4TH ANNUAL
UOP RESEARCH DAY
& STUDENT RESEARCH
COMPETITIONS

biomedical, clinical & educational research

PROGRAM &
ABSTRACTS

TUESDAY MAY 28 2002
ORTHO RESIDENT PRESENTATIONS

FACULTY & STUDENT TABLE CLINICS

SENIOR & ADA/DENTSPLY
RESEARCH COMPETITIONS
FOURTH UOP RESEARCH DAY
AND
STUDENT RESEARCH COMPETITIONS
PROGRAM AND ABSTRACTS

MAY 28, 2002
PROGRAM

PRESENTATIONS BY ORTHODONTICS RESIDENTS

12 - 2 pm

ROOM 103

12:00    Masoud Haghi* and Kenneth Snowdowne
EVIDENCE FOR THE EXISTENCE OF A pH-DEPENDENT
CALCIUM CHANNEL IN OSTEOBLASTIC-LIKE MC3T3-E1
CELLS

12:20    Scott Chong* and Sean Carlson
DIFFERENCES IN TOOTH DIMENSION MEASUREMENTS
BETWEEN PERiapICAL X-RAYS AND PANORAMIC X-RAYS

12:40    Paul Lund
RELIABILITY OF MEASUREMENTS ON PLASTER AND
ELECTRONIC MODELS

1:00     Steve O’Hara*, Kenneth Snowdowne, Alexander Vakula and Casimir Leknius
LIPOSOME CONTROLLED DELIVERY OF ANTIBIOTICS USING A
STABILIZING MATRIX

1:20     Jeff Roberts
EVALUATION OF TEN YEAR POST TREATMENT RECORDS
WITH THE PAR INDEX

1:40     Shahryar Sefidpour*, Sean K. Carlson, Sheldon Baumrind
THE RELATIONSHIP BETWEEN MANDIBULAR LENGTH AND
CHIN PROMINENCE
FACULTY POSTERS AND TABLE CLINICS

2 - 4 pm

CAFE PACIFIC

Leigh C. Anderson* and John R. Garrett

FLOW IN THE STREPTOZOTOCIN-DIABETIC RAT: EVIDENCE FOR IMPAIRED ENDOTHELium DEPENDENT VASODILATATION

Joel A. Cohen*, Per Lyngs Hansen, Rudi Podgornik and V. Adrian Parsegian

SURFACE-Grafted PEG POLYMERS Osmotically COMpressed BETWEEN PHOSPHolIPID MULTILAYERS OBEY BRUSH SCALING LAWS

Barbara Dorocka-Bobkowska, Krystyna Konopka and Nejat Düüzgunes*

THE INFLUENCE OF ANTIFUNGAL POLYENES ON IN VITRO ADHERENCE OF CANDIDA TO EPITHELIAL CELLS

James S. Dower, Jr.*, Brian Kenyon and Kenneth Louie

PREPARATION PRACTICAL EVALUATIONS WHEN TWO FACULTY EVALUATE EACH PREPARATION

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DERMATOLOGIC REACTION FROM BONDING AGENT ON A DENTIST’S GLOVED HAND

Nejat Düüzgunes*, Elizabeth Pretzer, Hanna Cho, Khaja Azhar Hussain and Arun K. Ghosh

INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 REPLICATION BY UIC-PI, A NOVEL HIV-1 PROTEASE INHIBITOR

Stefan Highsmith*, Will Bernt and Bruce Weiner

ACTIVE SITE CONTROL OF MYOSIN CROSSBRIDGE ZETA POTENTIAL

Hee Soo Oh*, Viviana Armentano, Terezie Mosby, Javier Mir and Marie Tolarova

OBLIQUE FACIAL CLEFTS
Nancy R. Shine, Susan C. Wang, Krystyna Konopka, Elizabeth A. Burks, Nejat Düzgünés* and
Christian P. Whitman

NEWLY CLONED SECRETORY LEUKOCYTE PROTEASE INHIBITOR (SLPI)
INHIBITS HIV-1 INFECTION OF MONOCYTIC THP-1 CELLS
Sheldon Baumrind, Kevin Noris, Katie Bales* and Rebecca Doucet*
DIGITAL ANALYSIS OF TOOTH MOVEMENT WITH RESPECT TO SKELETAL STRUCTURES DURING ORTHODONTIC TREATMENT

Leif Cobain*, Viviana Armentano, Hee Soo Oh, Javier Mir, Terezie Mosby, Amaya Bustinduy and Marie Tolarova
CLEFT LIP AND PALATE IN CHILLAN, CHILE

Leif Cobain*, Shahram Nabipour* and Gary D. Richards
THE ROLE OF SUTURES IN FRONTOFACIAL GROWTH: EVIDENCE FROM THE METOPIC SUTURE

Joshua Erickson*, Alex Vakoula and Leigh Anderson
CHRONIC CONSTRICTION OF THE ION AND THE EFFECTS OF NEUROPEPTIDE FF IN THE NUCLEUS CAUDALIS

Gil Grio*, Barbara Plowman and Leigh Anderson
MORPHOLOGIC ANALYSIS OF THE INFRAORBITAL NERVE FOLLOWING INJURY: LONG-TERM EFFECTS OF CHRONIC CONSTRICTION

Alexander J. Kim*, JoMarie Monzon, Krystyna Konopka and Nejat Düzgünes
GENE DELIVERY TO ORAL CANCER CELLS BY LIPID-DNA COMPLEXES CONTAINING HUMAN SERUM ALBUMIN AND PROTAMINE

Matt Milnes*, Krystyna Konopka and Nejat Düzgünes
TRANSCRIPTION FACTOR NF-κB BINDING SITES IN THE CYTOMEegalovirus PROMOTER AS AN INHIBITOR OF HIV REPLICATION

Tokiko Mito Long*, Terezie Mosby, Javier Mir, Amaya Bustinduy and Marie Tolarova
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Peter Shelley*, Alexander Vakoula and Leigh Anderson

GLIAL CELLS AS POTENTIAL THERAPEUTIC TARGETS IN OROFACIAL NEUROPATHIC PAIN

Ruth Veinote*, Leigh Anderson and Alexander Vakoula

INFLAMMATORY HYPERSENSITIVE IN A RAT MODEL OF OROFACIAL NEUROPATHIC PAIN IN SYMPATHETICALLY DEPENDENT
Tamer Alpagot, Kerri Font* and Aaron Lee
LONGITUDINAL EVALUATION OF GCF IFN-GAMMA LEVELS AND PERIODONTAL STATUS IN HIV+ PATIENTS

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

Sydney Moore*, Madelyn Olson* and Gary D. Richards
MENTAL FORAMEN POSITION: ONTOGENETIC CHANGES AND RELATIONSHIP TO CRANIOFACIAL GROWTH THEORY

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

ORTHODONTICS RESIDENTS
EVIDENCE FOR THE EXISTENCE OF A pH-DEPENDENT CALCIUM CHANNEL IN OSTEOBLASTIC-LIKE MC3T3-E1 CELLS

Masoud Haghi* and Kenneth Snowdowne

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

Several years ago Dr Snowdowne’s laboratory published an observation that a mild rise in extracellular pH causes a rise in cytoplasmic calcium (Ca$_i$) in MC3T3-E1 pre-osteoblasts. To do this they loaded the mouse osteoblastic-like cell MC3T3-E1 with aequorin, a calcium-sensitive luminescent protein and then entrapped the cells in a flow-through cuvette of a photometer. Ca$_i$ increased when the perfusate around these cells was made mildly alkaline & decreased when it was made mildly acidic.

Our goal was to identify the molecular mechanism(s) involved in this evoked rise in Ca$_i$. I followed the changes in the rate of calcium efflux from MC3T3-E1 cells occasioned by the change in pH. I also used well-known blockers of various types of calcium channels to attempt to identify the pH sensitive channel.

Our calcium efflux data showed that alkalinity does not influence the activity of the calcium pump. The major surprise in our finding was that Cobalt (one of the Lanthanides we used and a known blocker of L-type channels) blocked the L-type membrane channel of our cells, thus inhibiting the alkalosis-evoked rise of Ca$_i$. At the same time in a second experiment Nifedipine (also a known blocker of the L-type channel) failed to block the channel and had no inhibitory effect on the rise of Ca$_i$. Further investigation will be necessary to further clarify the nature of the pH-dependant calcium-conducting channel.
DIFFERENCES IN TOOTH DIMENSION MEASUREMENTS BETWEEN PERIAPICAL X-RAYS AND PANORAMIC X-RAYS

Scott Chong* and Sean Carlson

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

Periapical and panoramic x-rays are used regularly for dental diagnosis and treatment planning. Each x-ray creates a different type of image, and exposes the patient to a different amount of radiation. Although both types of x-ray are capable of visualizing the dentition, it is unclear whether the two may be substituted for each other. In this study, twenty-four orthodontic cases with post-treatment study casts, panoramic x-rays, and full mouth series of periapical x-rays were compared for length and width measurements. The two different types of x-ray showed differences in dimensional measurements that varied significantly both by location and by magnitude. The variability of dimensional measurements found in this study suggests that using panoramic x-rays to estimate actual tooth dimension is not advised.
LIPOSOME CONTROLLED DELIVERY OF ANTIBIOTICS USING A STABILIZING MATRIX

Steve O’Hara, Kenneth Snowdowne, Alexander Vakula and Casimir Leknius

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The present study was designed to investigate the feasibility for controlling the release of antibiotics from liposomes using a stabilizing matrix. We are also interested in finding which liposomes remain intact at 37 degrees C for one week and could therefore maintain therapeutic levels of antibiotic in vivo. Liposomes are spheres of phospholipids that can be used to carry antibiotics without changing the efficacy of the drug. Matrices such as agarose, alginate and atrigel can form a physical barrier to liposomal breakdown by macrophage and maintain the antibiotic in the area of infection for an extended period of time. A solution was heated to 37 degrees C and passed over one of three matrices containing four different liposomes with antibiotics bound inside. This “crevicular flow” was captured and the kinetic loss of antibiotic from each matrix was calculated. The kinetic loss of an antibiotic substitute from each of these matrices using various liposomes proved that the rate of loss could be changed dependant on the type of matrix used. The Agarose matrix was successful in maintaining therapeutic levels of antibiotic for an average of 20 days and I feel that this should be investigated further for clinical use in localized treatment of oral infection and periodontitis. Alginate and Atrigel had rapid kinetic loss of antibiotic indicating that they would not be able to maintain a therapeutic level necessary for treatment of infection.
THE RELATIONSHIP BETWEEN MANDIBULAR LENGTH AND CHIN PROMINENCE

Shahryar Sefidpour*, Sean K. Carlson and Sheldon Baumrind

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Cephalometric measurements of mandibular size and chin prominence have commonly been used to describe the lower jaw in the sagittal dimension. Several studies of functional appliance therapy to “encourage” mandibular growth have reported positive changes in these two measurements as evidence of successful treatment. Therefore it is easy to assume that an increase in mandibular length would be reflected by a proportional increase in chin prominence. However, other investigators have demonstrated that the relationship between mandibular length and horizontal position of the chin is unclear. In this study, pre and post-treatment cephalograms from a sample of 181 growing class I and class II patients were analyzed to develop a better understanding of the relationship between mandibular size and horizontal chin position, and to determine whether there are significant differences between males and females or Class I and Class II malocclusions. This study provides evidence that there is an association between static measurements of mandibular length and chin prominence. However, an increase in mandibular length is not necessarily associated with a corresponding increase in chin prominence. The results showed that there are no statistically significant differences in increased chin prominence between boys and girls or Class I or Class II malocclusions. This study also showed that there is strong static and dynamic relationship between soft tissue chin prominence and the underlying hard tissue prominence. Our data shows that horizontal movement of soft tissue pogonion followed the hard tissue pogonion with nearly a 1:1 ratio. This observation is in agreement to findings of other investigators.
ABSTRACTS

FACULTY
NEURAL REGULATION OF SUBMANDIBULAR GLAND BLOOD FLOW IN THE STREPTOZOTOCIN-DIABETIC RAT: EVIDENCE FOR IMPAIRED ENDOTHELIUM DEPENDENT VASODILATATION

Leigh C. Anderson* and John R. Garrett

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OBJECTIVES: Blood flow in the rat submandibular gland is under the control of sympathetic and parasympathetic nerves, but neural regulation of vascular tone is mediated, in part, via endothelium-dependent mechanisms. Given the central role of endothelium-derived relaxing and constricting factors and the deleterious effects of diabetes on endothelial cell function, we hypothesized that diabetes would significantly impair neural regulation of submandibular gland blood flow in the rat. METHODS: A total of 39 male Wistar rats (initially weighing 150-200 grams) were used. Three weeks after the induction of streptozotocin-diabetes relative blood flow through the glandular tissue was measured in perfusion units (p.u.) using laser Doppler flowmetry. Parasympathetic stimulation (2, 5 and 10 Hz) was delivered via the chorda-lingual nerve. For sympathetic stimulation, the right sympathetic nerve trunk was stimulated at 2 Hz continuously or 20 Hz in bursts of 1s every 10 s. RESULTS: Mean resting blood flow was higher in diabetic than in control rats (148 ± 21 p.u. and 113 ± 26 p.u., p<0.005). Continuous 2 Hz sympathetic stimulation resulted in similar reductions in blood flow in both control and diabetic rats (-31% and -22%, respectively). However, the magnitude and the duration of the after-dilatations were significantly reduced in diabetic animals. The same number of impulses delivered at 20 Hz in bursts also resulted in vasoconstriction, but unlike the effects of burst stimulation in control rats, the initial vasoconstriction was not converted to a net vasodilatation. Parasympathetic stimulation resulted in vasodilatation, but the initial response was delayed in diabetic rats and the maintained phase was also significantly reduced in magnitude (P<0.02). CONCLUSIONS: Blood flow responses to sympathetic and parasympathetic stimulation are altered in streptozotocin-induced diabetic rats. The vasoconstriction evoked by sympathetic impulses was unaffected, but vasodilatory responses, particular those associated with endothelium-derived NO, are significantly impaired.
SURFACE-GRAFTED PEG POLYMERS OSMOTICALLY COMPRESSED BETWEEN PHOSPHOLIPID MULTILAYERS OBEY BRUSH SCALING LAWS

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Membranes with “fuzzy” or “hairy” surfaces are attracting increasing interest in biology, medicine, dentistry, and therapeutics. Many biological cells, particularly red blood cells, have fuzzy glycosylated surfaces. The surfaces of intracellular macromolecules such as microtubules have peptide hairs that are essential to their function. Stealth® liposomes employ surface-grafted hydrophilic polymers such as polyethylene glycol (PEG) to create a fuzzy coating that protects them from immune-system recognition. These liposomes are in current use as intravenous drug-delivery vehicles, and are being considered for dental antibiotic applications.

A physical understanding of the behavior of hydrophilic polymers end-attached to biological surfaces is thus important. When the attached polymers are long enough and close enough, they are said to form a surface “brush”. A physical theory of surface-polymeric brushes, known as brush scaling theory, is currently well-accepted (Alexander, 1977; de Gennes, 1978). The applicability of brush scaling theory to polymer-grafted phospholipid membranes has been tested by surface-force measurements (Kuhl et al., 1994) and osmotic-stress measurements (Kenworthy et al., 1995). However, fits of established scaling laws to these data were not successful. Until now there has been no explanation for this failure.

In the current work, we provide the sought-for explanation by analyzing the criteria for validity of the brush scaling laws. We show that in order for the scaling laws to work, the polymer concentration must be high enough that the polymers are in the so-called “semi-dilute regime”, where polymer chains interpenetrate and their physical properties become independent of molecular weight. By analyzing bulk osmotic pressure data for PEG polymers free in solution, we are able to determine, for each-size polymer, the brush grafting density at which semi-dilute behavior sets in. It is shown that most data reported by the previous authors do not meet the semi-dilute criterion, thus lie outside the scaling regime. By re-analyzing those few data points that do meet the correct criterion, we find that scaling theory does give successful fits. Moreover, these fits provide new information on the distances between grafting sites on the membrane surface. Our new fits, now based on rigorous theory, indicate that the packing densities of grafted polymers on phospholipid membrane surfaces approach a polymer-dependent saturation limit -- an extremely useful result.

By careful analysis of the physics underlying polymer-brush scaling theory, we have shown that PEG polymers grafted on membrane surfaces indeed obey established brush scaling laws.

This work was presented at the 46th annual meeting of the Biophysical Society, San Francisco CA, February 2002, and has been submitted for publication in the Biophysical Journal.
THE INFLUENCE OF ANTIFUNGAL POLYENES ON IN VITRO ADHERENCE OF CANDIDA TO EPITHELIAL CELLS

Barbara Dorocka-Bobkowska¹,², Krystyna Konopka¹ and Nejat Düüzgünès¹*

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Adherence of Candida species to mucosal surfaces is implicated as the first step in the pathogenesis of oral candidiasis. We examined the adherence of Candida albicans and Candida glabrata to HeLa cervical carcinoma and HSC-3 oral squamous cell carcinoma cell monolayers, following a brief exposure to sublethal and minimum inhibitory concentrations (MICs) of three polyenes, amphotericin B, nystatin and natamycin. The adherence of C. glabrata to these cells was significantly less than that of C. albicans. Significant reduction of adherence was observed for the tested polyenes at the MICs and sublethal concentrations, except for the effect of nystatin on the adherence of C. glabrata to HeLa cells. Amphotericin B was the most effective against both Candida species, reducing the adherence by ~50 and ~60%, at the MICs and sublethal concentrations, respectively. Our data suggest that sub-therapeutic levels of polyenes that are likely to persist in the oral cavity following topical treatment may modulate candidal colonization.

Supported by a grant from the Kosciuszko Foundation (New York). Presented at the 31st Annual Meeting of the American Association for Dental Research, March 6-9, 2002, San Diego, CA.
PREPARATION PRACTICAL EVALUATIONS WHEN TWO FACULTY EVALUATE EACH PREPARATION

James S. Dower, Jr.*, Brian Kenyon and Kenneth Louie

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Our preclinical operative dentistry course allows students to appeal the evaluation of their practical examinations on amalgam preparations. Previously one faculty member evaluated the practical by scoring the student’s handling of 22 criteria and initialed the form so that the student and course director knew who did the evaluation. If the student disagreed with the evaluation given they had an opportunity to appeal the evaluation and have it reevaluated by three instructors with the average of the new evaluations becoming the “final evaluation”. Feeling there was too large a number of evaluations being appealed, the following year we had two faculty members independently review the preparations, come to an agreement on the evaluation given, and place their initials on the form. It was hoped this would increase student confidence in the faculty evaluations and improve the calibration of faculty doing the evaluations. There were nine practical examinations each year with eight of the practicals on the same teeth and surfaces. With one instructor evaluating the preparation there were 177 appeals from 135 students for a ratio of 1.31 appeals per student. With two instructors evaluating the preparation there were 165 appeals from 139 students for a ratio of 1.18 appeals per student. This represents a 9.5% decrease in the number of appealed preparations. We believe the decreased number of appeals is based on increased student confidence in the accuracy of the evaluations and on the faculty being better calibrated for evaluating practical preparations. Another measure of student preference for having two evaluators was found on the end of course survey where 94% of the class recommended the two evaluator system be continued in the course.

This abstract has been presented at the ADEA Meeting, March 2002, San Diego, CA, and the University of the Pacific Celebrate Research & Creativity Poster Session, April, 2002, Stockton, CA
DERMATOLOGIC REACTION FROM BONDING AGENT
ON A DENTIST'S GLOVED HAND

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Several articles have been written on dentists having allergic reactions from direct contact with latex gloves and bonding agents. Some dentists place bonding agent on their non-dominant gloved hand for use on instruments to keep the composite restorative material from sticking to the instruments. This case report is the first documentation of a dermatologic reaction from bonding agents placed on the dentist’s gloved hand. It is not currently known if this reaction can cause a sensitization to either latex or bonding agents.

This table clinic has been presented at the 2002 UOP Asilomar Conference in Pacific Grove.
INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 REPLICATION BY UIC-PI, A NOVEL HIV-1 PROTEASE INHIBITOR

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1Department of Microbiology, University of the Pacific School of Dentistry, San Francisco, CA and 2Department of Chemistry, University of Illinois at Chicago, Chicago, IL

UIC-PI (1) is an inhibitor of the human immunodeficiency virus type 1 (HIV-1) protease, and was developed via structure-based design. The EC50 and EC90 of the compound in acutely-infected H9 cells were <1 nM and ~1 nM, respectively. In chronically infected H9/HIV-1IIIB cells, the EC50 and EC90 were 20 nM and 50 nM, respectively. The efficacies of UIC-PI and the currently used protease inhibitor saquinavir (2) were compared in H9/HIV-1IIIB cells. Viral p24 levels in culture supernatants were an order of magnitude lower with UIC-PI than with saquinavir.
ACTIVE SITE CONTROL OF MYOSIN CROSSBRIDGE ZETA POTENTIAL

Stefan Highsmith1*, Will Bernt2 and Bruce Weiner2

1Department of Biochemistry, University of the Pacific, School of Dentistry, San Francisco, CA and 2Research Division, Brookhaven Instruments Corporation, Holtsville, NY

The electrical properties of contractile proteins contribute to muscle structure and perhaps function, but have not been characterized adequately. Electrophoretic mobility, $m_e$, is sensitive to the net electric charge and hydrodynamic size of a molecule in solution. We measured $m_e$ for nucleotide complexes of skeletal muscle heavy meromyosin (HMM) and subfragment 1 (S1). The results indicate that $m_e$ for HMM changes depending on the ligand bound in the active site. The changes in electric charge appear to occur mainly on the S1 moieties. For HMM(MgATP)$_2$ and HMM(MgADP.Pi)$_2$ the values of $m_e$ are -0.077 and -0.17 (mm/s)/(V/cm), respectively. When Pi dissociates from HMM(MgADP.Pi)$_2$ to form HMM(MgADP)$_2$, $m_e$ decreases to -0.61 (mm/s)/(V/cm). Increasing [Pi] increases $m_e$ for HMM(MgADP)$_2$ to values near those observed for HMM(MgADP.Pi)$_2$. For HMM alone, $m_e = -0.34$. MgADP binding to HMM decreases $m_e$ to -0.57 (mm/s)/(V/cm), and the dissociation constant is 9 mM. Taken together, these data indicate $m_e$ is controlled by ligand binding to the active site. Possible roles that the observed nucleotide-dependent changes in cross-bridge electric charge might have in the contractile cycle in muscle are considered.
OBlique FACIAL CLEFTs

Hee Soo Oh*, Viviana Armentano, Terezie Mosby, Javier Mir and Marie Tolarova

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

Congenital anomalies of the face, oral cavity and cranium belong to the most common - and due to the affected region - the most serious congenital anomalies. Among them, atypical facial clefts form a small subgroup (0.3%; Tolarova, 1998). Their prevalence has been estimated as 1 per 20 000 to 70 000 births (Kawamoto, 1976). The most common type is an oblique facial cleft constituting approximately 0.22% of all facial clefts (Natsume, 1999).

Atypical facial clefts - in contrary to typical orofacial clefts (cleft lip, cleft lip and palate, and cleft palate alone) - usually are not due to a failure of fusion of facial embryonic processes. These rare anomalies are very probably the result of some environmental insult to the developing embryo. In other words, atypical facial clefts are caused by exogenous (environmental) factors that compromise a normal genetically programmed development of the face in an early embryonic stage. Many reports suggest that an amniotic band can cause an oblique facial cleft. Atypical facial clefts are sporadic, with exception of midline facial anomalies, in which familial occurrence has been observed.

The treatment of severe facial anomalies, and atypical facial clefts specifically, has been significantly advanced by French plastic surgeon, Paul Tessier, who developed their classification (Tessier, 1976) and several surgical procedures for their repair. Atypical clefts belong to craniofacial anomalies that are most challenging for a treatment. Their treatment is always complex. In general, it follows basic principles of reconstructive surgery. However, it definitely presents real challenges for reconstruction. A multidisciplinary approach is the key to the successful treatment outcome. Among other specialists, dental professionals together with surgeons are usually the key players in the long term medical care of these patients.

We are presenting five individuals (3 males and 2 females) affected with oblique clefts. Four patients (3 males and 1 female) were from Caracas, Venezuela, and one patient (female) was from Guatemala City, Guatemala. All patients were seen during Rotaplast medical missions to South and Central America. Interestingly, all patients showed oblique facial clefts on the left side of face. In four cases, the facial cleft was accompanied by cleft lip and palate. Two patients showed a combination of oblique facial cleft (Tessier type 3 and 4) and transversal cleft (Tessier type 7). No family history of congenital anomalies was reported in four patients. No history of smoking, drugs, and alcohol consumption during pregnancy was reported in three patients; however, no information was available in other two.

Further studies of atypical facial clefts are necessary to find out more about causes of these rare anomalies. Also, long term follow up studies are needed to choose a treatment protocol with the best functional and esthetical outcome.

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.
NEWLY CLONED SECRETORY LEUKOCYTE PROTEASE INHIBITOR (SLPI) INHIBITS HIV-1 INFECTION OF MONOCYTIC THP-1 CELLS

Nancy R. Shinel, Susan C. Wang\textsuperscript{2}, Krystyna Konopka\textsuperscript{1}, Elizabeth A. Burks\textsuperscript{2}, Nejat Düzgünes\textsuperscript{1*} and Christian P. Whitman\textsuperscript{2}

\textsuperscript{1Department of Microbiology, School of Dentistry, University of the Pacific, San Francisco, CA and \textsuperscript{2Medicinal Chemistry Division, College of Pharmacy, The University of Texas, Austin, TX.}

The ability of the salivary protein SLPI to inhibit HIV-1 infection in vitro was reported previously and led to the suggestion that SLPI may be partially responsible for the low oral transmission rate of HIV-1. Contradictory results were also published, however. These discrepancies can be attributed to factors ranging from the variability of macrophages to HIV infection to the quality of commercially available SLPI. To resolve these differences and to study further the anti-HIV-1 activity of SLPI, the protein was expressed from a synthetic gene, re-folded and purified. This newly cloned SLPI reduced HIV-1Ba-L infection of differentiated human monocytic THP-1 cells, in contrast to observations with commercially available SLPI. While the two proteins displayed different anti-HIV effects, they had comparable anti-protease activity. The identification of THP-1 cells as a system that supports HIV replication inhibitable by a new preparation of SLPI, sets the stage for a thorough investigation of the molecular and structural basis for the anti-HIV activity of SLPI.

\textit{Supported by a Grant from the Pacific Dental Research Foundation. Presented at the 46\textsuperscript{th} Annual Meeting of the Biophysical Society, February 23-27, 2002, San Francisco, CA.}
LIPOSOME-MEDIATED MACROPHAGE DEPLETION

Alex Vakoula* and Leigh Anderson

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

Neuropathic pain is associated with structural damage to primary afferent axons and their ensheathing glia, and with the accompanying inflammatory process. Macrophages play an important role in the inflammatory response through the production of neuroactive and neurotoxic substances, and suppression of macrophage function has been shown to attenuate the degree of inflammation. Several methods for depletion of macrophages have been proposed. The most effective method in terms of completeness of the depletion and lack of unwanted effects on non-phagocytic cells is based on liposome-mediated delivery of dichloromethylene diphosphonate (clodronate). Free clodronate does not enter cells in amounts sufficient to disturb their metabolism. However, once delivered into the phagocytic cell by means of liposomes, clodronate becomes cytotoxic. The purpose of this study was to replicate previously published experiments describing the systemic depletion of macrophages. Natural phosphatidylcholine—cholesterol liposomes loaded with clodronate were prepared and administered to adult rats by injection through a tail vein. Macrophage depletion was assessed by immunohistochemistry. Liver, spleen, and lymph node tissues were stained with anti-rat monoclonal antibodies ED-1 and ED-2. ED-1 stains tissue macrophages and also monocytes and dendritic cells, whereas ED-2 is specific for macrophages. A single-dose administration of clodronate-containing liposome preparation resulted in an almost complete elimination of macrophages from the liver, and in dramatically reduced macrophage counts in the spleen and lymphatic node. The future aim of this study is to test a similar protocol to eliminate or reduce the number of macrophages invading the injury site after constriction of infraorbital nerve. Reduced macrophage infiltration to the site of constriction should moderate the inflammatory response, which is thought to play a role in the development of neuropathic pain. This approach should provide new insights into the molecular mechanisms of neuropathic pain.
ABSTRACTS

ADA/DENTSPLY STUDENT RESEARCH COMPETITION
This study was designed to create a digital database that can be used to investigate and quantify tooth movement with respect to skeletal structures during orthodontic treatment. Pre-treatment and end-of-treatment cephalometric X-ray images from 24 males and 24 females between the ages of 10 and 14 were obtained from the orthodontic practice of Dr. Arthur Dugoni. Equally represented in this sample are Angle Class I subjects treated with and without extractions and Angle Class II subjects treated with and without extractions. The data will allow us to measure total tooth displacement with respect to the Anterior Cranial Base. We will also attempt to differentiate between tooth displacements within the jaws and tooth displacements associated with the general growth of the rest of the skull.

* Co-presenters
CLEFT LIP AND PALATE IN CHILLAN, CHILE

Leif Cobain1*, Viviana Armentano3, Hee Soo Oh1,2, Javier Mir2, Terezie Mosby2, Amaya Bustinduy2 and Marie Tolarova2

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Our previous study of orofacial cleft anomalies in the Chilean province of Antofagasta (Ellerhorst et al, 2001) showed that the young parental age played an important role in the etiology of these birth defects. Our goal was to make a similar case control study on genetic and environmental factors causing clefts in Chillan, another location in Chile. Ultimately, the results of such studies will help to focus our efforts towards primary prevention of these serious anomalies. We studied a clinical sample of 203 individuals (97 males and 106 females) from Chillan, Chile, who were affected with orofacial cleft and examined during the Rotaplast medical missions.

Results. Diagnosis. The total of 182 individuals had a nonsyndromic orofacial cleft (90 males and 92 females), 3 children (2 males and 1 female) were affected with Van der Woude syndrome, 3 children (1 male and 2 females) with Stickler syndrome, 1 female with EEC syndrome, 1 male with Down syndrome, and 1 female with Velocardiofacial syndrome. Robin sequence was observed in 2 females and holoprosencephaly sequence in 1 female. One female was affected with frontonasal dysplasia and one another female with skeletal dysplasia. Hemifacial microsomia was diagnosed in two males and 1 female and MCA of unknown etiology in one male and 3 females.

The vast majority of nonsyndromic cases (N=160) had cleft lip with or without cleft palate. Only 7 males and 15 females were affected with isolated cleft palate. The majority of patients were affected with a cleft lip and palate (75.27%). Isolated cleft lip occurred in 12.64% and isolated cleft palate in 12.09%. In the majority of cleft lip cases, the defect occurred on one side only. The cleft occurred on the left side almost twice as often as on the right side (63.7% vs. 36.3%). We observed practically the same proportion of males and females (sex-ratio, i.e. males to females ratio = 1.08) in our sample of patients. A slightly higher sex-ratio was observed for patients with cleft lip and palate (1.21). The usual predominance of females was seen in our sub-sample of isolated cleft palate patients (sex-ratio = 0.43).

The month of birth was evaluated for 161 patients. The lowest percentage of cleft patients were born in May (4.97 %). The highest frequencies fell into the period from October till February. Those probands were conceived in the first months of the calendar year (January through May).

Birth order. A proband was more likely to be the second or the first born child. There was no difference (p=0.096) in the mean birth order between unilateral (mean=2.94, SE=0.26) and bilateral cases (mean=2.4; SE=0.22). The mean birth weight of the patients was 3235.7 grams (SE=55.4). We found a higher birth weight for bilateral cases (mean = 3414.1 grams; SE=95.9) than for unilateral cases (3155.9 grams; SE=82.2). This difference was significant (p=0.026).

The mean maternal age at the birth of a child with an orofacial cleft was 26.5 years (SE=0.67). The youngest mother was 10 years old and the oldest 55 years old. A higher proportion of case mothers fell into the age category of 26-30 years. The mean paternal age at birth of cases was 30.2 years (SE=0.94). The youngest father was 14 years old and the oldest 61 years old.

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 ROLE OF SUTURES IN FRONTOFACIAL GROWTH: EVIDENCE FROM THE METOPIC SUTURE

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The life history of the metopic suture presents a unique opportunity to investigate the role of sutures in craniofacial growth. Firstly, the metopic suture differs from other neurocranial sutures by spanning the neural and facial skull and by its early fusion, as normally expressed. Secondly, this loss of sutural patency in early postnatal stages raises questions about the role(s) of sutures during neurocranial expansion and about the rate of intramembranous bone growth during this period of rapid neurocranial expansion.

To delineate this suture’s life history and address the above questions, we compiled a sample of 128 infants with developmental ages ranging from late fetal/newborn to 3.0 years of age. We measured 11 dimensions of the frontofacial region and quantified the fusion sequence of the suture. We also compiled data: (1) on the fusion sequence of the mendosal suture from individuals in the late fetal to early postnatal period; and (2) from individuals (N = 35) who retain a patent metopic suture in later growth stages.

We found that in most individuals the metopic suture begins its closure sequence endocranially in the mid-frontal region with ossification proceeding ectocranially and then posterosuperiorly and anteroinferiorly from this point. We recorded this fusion sequence in 28.5, 42.9, and 79.0% of infants aged as late fetal/newborn, 0.5 years, and 0.75 years of age, respectively. We observed that fusion is initiated at the point of maximum curvature of the frontal bone in the sagittal plane. A normally occurring variant of this pattern is that found in skulls which retain the suture into later growth stages. We determined that the latter variant results from a modification in the configuration of the dura mater which redirects forces to the sutural margins, rather than directly to the suture’s center as in most cases. In all cases, fusing or non-fusing, there was a rapid expansion of the frontal bone. We also found that maximum and minimum frontal breadth increase equally during this period. We conclude that: (1) the suture presents with a wide age range for the initiation of fusion; (2) in many individuals the metopic suture normally becomes functionally fused at or near birth; (3) cranial shape does not appear to modify the initial location or sequence of sutural obliteration; (4) sutural fusion does not appear to affect normal lateral cranial expansion; (5) remodeling rates in intramembranous bone can be very high, most probably equaling those found in endochondral bones of the cranium; and (6) mechanical strains induced in the suture by the combination of neural growth and dural attachment positioning provide a biomechanical trigger for the production of fibroblastic growth factor, the biomolecular precursor to sutural fusion.

Presented at the 80th General meeting of the IADR, March 6-9, 2002, San Diego, CA.

* Co-presenters
CHRONIC CONSTRICTION OF THE ION AND THE EFFECTS OF NEUROPEPTIDE FF IN THE NUCLEUS CAUDALIS

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**Objectives:** Neuropeptide FF (NPFF) is a non-opiate peptide that is associated with antinociceptive activities in the descending pain pathways of the central nervous system. It can be found in the brainstem primarily in two locations, the nucleus caudalis and tractus solitarius, both of which are involved in pain transmission. In the rat, chronic constriction injury of the infraorbital nerve leads to hyperalgesia and allodynia. Therefore, the purpose of this study was to determine whether neuropathic pain is associated with changes of NPFF in the trigeminal nucleus caudalis. Methods: Male, Sprague-Dawley rats (N=10) were divided into three groups: 3 day CCI, 7 day CCI, and 7 day sham injuries. A unilateral constrictive injury to the infraorbital nerve was accomplished by placing a single loose ligature (5-0 chromic gut) around the IoN distal to the infraorbital groove. Brainstems were fixed and 50 μm sections were stained using an antibody for NPFF. The sections were mounted on glass slides and counterstained. The data were collected from digital photos of the sections. Discrete nerve terminals containing NPFF were counted in both the ipsilateral and contralateral nucleus caudalis.

**Results:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Upper</th>
<th>Middle</th>
<th>Lower</th>
<th>Average</th>
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<td>Left</td>
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<tr>
<td>3 Day (n=3)</td>
<td>134 ±32</td>
<td>143±24*</td>
<td>146±50</td>
<td>128±7*</td>
</tr>
<tr>
<td>7 Day (n=4)</td>
<td>97±24</td>
<td>90±20</td>
<td>90±16</td>
<td>77±25</td>
</tr>
<tr>
<td>7 Day Sham</td>
<td>99±14</td>
<td>93±10</td>
<td>95±9</td>
<td>89±18</td>
</tr>
</tbody>
</table>

Mean ± SD
* p <0.05, 3 day CCI vs Sham

**Conclusion:** Preliminary results suggest that NPFF increases 3 days after CCI, but that it decreases to sham injury levels by day seven. Data will need to be confirmed by increasing the number of animals tested. In addition, we will continue our research by looking at both earlier and later time points.
MORPHOLOGIC ANALYSIS OF THE INFRAORBITAL NERVE FOLLOWING INJURY: LONG-TERM EFFECTS OF CHRONIC CONSTRICTION

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OBJECTIVES: Partial deafferentation of peripheral sensory nerve territories is often associated with chronic pain conditions (i.e., neuropathic pain), and animal models, such as chronic constrictive injuries (CCI) of the sciatic nerve are used to study this phenomenon. Chronic pain behaviors (allodynia and hyperalgesia) also develop after constriction of the infraorbital nerve (IoN). However, unlike the sciatic nerve, which is a mixed sensory/motor/autonomic nerve, the IoN is purely sensory. The objective of this study is to determine whether partial deafferentation of the IoN nerve territory is associated with the maintenance of neuropathic pain behaviors. The purpose of this presentation is report on the use of applicable morphometric methods.

METHODS: Ten male Sprague-Dawley rats (initially weighing 250-300 grams) were used for these studies. Five rats received a CCI of the right IoN. A sham injury (exposure of the IoN) was performed in another 5 rats. A single ligature (5-0 chromic gut) was then tied loosely around the IoN as close to the infraorbital groove as possible. 12 weeks after injury, the nerves (injured and non-injured) were fixed for light and electron microscopy, and sections are being analyzed for nerve fiber density and size distribution.

RESULTS: One µm sections will be photographed at 400x magnification. Two-dimensional quantitative stereology will be carried out to determine nerve profiles per area (QA), the ratio of axonal to total area (AA) and the mean area of each nerve profile (AA/QA). Electron micrographs (1500x mag) will be used to determine the distribution of C, Aδ and Aβ fibers based on fiber diameter. The diameters of 150-200 nerve profiles will be determined using ImageTool© (University of Texas).

CONCLUSIONS: The stereological methods described here will be used for the quantitative analysis of nerve damage following IoN constriction.
GENE DELIVERY TO ORAL CANCER CELLS BY LIPI-DNA COMPLEXES CONTAINING HUMAN SERUM ALBUMIN AND PROTAMINE

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Oral squamous cell carcinoma (OSCC) is the most prevalent cancer involving the oral cavity and oropharynx. Our long-term goal is to deliver the Herpes Simplex Virus thymidine kinase (HSV-tk) “suicide gene” and other therapeutic genes for the treatment of OSCC. Cationic lipid-DNA complexes (lipoplexes) are being used increasingly as reagents for gene delivery both in vitro and in vivo. Advantages of lipoplexes include protection of DNA from degradation, delivery of large sizes of DNA up to chromosomal size, relative safety when compared to viral vectors, and ease of production on a large scale. Human squamous cell carcinoma (HSC-3) cells were transfected for 4 hours at 37°C with DOTAP/DOPE (Escort) + Albumin (“EA”), or Escort + Albumin + Protamine (“EAP”) in the presence of increasing concentrations of fetal bovine serum (FBS). The medium was replaced and the cells were incubated for another 48 hours in DME/10 complete medium. Efficiency of transfection was determined as luciferase activity using a luminometer (TD-20/20 Turner Designs). The data were expressed as relative light units (RLU) per ml of cell lysate. The use of EA, or EAP lipoplexes yielded increased transfection efficiency compared to plain Escort (“E”) lipoplexes. The EA and EAP complexes at higher charge ratios facilitated more efficient transfection in the presence of FBS. Experiments utilizing dynamic light scattering in a Coulter N4 Plus instrument indicated that Protamine condensed the DNA, thereby reducing the lipoplex size. Overall, results of our experiments indicate that i) transfection in the presence of serum can be enhanced by EA, and EAP lipoplexes compared to E lipoplexes, ii) higher charge ratios facilitate more efficient transfection in the presence of serum, and iii) Protamine condensation of DNA does not significantly increase the transfection efficiency.

Supported by a Research Committee Grant from the University of the Pacific School of Dentistry and a Grant from the Pacific Dental Research Foundation
TRANSCRIPTION FACTOR NF-κB BINDING SITES IN THE CYTOMEGALOVIRUS PROMOTER AS AN INHIBITOR OF HIV REPLICATION

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Human immunodeficiency virus (HIV) replication is regulated at the transcriptional level through the specific interaction of the viral regulatory protein Tat, binding to HIV mRNA, and cellular transcription factors (TFs) that bind to a variety of cis-acting DNA elements in the HIV-1 5' long terminal repeat promoter (LTR). A key cellular transcription factor is NF-κB, a major positive regulator of transcriptional activity at the HIV LTR. It has been shown that NF-κB-mediated transcriptional activation is necessary for the production of primary viral mRNAs, the first step in viral replication. Therefore, NF-κB is a potential target for anti-HIV therapy. Current decoy-based anti-HIV gene therapy models make use of an RNA decoy element to sequester RNA-binding viral factors. We propose an alternative approach, using the Cytomegalovirus (CMV) promoter element as a DNA decoy for cellular transcription factors co-opted by the HIV LTR promoter (Konopka et al., Gene 255, 235-244; 2000). Structural analysis of the CMV promoter element using the transfac database (http://bioinformatics.weizmann.ac.il/transfac/) demonstrates the presence of three NF-κB binding sites, suggesting that the CMV promoter may be capable of sequestering sufficient NF-κB to interfere with NF-κB-mediated transcriptional activation at the HIV LTR promoter.

To evaluate the possibility of NF-κB mediated competition specifically between the CMV and LTR promoter elements, we have co-transfected into HeLa cells the HIV pro-viral clone HXBt∆Bgl, and various effector plasmids containing the: 1) CMV promoter, 2) RSV promoter, 3), and 4) anti-NF-κB ribozyme under the transcriptional regulation of the CMV promoter. Viral p24 protein concentration in the supernatant was assayed by ELISA to determine the expression of HXBt∆Bgl. We also evaluated these effector plasmids using an LTR-Luciferase reporter plasmid, to validate that the effect of the effector plasmid on HIV production was mediated by the LTR promoter, rather than post-transcriptional regulation. Analysis of p24 and luciferase levels as a measure of HIV replication in the presence of the effector plasmids indicated that CMV promoter-containing plasmids reduced LTR-mediated expression, when compared to an RSV promoter-containing plasmid. Whether the anti-NF-κB ribozyme generated an additive effect, beyond the reduction in HIV expression mediated by the CMV promoter itself, was not evident.

To further evaluate the effect of NF-κB specific blockade on LTR-mediated transcription, we propose to co-transfect both HXBt∆Bgl and an LTR-Luciferase reporter plasmid with either (1) a mutant plasmid lacking the NF-κB DNA recognition sequences in the CMV promoter, and 2) an anti-NF-κB ribozyme plasmid under the transcriptional regulation of the RSV promoter.

Supported by a Student Research Fellowship from the American Association for Dental Research and a Research Committee Grant from the University of the Pacific School of Dentistry.
DESCRIPTIVE EPIDEMIOLOGY OF OROFACIAL CLEFTS. SANTIAGO DEL ESTERO, ARGENTINA

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The prevalence of orofacial clefts in Argentina is 19.2/10,000 births (1 in 520) according to WHO data. According to birth records from one large public hospital, one per 120 newborns had an orofacial cleft in the Northern Argentinean province of Santiago del Estero.

PURPOSE. Our goal was to conduct in that province a case control study in order to increase our knowledge on genetic and environmental causes of clefts and to focus our effort towards their primary prevention.

SAMPLE CHARACTERISTICS. This study is based on a clinical sample assembled during the Rotaplast medical missions. Our sample of cases consists of 165 males and 103 females affected with nonsyndromic orofacial cleft. The vast majority of cases (N=239) had cleft lip with or without cleft palate (CL/P). Only 15 males and 14 females were affected with an isolated cleft palate (CP). The sample of controls consists of 188 individuals (85 males and 103 females) from the same area.

RESULTS. Diagnosis. The majority of patients were affected with cleft lip and palate (CLP; 73.51%). Isolated cleft lip (CL) occurred in 15.67% and isolated cleft palate in only 10.82%. In the majority of cases with cleft lip, the defect occurred on one side only, with a predominance on the left side (61.4%). There were more bilateral than unilateral cases, and the ratio of bilateral to unilateral cases was higher for CLP than for CL (5.57 for CLP vs. 2.44 for CL). We observed more males than females (males to females ratio = 1.7) affected with CLP.

The month of birth was evaluated for 216 patients and for 122 controls. The highest percentage of patients were born in May through August (41%), having been conceived in the four last months of a year, the Spring in that region (September through December). In contrary, in the control sample, the same proportion of children were born in earlier months of the year (March – June), having been conceived during the Winter season. Cases tended to have a higher birth order than controls. Probands in our sample were most likely to be first born (24%). The proportion of first-borns in the control sample was higher (38.6%). The mean birth orders were significantly different when cases (mean=3.37, SE=0.15) and controls (mean=2.67; SE=0.18) were compared (P=0.0015). The mean birth weight of the cases was 3297.6 grams and of the controls was 3393.72 grams. The difference in the mean birth weight between the cases and controls was not significant (p=0.08). The mean maternal age at birth for children with an orofacial cleft was 26.7 years (SE=0.46). The youngest mother was 13 years old and the oldest 44 years old. The mean maternal age at birth for control children was 24.11 years (SE=0.49). There was a significant difference in mean maternal age between cases and controls (p=0.00008). The majority of our case mothers (53.3%) fell into age categories 26 years and older, compared to controls where only 33.5% fell into the same age groups. The mean paternal age at birth for cases was 31.2 years (SE=0.62). The youngest father was 11 years old and the oldest 63 years old. In the control group, the mean paternal age was 28.3 years (SE=0.63), with the youngest father being 14 and the oldest 52 years old. The difference in mean paternal age between cases and controls was highly significant (p=0.0006).

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.
GLIAL CELLS AS POTENTIAL THERAPEUTIC TARGETS IN OROFACIAL NEUROPATHIC PAIN

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Objectives: Patients presenting with orofacial neuropathic pain represent a significant challenge to the clinician. Current treatment methods include surgical, non-surgical, and pharmacological approaches that have shown only moderate success. Recent studies implicate microglia in the development and maintenance of chronic pain associated with spinal nerve injury (sciatic nerve). The purpose of this study was to determine whether glial cell activation also occurs in the brainstem following trigeminal nerve injury.

Methods: Male, Sprague-Dawley rats (n=8) were divided into 2 groups: constrictive injury (CCI) to the right infraorbital nerve (IoN) and sham-injury. Injury to the IoN was accomplished by placing a single loose ligature (5-0 chromic gut) around the nerve distal to the infraorbital groove. At 3 and 7 days, brainstems were fixed and activated microglia were visualized using an antibody (anti- RT1a) specific for microglial cells.

Results: Microscopic examination and analysis indicated a marked increase in microglial associated RT1a activity in all the trigeminal sensory subnuclei on the ipsilateral side of CCI rats at both 3 and 7 days. Only a diffuse, nonspecific activity was observed on the contralateral side of the brainstem. Sham injury rats showed no up-regulation of RT1a staining on either side.

Conclusions: The results of this study demonstrate that microglial activation occurs in the brainstem following trigeminal nerve injury. Thus, glial cells may also play a role in orofacial pain, and microglial cells may be potential therapeutic targets in treating neuropathic pain secondary to trauma.

*Presented at the Annual Meeting of the California Dental Association, April, 2002, Anaheim, CA.
INFLAMMATORY HYPERSENSITIVE IN A RAT MODEL OF OROFACIAL NEUROPATHIC PAIN IN SYMPATHETICALLY DEPENDENT

Ruth Veinote\textsuperscript{1*}, Leigh Anderson\textsuperscript{2} and Alexander Vakoula\textsuperscript{2}

\textsuperscript{1}Doctor of Dental Surgery Program and \textsuperscript{2}Department of Anatomy, University of the Pacific School of Dentistry, San Francisco, CA

Objectives: Injury to peripheral nerves often results in neuropathic pain, which manifests as chronic hyperalgesia and allodynia in humans and pain related behaviors in animals. The pain can be exacerbated by increased activity of sympathetic postganglionic neurons (sympathetically maintained pain). Recently, chronic constriction (CCI) of the Infraorbital nerve (ION) was shown to result in a hypersensitivity to a chemogenic challenge (formalin). The purpose of this study was to determine the effects of a sympathectomy on the response to inflammatory challenge following a ION injury. Methods: Male, Sprawley rats (N=21) were divided into two groups: Chronic constriction injury and sham-injury. An additional surgical sympathectomy took place on 5 of the animals with CCI and on 5 of the sham-injury animals. Injury to the ION was accomplished by placing a single loose ligature (5-0 chromic gut) around the ION distal to the infraorbital groove. After 21 days the responses to 50\textsubscript{1} of either saline or 2.5\% formalin injected into the right vibrissae pad were monitored continuously for 45 minutes. Behaviors were scored as Normal, Directed Rubbing, or Other (flinching, etc.). Results: In the sham injury rats there was a brief latency with an initial period of face rubbing, then a quiescent period, and finally a prolonged period of unilateral rubbing (525±69s). Other pain related behaviors were rarely observed. In contrast, CCI rats had an immediate response to formalin that involved both rubbing and flinching behaviors. As in the sham-injury rats this was followed by a quiescent period, and then by a prolonged period of rubbing (309±53s), and flinching and other pain related behaviors (799±153s). Thus, the total time recorded for pain-related behaviors was significantly greater in CCI rats than in sham-injury animals (p<.001). No pain behaviors were observed after saline injection in either CCI or sham-injury rats. In the animals that received a sympathectomy as well as a CCI, pain behaviors were similar to those seen after sham-injury. Conclusions: The data demonstrate that the hypersensitivity to the inflammatory challenge, which develops after constriction of the ION, is sympathetically dependent.
ABSTRACTS

SENIOR RESEARCH COMPETITION
LONGITUDINAL EVALUATION OF GCF IFN-GAMMA LEVELS AND PERIODONTAL STATUS IN HIV+ PATIENTS

Tamer Alpagot1,3, Kerri Font2* and Aaron Lee2

1Department of Periodontics, and 2Doctor of Dental Surgery Program, University of the Pacific School of Dentistry, San Francisco, CA and School of Dentistry, University of California, San Francisco, CA

Objectives: Loss of periodontal support and related tooth loss is a common finding among HIV+ patients. The etiology of this destruction may be an increase in the levels of pro-inflammatory cytokines and subsequent increase in periodontal disease activity. The purpose of this study was to investigate the association between gingival crevicular fluid (GCF) interferon (IFN)-gamma levels and measures of periodontal status in HIV+ patients.

Methods: Thirty-three HIV+ patients were recruited from the CARE clinic at the UOP School of Dentistry. Clinical measurements (gingival index, plaque index, bleeding index, probing depth and attachment loss) and GCF samples were taken from 8 sites of each patient at baseline and 6-month visits by means of sterile paper strips. GCF levels of IFN-gamma were determined by sandwich ELISA assays.

Results: Significantly higher GCF levels of IFN-gamma were found at progressing sites than in nonprogressing sites (p<0.001). A progressing site was defined as a site which had 2mm or more attachment loss during 6-month study period. GCF levels of IFN-gamma were highly correlated with viral load and clinical measurements taken at baseline and 6-month visits (0.001<p<0.01).

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

This study was supported by NIDCR grant DE12417 and UOP School of Dentistry DRE503-011.
SERUM INHIBITION OF FUGENE- AND TRANSFERRIN LIPOPLEX-MEDIATED GENE DELIVERY TO HSC-3 AND HEK293 CELLS

Alexander Lim1,2*, Aaron Lee1,2, Krystyna Konopka2 and Nejat Düüzgunes2

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Cationic lipid-DNA complexes (lipoplexes) are being used increasingly as reagents for gene delivery both in vivo and in vitro. Lipoplexes have been proven to be efficient vectors for the delivery of large sizes of DNA (up to chromosomal size) into various epithelial cell types. One limitation to the application of lipoplexes in vivo is the inhibition of gene delivery by serum. Our long-term goal is to deliver the plasmid-based HSV-tk “suicide gene” to Oral Squamous Cell Carcinoma (OSCC). With a high rate of morbidity, OSCC is the most common tumor involving the oral cavity and oropharynx. Human Squamous Cell Carcinoma (HSC-3) was obtained from Drs. R. Kramer and R. Stern (UCSF), and Human Embryonal Kidney (HEK293) from ATCC. HSC-3 and HEK293 cells were transfected with the pCMVLuc plasmid encoding luciferase using either Escort+ Tf or Fugene. Transfection was performed for 4 h at 37°C in the presence of various fetal bovine serum (FBS) concentrations. The medium was replaced and cells incubated for another 48 h in medium containing 10% FBS. Cell viability was quantified by the Alamar Blue assay and luciferase activity was determined. In this study, we have shown that transferrin (Tf)-lipoplexes had a higher transfection efficiency than plain lipoplexes (-Tf) in the presence of 6% FBS. The Tf-lipoplexes used in this study composed of transferrin complexed with DOTAP/DOPE (Escort). In addition, we have shown that the optimal condition of transfection was with a ratio of 6μl Fugene/2μg DNA for HSC-3 in the presence of 20% FBS. We have also determined that increasing the concentration of FBS from 20% to 60% did not result in further inhibition of Fugene-mediated transfection of HEK293. Our results indicate that (i) TF-lipoplexes facilitate transfection in the presence of serum; and (ii) substantial transfection can be achieved even at serum concentrations as high as 60% FBS.

Presented at the 31st Annual Meeting of the American Association for Dental Research, March 6-9, 2002, San Diego, CA.
MENTAL FORAMEN POSITION: ONTOGENETIC CHANGES AND RELATIONSHIP TO CRANIOFACIAL GROWTH THEORY

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Knowledge of mental foramen location is of import both to clinical dentistry and to understanding craniofacial growth. Clinically, it is essential to identify the foramen’s location during: (1) mental nerve anesthesia; (2) evaluating images of the mandible for the presence of pathological processes; and (3) surgical intervention in the canine-to-first-molar region. In craniofacial growth studies: (1) the foramen is employed as a registration point in quantitative studies; and (2) changes in foramen position and direction of opening are employed as supporting evidence for a periosteal sleeve model of mandibular growth. Observations on the mental foramen have been documented: (1) on dry bone samples; (2) on gross dissection of the neurovascular bundle in cadavers; and (3) by radiographic studies employing both dry bones and living and non-living individuals. The majority of this work documents the patterns observed in adults. Studies which document the growth trajectory of this foramen are few, and available works have very limited samples and employ age groupings which span broad ranges of time.

To address ontogenetic changes in the mental foramen and evaluate their applicability to models of mandibular growth we evaluated a large ontogenetic series of human mandibles (N = 429) which range in age from 6 months in utero to adults. Developmental ages were determined from radiographs and all ages were assigned on the basis of tooth calcification patterns. We employ seven metric measures and tabulate two non-metric features: foramen position relative to the developing dentition and direction of foramen opening.

We found that: (1) the mandible inferior to the foramen is more stable during growth than the portion superior to it; (2) there is little correspondence between the growth trajectories of regions superior and inferior to the foramen; (3) foramen breadth is less variable than the foramen height; (4) foramen positioning is not generally at the mid-point of mandibular corpus height; (5) direction of foramen opening varies from anterior to posterior; (6) direction of foramen opening does not shift continuously during growth but shows abrupt changes, especially in the 2.0-4.0 year-old age range; (7) neither corpus height nor breadth at the foramen are correlated with the direction of opening; and (8) position of the foramen relative to the dentition changes considerably during growth.

From these preliminary data we conclude that there appears to be a disjunction between the direction of opening of the foramen and the foramen’s position relative to the dentition. The direction of opening appears to be related to early postnatal growth of the mandible, whereas the position of the foramen becomes more posterior with age. These data raise questions about the applicability of the periosteal sleeve model to mandibular growth and indicate the possibility that a neutral zone exists in the mandibular corpus.

* Co-presenters
HERPES SIMPLEX VIRUS THYMIDINE KINASE / GANCICLOVIR-BASED NON-VIRAL GENE THERAPY IN HUMAN ORAL CANCER CELLS

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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. The purpose of this study was to deliver the plasmid-based Herpes Simplex Virus thymidine kinase (HSV-tk) "suicide gene" to OSCC cells in culture using cationic lipid-DNA complexes, which may have fewer safety concerns than viral vectors. Transfection of human oral cancer cell lines, HSC-3, H357, H413 and H376 was optimized using β-galactosidase (β-gal)-expressing plasmids and four transfection reagents: Fugene, Escort, Lipofectamine 2000, and GenePORTER. Two days after transfection, the cultures were fixed in parafformaldehyde and the number of cells expressing β-gal was determined. Fugene and Escort+Transferrin (Tf) provided the highest efficiency of transfection in oral cancer cells. About 40% and 10% of HSC-3 cells were positive for β-gal staining after transfection with Fugene and Escort+Tf, respectively. The delivery of the HSV-tk gene to HSC-3 cells by Fugene or Escort+Tf, followed by ganciclovir treatment for 8 days, resulted in >80% cytotoxicity, determined by the Alamar Blue assay. In contrast, only less than 5% of H357, H413 and H376 cells were positive for β-gal staining. Nevertheless, in H357 cells about 80% and 50% cytotoxicity was observed with Fugene and Escort+Tf, respectively, after 9 days of ganciclovir treatment. In H413 cells, viability was 40% with Fugene and 20% with Escort+Tf, after 9 days of treatment, while in H376 cells, viability was reduced to 60% only with Fugene. Thus, we observed the killing effect in the presence of ganciclovir, despite the very low efficiency of transfection. We conclude that (i) lipid-DNA complexes may be used for suicide gene therapy of OSCC and (ii) a high percentage of cell killing can be achieved despite the low efficiency of transfection, possible due to diffusion of phosphorylated ganciclovir into neighboring cells via gap junctions.

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