appropriate management of these patients.

METHODS: In this study, second year dental students from the University of the Pacific School of Dentistry were asked to evaluate the anxiety level of their pediatric dental patients in the school's Pediatric Clinic. Anxiety was assessed in both patients and dental students using a validated facial image scale (FIS). Student participants were also asked to rate their own anxiety around three issues: parent interaction, patient behavior management, and technical procedure. Meanwhile, pediatric dental patients selected for participation in the study were asked to choose the one emotionally representative face from a set of five using the FIS which represented how they were feeling about their appointment.

RESULTS: Initial review of the data suggests the following: one quarter of the student participants in the study predicted their patients' anxiety accurately (matching FIS scores). The remaining students showed varying degrees of inaccuracy in predicting their patients' anxiety levels. Nearly half of students were within one FIS score of their patients' reported anxiety, with students tending to over-estimate their patients' anxiety. Additionally, students who planned local anesthesia, had a previous behavior management case, and/or increased technical difficulty of procedure were less accurate.

CONCLUSION: Initial results suggest that students do not always accurately assess their pediatric patients' anxiety levels, especially in more stressful situations. Instructor awareness of planned procedures, students' previous patient behavior management experiences, and students' own anxiety about the technical procedure should trigger discussion and instruction around probable patient anxiety levels.

Further study is needed to clarify trends suggested by the data and other possible relationships between variables evaluated in this study, and may suggest areas for future investigation. Data analysis is ongoing.

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IDS STUDENT RESEARCH PRESENTATIONS

SELF-ETCH BONDING: REVOLUTION WITH DRAWBACKS

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INTRODUCTION: There are several options in adhesive systems for composite restorations. In recent years, the tendency has been towards self-etch bonding systems. These systems have some advantages and have grown in popularity. Since no system is devoid of drawbacks, we decided to prepare a literature review on the subject.

PURPOSE: To search within the available scientific literature to establish whether or not the advantages of self-etch composite bonding systems outweigh any inherent drawbacks these systems may possess.

METHOD: Our group researched within the literature for any relevant study on the subject within the last decade with greater emphasis in the more recent studies. Care was taken to find consensus within the literature as we often found contradictions from one peer reviewed journal to another. Some researchers' results showed great variability from one sample group to another. After much review, many patterns of similarity were recorded in key areas of our topic and thus, our paper focused on the common ground found among these key properties detailed in the different studies.

RESULTS: After carefully reviewing many studies from peer review journals, manufacturers' data and carefully selected internet search results, we found that the fifth generation systems repeatedly outperformed the later generations in adhesion to dentin and enamel. They provide longer lasting restorations with fewer problems of sensitivity reported in later systems. One very important and consistent finding was the more predictable quality of adhesion to enamel and the variable adhesion due to the many factors challenge adhesion to dentin. This is true of any bonding system but the current data show self-etch systems fair poorly to total etch systems in terms of decreased bond strength and micro leakage.

CONCLUSIONS: Ultimately, post operate sensitivity is a major issue in composite restorations. Success with adhesion systems is largely technique sensitive regardless of generation. Self-etch systems are developing momentum and popularity in the marketplace due to their ease of use in fields like pediatric dentistry and in orthodontics where they are being employed to attach brackets. Despite the greater convenience and relative insignificance of drawbacks in these clinical scenarios, general dentistry as a whole need not rush to switch to self-etch systems. At present, the downsides of the technology available outweigh the benefits for the average practitioner to whom the best possible integration and seal to the tooth structure is of the utmost importance.

IS CAD/CAM INLAY OR ONLAY AN ALTERNATIVE TO AMALGAM RESTORATION?

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INTRODUCTION: Patients are presenting to our clinic as well as private practice with more esthetic concerns than ever before demanding tooth colored restorations. We asked ourselves whether CAD/CAM fabricated inlays and onlays would be a good alternative to present to the patient. Based on this question, we performed a literature review of various articles published in peer reviewed journals.

PURPOSE: To search the literature regarding advantages and disadvantages of CAD/CAM fabricated inlays and onlays, as well as direct amalgam restorations. Our focus was on esthetics, preparation design, biocompatibility/toxicity, reported failures, cost and integration into clinical practice.

METHOD: Peer reviewed journals were examined with priority on recently published articles, as well as reliable manufacturer's websites where applicable. Our results were compiled for presentation.

RESULTS: Many trends in dentistry are without a doubt driven by patients and their desire for esthetic dentistry, driven by clever marketing campaigns. CAD/CAM systems such as CEREC enable the patient to be treated in one visit and deliver a fine esthetic result. While there have been reports in the literature about tissue reactions to amalgam, ceramics seem to have superior biocompatibility with no adverse events reported. Even though the failure rate is still higher for indirect restorations compared to amalgam, this continues to improve with further advances in technology. There are many factors a dentist must consider when purchasing large equipment and innovative technology, not the least of which is the potential financial return of such a purchase. The learning curve must also be considered. The majority of dentists achieve a predictable comfort level after placing approximately 20-30 restorations. While the prep design is similar as for laboratory fabricated ceramic restorations, the restoration design can be accomplished in less than five minutes with CEREC's latest software.

CONCLUSIONS: New FDA statement regarding amalgam toxicity should give the clinician pause to consider an alternative to offer the patient. Amalgam reactions have been observed, ceramics are extremely biocompatible. Decisions to use one over the other should be based on patient needs. CAD/CAM systems offer the clinician the chance to combine computer technology with artistry.

VENEERS: A LITERATURE REVIEW

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INTRODUCTION: In the last two decades, patients' esthetic dental concerns have become more important if not surpassing other oral health concerns. Among different treatment options, Veneers were looked at as a very promising treatment modality because they allowed for minimal removal of tooth structure as compared to crowns, they also had the advantage of less discoloration and greater surface smoothness as compared to direct composite restorations. Today veneers are considered to be a very good option for esthetic smile rehabilitation.

PURPOSE: To search the literature on the different preparation designs of veneers, and to learn about the effect of each design on impressing and seating procedures as well as on the longevity of the final restoration.

RESULTS: The most common designs are generally focusing on incisal and interproximal reductions with the primary objective being preserving as much tooth structure (enamel) as possible. It is recommended to place interproximal margin of the preparation either short of the proximal contact or extending beyond it; this position is easier for impressing and for seating procedures. Regarding incisal reduction there are four main types of preparation designs: 1- Window preparation which involve preparing the labial surface and placing the margin short of the incisal edge.
2- No incisal reduction with feathered incisal edge.
3- Incisal reduction without palatal chamfer (butt joint).
4-Incisal reduction with 1mm height palatal chamfers (incisal overlap).

CONCLUSIONS: In our review we found that there is no standard preparation design for veneers. Central and lateral incisors prepared with a lingual chamfer design showed a higher resistance to fracture than if they were prepared with window design. Canines prepared with a window design were more resistant to fracture than those prepared with a lingual chamfer design. Some studies reported that window preparations can withstand higher axial stresses than either feathered edge or overlapped design. Other studies reported that incisal overlapping design reduces stress in the veneer most effectively. Higher fracture load are required for failure of beveled and overlapping design than for feathered veneer designs.

BONDING AND MICROLEAKAGE IN DIRECT COMPOSITES

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INTRODUCTION: Microleakage and its result secondary decay is one of the main concerns in operative dentistry. The aim of this literature review is to compare the effect of different generation bonding systems on the microleakage in direct composite. Also we will analyze other factors and methods of decreasing microleakage and improving life of direct composite.

PURPOSE: To use the right methods and materials for decreasing microleakage in direct composites.

METHODS: Recently published articles and reliable websites of commercial products were revised and compiled.

RESULT: Incremental filling decreases shrinkage stress as a result of reduced polymerization material volume. Beveled enamel junction is resistant to aging, due to mineral nature of the tissue which ensures a better and more durable bond. Two-step self-etch adhesive exhibited better marginal sealing than an all-in-one adhesive at the enamel margins.

CONCLUSION: Reduced sealing ability was observed in dentin and significant differences were observed between materials.
In enamel, marginal leakage was prevented with phosphoric acid. Self-etching adhesives promoted slight occlusal leakage. Re-emergence of 4th- and 5th-generation bonding agents total-etch (etch and rinse) bonding agents have a long, successful record. Another measure is the careful control of polymerization shrinkage through an effective placement technique.

CENTRAL NON-ODONTOGENIC LESIONS IN CHILDREN FROM A DENTAL SCHOOL BIOPSY SERVICE

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INTRODUCTION: This report presents a review of 186 central non-odontogenic biopsy results from a total of 7,261 samples from patients 0-16 years of age received over 25 years at the University of the Pacific School of Dentistry (Pacific Oral and Maxillofacial Pathology Laboratory). Lesions of the jaws (Central) in a pediatric population can be divided into odontogenic and non-odontogenic types. The odontogenic lesions occur more frequently and are represented by lesions of endodontic origin (eg. Periapical granuloma, radicular cyst), dentigerous cyst and odontogenic keratocyst. The non-odontogenic lesions are less common and little has been reported on their prevalence in children. The purpose of our study is to review these non-odontogenic lesions occurring in children 0-16 years of age submitted to our biopsy service in the last 25 years. As central non-odontogenic lesions vary in their origin, we also wanted to evaluate and categorize the lesions using the MIND classification.

METHOD:The computerized data was retrieved and compiled for age, gender, location, race and diagnosis. The lesions were divided by the MIND classification system into 1)Metabolic 2)Inflammatory 3)Neoplastic 4)Developmental and 5)Idiopathic

RESULTS: The pediatric central non-odontogenic cases reviewed were 186 patients which represented 2.56% of total number of pediatric oral biopsies. We did not find any lesions in the Metabolic or Inflammatory category. The neoplastic category accounted for 46 lesions (24.73% of total pediatric central non odontogenic lesions) with 1 malignant neoplastic lesion (0.54% of total pediatric central non odontogenic lesions). The developmental category accounted for 32 lesions (17.20% %). One hundred and eight lesions were classified as Idiopathic which contributed 58% of the total pediatric central non-odontogenic lesions.

CONCLUSIONS:
(a) 3 most common central non-odontogenic lesions in children are (1) Traumatic bone cyst (2) Focal Osteosclerosis or (3) Central Giant Cell Granuloma which all made up 70% of all the biopsies.
(b) Location: A majority of the central non-odontogenic lesions occur in the mandible. (70.96% cases in mandible vs. 16.66% cases in maxilla).
(c) Age: Prevalence of cases increases with age.
(d) Gender: A majority occur in females. (44.62% in females, 32.25% in males)
(e) Race: A majority occurs in Caucasians. (51.07% in Caucasians, 8.06% in Asians, 4.30% in Hispanics and 2.68% in Blacks)

TOXICITY OF COMPOSITE MATERIALS: A LITERATURE REVIEW

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INTRODUCTION: Despite the obvious esthetic advantages of dental composites as restorative materials, there has been concern regarding the toxicity of some of the components of the polymer matrix. Based to some extent on these concerns, we decided to perform a literature review. This literature search presents a review of the results from various articles published in peer reviewed journals in recent years.

PURPOSE: To search the literature regarding controversial topic of possible toxicity of composite resin based materials and possible solutions to minimize the toxic effects.

METHOD: Peer reviewed Journals were reviewed to with priority to recently published up to date articles, as well as reference to reliable websites of commercial products. The similarities and differences from different articles along with facts were compiled for presentation.

RESULTS: In general Toxic effect of composite resin based materials in vitro can be categorized by long term and short term exposure, being the most important factor, the release of uncured or free monomer causing proliferation of bacteria and allergic reactions. In-vitro cytotoxicity, estrogenicity and mutagenicity has been reported for Bis GMA (component of Bisphenol A) as well as for UDMA, TEGDMA and others.
The range of increasing toxicity of these materials has been reported as follows: 1.HEMA 2. TEGDMA 3. UDMA 4. Bis-GMA. The toxicity of flowable composites due to its ratio of filler-monomer is also presented as an important parameter.

CONCLUSIONS:

- Amount of toxicity found in in-vivo and in –vitro subjects.
- Long term and systematic studies of amalgam and composite materials.
- Composites Resins are toxic to oral mucosa in susceptible individuals and in-vitro gingival fibroblasts
- Decision to use composite resin over dental amalgam or other material should be based on individual case need
- Adverse effects are more commonly seen in dental personnel than in patients, due to chronic and frequent exposure.
- Further independent research is required

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