3rd ANNUAL
UOP RESEARCH DAY &
STUDENT RESEARCH
COMPETITIONS

Tuesday, May 29, 2001
NOON PROGRAM

PRESENTATIONS OF ORTHODONTICS RESIDENTS

12-2 pm  Room 304

12:00 pm Brad Evans  STUDYING AN IN-VITRO MODEL TO DETERMINE THE FEASIBILITY OF USING LIPOSOMES FOR SUSTAINED ANTIBIOTIC RELEASE  (Mentors: Dr. Snowdowne & Dr. Leknius)

12:20 pm Jed Feller  DEVELOPMENT OF A LIPOSOME-BASED CARRIER SYSTEM FOR STIMULANTS OF BONE GROWTH  (Mentors: Dr. Snowdowne and Dr. Vakoula)

12:40 pm Brenda Caillouel  A QUANTITATIVE ANALYSIS OF THE DIFFERENCE BETWEEN RIGHT AND LEFT FACING ROENTOCEPHALOGRAMS  (Mentors: Dr. Schiff & Dr. Baumrind)

1:00 pm Hesham M. Amer  OUTCOME STUDY OF EXTRACTION CASES COMPARED WITH NON-EXTRACTION ONES USING THE ABO OBJECTIVE GRADING SYSTEM FOR DENTAL CASTS  (Mentor: Dr. Poulton)

1:20 pm Hossein Abarghouei  THE RELATIONSHIP BETWEEN LIP THICKNESS AND LIP DISPLACEMENT IN RESPONSE TO INCISOR MOVEMENT  (Mentors: Dr. Baumrind & Dr. Carlson)

1:40 pm Nitin Sallapudi  DEMONSTRATION AND DETERMINATION OF THE VALIDITY OF THE STEREO X-RAY AND 3D-METRICS SYSTEMS IN LOCATING CEPHALOMETRIC LANDMARKS ON DRY SKULLS  (Mentors: Dr. Baumrind & Dr. Carlson)
AFTERNOON PROGRAM

FACULTY AND STUDENT POSTERS AND TABLE CLINICS

4:30  Café Pacific

Miriam Gochin, Martin A. Case and George L. McLendon  IDENTIFICATION AND BIOPHYSICAL CHARACTERIZATION OF A PEPTIDE FROM THE GP41 EXTRACELLULAR COILED-COIL DOMAIN AS A TARGET FOR SMALL-MOLECULE DRUG DEVELOPMENT

Joel A. Cohen and Valentina Khorosheva  WATER FLOWS IN THE POLYMER COAT OF STEALTH® LIPOSOMES

W. Bernt and Stefan Highsmith  MYOSIN-NUCLEOTIDE ELECTRICAL PROPERTIES

Krystyna Konopka, Nancy R. Shine, S.C. Wang, Chris P. Whitman and Nejat Düzgünes  SECRETORY LEUKOCYTE PROTEASE INHIBITOR AND HIV-1 INFECTION OF THP-1 CELLS

Aaron Lee, Krystyna Konopka and Nejat Düzgünes  TRANSFECTION OF HELA, HEK293, AND HSC-3 CELLS BY TRANSFERRIN LIPOPLEXES: ROLE OF THE CHARGE RATIO

Dorothy Burk and Leigh C. Anderson  TRIGEMINAL GANGLION CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVITY IS NOT CORRELATED WITH THE MAINTENANCE OF NEUROPATHIC PAIN BEHAVIORS FOLLOWING INFRAORBITAL NERVE INJURY IN RATS

Hee Soo Oh, Charles Brodsky, Javier Mir, Terezie Mosby and Marie Tolarova  CLEFT LIP AND PALATE ANOMALIES IN PATAGONIA

Simona Tolarova, Laura Pastor, Terezie Mosby, Angelo Capozzi, Anne Boekelheide, Richard Spritz and Marie Tolarova  TWO CASES OF MARGARITA ISLAND ECTODERMAL DYSPLASIA

Charles Brodsky, Hee-Soo Oh, Javier Mir, Richard Spritz and Marie Tolarova  METHYLENETETRAHYDROFOLATE REDUCTASE: MUTATIONS IN SOUTH AMERICA

Thomas Ellerhorst, Cooper Owens, Hee Soo Oh, Javier Mir, Terezie Mosby and Marie Tolarova  AGE OF PARENTS AND THE RISK OF CLEFT LIP AND PALATE

Steven Pai, Hee Soo Oh, Chiyo Shidara, Terezie Mosby and Marie Tolarova  EDUCATION ABOUT CLEFT PALATE FOR ASIAN FAMILIES
EVENING PROGRAM

STUDENT RESEARCH COMPETITIONS

5:30-7 pm  Room 304

ADA/DENTSPLY STUDENT RESEARCH COMPETITION

Leah M. Walker, Sean K. Carlson and Sheldon Baumrind  DIFFERENCES IN TOOTH DIMENSION MEASUREMENTS BETWEEN STUDY MODELS, PERIAPICAL X-RAYS, AND PANORAMIC X-RAYS

Cory Costanzo, Tom Ellerhorst, Andrew Kouvaris, Robert Boyd and Vicki Vlaskalic  CLINICAL STUDY OF A REMOVABLE, CLEAR, ORTHODONTIC APPLIANCE SYSTEM

Alexander Lim, Aaron Lee, Krystyna Konopka and Nejat Düzgünes  SERUM INHIBITION OF FUGENE- AND TRANSFERRIN LIPOPLEX-MEDIATED GENE DELIVERY TO HSC-3 AND HEK293 CELLS

Sohail Saghezchi, Aaron Lee, Krystyna Konopka and Nejat Düzgünes  HERPES SIMPLEX VIRUS THYMIDINE KINASE/GANCICLOVIR-BASED GENE THERAPY IN HUMAN ORAL CANCER CELLS

Sydney Moore, Madelyn Olson, and Gary D. Richards  AN ONTOGENETIC PERSPECTIVE ON MENTAL FORAMEN POSITION: PRELIMINARY RESULTS

Charles Brodsky, Hee-Soo Oh, Javier Mir, Richard Spritz and Marie Tolarova  METHYLENETETRAHYDROFOLATE REDUCTASE: MUTATIONS IN SOUTH AMERICA

Thomas Ellerhorst, Cooper Owens, Hee Soo Oh, Javier Mir, Terezie Mosby and Marie Tolarova  AGE OF PARENTS AND THE RISK OF CLEFT LIP AND PALATE

Steven Pai, Hee Soo Oh, Chiyo Shidara, Terezie Mosby and Marie Tolarova  EDUCATION ABOUT CLEFT PALATE FOR ASIAN FAMILIES

SENIOR RESEARCH COMPETITION

Shaza Mardini, Kiran Rapal, Sydney Moore, Madelyn Olson, Gary D. Richards  SULCUS COLLI REGION ONTOGENY AND MANDIBULAR NERVE (V3) ANESTHESIA
STUDYING AN IN-VITRO MODEL TO DETERMINE THE FEASIBILITY OF USING LIPOSOMES FOR SUSTAINED ANTIBIOTIC RELEASE

Brad Evans, Ken Snowdowne and Casimir Leknius

Departments of Orthodontics and Fixed Prosthodontics, University of the Pacific School of Dentistry, San Francisco, CA

The purpose of this project was to determine if liposomes could be used to store and slowly release antibiotics that are useful against periodontal pathogens. Liposomes are bacterial-sized spheres of phospholipids that can sequester both hydrophobic and hydrophilic drugs and release them slowly over several weeks. Our laboratory has shown that certain liposomes bind to molecules and cells found in the periodontal pocket to provide enough antibiotic to protect the area for at least one week. These liposomes are negatively charged liposomes that contain a polyethylene glycol (PEG). PEG has been shown to greatly prolong the in vivo longevity of liposomes because of its ability to block removal by macrophage. We created an in vitro perfusion system to mimic the environment of the periodontal pocket including a constant flushing of crevicular fluid. We tested the ability of these liposomes to retain one of the following antibiotics: tetracycline, metronidazole or doxycycline. The rate of loss of the antibiotic was followed for 6 hrs. The kinetic analysis of this loss showed the rate of release from the liposomes was too quick to be useful to provide coverage. I concluded that these antibiotics are too hydrophobic to be retained by liposomes to make a useful liposome-based drug delivery system for periodontal diseases.
DEVELOPMENT OF A LIPOsome-BASED CARRIER SYSTEM FOR STIMULANTS OF BONE GROWTH

Jed M. Feller, Ken Snowdowne, Alex Vakoula

Department of Orthodontics, University of the Pacific School of Dentistry, San Francisco, CA

The goal of our laboratory is to develop tools to control the regeneration of oral tissues. We are currently developing a liposome sequestered cytokine system that will control the activity of fibroblasts. The purposes of the current studies were (1) to determine which liposomes are most stable; (2) to determine how much protein activity will be destroyed during the loading process and (3) to determine if lyophilization loading would be more efficient in loading proteins than passive loading and whether lyophilization would degrade the activity of the protein. Several types of liposomes were loaded with calcein and tested for retention in an in vitro model of a perfused interstitium. We found that artificially derivatized forms of phospholipids were much more stable. Unfortunately these phospholipid require a much higher annealing temperature which may destroy the loaded enzymes activity. To elucidate how destructive the loading process, we measured alkaline phosphatase (ALP) activity after two extreme aspects of the loading process, sonication and heat exposure. We then tested ALP loading efficiency for two loading methods, lyophilization and passive. Our results indicate that synthetic, anionic liposomes that are constructed with cholesterol are the best choice for protein loading. We showed that enzyme activity is not significantly damaged at the annealing temperature for the synthetic liposomes or with sonication. We also demonstrated that the lyophilization loading method is 14-17 times as efficient as the passive loading method. The results of these studies go a long way in developing a prototype to control tissue regeneration.

A QUANTITATIVE ANALYSIS OF THE DIFFERENCE BETWEEN RIGHT AND LEFT FACING LATERAL CEPHALOGRAMS.

Brenda Caillouel, Thomas Schiff and Sheldon Baumrind

Department of Orthodontics, School of Dentistry, University of the Pacific, San Francisco, CA

For initial diagnosis and evaluation of growth and/or treatment results, orthodontists use lateral cephalograms universally. In the United States, lateral cephalograms are conventionally taken with the left side of the patient’s head closest to the film. In Europe, however, the convention is to place the right side of the patient’s head closest to the film. This difference in patient orientation is important, in that the resultant lateral cephalograms attained from the two disparate techniques are not the same. Due to the divergent nature of x-rays, the structures of the craniofacial complex that are farthest from the film will be magnified more than those that are closer to the film. In this study, four dried skulls from the Spencer Atkinson Library of Applied Anatomy were employed 1) to determine the differences in landmark identification between right and left facing lateral cephalograms 2) to determine the magnitude of the differences for conventional angular and linear measurements used in clinical analyses 3) to visually exhibit the differences using the technique of subtraction radiography and 4) to compare Denoptics digital images with conventional analog images. The results showed small differences in landmark identification and their resultant angular and linear measurements. Subtraction images of the bilateral cephalograms, for each of the four dried skulls, were produced to visually exhibit differences between the right-left pairs. The final part of the study, however, was abandoned, as neither the Denoptics digital nor the analog image type was believed to be optimally exposed.
ORTHODONTIC TREATMENT OUTCOME USING THE ABO GRADING SYSTEM FOR DENTAL CASTS.

Hesham Amer and Don Poulton
Department of Orthodontics, University of the Pacific, School of Dentistry, San Francisco, CA

This study compared the ABO (American Board of Orthodontics) grading system to clinicians subjective assessments, the PAR (Peer Assessment Rating), and the HLD (Handicapping Labio-lingual Deviation index). Both intra-rater and inter-rater agreement of the scoring of 36 cases were calculated, and these were divided into 16 extraction and 20 non-extraction cases. For each of the 36 sets of final casts, an ABO score for tooth or contact was made by a group of orthodontists. Four orthodontists had been calibrated in its use, and a non-calibrated second year resident, graded the 36 final casts twice with at least two weeks interval between gradings. The PAR, HLD and the subjective assessment were taken from a previous study. There was a good correlation between ABO, PAR and clinicians subjective score, but no correlation between HLD and clinicians subjective score. A very good to excellent inter-rater reproducibility was found using the ABO grading system. Calibration helped to get more consistent intra-rater reproducibility. Extraction cases scored slightly higher (worse) than non-extraction ones.

THE RELATIONSHIP BETWEEN LIP THICKNESS AND LIP DISPLACEMENT IN RESPONSE TO INCISOR MOVEMENT

Hossein Abar, Sheldon Baumrind and Sean Carlson
Department of Orthodontics, University of the Pacific, School of Dentistry, San Francisco CA

Balance and harmony of the face relies on the morphologic relationships of the nose, lips, and chin, as well as the underlying skeletal components. Orthodontic treatment can alter the balance among these structures. Some studies have related the effect of orthodontic treatment on lip positions in response to the incisor retraction. However, the nature of correction between incisor retraction and lip adaptation is still controversial. The present study was undertaken to investigate the influence of lip thickness on the relationship between dental and integumental tissue changes in orthodontically treated patients. Our Sample was randomly selected from the orthodontically treated patients at University of the Pacific from 1988 to 1992. All patients were treated with edgewise appliances. Cephalometric headfilms of thirty late-adolescent and adult patients were digitized and analyzed for changes in the integumental profile with treatment. It was found that there was moderately significant correlation between the osseous changes and soft tissue changes of the four points measured; Point A to superior labial sulcus \( (r = 0.64) \), Maxillary incisors to upper lip \( (r = 0.82) \), Mandibular incisors to lower lip \( (r = 0.73) \), Point B to inferior labial sulcus \( (r = 0.78) \). However, no significant correlation was observed between the lip thickness and the ratio of the perioral soft tissue response to the hard tissue movement. These results suggest that gross tooth movement may not have a proportional effect on the profile contour, which further suggest that soft-tissue may be self-supporting.
ASSESSMENT OF THE ACCURACY OF THE CRIL STEREO X-RAY AND 3DMETRICS™ SYSTEMS IN LINEAR MEASUREMENT

Nitin Salapudi, Sheldon Baumrind and Sean Carlson

Department of Orthodontics, University of the Pacific, School of Dentistry, San Francisco CA

A study was undertaken to assess the accuracy of the CRIL Stereo X-ray and 3DMetrics systems in linear measurement. Several metal tie points and landmark identifiers were placed on a dry skull. Multiple measurements were then made between the tie points and also between landmarks. Three different methods of measurement, which included direct caliper measurement, the CRIL Stereo X-ray system and the 3DMetrics system were used. The direct caliper measurement was used as the gold standard, to which the other two systems were compared. The results of the experiment showed that the CRIL Stereo X-ray system was very accurate at measuring tie point measurements, but was very inaccurate at landmark measurement. The 3DMetrics system was not accurate in landmark measurements and was less accurate for tie point measurements. Future development of the CRIL Stereo x-ray system should focus in the area of landmark location.

IDENTIFICATION AND BIOPHYSICAL CHARACTERIZATION OF A PEPTIDE FROM THE GP41 EXTRACELLULAR COILED-COIL DOMAIN AS A TARGET FOR SMALL-MOLECULE DRUG DEVELOPMENT

Miriam Gochin1, Martin A. Case2 and George L. McLendon2

1Department of Microbiology, University of the Pacific, School of Dentistry, San Francisco CA and 2Department of Chemistry, Princeton University, Princeton, NJ

The extracellular domain of gp41 forms a trimer with a three-stranded α-helical coiled coil core near the N-terminus that is vital to the fusion mechanism. The coiled coil becomes transiently exposed upon gp120 binding to cells, following which a second α-helical region near the C-terminus packs in the reverse direction against the N-terminal core, forming the fusion active conformation of gp41. Biophysical and computational strategies are being employed to develop and test small molecules that can act as potential drugs by binding tightly to the N-terminal coiled coil, hence disrupting the six-helix bundle structure and inhibiting fusion. Peptides from the N-terminal core are extremely hydrophobic, due to their function in anchoring the C-terminal domain, and are therefore not readily amenable to study in solution by biophysical techniques. We have identified regions of the N-terminal core which show promise as possible drug-binding sites, and designed a corresponding peptide with an N-terminal capping bipyridyl group. Three bipyridyl groups ligate to a single transition metal ion, stabilizing the three-helix bundle structure in solution and improving solubility of the resulting complex. We have investigated biophysical methods for assaying binding to the target. We will show results of Nuclear Magnetic Resonance and Circular Dichroism studies of these complexes, as well as small molecules identified by computational docking procedures as having good shape and electrostatic complementarity to grooves in the coiled coil structure.

Supported by the Universitywide AIDS Research Program Grant R00-UP-092
WATER FLOWS IN THE POLYMER COAT OF STEALTH® LIPOSOMES

Joel A. Cohen and Valentina Khorosheva

Department of Physiology, University of the Pacific, School of Dentistry, San Francisco CA

Hydrodynamic properties of the surface PEG polymer layer on Stealth® liposomes have been studied by electrophoretic measurement of polymer-induced hydrodynamic drag. Classical analysis via the Smoluchowski equation permits determination of the location of an apparent shear surface, which defines the apparent hydrodynamic thickness ($L_{app}$) of the polymer layer. We investigated the dependence of $L_{app}$ on ionic strength. Since PEG is a neutral polymer, one naively expects $L_{app}$ to be relatively independent of ionic strength.

$L_{app}$ was determined for PEG-PE:PC (1:9 mol:mol) multilamellar liposomes, with PEG sizes ranging from 350 to 5000 Da, and NaCl ranging from 0.5 to 100 mM. Contrary to expectation, $L_{app}$ was found to be strongly ionic-strength dependent, especially for the longer polymers.

The ionic-strength behavior can be explained by accounting for water flow in the polymer layer, which the Smoluchowski equation does not do. In a simple model, the one-dimensional Navier-Stokes equation was solved analytically in the Debye-Hückel approximation assuming a rectangular polymer-segment density profile and Stokes friction in the polymer layer. $L_{app}$ is shown to approach the correct hydrodynamic thickness for low ionic strength and/or high friction in the polymer layer. The model accounts for the observed ionic-strength effects and provides a formula for extracting hydrodynamic thicknesses and effective frictional coefficients from the ionic-strength-dependent data.

Fitted hydrodynamic thicknesses for the 2000-, 3000-, and 5000-Da PEG coats vary linearly with polymer index, consistent with scaling theory for polymer brushes. The frictional decay lengths scale with the hydrodynamic thicknesses.

Valid electrophoretic determinations of hydrodynamic thicknesses of neutral polymer coats are shown to require Debye screening lengths significantly longer than the coat extensions.

This abstract and poster were presented at the Biophysical Society Annual Meeting, Boston, MA, February 17-21, 2001

MYOSIN-NUCLEOTIDE ELECTRICAL PROPERTIES

W. Bernt,* and Stefan Highsmith

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Electrophoretic mobilities, $\mu_e$, measure the rate ($\mu$m/s) at which a particle moves when it is in an electric field (V/cm). The more electrical charge on the particle, the faster it moves. We measured $\mu_e$ for of skeletal muscle heavy meromyosin (HMM) complexes of ATP and ATP analogs. HMM is a proteolytic fragment of myosin that has two ATP-hydrolyzing motor domains attached to a coiled-coil rod-like structure.

For HMM(ATP)$\gamma$S, HMM(ADP.Pi), and HMM itself, the values of $\mu_e$ are in the -0.077 to -0.17 ($\mu$m/s)/(V/cm) range. In contrast, when Pi dissociates from HMM(ADP.Pi) to form HMM(MgADP), $\mu_e$ decreases to -0.56 ($\mu$m/s)/(V/cm).

Particle charges, $Q_e$, were calculated from the $\mu_e$ values. The particle charge to mass ratios for the complexes are all in a range that falls within published values determined for a variety of other proteins, except for that of HMM(ADP). It has a higher charge to mass ratio. It is hypothesized that the high electric charge to mass ratio of HMM(ADP) creates electrostatic repulsion between the catalytic and light chain binding subdomains, providing free energy to elongate the motor domain and contribute to force generation during contraction.

Supported by NIH grant AR42895
SECRETORY LEUKOCYTE PROTEASE INHIBITOR AND HIV-1 INFECTION OF THP-1 CELLS

Krystyna Konopka 1, Nancy R. Shine 1, S.C. Wang 2, Chris P. Whitman 2 and Nejat Düzgüneş 1

1Department of Microbiology, University of the Pacific, School of Dentistry, San Francisco, CA and 2Medicinal Chemistry Division, College of Pharmacy, University of Texas, Austin, TX

Several studies have indicated that recombinant secretory leukocyte protease inhibitor (rSLPI), a serine protease inhibitor present in saliva, could inhibit HIV-1 infection. In this study we examined if rSLPI can inhibit HIV-1 infection in monocytic THP-1 cells expressing high levels of CCR5 and CXCR4 coreceptors. The expression of CD4, CCR5 and CXCR4 was examined using fluorescein isothiocyanate-conjugated Leu3/CD4, 2D7/CCR5, and 12G5/CXCR4 antibodies. The SLPI gene was synthesized by a PCR-based strategy using long oligonucleotides, and the protein was overexpressed in E. coli, purified, and successfully refolded. THP-1 cells were incubated with 10 μg/ml rSLPI for 2 h at 37°C prior to addition of the virus. rSLPI was also present during the 2 h infection period. Virus production in culture supernatants was monitored by an ELISA. THP-1 cells were efficiently infected by both T-cell tropic HIV-1NIIB and M-tropic HIV-1BaL isolates. Differentiation of THP-1 cells into adherent macrophage-like cells, induced by treatment with phorbol myristate acetate (PMA), markedly reduced CD4 surface expression (from >40% to <2%). In contrast, CCR5 and CXCR4 expression declined marginally, from 96% to 75% and from 94% to 78%, respectively. Differentiated THP-1 cells could be efficiently infected with HIV-1BaL but not with HIV-1NIIB. In differentiated THP-1 cells infected with HIV-1BaL, rSLPI reduced p24 production by 90% at 7 and 10 days and by 80% at day 13 post-infection. Based on these findings, we conclude that (i) downmodulation of CD4 expression in differentiated THP-1 cells results in a significant reduction in their susceptibility to HIV-1 infection with the T-cell tropic (NIIB) but not with the M-tropic (BaL) isolate, and (ii) rSLPI inhibits HIV-1BaL infection in differentiated THP-1 cells.

Partially supported by funds from the University of the Pacific School of Dentistry (DRES03-001). Presented at the 30th Annual Meeting of the American Association for Dental Research, March 7-10, 2001, Chicago, IL.

TRANSFECTION OF HELA, HEK293, AND HSC-3 CELLS BY TRANSFERRIN LIPOPLEXES: ROLE OF THE CHARGE RATIO

Aaron Lee, Krystyna Konopka and Nejat Düzgüneş

Department of Microbiology, University of the Pacific School of Dentistry, San Francisco, CA

Cationic lipid-DNA complexes ("lipoplexes") are being used as a therapeutic gene delivery vehicle both in vitro and in vivo. The limitations of lipoplexes include their low transfection efficiency, their potential cytotoxicity at high concentrations and the inhibition of transfection by serum. Previous studies have indicated that the efficiency of transfection can be enhanced by the complexation of human transferrin (TF) with cationic liposomes before lipoplex formation. TF is an iron-transporting serum glycoprotein, which binds to a receptor expressed on the surface of proliferating cells, with particularly high expression on erythroblasts and tumor cells. Here we examined the effect of TF on DOTAP/DOPE (ESCORT™)-mediated transfection of three different cell lines at various lipid/DNA (+/-) charge ratios, using luciferase as a reporter gene. In HEK293 human embryonal kidney cells, and HeLa human cervical carcinoma cells, the greatest enhancement of gene expression by TF was achieved at a charge ratio of 1.0 with a 3-fold enhancement. The TF-mediated enhancement of transfection in HSC-3 human squamous cell carcinoma cells was most significant with a charge ratio of 0.5 with a 10-fold enhancement. The level of gene expression was highly dependent on the cell type, with the HSC-3 cells being the most difficult to transfect. Enhancement of gene delivery of by TF has potential applications to the delivery of suicide genes into oral cancer cells.
TRIGEMINAL GANGLION CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVITY IS NOT CORRELATED WITH THE MAINTENANCE OF NEUROPATHIC PAIN BEHAVIORS FOLLOWING INFRAORBITAL NERVE INJURY IN RATS

Dorothy Burk and Leigh C. Anderson

Department of Anatomy, University of the Pacific School of Dentistry, San Francisco, CA

Partial injury to somatosensory nerves results in functional and cytochemical alterations in sensory neurons, and in pain-associated behaviors that resemble neuropathic pain states. Calcitonin gene-related peptide is a neuropeptide present in nociceptive neurons. The purpose of this research was to determine whether changes in CGRP immunoreactivity (CGRP-IR) within the trigeminal ganglia (TG) are associated with the maintenance of neuropathic pain behaviors after infraorbital nerve constriction (CCI) in adult rats. Male, Sprague-Dawley rats (N=6) were anesthetized, and CCI was accomplished by placing a single ligature (5-0 chromic gut) distal to the infraorbital groove. The appearance of mechanical hyperalgesia in CCI rats confirmed the effectiveness of the injury. Sham-injury rats exhibited no changes in behavior. After 21 days, the animals were perfused with fixative, and the TG were processed for immunocytochemistry. CGRP-IR in both the ipsilateral and contralateral TG was measured by determining the % CGRP-IR cells (Table 1) in areas corresponding to V1, V2 and V3. No significant differences were observed between either Sham-injury or CCI rats, or between ipsilateral and contralateral TG.

Table 1 (% CGRP-IR)

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<tr>
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<th>V1</th>
<th>V2</th>
<th>V3</th>
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<tr>
<td>SHAM (N=3)</td>
<td>23 ± 7</td>
<td>25 ± 9</td>
<td>31 ± 8</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>24 ± 7</td>
<td>19 ± 7</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>Contralateral</td>
<td>25 ± 7</td>
<td>23 ± 8</td>
<td>30 ± 6</td>
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Although these data are preliminary, they indicate that CGRP-IR in the trigeminal ganglion is not correlated with the maintenance of neuropathic pain following infraorbital nerve injury in adult rats.

Supported by grant DE12338 from NIDCR.

CLEFT LIP AND PALATE ANOMALIES IN PATAGONIA

Hee Soo Oh1, Charles Brodsky2, Javier Mir 3, Terezie Mosby3, and Marie Tolarova3

1AEGD Program; 2Doctor of Dental Surgery Program 2002, 3Department of Orthodontics, University of the Pacific, School of Dentistry, San Francisco CA

Cleft lip and palate anomalies represent a major problem in oral health worldwide. Every two minutes, a child affected with orofacial cleft is born somewhere in this world. On average, one out of every 500 newborns has a cleft. As a necessary step towards prevention, epidemiological studies evaluating etiological roles of environmental factors (including nutrition) and of genetic factors are performed. We studied 72 individuals affected with orofacial cleft and 38 unaffected (control) individuals from the Argentinean Patagonia province Chubut. A diagnostic spectrum, birth weight, birth order, age of parents and month of birth were evaluated. Results of an initial analysis of epidemiological characteristics of cases and controls examined in the city of Trelew are presented.

Mean age of our cleft patients was 17.8 years. The majority (83%) had cleft lip and palate. In unilateral cases, the left side was affected more often (69%). Children affected with cleft were more likely to be firstborn or of parity 4 or higher. The mean birth weight was 3209 grams. Evaluation of maternal age revealed a higher prevalence of mothers younger than 24 years for cases, but not for controls. Detailed analyses of epidemiological characteristics were performed in subcategories classified according to cleft severity and laterality. Ongoing analyses include environmental, nutritional and genetic data.

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, University of the Pacific School of Dentistry. The Table Clinic was presented in February 2001 at the UOP Asilomar Conference and at the CDA Convention in Anaheim, CA, in April 2001.
TWO CASES OF MARGARITA ISLAND ECTODERMAL DYSPLASIA

Simona Tolarova
Laura Pastor
Terezie Mosby
Angelo Capozzi
Anne Boekelheide
Richard Spritz
Marie Tolarova

Department of Orthodontics, University of the Pacific, School of Dentistry, San Francisco CA; University of Cuyo, Mendoza, Argentina; Shriner's Hospital, Sacramento, CA; School of Dentistry, UCSF, San Francisco, CA; Human Medical Genetics Program, University of Colorado, Denver, CO.

In 1987, Zlotogora et al. and later Bustos et al. (1991) described new autosomal recessive syndrome that involved clefting, syndactyly and ectodermal dysplasia (ED). The condition was found on the Margarita Island, Venezuela and was named Margarita Island Ectodermal Dysplasia or ED4 (MIM 225060). In 1998 Suzuki, Bustos and Spritz mapped the gene for this syndrome to 11q23. Recently, a detailed study on a mutation of PVRL1 was published in Nature Genetics. We demonstrate clinical features of two unrelated male probands affected with ED4. Both of them tested homozygous for the PVRL1 mutation. Probands were seen at the Rotaplast cleft screening clinic in 1998, in Carupano (case #1) and in Cumana (case #2). Case #1: 40 years old single man of short stature, developmentally delayed, never attended a school. He presents with bilat. cleft lip and palate (CLP), small head with just a few sparse hairs; with pigmentation on both scleras, red dry eyes with no eyelashes, no eyebrows, conjunctivitis, atresia ductus lacrimalis bilat. Thorax is narrow, back presents with scoliosis. His fingers and toes are short. On both hands, soft partial syndactyly 2+3 is present. Finger nails are dysplastic and very short, as well as toe nails. There is a mild heart murmur present. He has no teeth. His skin is light and very dry. His mother reports that he has had a very limited sweating and does not tolerate heat. He has dry, red complexion is dark, typical color for Moreno.

Case #2: 12 years old single man of short stature, developmentally delayed, never attended a school. He presents with bilat. cleft lip and palate (CLP), small head with just a few sparse hairs; with pigmentation on both scleras, red dry eyes with no eyelashes, no eyebrows, conjunctivitis, atresia ductus lacrimalis bilat. Thorax is narrow, back presents with scoliosis. His fingers and toes are short. On both hands, soft partial syndactyly 2+3 is present. Finger nails are dysplastic and very short, as well as toe nails. There is a mild heart murmur present. He has no teeth. His skin is light and very dry. His mother reports that he has had a very limited sweating and does not tolerate heat. Case #2: 12 years old boy with bilat. CLP and ED. He has been adopted and no history of his biological parents is available. His complex color is dark, typical color for Moreno. Skin is very dry; he has decreased sweating, but does tolerate moderate heat with just minimal problems. He has dry, red eyes, no eyelashes, no eyebrows and very thin, short scalp hair. He has very small, atypically shaped teeth in lower jaw and no teeth in upper jaw. He presents with syndactyly on both hands and feet and short and dysplastic nails. His development seems to be slightly delayed. Conclusion: Even if ED features are not difficult to diagnose, syndromic ED cases that follow Mendelian inheritance pattern may not be always correctly classified. Our presentation will help to get familiar with diagnostic features of a rare Margarita Island Ectodermal Dysplasia. Even if dysmorphology is not characteristic, gene analysis should confirm clinical diagnosis.

The field work for this study was supported by funding from ROTAPLAST International, Inc. Genotyping for PVRL1 mutation was done at the Human Medical Genetics Program, University of Colorado, Denver. Processing and analysis of the data were supported by the Department of Orthodontics, University of the Pacific School of Dentistry. The Poster has been presented at the 58th Annual Meeting of the American Cleft Palate-Craniofacial Association in Minneapolis, MN, April 2001.

DIFFERENCES IN TOOTH DIMENSION MEASUREMENTS BETWEEN STUDY MODELS, PERIAPOPICAL X-RAYS, AND PANORAMIC X-RAYS

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Periapical x-rays are considered more valid than panoramic x-rays in measuring tooth dimensions. However, quantitative values of their difference have yet to be reported. Due to the fact that orthodontic records often do not include periapical x-rays, a better understanding of the quantitative differences may allow similarly useful information to be gathered with reduced exposure to radiation. Sixteen pairs of periapical and panoramic x-rays taken on the same day of the same patient were selected and their tooth dimension measurements were compared to those taken from study models. Measurements of the mandibular right posterior quadrant were compared. Specialized computer software was used to locate tooth landmarks on each x-ray. Three independent judges made estimates of each landmark and their average was used for each measurement. The following comparisons were made: 1) Tooth width - study models vs. periapical x-rays 2) Tooth width - study models vs. panoramic x-rays 3) Tooth width - periapical x-rays vs. panoramic x-rays 4) Tooth length - periapical x-rays vs. panoramic x-ray. The was no statistical or significant difference between all canine measurements. All other measurements showed statistical and clinical significance (p < 0.05) with the exception of the periapical 1st and 2nd bicuspid width measurements (p = .182 and p = .169 respectively). Periapical width measurements compared to study models showed modest enlargement (canine = 104%, 1st bicuspid = 104%, 2nd bicuspid = 103%, 1st molar = 105%, and 2nd molar = 111%). Panoramic width measurements compared to study models show greater enlargement than periapicals, which consistently increased toward the posterior (canine = 105%, 1st bicuspid = 118%, 2nd bicuspid = 126%, 1st molar = 133%, and 2nd molar = 142%). These data suggest that periapical x-rays show greater validity than panoramic x-rays in measuring tooth size. The consistent enlargement of panoramic tooth measurements toward the posterior suggests that extrapolating estimates of actual tooth size from panoramic x-rays may be possible.
This clinical study researches the feasibility of a new, clear, removable orthodontic appliance system. Currently, 40 subjects are enrolled in the first clinical trial of the Invisalign System™ of orthodontic tooth movement. Treatment involves first creating a 3-dimensional computer image from a polyvinyl siloxane impression of the patient. This image is then manipulated to the clinician’s prescribed treatment plan. A series of clear, plastic, overlay appliances is then created for each increment of tooth movement. This clinical investigation is instrumental in refining the many processes involved in the production of, and treatment with the new orthodontic system. A 25-year-old female subject with a Class II division 1 malocclusion is presented to illustrate the orthodontic capabilities of this appliance.
HERPES SIMPLEX VIRUS THYMIDINE KINASE/GANCICLOVIR-BASED GENE THERAPY IN HUMAN ORAL CANCER CELLS

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Cationic lipids have proven to be efficient vectors for the delivery of therapeutic genes to various epithelial cell types. Oral Squamous Cell Carcinoma (OSCC) is a well-diagnosed epithelial malady with a high rate of morbidity due to insufficient early diagnosis and treatment. A number of gene therapy strategies using viral and non-viral vectors for the treatment of OSCC are being explored. Our long-term goal is to deliver the plasmid-based Herpes Simplex Virus thymidine kinase (HSV-tk) "suicide gene" to OSCC cells using lipid-DNA complexes. Human oral cancer cell lines, HSC-3, H357, and H413, were provided by Drs. R. Kramer and R. Stern (UCSF). Transfection efficiency was optimized first in HSC-3 and H357 cells, using different transfection reagents and either the pCMV.SPORT-β-gal or pCMVlacZ plasmid, expressing β-galactosidase (β-gal). Forty eight hours after transfection, the cultures were fixed in paraformaldehyde and the number of cells expressing β-gal, as demonstrated by conversion of the substrate (X-gal) to a blue product, was determined. Four cationic lipid formulations were used: Lipofectamine 2000 (GIBCO-BRL), Escort (Sigma), Fugene (Boehringer Mannheim), and GenePORTER (Gene Therapy Systems). Fugene and Escort+Transferrin (Tf) provided the highest efficiency of transfection. Less than 10% of HSC-3 and less than 5% of H357 cells were positive for β-gal staining. We then investigated if the delivery of the HSV-tk gene into these cells, via either Fugene or Escort+Tf, followed by ganciclovir treatment, would result in cell killing. Cell viability was quantified using the Alamar Blue assay. We observed the killing effect in the presence of ganciclovir, despite the low efficiency of transfection. In HSC-3 cells, viability was reduced to 20% with either Fugene or Escort+Tf following incubation with ganciclovir for 8 days. In H357 cells, viability was 20% with Fugene and 50% with Escort+Tf after 9 days of treatment. We conclude that (i) lipid-DNA complexes may be used for suicide gene therapy of OSCC and that (ii) the high percentage of suicide, despite the low efficiency of transfection, is likely due to diffusion of phosphorylated ganciclovir into neighboring cells via gap junctions (the "bystander effect").
METHYLENETETRAHYDROFOLATE REDUCTASE: MUTATIONS IN SOUTH AMERICA
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One in every 550 newborns is born with an orofacial cleft. This category of birth defects consists of cleft lip (CL), cleft lip and palate (CLP), and cleft palate only (CP). Orofacial clefts are not directly life-threatening conditions, however, the multidisciplinary treatment is complex, challenging, expensive, and lasts for many years. An average lifetime medical cost in the USA is ~$100,000 per individual born with an orofacial cleft.

It has been shown that folates play an important role in the etiology of dysraphic congenital anomalies (i.e. neural tube defects and orofacial clefts), and also in their prevention. Methylenetetrahydrofolate reductase (MTHFR) is one of the most important enzymes in the folate metabolic pathway. It is essential for converting homocysteine into methionine, which is a donor of a methyl group used for methylation of proteins and DNA.

The goal of our present study was to conduct a pilot study investigating the prevalence of the MTHFR C677T and A1298C mutations in a sample of individuals with nonsyndromic CL and CLP from South America. Our simultaneous goal was to learn the methodology of DNA extraction from dried blood spots on filter paper, PCR and MDE gel electrophoresis techniques used for analysis of MTHFR polymorphisms. Altogether, 70 specimens were analyzed, 14 specimens for the C677T polymorphism, 52 for the A1298C polymorphism, and 2 for both polymorphisms of the MTHFR gene.

The 677TT genotype was found in 12.5% of cases and the 677CT genotype in 25.93% of cases. However, results from unaffected controls are relatively evenly distributed in the remaining one quarter. Males were more often found to have cleft lip with or without cleft palate (CL±P) than females, but the reverse was true for CP.

The median age of cases and controls was the same (13.9 vs. 14.1 years). However, we found a significant difference between the mean maternal age of cases and controls (25.8 vs. 26.4; P=0.005), as well as between the mean paternal age of cases and controls (28.7 vs. 31.4; P=0.000003). Mothers who were 20 years old and younger and also fathers who were 20 years old and younger had the highest risk of having a child affected with NSCL/P. Detailed analysis of epidemiological characteristics was done for subgroups according to cleft diagnosis. A strong relationship was found between the young paternal age and unilateral NSCL/P (P=0.006). A similar association was found between the young age of mothers and unilateral NSCL/P (P=0.014) and bilateral NSCL/P (P=0.022). The data sets from two different South American Sites, namely Antofagasta and Chillan in Chile, were then compared. In both samples, one half of the mothers who gave birth to a child with bilateral NSCL/P were 24 years old or younger. Similar studies are currently being conducted in other areas of South America. It will be interesting to compare the data from Antofagasta and Chillan with the data from other sites, in order to evaluate general and specific relationships between environmental factors and NSCL/P prevalence in offspring.

These results will help us to understand etiology of cleft anomalies in South America and thus enable us to develop preventive measures to decrease the prevalence of these serious congenital anomalies.

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EDUCATION ABOUT CLEFT PALATE FOR ASIAN FAMILIES

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There is no better field of medicine than dentistry to show that prevention is the best investment, one that can go far beyond the prevention of dental caries. Recent gains in knowledge brought by the Human Genome Project moved us closer to understanding the etiology and thus prevention of birth defects. The most common birth defects in the orofacial region are cleft lip and palate anomalies. These serious birth defects affect 1 in every 550 newborns. In some populations, the prevalence is even higher, particularly among American Indians and Asians. The goal of our project was a building of public awareness in the field of orofacial cleft anomalies in Asian families living in the San Francisco Bay Area, California, specifically in Chinese who speak Mandarin, in Koreans, and in Japanese. We developed an educational material on etiology and prevention of cleft lip and palate anomalies for Mandarin, Korean and Japanese speaking families who had a child with orofacial cleft. We took into consideration differences in cultural background of each of these ethnically different populations. Culture differences. Even though there are obvious cultural differences between individual Asian populations, several common characteristics exist that need to be taken into consideration. Asian men are more active in social-economic activities and women are more responsible for family’s diet and health, children’s education, and all sorts of matters regarding family. Women are often blamed and also have guilty feelings, if they have a child with a congenital or acquired defect.

Prevalence of orofacial clefts among Asians in California. Cleft lip and palate anomalies are known to occur in a higher prevalence among Asian Populations based study of orofacial clefts in California (Croen et al, 1998) analyzed maternal- paternal combinations of race and ethnicity and showed that the effect of race and ethnicity is not modified by parental gender. The study also revealed, that foreign-born Chinese women had a slightly decreased risk of CLP compared to US-born Chinese women. Similar prevalence was observed among foreign-born and US-born Japanese and Korean women. Conclusions: It is extremely important to consider cultural differences, customs and taboos in any educational activities focused on prevention of cleft lip and palate anomalies in Asian families. Usual approach, successfully used in Caucasian families, must be modified in order to respect cultural background. Any issues related to family planning must respect leading men’s role in majority of Asian families. When prevention protocol requires changes in the life style and dietary pattern, sensitive approach - respecting often deep cultural roots - has to be taken to deliver the message. The development of the original pamphlet in English and Spanish was supported by March of Dimes Community Service Grant. Additional support for development of Asian versions was received from Department of Orthodontics, University of the Pacific School of Dentistry. This Table Clinic was presented in February 2001 at the UOP Anslmar Conference and at the CDA Convention in Anaheim, CA, in April 2001.

SULCUS COLLI REGION ONTOGENY AND MANDIBULAR NERVE (V3) ANESTHESIA

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Nerve block anesthesia is commonly employed in mandibular dental procedures for pain control. Injection methods allow one to serially block the inferior alveolar (IA), mylohyoid (MH), and lingual nerves. Clinically acceptable IA anesthesia can be as low as 80%, due in part to anatomical considerations. In countering this problem clinicians employ modified insertion methods or different techniques. Harvey (1970) summarized available methods, finding 11 suggested points of entry, 6 horizontal and 8 vertical bearings, 11 depths of penetration, 9 positions of the foramen, and 12 target areas! The same basic method is used in subadults and adults. In all cases the basis for successful IA block rests on an anatomical foundation. Review demonstrates that the literature on mandibular nerve anatomy is insufficient for providing appropriate levels of understanding of ontogenetic, geographic, or idiotype variation, particularly in the sulcus colli region. To delineate bony medial ramal anatomy we are acquiring dimensional data from a large ontogenetic series (N= 380), but here focus on our 8.0-10.0 year-old sample. With reference to literature on craniofacial growth, median ramal anatomy, and block procedures, we employ 39 measures to delineate morphology relevant to IA blocks. We also made distribution maps of IA-associated foramina. Bony observations are supplemented with dissection data, focused on the sphenomandibular (SM) ligament and associated cervical fascia and medial pterygoid muscle. We found that: (1) the SM ligament-fascia thickenings comprise a multi-layered sheet and numerous neurovascular related fibrous tubes, the latter having multiple bony attachments within and below the sulcus colli; (2) variation in the medial pterygoid muscle insertion results in ontogenetic changes which affect bony and soft tissue relations of the IA and MH nerves; (3) there is extensive variation in the position of the mandibular foramen relative to both anterior and posterior ramal landmarks used in IA injections; (4) the mandibular foramen is generally positioned anterior to the middle of the ramus. We conclude that: (1) SM ligament variation both isolates nerve branches and influences anesthetic flow; (2) attachment variation in the medial pterygoid muscle affects nerve position and access to the IA nerve; (3) bony variation of the medial ramus significantly affects needle insertion position and depth of needle penetration; and (4) mandibular foramen positioning is more anterior than generally recognized. Modification of IA block technique is required to address these anatomical variants.

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