7TH ANNUAL PACIFIC RESEARCH DAY & STUDENT RESEARCH COMPETITIONS

PROGRAM & ABSTRACTS

WEDNESDAY MAY 25 2005

FACULTY & STUDENT TABLE CLINICS
ORTHODONTICS RESIDENTS' ORAL PRESENTATIONS
ADA/DENTSPLY & SENIOR RESEARCH COMPETITIONS
7TH ANNUAL PACIFIC RESEARCH DAY

AND

STUDENT RESEARCH COMPETITIONS

PROGRAM & ABSTRACTS

WEDNESDAY MAY 25, 2005

Sponsored in part by a grant from Western Dental Services, Inc.
PROGRAM

12 – 2 PM  (ROOM 304)

ORTHODONTICS RESIDENTS’ PRESENTATIONS

3 – 5:30 PM  (CLINICS)

FACULTY AND STUDENT POSTERS AND TABLE CLINICS

ADA/DENTSPLY STUDENT RESEARCH COMPETITION

SENIOR RESEARCH COMPETITION

5:30 – 7:00 PM  (CAFÉ CAGNONE)

RECEPTION
ABSTRACTS

ORTHODONTICS RESIDENTS’ PRESENTATIONS

12 – 2 PM  ROOM 304
COMPARISON OF ROOT LENGTH AND ROOT CURVATURE FOR THREE ORTHODONTIC TREATMENT STRATEGIES FOR PATIENTS PRESENTING IN THE MIXED DENTITION

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Orthodontists have long debated the relative merits of starting treatment in the mixed dentition versus postponing intervention until the eruption of the permanent dentition. One factor that may influence a practitioner’s decision on when to treat is the affect early treatment might have on root development. In this randomly selected retrospective study three treatment groups are being evaluated: subjects for whom treatment was delayed, then completed in the permanent dentition (Group 1), subjects who received early treatment and did not need additional treatment (Group 2.1), and subjects who received early treatment and were treated again in the permanent dentition (Group 2.2). Periapical radiographs for the different treatment groups are being evaluated by objective measurement and clinical judgment.
THE ROLE OF THE INTERFERON REGULATORY FACTOR 6 (IRF-6) GENE VARIANT IN THE ETIOLOGY OF THE NON-SYNDROMIC CLEFT LIP AND PALATE ANOMALIES

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Approximately one out of every 500-700 newborns are affected with an orofacial cleft—20 new cases a day in the United States and more in Central and South America. Non-syndromic cleft lip and palate (NCLP) is caused by interactions of environmental and genetic factors. Interferon regulatory factor 6 (IRF-6) belongs to a family of transcription factors that regulate cell proliferation and immune response. Recent research has demonstrated high levels of IRF-6 mRNA along the medial edges of the developing palate and tooth buds in utero. Mutations in the IRF-6 gene have been shown to cause Van Der Woude Syndrome, the most common syndromic form of orofacial clefting. Very recently, IRF-6 polymorphisms were implicated in etiology of NCLP as well.

If data on prevalence of different candidate gene polymorphisms would be available for a certain location, preventative measures can be applied in families at a high risk of NCLP development in offspring.

Our research has been focused on prevalence of IRF-6 polymorphisms in a sample of patients diagnosed with NCLP and in controls.

Dry blood spot specimens on filter paper were prepared from blood collected from 98 cases diagnosed with NCLP and from 72 controls in Guatemala City, Guatemala, during the Rotaplast medical mission in 2004.

DNA isolation and amplification of a specific segment of DNA by polymerase chain reaction (PCR) were performed with each blood specimen. The PCR products can be used for identification of the single-nucleotide polymorphism in some cases by sequencing. The individual genotypes of the IRF-6 polymorphism will be diagnosed by the specific Taqman single-nucleotide polymorphism assay in summer 2005.

CONCEPTS OF “FACIAL ATTRACTIVENESS” IN THE ORTHODONTIC COMMUNITY: HOW WELL DO CONVENTIONAL PHOTOGRAPHIC “ANALYSES” REFLECT CLINICIAN’S JUDGMENTS

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Significance Majority of orthodontic diagnosis and treatment planning is based on hard tissue charting and classic Angle principles. Hard tissue measurements are inadequate alone in this day to provide excellent orthodontics. An overwhelming movement toward an aesthetic paradigm is spreading throughout the orthodontic community worldwide, and the average patient entering an orthodontic office is very conscious of what is currently considered “attractive”. The ideals being used have existed for decades and have not been reevaluated to determine applicability to today’s orthodontic patient. Testing these “ideals” against attractiveness indices established by different groups of people can determine if they are adequate.

Methods Frontal and lateral photos along with lateral cephalometric films will be digitized and traced according to guidelines presented by The American Academy of Facial Plastic and Reconstructive Surgery in the Proportions of the Aesthetic Face text from Stanford University. Various “norms and ideals” will be treated as testable hypotheses. Correlative and statistical significance tests will be run against “attractiveness indices” assigned by a group of judges to determine applicability to our sample.
THE DECISION TO EXTRACT: A COMPARISON OF GENERAL CRITERIA CITED BY CLINICIANS AND THE DECISIONS MADE ON SPECIFIC CASES

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Objective To examine the correlation between the general concepts on which clinicians say the use to make extraction decisions in orthodontic treatment. Clinicians were then shown complete pre-treatment records (models, facial and intra-oral photos, panoramic and cephalometric radiographs, and cephalometric tracings) from 8 specific cases and given a second questionnaire asking specific questions about their decision and preferred treatment for each case. The responses given for the specific cases were compared with the responses given in the open-ended questionnaire. The responses were also compared with a set of responses to similar questions about the same subjects that were obtained from clinical instructors at another teaching institution.

Results In progress.

Hypothesis The general criteria from which clinicians say they base their decisions to extract or not extract teeth during orthodontic treatment will differ from the criteria they use when presented with pre-treatment records for actual cases.

Introductions and Objectives: Orofacial clefts including cleft lip with or without cleft palate (CL+/-P) are one of the most frequent congenital anomalies. The world wide incidence of nonsyndromic CL+/-P is 1.2 per 1,000 births, whereas in Venezuela the incidence is 2.5 per 1,000 births. Many environmental and genetic factors have been implicated as possible etiologies. Previous findings indicate that folic acid deficiency was coincident with increased orofacial clefting. Our focus was on the missense mutations occurring on the Reduced Folate Carrier (RFCI) gene and the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene. We hypothesized that specific mutations (RFCI 80A->G and MTHFR 677C->T) individually and concurrently would represent genetic risk factors associated with nonsyndromic cleft lip and palate in Caracas, Venezuela.

Samples and Methods: Blood samples and orofacial cleft diagnoses were collected from affected individuals (n=120) during Rotoplast medical missions in 2001 and 2002 in Barquisimeto, Venezuela. Blood samples were also collected from individuals without orofacial clefts (n=92) during the same missions as well. The drawn blood was blotted on filter paper and shipped to University of the Pacific School of Dentistry. DNA extraction was then performed followed by polymerase chain reaction. Genotypes for both A80G RFCI and C677T MTHFR were then established using the purified and amplified gene samples through polyacrylimide gel electrophoresis.

Results and Conclusion: There was a slight increase in the occurrence of the RFCI 80GG mutation (34% vs. 27%) as well as a slight increase in the MTHFR 677TT mutation (14% vs. 12%) in the CL+/-P individuals vs. control individuals. However, the differences were not statistically significant for either RFCI ($\chi^2$, p = 0.511) or MTHFR 677 ($\chi^2$, p = 0.665). Similarly, there were no significant differences between the cleft severity and the control population RFCI ($\chi^2$, p = 0.342) or MTHFR 677 ($\chi^2$, p = 0.779). The total alleles were then compared, with no significant differences between controls and affected groups RFCI ($\chi^2$, p = 0.269) or MTHFR 677 ($\chi^2$, p = 0.386). The Genotype combinations were then compared and no significant differences were found ($\chi^2$, p = 0.437). We found no statistically significant genotype differences between a group of CL+/-P patients and un-affected controls for both RFCI A80G and MTHFR C677T in Barquisimeto, Venezuela. The etiology of CL+/-P is a very complex interplay between both genetic and environmental factors. It appears that the mutations occurring at both RFCI A80G and MTHFR C677T have little effect on cleft lip +/- palate incidence in our population study. Further investigation is needed to determine the role of these factors in the etiology of orofacial clefts in the current population.
Orthodontists use different types of physical records to make treatment decisions and to assess treatment outcomes. These include study casts, lateral cephalometric x-rays, and facial photographs. Merging these records into an integrated 3-dimensional system would allow for a much better understanding of treatment progress and growth. Such efforts have been on-going for several years at the Craniofacial Research Instrumentation Laboratory (CRIL) at the Arthur A. Dugoni School of Dentistry. This investigation focused primarily on the quantification of the errors associated with merging a 3D study cast model into a frame of reference defined on a 2D lateral cephalometric head film.

Introduction:
Nonsyndromic cleft lip and palate (NCLP) is among the most common congenital anomalies. The etiology of NCLP is complex. Both genetic and environmental factors are involved. Several "candidate" genes (TGFA, TGFB3, MTHFR, RFC1, MSX1, IRF6, PVRL1) have been identified. Based on several recent studies, approximately 15-20% of NSCP are strongly determined by four genes and their combinations: MSX1, RFC1, IRF6 and TGFB3.

Objectives:
The goal of our case-control study was to find whether an association between a mutation of MSX1 and NCLP exists in the Guatemalan population.

Material and Methods:
The total of 37 individuals affected with NCLP and 24 unaffected individuals from the same location were analyzed for a variable number of CA tandem repeats in the intron region of the MSX1 gene. Diagnosis of cleft was determined by physical examination of each individual. DNA was isolated from dry blood spots on filter paper. Genotyping of the MSX1 gene in respect to four alleles consisting of CA-repeats was performed by means of PCR amplification and sequencing.

Results:
Altogether 61 specimens were analyzed and MSX1 CA repeats allele frequencies were compared in cases and controls. The most common genotype observed in both cases and controls was A4A4 - i.e. homozygotes for 9 CA repeats. However, there was more than 1.5 times more cases (71.3%) than controls (41.7%) with A4A4 genotype. This difference was statistically significant (p=0.05). The second most common genotype was A4A2 with almost the same proportion in cases (21.6%) and in controls (29.2%). When distribution of alleles A1, A2, A3, and A4 was analyzed for cases and controls, there was found a significant difference between cases and controls (p=0.045). The frequency of A4 allele was significantly higher in cases compared to controls (p=0.024).

Acknowledgment:
Rotaplast International, Inc., supported the fieldwork for this study. Processing and analysis of data was supported by the Department of Orthodontics.
THE RELIABILITY OF LANDMARK LOCATION ON A LATERAL CEPHALOMETRIC IMAGE EXTRACTED FROM A NEWTOM DATA SET COMPARED TO A CONVENTIONAL LATERAL CEPHALOMETRIC IMAGE

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Introduction: Currently, most practicing orthodontists use lateral cephalometric radiographs for locating boney and soft tissue landmarks on patients to aid in the diagnosis and treatment planning of cases. Recently, there have been developments in the area of craniofacial imaging. These developments have led to units like the Newtom CT scan (made by QR) that allow us to gain volumetric images of a patient’s craniofacial complex. The information provided by such a survey can prove to be very useful in gaining insight into the location of impacted teeth as well as the shape and location of the head of the condyle. Practitioners who are utilizing this new technology are usually still taking a traditional lateral cephalometric radiograph to obtain necessary diagnostic information as well which exposes the patient to more radiation.

Significance: By utilizing an extrapolated lateral cephalometric image from a Newtom data set, the need to take a traditional lateral cephalometric image for diagnostic and treatment planning needs could be eliminated. This would reduce the amount of radiation exposure to patients in cases where a volumetric scan (Newtom) is going to be ordered for diagnostic purposes.

Sample: The sample that was used in this study was taken from records that were gathered in a commercial dental radiology lab (C Dental) from patients that were having both a Newtom and a traditional cephalometric head film taken on the same day. The sample size was the records of 15 (fifteen) patients.
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The etiology of orofacial clefts is multifactorial, involving genetic and environmental factors. A low folate intake and disturbances of the folate metabolic pathway have been shown to play a critical role in the etiology of dysraphic anomalies such as neural tube defects, orofacial clefts, conotruncal heart defects, and other birth defects.

Our pilot study is focused on the comparison of plasma folate levels (PFL) between two groups of women in Cebu City, the Philippines—those who had a child affected with cleft lip and palate, and those who had a healthy child. Our sample consisted of 57 mothers of patients affected with OFC and 57 mothers of unaffected children.

The Immulite 1000 analyzer and test kits supplied by the manufacturer were used for quantitative measurements of folate levels in plasma. The normal range of folate concentration in human plasma is 3-17 µg/L.

The mean PFL level for mothers of cases was 3.44 µg/L (SD=1.6), while for control mothers the mean was 4.04 µg/L (SD=1.43). The difference between the mean plasma folate level of case mothers and control mothers was statistically highly significant (t-test; p<0.0001).

When our sample was analyzed according to PFL groups, there was a tendency for the higher frequencies of case mothers in the low PFL groups and for higher frequencies of control mothers in the high PFL groups. Chi square test for trend confirmed that there was a dose dependent effect ($X^2 = 5.1389; p=0.0234$).

The purpose of our last analysis was to evaluate the association of PFL with the risk for having a child with a cleft. Proportions of case and control mothers in each of four PFL quartiles were calculated. This analysis confirmed a dose dependent effect and suggested that the highest risk for having a child with a cleft was for mothers whose PFL was below 2.68 µg/L.
THE ASSOCIATIONS BETWEEN MMP-9, TIMP-1 AND PERIODONTITIS IN HIV+ PATIENTS

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Objectives: The study aim was to determine whether matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinases-1 (TIMP-1) in gingival crevicular fluid (GCF) could serve as prognostic factors for the progression of periodontitis in HIV+ patients.

Methods: Clinical measurements including gingival index (GI), plaque index, bleeding index, probing depth (PD), attachment loss (AL) and GCF samples were taken from 2 healthy sites (including sites with gingival recession, GI=0; PD<4mm; AL<3mm), 3 gingivitis sites (GI>0; PD<4mm; AL=0) and 3 periodontitis sites (GI>0; PD>4mm; AL>2mm) of each patient at baseline and 6-month visits by means of paper strips. GCF MMP-9 and TIMP-1 levels were determined by sandwich ELISA assays.

Results: The mean amounts of MMP-9 and TIMP-1 in diseased sites were significantly higher in gingivitis and periodontitis sites than in healthy sites (p<0.0001). A progressing site was defined as a site which had 2mm or more attachment loss during 6-month study period. GCF MMP-9 levels were highly correlated with probing depth, attachment loss, age, pack years, viral load values at baseline and 6-month visits (0.0001<p<0.001). TIMP-1 levels were only correlated with CD4, viral load and MMP-9 (0.001<p<0.01). Repeated measures analysis of 11 active sites versus 269 inactive sites indicated that MMP-9 and TIMP-1 levels were significantly higher in active sites than in inactive sites (p<0.0001).

Conclusion: These data indicate that sites with high GCF levels of MMP-9 in HIV+ patients are at significantly greater risk for progression of periodontitis.

QUANTITATIVE ANALYSIS OF PATIENT COMPLIANCE DURING EARLY MIXED DENTITION ORTHODONTIC TREATMENT

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This study has been conducted based upon statistical analysis of data gathered from treatment records of 32 randomly selected patients from Dr. Steve Dugoni’s Orthodontic practice. This is an initial attempt to investigate the incidence of patient compliance and non-compliance in early mixed dentition treatment as compared with their incidence in single stage treatment in early adolescence.

Data for three different treatment groups are being collected and analyzed. All subjects originally presented for orthodontic evaluation between the ages of 7.5 and 10 years. On the basis of predefined criteria, treatment for some subjects was deferred until the eruption of the permanent buccal dentition while treatment for other patients was commenced immediately. The early treatment group was further divided into two sub-groups — one for which the initial phase of treatment in the mixed dentition was sufficient and the other which required a second phase of intervention in the full permanent dentition.

Each patient’s full treatment record is being coded in digital form in order to identify and enumerate all instances of non-compliance with respect to oral hygiene, appliance wear, late or missed appointments and miscellaneous other instructions at each of six treatment stages. Preliminary data will be presented.
VITAL TOOTH BLEACHING

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Tooth whitening has been used since the 1800s to achieve a more desirable tooth color. In history the one event that initiated the era of cosmetic dentistry was in the late 1960s with the introduction of "night guard bleaching technique". The bleaching agent currently used, carbamide peroxide was discovered by an orthodontist, who prescribed it as an antiseptic for gingivitis. The bleaching process takes place through a chemical reaction where hydrogen peroxide radicals react with the unsaturated bonds of the organic portion of enamel that causes a breakdown of molecules to simpler molecules that reflect less light so tooth appears whiter.

When choosing a product for bleaching it is important to consider the type of stain, patient selection, and concentration of bleaching agent for a successful outcome.

Different approaches to tooth whitening have been recognized they are:

- Dental administered bleaching using a high concentration of hydrogen peroxide (35 to 50 %) or carbamide peroxide (35 to 40 %), supplemented with a heat source such as light or argon laser or CO2 laser. This procedure also called power bleaching or in-office bleaching. Factors to be considered for this procedure would be, surface cleanliness of the tooth to be bleached, concentration of the agent, temperature, pH and time.

- Dentist –supervised bleaching in which the dentists provides a customized tray, bleaching agent, and instructions to the patient. Tray fabricated from a vacuum formed splint. Concentration of the bleaching agent may be about 10% to 15% of carbamide peroxide or hydrogen peroxide.

Over the counter bleaching agents like whitening toothpaste, whitening strips (6%, 14% hydrogen peroxide), boil and bite trays are widely used by people and have shown variable results.

Effects of tooth bleaching, most common one is tooth sensitivity which generally persists for 3-4 days after bleaching. Another side effect of bleaching is mucosal irritation which is caused by higher concentration of hydrogen peroxide (30% to 35 %) that comes in contact with mucosal membrane. Alteration of enamel surface also seen may lead to surface roughness. Other effects shown by studies are soft tissue irritation, orthodontic tooth movements, occlusal problems, sore throat, laxative effects, seen with the bleaching procedure where trays are employed.

MONTE CARLO EXPLORATION OF LEARNING EFFECTS

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Objective: Develop methodologically rigorous methods for exploring the effects of learning and groups of learners in research on teaching. Although student differences are traditionally regarded as error variance in studies of teaching, they may represent various patterns of explanatory interaction with teaching effects.

Methods: Seven Monte Carlo simulations were constructed to explore such effects. In each case a uniform improvement in average performance across four trials and random variation was held constant (Model T + e). Six patterns of learning effects were explored, always preserving the uniform overall improvement in average score (Model T + L + e, where T and e remain constant). The modeled patterns included significant random interventions other than teaching, parallel subgroups without interactions, interactions among subgroups with the teaching effect, sequential all-or-none learning, learning as reduction in variation across trials, and interactions with "floor" and "ceiling" effects (measurement constraints).

Results: Four methods for comparing the effects of learning subgroups were calculated for each of the simulations: graphing, conventional statistical significance using repeated measures ANOVA, Cronbach's generalizability analysis showing the proportion of variance attributable to each source of variance, and process capability, Cpk, used in engineering as a measure of proportion of duds generated by a process. These models represent extreme pure types and the theoretical results obtained are functions of the assumptions of the models. They signal issues in statistical assumptions that differ by model type despite identical results for teaching. Examples are presented from the literature showing such learning effects on teaching research and of analogues in clinical trials research where repeated measures are taken.

Conclusions: Theoretical templates can be used to illustrating potential underlying and interacting effects of learning on research in teaching leading to deeper understanding of ways such factors might be identified, analyzed, and interpreted.
CONNECTING THE DOTS: PEG OSMOTIC PRESSURES FOLLOW UNIVERSAL SCALING LAWS

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Poly(ethylene glycol) is a polymer of much current interest in biophysics, medicine and therapeutics. It makes surfaces biocompatible, endows liposomes with stealth, and exerts nearly ideal osmotic forces on nanoparticles such as proteins, vesicles, or DNA. PEG-coated materials are used for medical implants. Stealth® liposomes are used for intravenous drug delivery. PEG-lipid micelles are used as vectors for intracellular delivery of plasmids and steroids. PEG osmolytes are used to apply controlled compressive forces on macromolecules and membranes.

It is not fully understood why poly(ethylene glycol) works so well in so many arenas. It is said to endow surfaces with the ability to "look like water." Much work has been done to establish and understand the physical properties of this unusual polymer. A unique feature of PEG is its repeating -(CH2-CH2-O)- chain which has a balance of both hydrophilic and hydrophobic character.

We ask the important question: Does PEG behave as a "proper" flexible polymer, following well-established laws of polymer physics, or not? Osmotic pressure is a colligative property of polymer solutions that yields insight into polymer dynamics. We have analyzed existing osmotic pressure data of PEGs to determine whether they adhere to polymer scaling theory. This has not been done before. In doing so, we have discovered a new scaling law for polymer osmotic pressures which is applicable to any flexible polymer.

Osmotic-pressure data were analyzed for 12 PEGs ranging from 300 to 20,000 Da at concentrations from 1.5 to 67.5 wt%. The experimenters fitted these data empirically with 3 parameters for each PEG, i.e. 36 parameters. Later, Cohen & Highsmith (Biophys J., 1997) fitted these data with virial expansions, placing the fits on a physical footing and reducing the number of parameters to 2 per PEG, i.e. 24 parameters. Now we show, by proper scaling, that all the data can be fitted with only one parameter.

By first normalizing all the polymer concentrations to c*, a physically-important quantity known as the overlap concentration (where the polymers are concentrated enough to begin to interact with each other), then requiring proper limiting forms of osmotic pressure when cH c* (van't Hoff's law) and when cH c* (de Château behavior), we derive a scaling law which predicts a plot of IN95 vs c/e* should collapse all the data onto a single curve. It works.

We have shown that PEG behaves as a "proper" flexible polymer over a wide range of sizes and concentrations. The single-parameter fit is powerful. It yields much physical insight into PEG behavior and permits useful calculations of PEG osmotic pressures for osmotic stress and membrane brush experiments (Presented at the Biophysical Society, February 2005).

AGE OF MOTHERS AND THE RISK OF CLEFT LIP AND PALATE

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Several epidemiological studies suggested that the parental age at the time of conception (ascertained as parental age at child's birth) plays an important role in the etiology of congenital anomalies and some other diseases. The goal of our study was to explore the maternal age as a possible risk factor for nonsyndromic cleft lip and palate (NCLP) in Karaikal, India.

Materials and Methods: The study is based on the clinical sample of 35 females and 42 males affected with NCLP obtained during Rotaplast medical mission to Karaikal, India, in 2003. The sample of controls consisted of 46 individuals (26 males and 20 females) from the same area. A detailed interview was conducted with each mother and/or with an adult patient. Descriptive statistics was used for calculation of the mean and standard error (SE) values. The t-test was used for analysis of differences between samples of cases and controls.

Results: Isolated cleft lip (CL) occurred in 30.8 % and isolated cleft palate (CP) in 10.2 %. The majority of the patients (59.0 %) were affected with cleft lip and palate (CLP). In the majority of cases, the defect occurred on one side only (77.1 %). Much higher proportion of bilateral than unilateral cases was seen in CLP compared to CL, and the left side was significantly more often affected with cleft lip and/or palate (CLP: 64.8%) compared to the right side.

Sex Ratio: In population based studies, males are more often found affected with CL/P than females, and females are more often found affected with CP than males. Also in our Karaikal sample, more males were affected with CL and CLP (males to females ratio = 1.4 and 1.3, respectively).

Age of Mother: The mean maternal age at birth of a child with an orofacial cleft was 23.47 years (SE=0.54). The youngest mother was 15 years old and the oldest 39 years old. The mean maternal age at birth of a control child was 24.22 years (SE=0.78) with the youngest mother 14 years old and the oldest one 35 years old. There was a significant difference in the mean maternal age between cases and controls (p= 0.029). The majority of our case mothers fell into age categories younger than 22 years: when compared with control mothers, the curve of age group distribution was shifted to the left. The data also suggested a significant relationship between young maternal age and unilateral CL/P (p= 0.029), when compared to controls.

Conclusions: Maternal age is a known factor in etiology of orofacial anomalies such as CL/P. We found a significant difference in mothers' age distributions between cases and controls in our Karaikal sample. This difference was especially seen in a subgroup of mothers of children with unilateral CL/P.

Acknowledgements: The field work for this study was supported by funding from ROTAPLAST International, Inc. Processing and analysis of the data were supported by the Department of Orthodontics, UOP Arthur A. Dugoni School of Dentistry, San Francisco, CA.
GLASS Ionomers

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This is a literature review of Glass Ionomers. A number of thirty-nine articles were reviewed. The study has four chapters and it was confined to the following aspects:

1. Composition and characteristics of Glass Ionomers, Resin Modified Glass Ionomers and composites. A comparison of these different types of Glass Ionomers was outlined in this study.

2. Indications and limitations of Glass Ionomers are presented for both permanent and primary dentition.

3. Microleakage presents factors and methods to prevent microleakage with the use of Glass Ionomers.

4. Factors that can affect Glass Ionomer restorations longevity.

DIRECT RESTORATIVE MATERIALS: IMPACT OF SOCIETAL FACTORS ON USE

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There are many societal pressures and government regulations impacting the use of direct restorative materials. Societal pressures come from the media, patients, dentists, dental staff, and other people. In many areas there are regulations from regional agencies and the state and federal government. Some of these regulations include:

- Required manufacturer Class II labeling of mercury and amalgam products due to potential allergic reactions
- Required office signs of potential hazards of dental materials
- Required informing patients on the available restorative materials
- Requirements on mercury and amalgam waste
- OSHA workplace requirements on mercury vapor
- Government agency suggestions on certain fish consumption in certain areas
- Government agency suggestions on certain fish consumption for at risk people

Previously presented at the 2005 ADEA Operative Dentistry & Biomaterials Section Program.
INHIBITION OF PROLONGED BITTER TASTE: PARTITIONING OF BITTER TASTANTS INTO LIPOSOMES AND INHIBITION OF THEIR UPTAKE BY CULTURED CELLS

Diana Flasher 1, Shlomo Nir 2, Michal Naim 3 and Nejat Ditzglinetz 1

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Innate rejection of bitter taste by mammals is a phylogenetically developed mechanism for survival. Today, many bitter foods, such as flavonoids and terpenes, are not only safe for consumption, but diets rich in these constituents have been linked to lower rates of cancer and coronary heart disease. Bitterness, however, reduces the acceptance of certain foods. Bitterness is often produced during food processing, such as the production of limonin during juice extraction, and tomatine and naringin in tomato purées. Attempts to mask bitterness usually involve additives like sugar. Stimulation of taste tissue by certain bitter tastants, including limonin, naringin and the casein-derived cyclo(Leu-Trp), induce a lingering “second messenger” release in cells, resulting in the bitter aftertaste sensation.

Amphiphilic bitter tastants permeate rapidly into taste cells through the apical taste-bud pores and stimulate multiple receptors and signal pathways of bitter taste. We explored the ability of liposomes of different phospholipid composition to scavenge bitter tastants in an effort to prevent their access to bitter taste receptors. We investigated the capacity of liposomes to prevent the entry of bitter tastant molecules into cultured HSC-3 oral epithelial cells used as models of taste bud cells. Tastant partitioning into liposomes or cells was evaluated by fluorescence spectroscopy. For the bitter tastant quinine, the order of efficacy for partitioning into liposomes was PC:PS MLV > PC:PA LUV > BMI-40 > PC:PA MLV > PC:PI LUV (PC, phosphatidylcholine; PS, phosphatidylserine, PA, phosphatidic acid, MLV, multilamellar vesicle; LUV, large unilamellar vesicle; BMI-40, a proprietary compound composed of 50% branched dextrin, 40% PA and 10% other lipids). The sequence of effectiveness in preventing uptake into cultured cells was PC:PS LUV > PC:PA MLV > PC:PE LUV > BMI-40. We examined the uptake of the casein-derived cyclo(Leu-Trp) in a similar manner, and found the sequence of effectiveness: PC:PI LUV > BMI-40 > PC:PG MLV > PC:PA MLV = PC:PS LUV > PC:PE LUV > PC:PE MLV (PG, phosphatidyl-glycerol). Thus, liposome compositions can be found that are more effective than BMI-40 in inhibiting the uptake of bitter tastants by cells. Our results suggest that liposomes of particular composition can inhibit the uptake of specific bitter tastant molecules by taste receptor cells and prevent or minimize the sensation of prolonged bitter taste.

This work was supported by a grant (IS-3366-02C) from the United States-Israel Binational Agricultural Research and Development Fund.

TRAMADOL (ULTRAM), OPIOID WITH MONAMINERGIC INTERACTION, INDUCES PAIN RELIEF, INCREASES FAST EEG-POWER SPECTRA AND IMPROVES DISABILITY IN COGNITIVE FUNCTION IN ELDERLY PATIENTS WITH CHRONIC OSTEOARTHRITIS (OA)

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The opioid tramadol was able to reduce pain in elderly patients with chronic osteoarthritis. It also restored cognitive dysfunction as demonstrated in items of SCAG and increased vigilance as demonstrated in the EEG-power spectra. It therefore can be regarded as an analgesic, which has increased potentials in the elderly demential patient with reduced vigilance. Such beneficial effects very likely are due to the monoaminergic reuptake properties rather than those of an opioid-related effect. As the need for health systems for the aging patients continuously grows, it is an important obligation to provide effective yet safe pain control to those patients.
EFFECT OF 17ß-ESTRADIOL ON LPS-INDUCED ALTERATION OF CALCIUM HOMEOSTASIS IN HUMAN ENDOTHELIAL CELLS

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Our preliminary studies showed the atheroprotective effect of 17ß-estradiol (E2) in decreasing the lipopolysaccharide (LPS) mediated expression of adhesion molecules (AMs). Intracellular Ca²⁺ concentration ([Ca²⁺]i) may play a role in the enhancement of cytokine induced AMs expression. In the current study, the regulatory role of [Ca²⁺]i was investigated in the LPS (100 ng, 18 hr) stimulated human endothelial cells treated with E2. [Ca²⁺]i in EA.hy926 cells was measured by fluorescence spectrophotometry. In the presence of extracellular Ca²⁺, inhibition of the endoplasmic reticulum (ER) Ca²⁺-ATPase with thapsigargin (TG, 1 µM) caused a biphasic increase in [Ca²⁺]i in LPS stimulated and control cells. However, the extent of TG-induced initial [Ca²⁺]i increase was significantly higher in LPS stimulated cells than its control counterpart. Treatment of LPS stimulated cells with E2 (1 µmol/L, 48 hr), returned the Ca²⁺ response to the same level as it was in control cells. Removal of extracellular Ca²⁺ pool in LPS stimulated cells. Moreover, our results suggest that E2-regulated Ca²⁺ homeostasis may play a role in controlling the enhancement of LPS induced AMs involved in atherogenesis.

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EFFECTS OF 17ß-ESTRADIOL ON LPS-INDUCED ADHESION MOLECULE mRNA EXPRESSION IN HUMAN ENDOTHELIAL CELLS

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An early event in atherogenesis is the adhesion of monocytes to endothelium via adhesion molecules. The aim of this study was to investigate the atheroprotective effect of 17ß-Estradiol (E2) in decreasing the lipopolysaccharide (LPS) mediated expression of adhesion molecules and the potential roles of estrogen receptors in human endothelial cells (EA.hy926). EA.hy926 cells were pretreated with E2 (1 µmol/L) for 48 hours before stimulation with LPS (100 ng/mL) for 18 hours. The mRNA expression of intracellular adhesion molecule-l (ICAM-1) and platelet cell adhesion molecule (PCAM) were measured by real-time PCR. RT-PCR analysis revealed a significant increase in the amount of mRNA for ICAM-1 and PCAM in LPS-stimulated EA.hy926 cells. Furthermore, treatment of these cells with E2 (1 µmol/L) for 48 hours, significantly reduced LPS-induced ICAM-1 and PCAM mRNA expression (by 50%) as compared to their LPS-stimulated counterpart. This action of E2 was abrogated by estrogen receptor antagonist ICI 182,780 (10 µmol/L) demonstrating an estrogen receptor mediated effect. Our results indicate that antiatherogenic effect of estrogen on the vascular wall may, in part, be due to the downregulation of ICAM-1 and PCAM expression at the transcriptional level.

Supported by a grant from the NHLBI.
EFFICIENT, SERUM-RESISTANT TRANSFECTION OF MURINE SQUAMOUS CELL CARCINOMA CELLS BY METAFFECTENE AND GENEJAMMER: APPLICATION TO HSV-TK/GANCICLOVIR GENE THERAPY

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Current treatments of head and neck squamous cell carcinoma (HNSCC) are largely unsatisfactory, and the five-year survival rate has not improved over the last two decades. The genetic approach to the treatment of HNSCC is based on the hypothesis that expression of therapeutic genes in target cells will cause a cytotoxic effect or mediate apoptosis. Oral cancer is a particularly appropriate target for gene therapy, since direct injection of genes into most primary and recurrent lesions is possible. Although generally efficient in transducing cells, viral vectors suffer from problems of immunogenicity, toxicity; limits in the size of exogenous DNA, and the risk of inducing tumorigenic mutations and generating active viral particles through recombination. Synthetic cationic liposome-DNA (lipoplexes) or cationic polymer-DNA complexes (polyplexes) constitute a promising alternative to the use of viral vectors and provide a simple means of transferring DNA into target cells. The inhibitory effect of serum on transfection mediated by non-viral vectors represents a serious limitation for their use in vivo. The presence of serum proteins, however, is unavoidable in vivo, and is advantageous in vitro because it increases cell viability and reduces transfection-associated cytotoxicity. In this study, we examined the effect of high concentrations of fetal bovine serum (FBS) on the delivery of luciferase, B-galactosidase and Herpes Simplex Virus thymidine kinase (HSV-β) gene to murine squamous cell carcinoma cells, SCC-7, by the polycationic liposome, Metafectene, and the polyamine reagent, GeneJammer. Transfection was optimized using a luciferase-expressing plasmid. The level of gene expression in cell lysates was evaluated by measuring enzyme activity after a 48-h incubation. The optimal ratios for delivering the luciferase gene were 2 μl Metafectene:1 μg DNA and 3 μl GeneJammer:0.5 μg DNA. When transfection was performed in the presence of 20-60% FBS, both Metafectene- and GeneJammer-mediated luciferase expression were reduced only by about 20%. SCC-7 cells were also transfected with the B-galactosidase expressing plasmid in the absence or presence of 60% FBS. Approximately 60-70% of the cells were positive for β-gal staining. The expression of B-galactosidase was essentially not affected by serum. Cells transfected with the HSV-β plasmid were incubated in the absence or the presence of ganciclovir (GCV; 20 μg/ml) for the indicated periods of time. The Alamar Blue assay was used to determine GCV-mediated cytotoxicity. Mock-transfected cells served as controls. The delivery of the HSV-β gene by Metafectene in the absence and presence of 60% FBS, followed by GCV treatment for 3 days, resulted in 68% and 36% cytotoxicity, respectively. With GeneJammer, transfection in 0% and 60% FBS resulted in 60% cytotoxicity. After 6 days, 90-100% cytotoxicity was observed in the presence of 0% or 60% FBS. Our observations suggest that both GeneJammer and Metafectene may be useful for the gene therapy of OSCC in animal models.

THE ROLE OF REF1 A80G MUTATION IN ETIOLOGY OF CLEFT LIP AND PALATE ANOMALIES IN VALDIVIA, CHILE

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Introduction The reduced folate carrier 1 (RFC1) protein is involved in transportation of folate across the cell surface membrane. The RFC1 gene has been recently suggested as a candidate gene that may play an important role in etiology of cleft lip with or without cleft palate anomalies. Previous research done by Dr. Cory Costanzo (2004) showed a strong role of the polymorphism at nucleotide 80 (A80G) of this gene in etiology of nonsyndromic cleft lip and palate anomalies (NCLP) in Guatemala.

Objectives The purpose of this study was to find, if RFC1 A80C> G mutation is involved in etiology of NCLP also in Valdivia, Chile, as suggested by Costanzo’s findings in Guatemala population.

Methods We investigated a sample of individuals affected with NCLP (n=66) and a sample of unaffected individuals (n=21) from the same location. Cases and controls were identified through Rotaplast medical mission in Valdivia, Chile, in 2000. Diagnosis of cleft was determined by physical examination. DNA was isolated from dry blood spots on filter paper by the Chelex method. RFC1 A80G genotypes were established by PCR amplification followed by detection of the single nucleotide conformational polymorphism using polyacrylamide gel electrophoresis.

Results In cases, 13.64% of individuals had A80/A80 genotype, 45.45% had G80/G80 genotype, and 40.91% were heterozygotes (A80/G80). Proportions of genotypes in controls were 23.81% A80/A80, 42.86% G80/G80, and 33.33% A80/G80. The A allele frequency was 0.341 for cases and 0.405 for controls, while the G allele frequency was 0.660 for cases and 0.595 for controls. When compared with Costanzo’s study of Guatemala cleft population (G allele frequency 0.573), the G allele frequency in cases of the Valdivia study was even higher.

Conclusion Results of this study suggest that the G allele in nucleotide 80 of the RFC1 gene also contributes to the etiology of NCLP in Valdivia, Chile. Additional studies are in progress, especially genotyping of a larger group of controls.

Acknowledgements The field work for this study was supported by funding from ROTAPLAST Intl., Inc. Processing and analysis of the data and specimens were supported by the Department of Orthodontics, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA.
DNA-BINDING PROPERTIES OF TWO NICKEL COMPLEXES


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Metals play many roles in living organisms; some metals are beneficial such as enzyme cofactors or cellular signals, some are toxic, and some metal complexes such as cisplatin are used for therapeutic purposes. Nickel does not appear to be very toxic although there have been some reports claiming that it is carcinogenic. We are in the process of investigating two complexes, NiCR and Ni(CR-2H) that contain Ni^{2+} and a nitrogen-containing macrocyclic ligand. NiCR is synthesized according to published protocols and Ni(CR-2H) can be obtained by oxidation of NiCR. At first, it was hypothesized that these compounds differ in their ability to interact with DNA. Thus, intercalation into DNA was studied using fluorescent spectroscopy. The results obtained in the ethidium displacement assay were almost identical for NiCR and Ni(CR-2H). This was consistent with the almost identical structures obtained by molecular modeling. However, viability studies on human breast cancer cells showed significant differences: Ni(CR-2H) is much more toxic than NiCR. Thus, the ability of both compounds to induce DNA strand breaks was investigated using the "comet" or alkaline single cell electrophoresis assay. Again, Ni(CR-2H) caused more damage than NiCR leading to the hypothesis that although both compounds intercalate into DNA, only Ni(CR-2H) is reactive enough to either bind to or oxidatively damage DNA. This was further investigated by reacting both compounds with DNA in vitro and then treating the DNA with piperidine. This assay showed that Ni(CR-2H) degraded DNA in a dose-dependent fashion. The exact type of DNA damage caused by Ni(CR-2H) is currently under investigation. Techniques used include \textsuperscript{32}P postlabeling of DNA treated in vitro and studies on treated oligonucleotides. Mass spectrometry will be used for identification if individual products (modified nucleotides) can be retrieved in sufficient quantities. A better understanding of the mechanism(s) by which Ni(CR-2H) but not NiCR damages DNA may lead to development of Ni(CR-2H) for therapeutic purposes or as a research tool.

LASER DENTISTRY: TOMORROW’S DENTISTRY...TODAY

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Forty articles were reviewed by the authors considering the history and development, indications and contraindications, and advantages and disadvantages of lasers in dentistry. The ruby laser was the first laser developed in 1960 and the CO2 laser was the first laser to be used for intraoral use. The most commonly used lasers in clinical dentistry are the CO2 lasers, Nd: YAG, and Argon lasers.

Lasers are used for hard and soft tissue conditions. The various uses of lasers include treatment of gingival hyperplasia, gingivectomies, fibromas, periodontal pocket reduction, calculus removal, bleaching, caries detection (DIAGNOdent), caries removal, and curing composites. Contraindications for lasers include removal of metallic restorations—amalgam, porcelain and gold. Amalgam is contraindicated due to mercury vapors.

Lasers have several advantages over conventional methods for treating soft and hard tissues conditions. Soft tissue laser advantages include hemostasis, elimination or reduction of scar formation, sterilizing, reduced need for anesthesia, and reduction of post-operative pain. Hard tissue laser advantages include diagnosis of early pit and fissure caries, treating dentinal hypersensitivity, and assessing the vitality of human pulp tissue.

The disadvantages of soft tissue lasers include prolonged healing time, thermal damage to surrounding healthy tissue and they are not as effective as a scalpel when making full thickness flaps. Hard tissue laser disadvantages include the fact that they are not as effective as hand instruments in removing calculus and they are not as effective as NaOCl (sodium hypochlorite) in disinfecting root canals. Lasers if used carelessly may jeopardize pulp vitality. Safety is one of the most important aspects of lasers dentistry which includes a properly trained staff as well as eye protection for the operator, assistants, and patient.
DEVELOPMENT OF A HIGH THROUGHPUT REPORTER SYSTEM USING BETA-GALACTOSIDASE AND THE YEAST: PICHIA PASTORIS

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Pichia pastoris is a methylotrophic yeast gaining notoriety for its capabilities in heterologous protein expression. In contrast to other host organisms such as bacteria or mammalian cells, P. pastoris offers the advantages of being cost-effective, able to grow to high cell densities, and able to perform post-translational protein modifications. Here we report the development of a high throughput reporter system. By cloning a plasmid construct containing beta-galactosidase into P. pastoris, we were able to select for transformants using Zeocin as our antibiotic resistance marker and X-GAL to conduct a blue/white screen. Western blot analyses confirm the expression of beta-galactosidase and a colorimetric liquid assays further confirm enzymatic activity of the reporter protein. Our developments allow for the high throughput detection of potential super secreting mutant yeast strains.

DRUG DISCOVERY AND RESEARCH IN NEUROPHARMACOLOGY

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Our laboratory utilizes electrophysiological and molecular biological techniques to investigate drug effects on nerve cell receptors – the proteins that sub-serve communication between nerves and are also the target of many therapeutic and recreational drugs. The major aim of the lab is to contribute to an understanding of how drugs modulate receptors and alter brain function and secondarily to identify novel targets for the development of new medicines. Two unique features of the lab include a cell culture work station, which allows us to prepare the cells that are used in our experiments and two (patch-clamp) electrophysiological workstations that allow us to record small voltage and current changes across cell membranes in response to the opening of ion channels. Currently, our laboratory is involved in several projects including work with stem cells and anti-inflammatory drugs. The primary author is involved in all aspects of research including maintaining the cell cultures, preparation of DNA, electrophysiological recordings and most recently, developing a Xenopus laevis oocyte recording station. This project has been made possible through the School of Engineering and Computer Science Cooperative Education Program and funding from the National Institute of Health.
USE OF SALIVA FOR MOLECULAR GENETIC STUDIES

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Introduction: A modern approach to diagnosis and treatment planning for numerous conditions in dentistry will take more and more advantage from application of human molecular genetic diagnostic techniques. Not only in craniofacial anomalies, such as cleft lip and palate or craniosynostosis syndromes (for which polymorphisms of candidate genes have been of great interest recently), but also in more common conditions in dentistry, such as hypodontia or root resorption, a genetic part of etiology can be ascertained. There is still a certain resistance of dentists to collaborate on research studies involving DNA analysis. In order to help our students and faculty to overcome difficulties in obtaining specimens from patients, we conducted a study that compared the yields of DNA from saliva obtained by three types of sample collection and two types of DNA isolation techniques.

Material and Methods: In our study, 85 saliva specimens were collected from 36 healthy volunteers. Each individual was asked to provide saliva into a Falcon tube (S1) and into an Oragen container (S3). Approximately 2 ml of saliva were collected in each. Then, 50 µL of saliva from the Falcon tube was used to make a single large spot on a piece of filter paper and allowed to dry (S2). DNA from Falcon tube (S1) and from the filter paper (S2) was isolated using the Sigma REDExtract-N-Amp Tissue PCR kit. The Oragen protocol for DNA isolation was used for S3 specimens. From majority of individuals (N=20), all three kinds of specimens (S1, S2, S3) were collected. From 9 individuals, two specimens were collected and only one saliva specimen was obtained from 7 individuals. The concentration of DNA extracted from each type of a saliva sample (S1, S2 and S3) was measured using Nanodrop spectrophotometer. Each specimen was measured at least two times. When values differed by more than 10%, the third measurement was done. Average value of two closest measurements was calculated and used for calculation of a yield of DNA obtained by different isolation techniques.

Results: The highest yield of DNA was obtained from S2 specimens (N=21), i.e. dry saliva spots on filter paper (mean value 89.76 µg, SE 3.41). The average yield from S1 specimens - liquid saliva in Falcon tube - was 65.74 µg, SE=15.97). The lowest yield of DNA was obtained by using the Oragen saliva kit (mean value 7.52 µg (SE=0.88). The differences in yields of DNA between S2 and S3 specimens and between S1 and S3 specimens were statistically significant (p<0.0001 for both).

Conclusions: Our study demonstrated that saliva, which can be obtained quite easily from patients of any age, is a very good source of DNA. All three types of specimens that were used in our study have their advantages and disadvantages that are discussed in our presentation.

Acknowledgements: Department of Orthodontics, University of the Pacific Arthur A. Dugoni School of Dentistry provided funding and personnel support for this study.

DNA ISOLATION: THE FIRST AND THE MOST IMPORTANT STEP IN A GENETIC ANALYSIS

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Introduction: DNA diagnostic methods have been proven to be useful for criminal and natural disaster forensics, determining paternity, diagnosing existing disease, as well as many other uses. Studies of human DNA are uniquely sensitive to a contamination due to a continual presence of potential contamination sources like skin cells, hair and saliva. Thus, specific protocols have been developed to ensure quality sample collection and accurate DNA isolation.

Collection of Specimens and their Storage: Specimens used for this study were collected in Santiago del Estero, Argentina, during Rotaplast medical mission in 1999. Venous blood was drawn and 6-9 drops of blood (with EDTA) were put on a 1" x 2" piece of filter paper. The filter papers were let dry, stored individually in coin paper envelopes, and shipped to USA. During the whole process of preparing specimens on site, gloves, facial masks, head caps, and gowns were worn in order to prevent contamination with foreign DNA from people working with the specimens. The specimens used in this study were stored in ordinary cabinets at room temperature for 6 years before DNA was isolated.

Isolation of the DNA in out Laboratory: The protocol for isolation of DNA from blood spots has been derived from the technique described by Polski, et al (1998). The technique includes a use of a resin, Chelex-100, which captures bivalent cations. To determine concentration of isolated DNA (ng/µL), Nanodrop spectrophotometer was used. Throughout the DNA isolation procedure gloves, facial masks, head caps and gowns were worn and the Craniofacial Genetics Laboratory procedures for DNA isolation were followed.

Results and Conclusion: Typical yield of DNA isolated from 1cm x 1cm piece of filter paper with a dried blood spot was between 100 and 300 µg DNA/mL water. The mean value of extracted DNA from 53 specimens was 221.09 µg/mL (SE = 21.44; 95% Confidence level = 43.03) with the smallest value 37.28 µg/mL and the highest value 847.30 µg/mL. Median value was 185.20 µg/mL (Table 1, Chart 1).

This study demonstrates that dry blood spots on filter paper are very useful for studies, in which future DNA analyses are expected. The preparation and storage are simple and the yield of DNA, even after many years of storage at room temperature, is sufficient for testing polymorphisms of several genes.

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PORCELAIN VENEERS

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Overall Clinical Performance: Success/Failures: The failure rate increased when the finish line was on existing filling or when partially bonded to dentin. Occlusion played a major role in most failures. Major shortcomings of the porcelain veneers system were relatively large marginal discrepancy and an insufficient wear resistance of the luting composite.

Technical Considerations: Materials and Devices: Different laboratory techniques are available for making porcelain veneers, and each has its own advantages and limitations. Computer Assisted Design/Computer Assisted Manufacture (CAD/CAM) CEREC technology shows that this system can be successfully used for making porcelain veneers.

Consequences of Inadequate Tooth Reduction: The consequences of inappropriate tooth reduction for veneers are dentin exposure, periodontal/esthetic complications and technical difficulty in placing margins of veneers without adequate tooth preparation. Studies have demonstrated that the 'window' type of preparation was the strongest. It also confirmed that the strength of the veneer was not proportional to its thickness.

Report of a Case: Treatment of a Severe Amelogenesis Imperfecta Patient: Metal ceramic fixed partial dentures were placed on posterior teeth to modify the occlusion, and porcelain laminate veneers were placed to improve the esthetics of the maxillary anterior teeth. Clinical examination twelve months after treatment revealed no evidence of disorders associated with the restored teeth or their supporting structures.

HUMAN EMBRYONAL CARCINOMA STEM CELLS EXPRESSING GREEN FLUORESCENT PROTEIN FORM FUNCTIONAL NEURONS IN VITRO: A RESEARCH TOOL FOR CO-CULTURE STUDIES

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Neural differentiation is controlled by complex molecular mechanisms that determine cell fate and diversity within the nervous system. Interactions between developing tissues play an important role in regulating this process. In vitro co-culture experiments offer a method to study cell differentiation and function under controlled conditions, with the additional benefit of investigating how interactions between populations of cells influence cell growth and behavior. However, it can often be difficult to distinguish between populations of co-cultured cells. Here we report the development of a human embryonal carcinoma (EC) stem cell line (named TERA2.cl.SP12-GFP) that expresses the genetic marker, green fluorescent protein (GFP). We demonstrate that TERA2.cl.SP12-GFP stem cells stably express GFP and that this remains detectable during retinoic acid-induced differentiation. Regulated expression of neural markers during cell development correlated with the formation of morphologically identifiable neurons. Populations of post-mitotic GFP-positive neurons were readily purified and electrophysiologic characterization confirmed that such neurons were functionally active. Thus, cultured TERA2.cl.SP12-GFP cells can be readily distinguished from alternative cell types in vitro and provide an amenable system for live cell imaging to study the development and function of human neurons in isolation, and in co-culture with other tissue types.
ABSTRACTS

ADA/DENTSPLY STUDENT RESEARCH COMPETITION

3 – 5:30 PM CLINICS
DID I GET IT FROM MOM?
SEARCHING FOR MATERNAL CONTRIBUTION TO GENETIC SUSCEPTIBILITY FOR CLEFT LIP AND PALATE THROUGH RFC1 GENE

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Approximately 4000 children are born with orofacial clefts in the Philippines every year, putting cleft lip and/or palate (CL/P) among the top 12 birth defects in the country. The etiology of nonsyndromic cleft lip and palate anomalies (NCLP) has been attributed to both genetic and environmental factors. Studies have shown that folate is essential for cell reproduction and maintenance, and that deficiencies of folate are linked to congenital defects such as anencephaly, spina bifida, and NCLP. However, birth defects may still occur even with normal intake of folate during pregnancy.

Objective: To answer the question how mutations of the reduced folate carrier-1 gene (RFC1) at nucleotide 80 in mothers contribute to genetic susceptibility for NCLP in their children.

Material and Methods: Our study population came from the island province of Cebu, Philippines. Cases (patients affected with NCLP), their mothers (case mothers) and controls (individuals from the same location without a congenital anomaly) were identified during Rotaplast medical mission in Cebu City, Philippines, in 2003. Descriptive epidemiology of this sample was presented previously (Pawar et al, 2004). In the present study, we ascertained distribution of RFC1 A80G genotypes in 80 mothers of children with NCLP (case mothers). We compared it with genotype distribution in 75 of their children (cases) and in 69 controls. DNA was isolated from dry blood spots on filter paper by the Chelex method. RFC1 A80G genotypes were established by PCR amplification followed by detection of the single nucleotide conformational polymorphism using polyacrylamide gel electrophoresis.

Results: The distribution of genotypes in case mothers was: AA - 27.5%, AG - 38.75%, and GG - 33.75%. In corresponding allele frequencies, G allele slightly prevailed (A-46.88%, G-53.13%). The distribution of genotypes in cases showed higher proportion of heterozygotes (AA -17.3%, AG -56%, GG -26.7%) than in mothers and this difference was statistically significant ($\chi^2=3.95$, $p=0.046$). Frequencies of A and G alleles were very similar (A-45.33%, G-54.67%), with no statistically significant difference. When case mothers were compared with controls, there was no statistical difference found neither for genotype distribution (AA -31.9%, AG -44.9%, GG -23.2%) nor for allele proportions (A-54.35%, G-45.65%).

Conclusions: Our study showed the highest proportion of homozygotes with mutation (GG) in case mothers. Cases, individuals affected with NCLP, had the highest proportion of heterozygotes. As proportions of A and G alleles remained similar, we can speculate that alleles A and G contributed by fathers decreased frequencies of maternal homozygotes GG and AA, respectively. The final answer to this question will be obtained from a study of mother-father-child trio that is in

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GENDER DIFFERENCES IN RAT SMG BLOOD FLOW AND SECRETION

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Objectives: Parasympathetic stimulation elicits a copious secretion of fluid from the rat SMG and an accompanying increase in glandular blood flow. Previous studies in male rats have shown that endothelium-derived relaxing factors, such as NO, prostaglandins (PG) and endothelium-derived hyperpolarizing factor (EDHF), are involved in parasympathetic vasodilatation. Gender differences in the regulation of vascular tone are well documented, and there is evidence in humans suggesting that gender affects salivary flow rate. However, few data are available on the effects of gender with respect to either salivary gland blood flow or secretion in experimental animals.

Methods: Male (n=31) and female (n=31) rats were anesthetized and parasympathetic stimulation was delivered via the chorda-lingual nerve (2, 5 and 10 Hz (5-6 V, 2 ms). Laser-Doppler flowmetry was used to measure blood flow (perfusion units) in the presence or absence of inhibitors of NO synthase (L-NAME) and PG synthesis (indomethacin). Blood pressure was also monitored. All data were collected and analyzed using PowerLab software.

Results: Basal perfusion was higher in females than in males (10,984 ± 1,771 and 8,462 ± 2,653, p < 0.01), but it was unaffected by either L-NAME or indomethacin. L-NAME and indomethacin both inhibited parasympathetic vasodilation. However, their specific effects depended on both gender and order of administration, i.e. whether L-NAME or indomethacin was first. Further, the data suggest that EDHF plays a greater role in females than male. With respect to salivary flow, the total volume of saliva collected from females was less than that from males. Nonetheless, when normalized to gland weight no gender differences in flow rates were observed were observed. Finally, L-NAME had no effect on flow rate, whereas indomethacin reduced salivary flow (p<0.05).

Conclusions: The relative contributions of NO, PG and EDHF to parasympathetic vasodilation in the rat SMG are gender dependent. In contrast, salivary flow is not influenced by gender, and PG play a role in parasympathetic salivary flow.

INFECTION OF ORAL EPITHELIAL CELLS BY PORPHYROMONAS GINGIVALIS: A DNA MICROARRAY ANALYSIS OF HOST CELL GENE expression

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Periodontal disease affects many millions of individuals in the United States, one estimate being about 36 million. While there is no specific causative agent of periodontal disease, Porphyromonas gingivalis is known to be one of the main pathogens involved. While many studies have defined various virulence factors expressed by P. gingivalis there have been few studies on the oral epithelial cells’ response to infection by this microorganism. We examined the infection of KB oral epithelial cells with P. gingivalis (strain 332177) using bacteria labelled with a vital fluorescent probe (Cyto5). Cells were infected at a bacterium/cell ratio of 10, incubated for 4 hours, fixed in parafomaldehyde and examined by confocal fluorescence microscopy. The results indicated extensive infection over this time period. A 24 hour incubation resulted in extensive cytotoxicity. To examine the gene expression profile of KB cells upon infection, in parallel experiments, RNA was extracted from the KB cells using an RNeasy kit from Qiagen. The RNA will be converted to complementary DNA and labeled with the fluorescent probes Cy3 and Cy5. The labeled cDNA will be analyzed using DNA microarrays containing hybridization spots for 17,000 human genes. Based on previous microarray analyses with macrophage-like cells, and histochemical analysis of KB cells, we expect to see an increase in certain clusters of related genes. These include genes controlling the expression of extracellular communication proteins (e.g. hepatocyte growth factor, fibroblast growth factor, transforming growth factor-beta 1, interleukin 1 beta, interleukin 6 and interleukin 8) and cell receptors (e.g. interferon receptor 2, interleukin 8 receptor alpha, and interleukin 2 receptor). An increase in certain groups of genes, for example, cytokines or receptor signaling proteins, will allow us to better understand the inflammatory response of oral epithelial cells to infection with oral pathogens.

This work was supported by Research Funds (038) from the Arthur A. Dugoni School of Dentistry.
models have been shown to displace each other in discriminatory fashion, and more importantly, utility of this assay.

peptide’s binding sites. The success with competitive inhibition shows promise for the practical functions as designed and may very soon be ready for limited screening. The various C-peptide molecules that can displace the one model gp41 peptide from the other in a laboratory setting with results that can be duplicated in vivo. Drugs of this class are already in circulation particularly N- ( env-) peptide that we anticipate is sufficiently reliable to create an assay designed to DRUG SCREENING.

Specifically, we have identified a C-heptad peptide that shows a level of affinity for a host cell. By using Fluorescence Resonance Energy Transfer methods, we have been able to label one of these HIV peptide analogs and measure its binding affinity/dissociation constant for the other. Specifically, we have identified a C-heptad peptide that shows a level of affinity for a particular N- (env-) peptide that we anticipate is sufficiently reliable to create an assay designed to rapidly screen volumes of pharmaceutical molecules. This assay is intended to detect molecules that can displace the one model gp41 peptide from the other in a laboratory setting with results that can be duplicated in vivo. Drugs of this class are already in circulation (Enfavir tide, T-20), but because of their peptide composition, a compensatory high cost and strict parental delivery are drawbacks. Our experiments have been carried out with the purpose of designing an assay for the screening of drug libraries for non-peptide scaffolds that may be developed as orally available HIV anti-fusion therapeutics.

Results and Conclusion: Recent tests results have proven to be encouraging that the assay functions as designed and may very soon be ready for limited screening. The various C-peptide models have been shown to displace each other in discriminatory fashion, and more importantly, a known competitive inhibitor D-peptide is able to displace the C-peptide from the envelope peptide’s binding sites. The success with competitive inhibition shows promise for the practical utility of this assay.

Introduction: The HIV-1 virus is responsible for AIDS and the patient’s accompanying susceptibility to opportunistic pathogens that cause systemic disease. Of direct dental concern, the immunocompromised HIV+ patient manifests a myriad of infections ranging from Candidiasis to Necrotizing Ulcerative Periodontitis. The most effective method of prevention of these conditions is via suppression of the HIV viral load. Among several with this purpose, a newer category of HIV drugs with room for improvement and advancement is the anti-fusion class.

Objective s: In our study, we have constructed various models of two peptide sequences on HIV-1 gp41 protein that, upon binding in vivo, are responsible for the fusion of the virus to the human host cell. By using Fluorescence Resonance Energy Transfer methods, we have been able to label one of these HIV peptide analogs and measure its binding affinity/dissociation constant for the other. Specifically, we have identified a C-heptad peptide that shows a level of affinity for a particular N- (env-) peptide that we anticipate is sufficiently reliable to create an assay designed to rapidly screen volumes of pharmaceutical molecules. This assay is intended to detect molecules that can displace the one model gp41 peptide from the other in a laboratory setting with results that can be duplicated in vivo. Drugs of this class are already in circulation (Enfavir tide, T-20), but because of their peptide composition, a compensatory high cost and strict parental delivery are drawbacks. Our experiments have been carried out with the purpose of designing an assay for the screening of drug libraries for non-peptide scaffolds that may be developed as orally available HIV anti-fusion therapeutics.

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SURFACE ELECTRICAL CHARGE DURING REVERSIBLE BINDING OF S. MUTANS TO HYDROXYAPATITE

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Our goal is to characterize the contributions of electrostatic interactions during the earliest stages of dental biofilm formation. Zeta potentials for HA beads, S. mutans-C (SM) (a generous gift from Professor William Bowen, University of Rochester) and HA-SM complexes at 20 and 37°C in 20 mM ionic strength solutions are calculated from electrophoretic mobility \( \nu_c \) measurements. The greater the electric charge on a particle, the faster it moves in an electric field. Preliminary data indicate SM binding to HA is reversible. Binding is complete in less than 60 minutes. Dissociation is much slower, requiring at least 600 minutes. This result is corroborated by parallel measurements of free and bound total SM protein. The net electric charge of SM appears to be unchanged by reversible binding. The effects of time, temperature, and ionic strength on the electrical charge SM are reported and discussed.

POLYMORPHISM OF MTHFR677CT IN UNAFFECTED FATHERS OF CHILDREN WITH OROFACIAL CLEFTS

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1 Doctor of Dental Surgery Program and 2 Craniofacial Genetics Laboratory, Department of Orthodontics, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA; 2 Pediatric Clinical Research Center, PCRC Core Laboratory, Children's Hospital, University of California San Francisco, San Francisco, CA

Non-syndromic cleft lip and palate (NCLP) is one of the world's most common birth defects. Both genetic and environmental factors are involved in the etiology. Clinical genetic studies offer an increasing evidence that the methylenetetrahydrofolate reductase gene (MTHFR) 677CT polymorphism plays a significant role in the developmental process of cleft lip and palate and thus a thorough analysis of this gene appears crucial for our understanding. The majority of studies follow the genotypes of mothers of affected children with scarce data on the contribution of fathers. Recent research (Christensen and Fletcher, 2004), however, has indicated that father's genetic contribution may be significant.

Objective: To increase a sample size of the previous pilot study on paternal genetic contribution of MTHFR 677CT polymorphism to the etiology of NCLP in Guatemala and to analyze this polymorphism in samples of fathers of NCLP children from different locations.

Methods: Samples were collected in Philippines, Guatemala, and Chile during Rotaplast medical missions in the years 1999-2004. We selected fathers from 53 families: 23 from Philippines, 26 from Chile, and 4 from Guatemala (added to the sample of 36 fathers of Christensen and Fletcher, 2004). We performed DNA isolation from blood specimens, PCR and PAGE (polyacrylamide gel electrophoresis) to determine the MTHFR 677CT genotypes.

Results: Analysis of specimens from all 89 fathers revealed the following distribution of genotypes: CC 40.45%, CT 35.96%, and TT 23.60% (ratio of frequencies of C to T alleles = 58.43% to 41.57%). However, there was a striking and highly significant difference in genotype distributions and in allele proportions between samples from different locations (genotype distribution \( p<0.0001 \), allele distribution \( p=0.0001 \)). Guatemala sample showed the highest proportion of the mutated T allele (66.25%), Philippines sample the lowest (4.35%), with the middle value in Chile sample (40.45%).

Conclusion: This study revealed very interesting results. First, it has shown, that MTHFR polymorphism may have a varied involvement in etiology of NCLP – a very strong contribution in some locations (as in Guatemala) and a little or none in others (Chile, Philippines). Second, it has substantiated a hypothesis that a parental genetic contribution (mother vs. father) may vary by location and ethnicity. Overall, the results strongly support our hypothesis that frequencies of candidate gene polymorphisms that are creating susceptibility for NCLP are location specific.

Acknowledgements: The field work for this study was supported by funding from ROTAPLAST Intl., Inc. Processing and analysis of the data and specimens were supported by the Department of Orthodontics, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA.
LONGITUDINAL EVALUATION OF TRANSFORMING GROWTH FACTOR-BETA AND PERIODONTAL STATUS IN HIV+ PATIENTS

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Objectives: We hypothesize that the elevated GCF levels of TGF-beta in HIV+ patients with periodontitis are significantly associated with attachment loss and immune status (CD4 and viral load values).

Methods: Thirty-five HIV-infected patients will be randomly selected from the database of an ongoing epidemiologic project. The demographic, clinical, and immunologic data related to these thirty five HIV+ patients will be used from the same database. The HIV+ patients were recruited from the CARE clinic at the University of the Pacific, Arthur A. Dugoni School of Dentistry. Following extrarad and intraoral examinations, the most diseased sextants (determined radiographically) in each subject were evaluated including the mesiobuccal sites of two premolars and two molars in that sextant including third molar. The plaque index (17), gingival index (18), probing depth, attachment level, and bleeding on probing were recorded for each experimental site including four posterior teeth and four lower anterior teeth by a calibrated examiner. The study sites in the lower anterior sextant were the right incisors. GCF sampling was carried out using sterile paper strips. The clinical mesiobuccal sites of the two lower left incisors and the distobuccal sites of two lower right incisors. GCF sampling was carried out using sterile paper strips. The clinical examinations and GCF sampling from the study sites were repeated at six month visits. GCF TGF-beta levels will be determined by ELISA assays. The associations between the demographic variables, clinical measurements, MMP-9 and TIMP-1 values will be determined by Spearman correlation coefficients. Analysis of variance (ANOVA) for repeated measures and paired t-tests. To develop regression models for the prediction of probing depth and attachment loss increases in subjects with active sites, TGF-beta, age, smoking packyears, clinical measurements including plaque index, gingival index, bleeding on probing will be entered into step-wise multiple regression analysis.

EVALUATION

POLYMORPHISM OF RFC1 GENE AMONG UNAFFECTED SIBLINGS OF PATIENTS AFFECTED WITH NONSYNDROMIC CLEFT LIP AND PALATE

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Introduction: Cleft lip and palate anomalies are common congenital anomalies, affecting one out of every 500 - 700 newborns worldwide. The vast majority of orofacial cleft anomalies are caused by a combination of genetic and environmental factors. Our target gene is RFC1 that is involved in a transport of folate across cell surface membranes. The gene product mediates an absorption of folate in the small intestine and plays a role in a maintenance of intracellular concentrations of folate. Point mutations and alterations in RFC1 result in downregulation of carrier activity.

Materials and Methods: We investigated a sample of unaffected individuals (n=21) who had at least one sibling affected with nonsyndromic cleft lip and palate (NCLP). All of them were identified during Rotaplast medical missions at Antigua and Guatemala City, Guatemala. DNA was isolated from dry blood spots on filter papers at the Craniofacial Genetics Laboratory. Polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis (PAGE) were used for genotyping.

Results: Twenty one affected individuals (cases) and their 21 unaffected siblings were analyzed for the RFC1 AG point mutation at the nucleotide 80. There was no wild-type homozygous (AA) genotype found in cases; 7 (33.3%) were heterozygous (AG), and 14 (66.7%) were homozygous (GG) for the mutation. G allele prevails in the case group (A allele 16.7%, G allele 83.3%). In the genotype found in cases, 7 (33.3%) were heterozygous (AG), and 14 (66.7%) were homozygous with mutation (AA- 9.5%, AG- 47.6%, GG- 66.7%). Allele frequencies for the sibs group were 33.3% for the A allele and 66.7% for the G allele. We compared results from our pilot study with another RFC1 study on a sample from Guatemala (Costanzo et al, 2003) showing the highest proportion of homozygotes GG as well as the highest proportion of mutated allele G was found in our case group, followed by the sibs group, and then by the case group from the Costanzo study. The highest proportion of homozygotes GG as well as the highest proportion of mutated allele G was found in our case group, followed by the sibs group, and then by the case group from the Costanzo study.

Conclusion: The A80G variant of the RFC1 gene was found to be significantly more prevalent in NCLP patients and in their unaffected siblings indicating that this mutation has to be seriously considered as a contributing factor to the etiology of NCLP in Guatemala. The higher prevalence of mutated G allele in unaffected siblings from Antigua compared to NCLP individuals from Costanzo study may represent another support for a “location specific” prevalence of genetic and environmental factors (Tolarova 2003) participating in etiology of these serious congenital anomalies.

Acknowledgment: Rotaplast International, Inc., supported the fieldwork for this study.

Processing and analysis of data was supported by the Department of Orthodontics, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA.
GENE DELIVERY TO MURINE ORAL CANCER CELLS IN THE PRESENCE OF MOUSE SERUM: APPLICATION TO HSV-TK/GANCICLOVIR SUICIDE GENE THERAPY

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Current treatments of head and neck squamous cell carcinoma (HNSCC) are largely unsatisfactory, and the five-year survival rate has not improved over the last two decades. Gene therapy is a new therapeutic modality in which defective genes are replaced with functional ones, or genes are delivered that can specifically kill cancer cells. Due to the accessibility of primary and recurrent lesions to direct injection, oral cancer may be amenable to gene therapy. Although generally efficient in transducing cells, viral vectors suffer from problems of immunogenicity, toxicity, limits in the size of exogenous DNA, and the risk of inducing tumorigenic mutations and generating active viral particles through recombination. Synthetic cationic liposome-DNA complexes (lipoplexes) constitute a promising alternative to the use of viral vectors and provides a simple means of transferring DNA into target cells. However, transfection mediated by non-viral vectors is inhibited by serum components, and would therefore limit the efficiency of gene delivery in vivo. Thus it is useful to identify non-viral vectors that are resistant to the inhibitory effects of serum. In this study, we examined the effect of high concentrations of mouse serum on the delivery of the luciferase and Herpes Simplex Virus thymidine kinase (HSV-tk) genes to murine squamous cell carcinoma cells, SCCVII, by the cationic liposome, Metafectene and the cationic polymer GeneJammer. The level of gene expression in cell lysates was evaluated by measuring enzyme activity after a 48 hour incubation. The optimal ratios for delivering the luciferase gene were 2 μL Metafectene:1 μg DNA and 3 μL GeneJammer:0.5 μg DNA. Metafectene-mediated luciferase expression was reduced by 70-80% in 20% serum and 25-70% in 60% serum. Gene delivery via GeneJammer was inhibited by 50-60% in serum-containing media in one experiment and enhanced in 20% and 60% serum in a second experiment. SCCVII cells transfected with the HSV-tk plasmid were incubated in the absence or the presence of ganciclovir (GCV; 20 μg/ml) for 7 days. The Alamar Blue assay was used to determine GCV-mediated cytotoxicity. Mock-transfected cells served as controls. The delivery of the HSV-tk gene by Metafectene in the absence and presence of 60% mouse serum, followed by GCV treatment for 3 days, resulted in 0% and 36% cytotoxicity, respectively. After 7 days, 90% and 82% cytotoxicity were observed in 0% and 60% mouse serum, respectively. Our observations suggest that Metafectene may be useful for the gene therapy of OSCC in an orthotopic murine model involving the generation of oral tumors by SCCVII cells.

ABSTRACTS

SENIOR RESEARCH COMPETITION

3 – 5:30 PM CLINICS
SUICIDE GENE THERAPY FOR ORAL CANCER: APOPTOSIS OR NECROSIS?

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Oral squamous cell carcinoma (SCC) kills 31,000 people a year in the U.S. and is still without an efficient, effective cure. Gene therapy approaches are being explored to treat oral SCC. "Suicide" gene therapy involves the delivery of the Herpes Simplex Virus thymidine kinase (HSV-tk) gene to cancer cells followed by the administration of the nucleoside analog antiviral drug, ganciclovir. tk monophosphorylates ganciclovir, which is then di- and tri-phosphorylated by cellular kinases. Tri-phosphorylated ganciclovir incorporates into replicating DNA during cell division, and causes chain termination and cell death. We examined whether the mode of cytotoxicity induced by HSV-tk + ganciclovir in oral SCC cells is via programmed cell death (apoptosis), or by necrosis. Human HSC-3 SCC cells were transfected with the plasmid pCMV-HSV-tk, using the cationic lipid reagent Metafectene, and incubated with or without ganciclovir up to 9 days. Cell viability was ascertained by the spectroscopic Alamar Blue assay on days 3, 6 and 9. The extent of necrosis was demarcated by the entry of the dye, Propidium Iodide, into cells and its ability to stain nuclei red. Apoptosis was ascertained by the binding of AlexaFluor 488-labelled Annexin V to phosphatidylserine exposed on the outer leaflet of the plasma membrane, resulting in bright green fluorescence. While significant ganciclovir-dependent cytotoxicity was observed with Alamar Blue, ganciclovir-independent cytotoxicity was also noted. The latter may be attributed to cytotoxicity caused by the transfection procedure. HSV-tk + ganciclovir-mediated cytotoxicity was dominated by apoptosis (42%), rather than necrosis (27%).
GENE DELIVERY TO HUMAN AND MURINE ORAL CANCER CELLS: EFFECT OF SERUM ON NEOPHECTIN- AND TROJENE-MEDIATED TRANSFECTION

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¹Doctor of Dental Surgery Program and ²Department of Microbiology, Arthur A. Dugoni School of Dentistry, University of the Pacific, 2155 Webster Street, San Francisco, CA 94115

Oral squamous cell carcinoma (SCC) is the most common type of oral cancer. Nine out of ten oral cancer malignancies are SCCs. Our long-term goal is to develop gene therapy for oral SCC based on the delivery of the Herpes Simplex virus thymidine kinase (HSV-tk) gene to SCC cells, followed by ganciclovir treatment to mediate cytotoxicity. As a first step towards testing this strategy in an animal model of oral SCC, we investigated the ability of novel transfection reagents to deliver a reporter gene (luciferase) into human HSC-3 and murine SCCVII cells. For HSC-3 cells, the best reagent was found to be Trojene with an optimal ratio of Trojene:DNA (µl:µg) of 4:1. GeneJammer, NeoPhectin and JetPEI mediated much lower levels of gene expression. For SCCVII cells Trojene was found to be superior to Metafectene and the optimal reagent:DNA ratio was 2:1 (µl:µg) for both. Since our ultimate aim is to deliver the HSV-tk gene to oral tumors in animal models, where macromolecules in biological milieu can inhibit gene delivery, we examined the effect of serum on transfection activity. The presence of 60% fetal bovine serum completely inhibited Trojene-mediated transfection of SCCVII cells, while NeoPhectin-mediated transfection was inhibited by 86-94%. Delivery of HSV/tk to SCCVII cells via NeoPhectin at 0% serum, followed by ganciclovir treatment, resulted in 24 and 32% cytotoxicity on days 3 and 6, respectively. Transfection in 60% serum resulted in only 8 and 16% cytotoxicity, respectively. Our results indicate that transfection reagents must be tested in vitro in high concentrations of serum before experiments are undertaken in animal models of SCC. Our observations suggest that Metafectene may be useful for the gene therapy of OSCC in an orthotopic murine model involving the generation of oral tumors by SCCVII cells.

ROOT CANAL PREPARATION AND DECONTAMINATION USING ER,CR:YSGG LASER IRRADIAITION

Caton State¹, Nathan Overlid² and Douglas A. Young³

¹Doctor of Dental Surgery Program, ²Department of Microbiology and ³Department of Diagnosis and Management, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

The purpose of this study is to assess decontamination when a commercially available Er,Cr:YSGG laser (Waterlase, Biolase Technology Inc., San Clemente, CA) is used to ablate and prepare the canal, using the crown down root canal preparation technique. Sixty human teeth were instrumented to a 25mm file, sterilized, then inoculated with Streptococcus anginosus and randomly divided into five groups: 1) 2 positive control groups (in and out of chamber with bacteria added and no treatment), 2) Conventional files and hypochlorite group, 3) laser group without NaOCl irrigation, 4) laser group without NaOCl or irrigation, 5) laser group with NaOCl irrigation. After incubation the colony-forming-units were quantified and cultured by removing the broth with a micropipette and plating onto Brain Heart Infusion plates. Results show a decrease in CFU in all treatment groups; however, the laser group without NaOCl or irrigation has the best decontamination potential.