PACIFIC RESEARCH DAY & STUDENT RESEARCH COMPETITIONS

BSTR

PROFIL

TUESDAY JUNE 3 2003 ORTHO RESIDENT PRESENTATIONS

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FAGULTY & STUDENT TABLE GLINICS

SENIOR & ADA/DENTSPLY RESEARCH COMPETITIONS

PROGRAM

PRESENTATIONS BY ORTHODONTICS RESIDENTS

12:10 – 1:30 pm

Room 103

- 12:10 David S. Gilmore and Sean K. Carlson DIFFERENCES IN PERCEPTION OF MESIODISTAL ROOT ALIGNMENT BETWEEN EVALUATION ON STUDY MODELS AND PANORAMIC RADIOGRAPHS
- 12:30 Iris Kohlmann–Erdell and Marie M. Tolarová MATERNAL NUTRITION, FOLATE STATUS, MTHFR POLYMORPHISMS, AND OROFACIAL CLEFT ANOMALIES IN OFFSPRING
- 12:50Jason Larkin, Sheldon Baumrind, S. Curry and Sean Carlson3 DIMENSIONAL DIFFERENCES BETWEEN A STANDARDBOLTONPAIR AND TWO CRIL STEREO PAIRS
- 1:10 Brian Steinhoff and Sean Carlson DIFFERENCES IN THE RELIABILITY OF TOOTH LONG AXIS DETECTION BETWEEN PERIAPICAL AND PANORAMIC RADIOGRAPHS

FACULTY POSTERS & TABLE CLINICS

3:00 – 7:30 pm

Café Pacific

Tamer Alpagot EFFECTS OF CIGARETTE SMOKING AND ALCOHOL USE ON HIV-RELATED **ORAL LESIONS**

David W. Chambers THREE APPROACHES TO ANALYZING AND REPORTING THE RESULTS OF CLINICAL TRIALS

James S. Dower, Jr., Brian Kenyon, Kenneth Louie, Christine Tomaszewski and Edward Y. Chan

FEWER APPEALS OF PRECLINICAL OPERATIVE PRACTICAL EVALUATIONS

James S. Dower, Jr. PASSING THE AMALGAM SECTION OF THE CALIFORNIA STATE BOARD **EXAMINATION**

Miriam Gochin, Martin A. Case and R. Kip Guy A HIGH-THROUGHPUT BIOPHYSICAL ASSAY FOR DETECTION OF DRUG **BINDING TO THE HIV-1 GP41 COILED COIL CORE**

Mark S. Hagge, Ralan D.M. Wong, James S. Lindemuth and Walter C. Daniels FIVE CEMENTS' POST RETENTION FOLLOWING CANAL OBTURATION USING **ZOE SEALER**

Mark S. Hagge

USE OF SURGICAL TELESCOPES BY SENIOR DENTAL STUDENTS: A SURVEY

Mark S. Hagge, Scott C. Di Lorenzo, James S. Lindemuth, Mark A. Latta and James W. Smith **RETARDING COMPOSITE RESIN POLYMERIZATION WITH YELLOW PLASTIC FOOD WRAP**

Mark S. Hagge, James F. Simon, Gabriela Pitigoi-Aron and Larry G. Loos MODIFIED CLINICAL TEST CASE PROGRAM IMPROVES OPERATIVE DENTISTRY PERFORMANCE ON CALIFORNIA STATE DENTAL BOARD **EXAMINATION**

Valentina A. Khorosheva and Joel A. Cohen WHAT IS A MEMBRANE BRUSH?

Krystyna Konopka and Nejat Düzgüneş THE SV40 PROMOTER/ENHANCER ELEMENT IN TRANS STIMULATES **HIV-1 LTR-DRIVEN GENE EXPRESSION**

Krystyna Konopka, Nancy R. Shine, S.C. Wang, Nejat Düzgüneş and Chris P. Whitman ANTI-HIV ACTIVITY OF NEWLY CLONED SECRETORY LEUKOCYTE **PROTEASE INHIBITOR (SLPI)**

Alexander Vakoula and Leigh Anderson EVIDENCE FOR THE ROLE OF MACROPHAGES IN THE DEVELOPMENT OF **OROFACIAL NEUROPATHIC PAIN**

ADA / DENTSPLY STUDENT RESEARCH COMPETITION

5:00 - 7:30 pm

Cafe Pacific

Adrienne Brugos-Gunstream and Casimir Leknius A SYSTEM FOR THE QUANTITATIVE ANALYSIS OF SURFACE ROUGHNESS

Sherry A. Caraveo, Eric Fillmore, Baokhanh Nguyen, Laura Nichols and Marie Tolarova ASSOCIATION BETWEEN MATERNAL SMOKING AND ALCOHOL **CONSUMPTION AND CLEFT LIP AND PALATE ANOMALIES**

David J. Crippen and A. Jeffrey Wood **INITIAL PLAQUE SCORE AS AN INDICATOR OF PATIENT APPOINTMENT** COMPLIANCE

Basma Fallah, JoMarie Monzon-Duller, Krystyna Konopka and Nejat Düzgünes SERUM-RESISTANT HSV-TK/GANCICLOVIR GENE THERAPY IN ORAL CANCER **CELLS VIA NONVIRAL VECTORS**

Eric Fillmore, Sherry Caraveo, Baokhanh, Nguyen, Laura Nichols and Marie M. Tolarova THE EVALUATION OF SEASONAL INCIDENCE OF CHILDREN BORN WITH **CLEFT LIP AND PALATE IN BARQUISIMETO, VENEZUELA**

Joseph R. Kolody, David R. McDonough and Nejat Düzgünes THE EFFICIENCY OF ECOTRU AND TRICIDE DISINFECTANTS ON HARD SURFACES OF DENTAL UNITS IN A LARGE DENTAL SCHOOL CLINIC

Dave Martin, Dallen Phillips and Leigh Anderson **EFFECTS OF NITRIC OXIDE AND PROSTAGLANDIN INHIBITION ON** PARASYMPATHETIC VASODILATATION IN THE RAT SUBMANDIBULAR GLAND

Baokhanh Nguyen, Sherry Caraveo, Eric Fillmore, Laura Nichols and Marie M. Tolarová YOUNG OR OLD, WHEN TO HAVE KIDS? ASSOCIATION BETWEEN MATERNAL AGE AND NON-SYNDROMIC CLEFT LIP AND PALATE ANOMALIES

Laura Nichols, Sherry Caraveo, Eric Fillmore, Baokhanh Nguyen and Marie Tolarova **IS THE FIRST ALWAYS BEST?** A STUDY OF THE ASSOCIATION OF INCREASED BIRTH ORDER WITH **INCREASED INCIDENCE OF CLEFT LIP AND PALATE**

Reza Riahi, JoMarie Monzon-Duller, Krystyna Konopka and Nejat Düzgüneş PROTAMINE ENHANCES TRANSFERRIN-LIPOPLEX-MEDIATED GENE CANCER CELLS

Antonio Vera, Jonathan Wu, Mark Macaoay and Alan H. Gluskin A COMPARISON OF FIVE MOTORIZED FILING SYSTEMS INVESTIGATING AND CURVED CANALS

Brian J. Kenyon, Mark S. Hagge, Walter C. Daniels, Casimir Leknius and Scott T. Weed* DIMENSIONAL ACCURACY OF SEVEN DIE MATERIALS

Andrew Yap and Marie Tolarova SEARCHING FOR ENVIRONMENTAL FACTORS CAUSING CLEFT LIP AND PALATE IN VALDIVIA, CHILE

Maryam Aghchay[§] and A. Jeffrey Wood A PEDIATRIC PATIENT: CASE REPORT

* Presenter [§] 2nd year Student presentation, not participating in the ADA/Dentsply Competition

DELIVERY AND HSV-TK/GANCICLOVIR-MEDIATED CYTOTOXICITY IN ORAL

APICAL TRANSPORTATION WHEN TAKEN BEYOND THE APEX IN STRAIGHT

MANAGEMENT OF A MUCUS EXTRAVASATION PHENOMENON (MUCOCELE) IN

SENIOR RESEARCH COMPETITION

5:00 – 7:30 pm

Cafe Pacific

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

Alex Kim, JoMarie Monzon-Duller, Krystyna Konopka and Nejat Düzgüneş GENE DELIVERY TO ORAL CANCER CELLS BY LIPID-DNA COMPLEXES CONTAINING HUMAN SERUM ALBUMIN

Matt Milnes, Krystyna Konopka and Nejat Düzgüneş TRANSCRIPTION FACTOR NF-KB BINDING SITES IN THE CMV PROMOTER AS AN INHIBITOR OF HIV REPLICATION

Mark S. Hagge, Walter C. Daniels, Devin L. Nelson* and Marc J. Geissberger FRACTURE STRENGTH OF EMPRESS CERAMIC INLAYS UNDER THREE CONDITIONS

Peter Shelley, Alexander Vakoula and Leigh Anderson GLIAL CELLS AS POTENTIAL THERAPEUTIC TARGETS IN OROFACIAL NEUROPATHIC PAIN

Ruth Veinote, Alexander Vakoula and Leigh Anderson INFLAMMATORY HYPERSENSITIVITY IN A RAT MODEL OF OROFACIAL NEUROPATHIC PAIN IS SYMPATHETICALLY DEPENDENT

Yasmine Zaeni, Sean K. Carlson, Sheldon Baumrind and Robert L. Boyd DIFFERENCES IN THE REPRESENTATION OF MESIODISTAL ROOT ALIGNMENT BETWEEN PERIAPICAL AND PANORAMIC X-RAYS

ABSTRACTS

ORTHODONTICS RESIDENTS

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* Presenter

DIFFERENCES IN PERCEPTION OF MESIODISTAL ROOT ALIGNMENT BETWEEN EVALUATION ON STUDY MODELS AND PANORAMIC RADIOGRAPHS

David S. Gilmore¹ and Sean K. Carlson²

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This study looked at the reliability among five judges when evaluating root position upon examining two different record types. We set out to determine if a significant difference exists between clinicians' ability to diagnose root parallelism discrepancies using study casts or panoramic radiographs. Judges evaluated the mesiodistal root angulation for 24 patients. The judgments were made twice. We studied the intra- and inter-judge reliability. We found that the highest intra-judge reliability occurred when the judges looked at the radiographs only. Looking at the study models resulted in the next highest reliability, while comparing the findings when looking at both record types was the lowest. There was a wide variation in inter-judge reliability. Additionally, we found that the upper lateral incisors represented the region where the highest disagreements were found when looking at the two record types. These teeth were more commonly viewed as being either mesial or distal when evaluated radiographically, compared with evaluation of the study models.

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RELATIONSHIP BETWEEN DENTAL STATUS AND NUTRITION IN CHILDREN AFFECTED WITH OROFACIAL CLEFTS

Rebecca Keller¹ and Marie Tolarová^{2, 3}

¹Orthodontics Residency Program, ²Department of Orthodontics and ³Craniofacial and Cleft Prevention Program, School of Dentistry, University of the Pacific, 2155 Webster St., San Francisco, CA 94115

The purpose of this study is to describe the dental and nutritional status of a population of children affected with orofacial clefts. All data was collected during medical missions to Guatemala in March 2001 and 2002. Dental variables were assessed for a sample of 112 cases and 39 controls. Cases showed a significantly higher level of caries in permanent teeth (p=0.053) and primary teeth (p=0.059), though not to the level of statistical significance. Cases also displayed a significantly higher level of dysplasia in primary teeth (p=0.049). Nutritional variables were assessed for a sample of 100 cases and 43 controls subjects with completed nutritional questionnaires. Cases reported a statistically significant (p=0.040) lower level of fat in their diet than controls. Cases also reported a higher level of carbohydrates in their diet than controls, though this was not statistically significant (p=0.051). No other dental or nutritional variables were found to be significant.

MATERNAL NUTRITION, FOLATE STATUS, MTHFR POLYMORPHISMS, AND OROFACIAL CLEFT ANOMALIES IN OFFSPRING

Iris Kohlmann-Erdell¹ and Marie M. Tolarová²

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Genetic and environmental factors play important roles in the etiology of non-syndromic orofacial clefts (NSC). In order to increase our understanding of causes of NSC, we studied nutritional characteristics and two mutations affecting the key-enzyme methylenetetrahydrofolate reductase (MTHFR) in folate metabolism in 175 mothers who had a child affected with NSC and in 59 control mothers from Cumaná, Venezuela.

A personal interview with each mother was conducted using two interview instruments: the General Genealogical Questionnaire (information about genetic, medical and pregnancy history, lifestyle, and demographics), and the Food Frequency Questionnaire (specifically developed for this population and compatible with DietSys nutrition software). Blood specimens were collected, preserved on filter papers and analyzed for 677CT and 1298AC polymorphism of the MTHFR gene. Descriptive statistics, t-tests, Mann-Whitney U tests, ANOVA, x2 test, and logistic regression were used for statistical analysis. In all statistical tests, we accepted a p level of significance ≤ 0.05 .

For nutritional analyses daily caloric intake and 8 nutrients were evaluated in 54 case and 31 control mothers. Daily caloric intake of case mothers was significantly lower than that of controls. We observed a significantly higher intake of retinol, zinc, vitamin B1, vitamin B2, vitamin B3, vitamin B6, and folate in controls compared to cases. In case mothers, only values for vitamin B2 and vitamin A reached or exceeded RDI values (Recommended Daily Intake as specified by the American Dietetic Association). In control mothers, mean nutrient values except folate and vitamin B3 were above RDI. Nutritional parameters differed among cleft types: mothers of children with bilateral NSC showed a significantly lower intake of retinol, zinc, vitamin B1, vitamin B2, and folate than mothers of children with unilateral clefts.

Blood specimens were available for genotyping from 95 case mothers. Analysis of the 677CT and 1298AC MTHFR mutations revealed no statistically significant differences of frequencies of specific genotypes between case and control mothers (p = 0.1125 for 677CT and p = 0.1734 for 1298AC; χ^2 test).

Folate intake did not vary by mothers' genotype and we did not detect any significant interactions between the two mutations in our samples (p>0.05; ANOVA).

Based on this study, we suggest that nutritional factors may have a stronger impact on etiology of NSC in Cumaná, Venezuela, than two MTHFR mutations (677CT and 1298AC) analyzed in our research. Further studies, preferably on larger samples, including other candidate genes and focused on different populations, are needed to investigate the role of potential associations and interactions between nutritional and genetic factors in etiology of NSC.

3 DIMENSIONAL DIFFERENCES BETWEEN A STANDARD BOLTON PAIR AND TWO CRIL STEREO PAIRS

Jason Larkin¹, Sheldon Baumrind², S. Curry² and Sean Carlson²

¹Orthodontics Residency Program and ²Department of Orthodontics, School of Dentistry, University of the Pacific, San Francisco, CA 94115

This project compared the differences between the 3 dimensional cephalometric data acquired from 2D measurements using a co-planar lateral pair, a co-planar frontal pair, and a biplanar pair (standard Bolton pair) of x-ray images. The hypothesis was that there would be very little difference in the 3D data. All of the cephalometric x-rays for this study were taken using a calibrated stereo x-ray machine located in the Craniofacial Research Instrumentation Laboratory (CRIL). Metallic tie points were placed on the patient's face to facilitate merging data from the film pairs to create the 3D maps. Two sets of 3D data were collected from the bi-planar images. The root mean squares (RMS) of the X, Y, and Z coordinates were 2.395, 3.683, and 35.160 mm's respectively for the 1st set. The 2nd set of data was calculated by allowing the emitter location to float in space. The RMS of the 2nd set of data are 0.172, 2.204, and 2.530 mm's. After re-examining the calibrated stereo x-ray machine located in CRIL it was determined that the ear rod holder does not rotate around its center. This explains the large difference in the Z coordinate. This can only be determined in a calibrated system. The 3D coordinates for x, y, and z calculated from the co-planar pairs were within .2 mm. Although the differences in the 3D coordinates of the bi-planar pair are large compared to the co-planar pair, the data shows that a 3 dimensional map of a patient can be constructed using bi-planar images. However, the geometry of the system would have to be well known and it would need to be calibrated.

SKELETAL, DENTAL, AND SOFT TISSUE PROFILE CHANGES FOLLOWING HERBST APPLIANCE TREATMENT

Tyler Robison¹ and Donald Poulton²

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The Herbst appliance is a tooth-borne functional appliance for use in patients with Class II malocclusions; it positions the mandible forward into a Class I occlusion. The aim of this study was to investigate the Herbst's dental, skeletal and soft tissue effects on the profile of 21 Class II patients. The treatment group consisted of 9 males and 12 females between 8 and 14 years of age upon treatment initiation (average age 11.10 years). The radiographic data was collected before treatment, immediately following Herbst removal, and at one-year posttreatment. Cephalometric measurements of the treatment group were compared to an untreated contrast group. The results of the study showed that the Herbst produced measurable anterior repositioning of the mandible. Effects of the appliance also included a forward movement of the mandibular incisors and a decrease in the facial contour angle. Herbst appliance therapy has shown to be an effective instrument in the correction of Class II malocclusions. Conclusions coincided with results of previous studies.

DIFFERENCES IN THE RELIABILITY OF TOOTH LONG AXIS DETECTION BETWEEN PERIAPICAL AND PANORAMIC RADIOGRAPHS

Brian Steinhoff¹ and Sean Carlson²

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This study investigated if a tooth's perceived long axis was more reliably located on a panoramic radiograph or a full mouth series (FMX). Post-treatment radiographs from 47 patients were acquired from a private practice and were evaluated by three judges. The radiographs were digitized and the judges independently drew their perceived long axes using a computer program. Overall average angular differences were calculated for each tooth studied. We found that the panoramic film's difference was statistically significant when compared to the FMX for the following teeth: upper first and second bicuspid, lower second bicuspid, and lower central and lateral incisors. The poorest reliability between judges was 3.83° +/- 2.13 (SD) for the maxillary first molar on the panoramic radiograph, and the greatest reliability was 0.89° +/- 0.52 (SD) for the mandibular central incisor on the FMX. The greatest difference in reliability between the panoramic film and FMX was 1.17° +/- 2.16 (SD) for the maxillary first premolar, and the smallest difference in reliability was 0.06° +/- 2.53 (SD) for the maxillary canine. The results of this study suggest that even though there were statistically significant differences, these differences were clinically negligible; therefore the long axes can be reliably located on both a panoramic radiograph and a FMX.

FACULTY

ABSTRACTS

EFFECTS OF CIGARETTE SMOKING AND ALCOHOL USE ON HIV-RELATED ORAL LESIONS

Tamer Alpagot

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Objectives: The study aim was to identify the effect of smoking and alcohol use on HIVrelated oral lesions in a cross-sectional study. Methods: Oral examinations were performed to assess the prevalence of oral lesions in 152 HIV+ patients. Results: Oral candidiasis, pseudomembranous and/or erythematous, was the most common oral lesion among HIV+ patients (27.6%), followed by hairy leukoplakia (18.4%), exfoliative cheilitis (13.8%), linear gingival erythema (LGE) (11.1%), aphtous ulceration (9.8%). After adjusting for CD4 cell count and viral load, current smokers were significantly more likely to have oral candidiasis (OR = 2.45 and 95% CI = 1.94-3.52), hairy leukoplakia (OR = 2.19 and 95% CI = 1.45-3.14), exfoliative cheilitis (OR = 1.92 95% and CI = 1.42-2.61), LGE (OR = 1.71 and 95% CI = 1.33-2.29) and aphtous ulceration (OR = 1.68 and 95% CI = 1.31-2.24) than were current nonsmokers in HIV+ group. Alcohol consumption was a significant risk factor for the presence of oral candidiasis (OR = 2.27 95% CI = 1.62-3.25). Conclusion: The results suggest a strong association between cigarette smoking/alcohol use and the presence of specific oral lesions.

This study was supported by NIDCR grant DE12417

THREE APPROACHES TO ANALYZING AND REPORTING THE RESULTS OF CLINICAL TRIALS

David W. Chambers

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Traditionally, clinical trials are designed, analyzed, and reported based on the difference in means on a single dependent variable between experimental and control groups. If no difference is detected in means, no conclusion can be drawn; if a difference is found at a conventional statistical level (and the design is rigorous), it can be concluded that the principle or technology involved is effective.

Increasingly, the dental literature is also reporting some measure of effect in clinical trials. This is an advance over the dichotomous "significant/insignificant" designation of hypothesis testing. Confidence intervals allow for estimates of the likely range of results of outcome averages or of odds comparing the effects of treatments. This represents a higher level of usable information for practitioners who can determine the probability that a principle or technology will perform at a satisfactory level, when considered as groups of treatments to be averaged.

A third level of analysis is beginning to emerge. Called generalizability analysis, these statistical procedures answer two further questions: (a) what is the likely outcome range for a single application? and (b) what are the components of the result that most heavily influence the outcome?

Traditional and confidence interval approaches are prone to contain hidden sources of variation. A technology reported effective in controlled circumstances where one operator performed the procedures cannot support any claims about the relative magnitude of outcomes attributed to the technology, the operator, or the circumstances. Similarly, no claims can be made regarding potential interactions between the technology and various types of operator or various circumstances. Consequently, both traditional and confidence interval approaches to analyzing and reporting clinical trials tend to overestimate the impact of technology – commit Type I Error. This bias is reinforced by industry-sponsored clinical trials that must follow FDA guidelines and ADA advertising standards concerning comparative product claims.

A measure of technology robustness is proposed: Variance in outcome attributable to technology alone divided by all sources of variability that include the technology component.

FEWER APPEALS OF PRECLINICAL OPERATIVE PRACTICAL EVALUATIONS

James S. Dower, Jr., Brian Kenyon, Kenneth Louie, Christine Tomaszewski and Edward Y. Chan

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One of the interfaces between the students and faculty in preclinical operative dentistry courses is the evaluation of practical examinations. Our preclinical operative dentistry course has allowed students to appeal the evaluation of their practical examinations. The appeal process involves three faculty members independently evaluating the preparation without knowledge of the prior evaluation. After reexamining the preparations, recording three new evaluations and then seeing the original evaluation form and score, it was inconceivable why many were appealed. At the conclusion of the course the students were surveyed concerning why they appealed their preparations. 16% indicated they appealed because they did not believe the score could go lower and 8.5% appealed because the instructor, who was identified by their initials on the form, had a reputation for being "hard". In those cases the students were not appealing the evaluation based on criteria and inaccurate grading, and this in turn was frustrating to the faculty. These unwarranted appeals were also taking the faculty from their primary responsibility of direct student instruction. To remove these reasons for having an evaluation appealed, the next year the practical exams and appeal process was changed to provide anonymity of the grader and cause the person to lose a point if they appealed an evaluation and the score did not go higher. These changes resulted in significantly fewer "appeals". Although both classes had nine practical examinations on the same teeth and surfaces, there was a 45% reduction in the number of appeals. Prior to the changes there were 165 appeals from 139 students for a ratio of 1.18 appeals per student. The following year there were 89 appeals from 137 students for a ratio of 0.65 appeals per student.

(Presented at the ADEA Meeting, San Antonio, March, 2003)

PASSING THE AMALGAM SECTION OF THE CALIFORNIA STATE BOARD **EXAMINATION**

James S. Dower

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The state/regional board examination is one of the most important interfaces a person encounters on the road to practicing dentistry. The School of Dentistry has gone to great lengths to prepare the students to pass the California State Board Examination. The results of the examination have usually been very good to excellent yet varied through the years. A new "Practice for State Board Examination" course for the amalgam section was instituted for the Class of 2001 that resulted in the dental school's highest pass rate. The new course had two sessions on simulators within three months of the board examination and attendance was mandatory. In the first session, the students all prepared the same tooth for standardization of grading by the faculty. At some point in the preparation a faculty member made a defect into the axial wall that the student based to ideal. After the evaluation of the preparation the student placed an amalgam restoration for evaluation. The second session was within six weeks of the state board examination, and the student prepared and restored the tooth and surfaces they expected to do for the state board examination. The state board results on the amalgam section for the class prior to the new course were 25% (29/117) failures, a mode of 71, mean of 80.5 and standard deviation of 5.4. The Class of 2001 had 13% (16/123) failures, a mode of 75, mean of 78.5, and standard deviation of 4.6.

(Presented at the ADEA Meeting, San Antonio, March 2003)

A HIGH-THROUGHPUT BIOPHYSICAL ASSAY FOR DETECTION OF DRUG **BINDING TO THE HIV-1 GP41 COILED COIL CORE**

Miriam Gochin^{1, 2}, Martin A. Case³ and R. Kip Guy²

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The HIV-1 envelope protein gp41 plays a critical role in viral entry. It contains a rod-like coiled coil domain (HR1), which acts as a scaffold for the correct folding of a second (HR2) domain in formation of fusion-competent gp41. Drug or peptide binding to HR1 has been implicated in the prevention of the conformational change of gp41 to its fusogenic state. Thus, the peptide T-20 (Fuzeon) works by such a mechanism.

The search for non-proteolytic alternatives to T-20 can be greatly enhanced by the availability of a rapid test for drug binding to the coiled coil. We are developing a highthroughput fluorescence assay to monitor drug binding. The gp41 coiled coil core has been designed to contain a transition metal complex, which can act as a donor or acceptor in fluorescence resonance energy transfer (FRET). A peptide from HR2 which binds to HR1 has been labeled with a fluorophore at its C-terminal end. The close proximity between donor and acceptor that occurs during binding results in FRET and a reduction of fluorescence intensity of the donor. This change can be easily monitored, and adapted to a high-throughput screen using 396-well-plate technology. Then, large libraries of peptidic and organic compounds can be screened by competitive inhibition.

This work is supported by grants R00-UP-092 from the California University-wide AIDS Research Program and 106388-33-RGGN from the American Foundation for AIDS Research.

(Presented at the 16th International Conference on Antiviral Research, Savannah, GA, April 27-May 1, 2003)

FIVE CEMENTS' POST RETENTION FOLLOWING CANAL OBTURATION USING ZOE SEALER

Mark S. Hagge^{1, 2}, Ralan D.M. Wong¹, James S. Lindemuth³ and Walter C. Daniels²

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Objectives: To compare the retention of prefabricated posts luted with 5 different cements in post spaces prepared 4 weeks following gutta percha/ZOE sealer obturation. A previous study had been performed using a 1-week interval. In that study, the 4-META cement failed to polymerize. All remaining cements [Panavia-21 (P-21); Fleck's zinc phosphate (ZnP); Ketac-Cem (GIC); Parapost Bis-GMA/EMA (PC)] were significantly less retentive than P-21 used in unobturated controls. P-21 had significantly greater retention than ZnP and PC. GIC had retention not statistically different from other set cements. Although residual eugenol was suspected as an etiologic factor, the sealer had not completely set. Methods: Ninety-six singlerooted teeth were instrumented up to size 5 Gates-Glidden, and divided into 6 groups (n=16). Five groups were obturated using gutta percha/ZOE sealer. A control group remained unobturated. Post space preparation (10-mm deep; size 6 Gates-Glidden) and cementation (size 5 Parapost) were performed 4 weeks later. After 48 hours, the dowels were removed with an InstronÔ 5566 in tensile mode at 1 mm min-1. Data were analyzed using one-way ANOVA and Tukey test with significance set at p < .05. Results: The 4-META cement failed to polymerize. All (set) cements again had significantly lower retention strengths than unobturated controls (P-21). However, at 4 weeks, the relative strengths of P-21 and ZnP were reversed. ZnP demonstrated significantly greater retention than P-21 and PC. None of the other pairwise comparisons at 4 weeks were statistically significant. "4-week" ZnP was significantly more retentive than "1-week" ZnP. Conversely, "4-week" P-21 was significantly less retentive than "1-week" P-21. The ZOE sealer was completely set at 4 weeks. Conclusion: Residual eugenol and unset root canal sealer seem to have time-dependent and

separate adverse effects on the retentive capabilities of specific dental cements.

USE OF SURGICAL TELESCOPES BY SENIOR DENTAL STUDENTS: A SURVEY

Mark S. Hagge¹

¹School of Dentistry, University of the Pacific, San Francisco, CA 94115

Purpose: To examine the use of surgical telescopes (ST's) in a large senior dental student class where ST purchase is elective. Methods and Materials: The percentage of students who purchased ST's, factors that influenced those purchases, frequency/patterns of use, and purchase satisfaction were investigated. Results: The survey return rate was 85.3% (128/150). Among the respondents, 86% had purchased ST's; 14% had not. Only 38% (47/128) of students expressed complete satisfaction with the current level of instruction in ST selection and use. Forty-three percent (55/128) of respondents desired more instruction, while the remaining 20% (26/128) either did not recall or were unsure that any instruction had been provided. This finding should be considered by the appropriate dental school committee. The most frequent motive for ST purchase was advice received from others (43%), with ergonomic reasons (later determined to consist primarily of neck pain) cited by 21% of users. Fixed lens designs were selected more frequently (70%) than flip-down systems (30%). Nearly all ST purchases were made during the first year of dental school (95%), and low-power magnification (2-2.5X) was chosen by 88% of purchasers. ST's were used more often for operative dentistry, fixed prosthodontics, endodontics, and pediatric dentistry than for practice in other disciplines. ST users expressed a high level of satisfaction with their purchases (84%). The most common reason given for not purchasing ST's was being able to see well enough without them (9/18; 50%).

RETARDING COMPOSITE RESIN POLYMERIZATION WITH YELLOW PLASTIC FOOD WRAP

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Objectives: Dental unit-emitted light can rapidly polymerize highly light-reactive composite resins beyond the point of workability, preventing adequate time for the shaping and sculpting of large direct composite resin restorations. The purpose of this study was therefore to determine if the use of a yellow plastic food wrap over a dental unit light would retard the polymerization rate of a very photosensitive composite resin (Esthet-X, Shade A1, L.D. Caulk). Methods: 0, 1, and 2 thicknesses of yellow plastic food wrap (Reynolds Metals Company) were, in turn: (A) placed in a spectrophotometer and compared for percentage transmission of 470 nm light; (B) placed over a curing unit light wand and compared for mW output on a radiometer; (C) placed over a dental unit light positioned 25 inches away from .2 mm thick composite resin specimens, with FTIR readings then made every 40 seconds to measure degree of composite polymerization; (D) placed over a dental unit light positioned 25 inches away from a direct veneer preparation (clinical simulation) that was then restored by four experienced clinicians who reported working times. Results: (A) 0 sheets, 100%; 1 sheet, 34%, 2 sheets, 6%; (B) 0 sheets, 580 mW; 1 sheet, 190 mW, 2 sheets, 20 mW; (C) Percentage of carbon bonds formed: (at 1m20s) 0 sheets, 8%; 1 sheet, 0.65%; 2 sheets 0.76%; (at 2m00s) 0 sheets, 19.11%, 1 sheet, 12.22 %, 2 sheets, 0.42%; (D) Mean working times: 0 sheets, 34s; 1 sheet, 1m12s; 2 sheets, 2m35s. Conclusions: yellow plastic food wrap placed over a dental unit light markedly extended the working time of a highly light sensitive composite resin in each of four assessments performed. Two sheets of plastic wrap were more than twice as effective as 1 sheet.

MODIFIED CLINICAL TEST CASE PROGRAM IMPROVES OPERATIVE DENTISTRY PERFORMANCE ON CALIFORNIA STATE DENTAL BOARD EXAMINATION

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Purpose: To see if several modifications of an operative clinical test case program resulted in measurable changes in scores and pass rates of two operative dentistry procedures during the California State Dental Board Examination (CDBE). Methods and Materials: Overall CDBE pass rates as well as pass rates and distribution of scores for Class III composite resin and Class II silver amalgam preparations/restorations were examined for two consecutive graduating classes (2000 and 2001) from one dental school. As senior students, the class of 2000 performed operative test cases (OTC's) that had worked well for ten years. For the class of 2001, several changes were made to the OTC design. The number of required OTC's was decreased from 8 to 5; However, correspondingly increased attention was made with regard to: 1. structure [change to adjacent chairs in same clinic bay from open seating, limit to 8 students per session; change to limited clinic time (total of 4 half-day sessions from any open clinic)]; 2. grading standardization (possible instructor grading-pair combinations reduced from 30 to 3), and; 3. instructional techniques (increased intra-procedural observation with more feedback/error correction) used during clinical OTC's. Results: The overall CDBE pass rate increased from 91.66% (2000) to 94.66% (2001). The 2001 graduates demonstrated very slight improvements in both mean score and overall pass rate for composite resins (2000: mean score 80.30; pass rate 85.6%); (2001 mean score 80.37; pass rate 86.3%). Notably, however, while the mean score for amalgam preparations/restorations was slightly lower for 2001 graduates (2000: 78.40); (2001: 78.21), the 2001 graduates displayed a statistically significant increase (z = 1.685; p < .10) in the amalgam procedure pass rate (2000: 76.5%); (2001: 84.7%). Conclusion: A 30 percent reduction in numerical requirements for a clinical operative test case program did not adversely affect recent graduates' performance of two procedures during a state dental board examination. Concurrent quality improvements to the program were believed to have offset any potential negative effects from reduced numerical requirements.

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WHAT IS A MEMBRANE BRUSH?

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Virtually all eucaryotic cells have "hairy" or "fuzzy" surfaces. Fuzzy liposomes created with covalently-attached polymers have found therapeutic use as drug-delivery vehicles able to evade immune-system recognition. Dental applications for antibiotic therapy are currently being evaluated. However, a physical description of such coats on cell and liposome surfaces is lacking. Some unanswered questions are: How thick is a surface coat? What is the coat density profile in both the transverse and perpendicular directions? What are the hydrodynamic frictional properties of the coat? What degree of surface "coverage" does the coat provide?

We have undertaken a highly-controlled electrophoretic study of the surface hydrodynamic properties of liposomes bearing surface-grafted polymers of poly(ethylene glycol), i.e., $(-CH_2-CH_2-O)_N$, also known as PEG. The polymer lengths and grafting densities span the range of conditions known to produce "mushrooms" (where individual polymers are far apart and surface coverage is low) to those expected to form "brushes" (where the polymers are close together, coverage is high, and the coat forms a uniform surface layer). Previously we reported data and analysis for liposomes with surface-grafted PEG polymers ranging in size from N = 7 to 113 at a fixed grafting density of 10 mol% PEG-grafted lipids (Cohen and Khorosheva, *Colloids & Surfaces A* (2001) 195, 113-127). Electrophoretic mobilities were measured as a function of ionic strength between 0.5 mM and 100 mM.

The analysis showed the coat thickness in the expected brush regime to scale with N, which is consistent with scaling theory for surface brushes. However, the hydrodynamic frictional parameter f scaled with 1/N, which is *not* consistent with standard brush scaling theory: the theory predicts f = constant, independent of N. What is wrong?

The standard brush theory assumes a very simple monomer density profile in the brush: monomer density = constant out to a distance L (the brush "thickness"), where it abruptly drops to zero. To date, there have been no data to suggest that this picture is inadequate. We now believe we have such data. A recent theory suggests the monomer density profile in a brush is *parabolic*, not constant. At stake is the answer to the question: what is a membrane brush?

We have now extended measurements to different grafting densities. In terms of mol% PEG-grafted lipids: 10%, 5%, 2%, 1%, 0.5%. Analysis is in progress to determine the behavior of the parameters L and f vs. N at the various grafting densities. Valuable information regarding the "mushroom to brush transition" is also provided by plots of the electrophoretic mobility vs grafting density for fixed polymer sizes N.

An accurate physical description of fuzzy coats on membrane surfaces will constitute a major advance in the understanding of cell and liposome behavior *in vivo*.

THE SV40 PROMOTER/ENHANCER ELEMENT IN *TRANS* STIMULATES HIV-1 LTR-DRIVEN GENE EXPRESSION

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Mammalian expression vectors often utilize viral promoter/enhancer elements, including cytomegalovirus (CMV), Rous sarcoma virus (RSV), and simian virus 40 (SV40). Such constructs containing reporter genes are co-transfected together with effector gene constructs. The relative strength of viral promoters, however, may affect the expression of the tested construct. We have previously shown that the HIV-1 LTR- and SV40-driven expression is greatly reduced by the strong CMV promoter (Konopka et al. (2000) GENE 255: 235-244). Here we examined whether the SV40 promoter could influence HIV-1 LTR-driven gene expression. HEK293 cells were co-transfected with either the CMV plasmids [pCMV.SPORT-ßgal, pCMVlacZ, pCMV-luciferase, and pNGVL1-tk] or the SV40 luciferase plasmids [pGL3-Control Vector (CV), pGL3-Basic Vector (BV), pGL3-Enhancer Vector (EV), and pGL3-Promoter Vector (PV)] and the HIV-1 proviral clones HXB∆Bgl or pNL4-3, complexed with the transfection reagent Fugene. Viral p24 expression and luciferase activity were determined 2 days after transfection. Co-transfection of the CMV plasmids with both proviral clones, at a weight ratio of 4:1 (1.6 µg CMV plasmid, 0.4 µg HXBABgl) significantly reduced viral production when compared to co-transfection with the control Pol III U6 promoter. In contrast, under the same conditions, co-transfection of the SV40-CV with HXB∆Bgl, or pNL4-3 enhanced p24 production by 6- or 10-fold, respectively. The SV40 luciferase plasmids lacking enhancer and promoter sequences did not enhance the HIV-1 LTRdriven expression, as determined by p24 production. Transfection with any of the plasmids complexed with Fugene was not cytotoxic. The stimulation of HIV-1 LTRdriven expression by the SV40 enhancer/promoter may be explained by competition for negatively acting cellular transcription factors that downregulate HIV-1 transcription by binding to the negative regulatory element (NRE) of HIV-1 LTR. This observation points to the potential for misleading results in co-transfections involving SV40 constructs.

(Presented at the 42nd Annual Meeting of the American Society for Cell Biology, San Francisco, CA, December 14-18, 2002)

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ANTI-HIV ACTIVITY OF NEWLY CLONED SECRETORY LEUKOCYTE PROTEASE INHIBITOR (SLPI)

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Secretory leukocyte protease inhibitor (SLPI), a serine protease inhibitor present in saliva, has been shown to inhibit HIV infection of macrophages and primary T-cells. However, contradictory results have also been published and variable results were observed in our laboratory. All these studies have been performed using the same, commercially available, preparation of recombinant SLPI obtained from Synergen and/or R&D Systems. We examined the anti-HIV activity of a newly cloned SLPI using human macrophages and differentiated THP-1 cells. THP-1 cells were induced to differentiate by treatment with phorbol 12-myristate 13acetate (PMA). Macrophages and THP-1/PMA cells were infected with HIV-1BaL, in the absence or presence of SLPI. Cells were incubated with SLPI, for 30 min at 37°C prior to addition of the virus. SLPI was also present during the 2-h infection period. Over 90% inhibition of p24 production was observed in macrophages infected in the presence of 5, 10 or 20 μ g/ml of SLPI. At 0.5 or 1 µg/ml, SLPI reduced the p24 levels by ~70 to 80%. This potent inhibition of virus production was sustained for two weeks of culture. SLPI also significantly reduced p24 production in THP-1/PMA cells. At 5 or 10 µg/ml SLPI, ~95% inhibition of p24 production at 7 day and ~90% at 11 day post-infection was observed. The p24 viral protein was undetectable after 3 and 5 days. The availability of an active SLPI will enable the investigation of the biochemical and structural basis for its anti-HIV activity.

(Presented at the 16th International Conference on Antiviral Research, Savannah, GA, April 27-May 1, 2003)

EVIDENCE FOR THE ROLE OF MACROPHAGES IN THE DEVELOPMENT OF OROFACIAL NEUROPATHIC PAIN

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OBJECTIVES: Macrophage activation at the site of injury may play an important role in the development of neuropathic pain behaviors through the production of inflammatory mediators (TNFa, IL-1β, IL-6). This has led to the hypothesis that suppression of macrophage function and the attenuation of the inflammatory response will limit or abolish the development of neuropathic pain behaviors. The purpose of this study was to determine the effect macrophage depletion, using liposomally-delivered clodronate to induce apoptosis, on the development of hyperalgesia after infraorbital nerve (IoN) constriction in the rat. METHODS: Sprague-Dawley rats (240-260 grams, N=8) were anesthetized with pentobarbital (50 mg/kg i.p.). The right IoN was exposed and a single 5-0 chromic gut ligature was tied loosely around the nerve proximal to the infraorbital groove. In 5 animals, clodronate-loaded liposomes were administered via the tail vein: 1) Natural phosphatidylcholine-cholesterol liposomes were injected on the day of the surgery and 2) PEG-ylated liposomes were given 3 days later. Rats with constrictive injuries, but not receiving clodronate, served as controls. After 8 days, control and clodronate-treated rats were tested for the development of mechanical hyperalgesia. Macrophage depletion (spleen and injury site) was assessed by immunocytochemistry using an anti-rat monoclonal antibody ED-1. Analysis of the tissue sections was accomplished using NIH Image analysis software (Scion Corp.). RESULTS: Prior to injury, all rats exhibited only mild nocifensive responses to pin prick (1 or 2 quick face rubs and a sharp withdrawal of the head). After injury, control rats demonstrated an initial period of complete analgesia (3-4 days) followed by a conversion to hyperalgesia (vigorous directed grooming, escape and attack). In contrast, clodronate-treated animals did not experience complete analgesia and 4 of 5 treated animals failed to develop a mechanical hyperalgesia. In a 5th clodronate-treated rat nocifensive behaviors were observed but they were less exuberant than those seen in control rats. There was a dramatic decrease in the number of splenic macrophages in clodronate-treated rats compared with controls, and at the site of injury there was approximately a 50% reduction in the number of ED-1 reactive cells (p<0.02). CONCLUSIONS: Our results demonstrate a correlation between macrophages at a site of IoN constriction and the development of mechanical hyperalgesia. Thus, our data support the hypothesis that macrophages play an important role in the development of neuropathic pain behaviors following nerve injury

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ABSTRACTS

ADA/DENTSPLY STUDENT RESEARCH COMPETITION

A SYSTEM FOR THE QUANTITATIVE ANALYSIS OF SURFACE ROUGHNESS

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The use of an explorer for identifying and locating sub-gingival calculus is based on the qualitative evaluation of the dentist. This method, if done correctly can be effective. However, the qualitative nature of the test lends itself to large variations among dentists. If calculus is undetected or improperly removed periodontal defects will have impaired healing. For these reasons a quantitative detection system has been developed to determine the roughness of surfaces. The system analyzes sound waves from vibrations transmitted through an explorer moving across a surface. Correlation between surface roughness and the system analysis has been demonstrated. This system allows for accurate determination of surface roughness while recording the information and displaying it graphically on a computer.

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ASSOCIATION BETWEEN MATERNAL SMOKING AND ALCOHOL CONSUMPTION AND CLEFT LIP AND PALATE ANOMALIES

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The purpose of this study was to analyze the impact of five environmental factors (maternal smoking, alcohol consumption, socioeconomic status, place of residence and birth weight) on the occurrence of cleft lip and palate anomalies in their children. We studied 245 cases and 123 controls in Barquisimeto, Venezuela. We found a positive correlation between maternal smoking (p=0.006) and maternal alcohol consumption (p=0.002) during pregnancy and occurrence of cleft in their child. More case mothers smoked during pregnancy (19.8%) compared to controls (10.6%) and a higher percentage of case mothers reported alcohol consumption during pregnancy (12.9%) than controls (8.6%). We also found that case families were poorer, tend to live in villages or countryside, and their children's birth weight was significantly lower than in control children. The results of this study suggest that environmental factors play an important role in etiology of cleft abnormalities in Barquisimeto.

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INITIAL PLAQUE SCORE AS AN INDICATOR OF PATIENT APPOINTMENT COMPLIANCE

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High plaque score is widely recognized as predictive of a patient's likely restorative needs and future caries risk. This study evaluated high plaque scores as being predictive of patient compliance behaviors. We found that high initial plaque scores can be predictive of poor appointment compliance for subsequent recall appointments.

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SERUM-RESISTANT HSV-TK/GANCICLOVIR GENE THERAPY IN ORAL CANCER CELLS VIA NONVIRAL VECTORS

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Oral Squamous Cell carcinoma (OSCC) is the most prevalent cancer involving the oral cavity and oropharynx. Our goal is to use a gene therapy approach to treat OSCC. We examined the ability of a proprietary polyamine reagent, Gene Jammer, and a polycationic liposomal reagent, Metafectene, to deliver a licuferase-expressing plasmid (pCMVluc) to HSC-3 human squamous cell carcinoma cells in the presence and absence of fetal bovine serum (FBS), since it is important to achieve efficient gene delivery in the presence of biological milieu. We assessed gene delivery by measuring luciferase activity in cell lysates, expressed as relative light units (RLU)/ml of lysate. In initial experiments, we determined the optimal ratios of GeneJammer:DNA and Metafectene:DNA (µl:µg), which were 4 µl Metafectene:µg DNA and 6 or 12 µl GeneJammer:µg DNA. The presence of 10 % FBS was not inhibitory to gene expression in either system. We then examined the ability of Herpes Simplex virus thymidine kinase, expressed from its delivered gene (HSV/tk), to specifically kill HSC-3 cells in the presence of the antiviral drug ganciclovir. In the absence of serum, 100% cytotoxicity was achieved with both reagents. In the presence of 10% FBS, GeneJammer and Metafectene mediated 70% and 100% cytotoxicity, respectively. Metafectene caused significant cytotoxicity even in the presence of 60% FBS present during the transfection step. This observation suggests that Metafectene may be useful for the gene therapy of OSCC in animal models.

(Presented at the CDA Scientific Session, Anaheim, April, 2003)

THE EVALUATION OF SEASONAL INCIDENCE OF CHILDREN BORN WITH CLEFT LIP AND PALATE IN BARQUISIMETO, VENEZUELA

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Our study found seasonal variations in occurrence of cleft lip and palate anomalies in the Barquisimeto, Venezuela population. Our sample consisted of 236 cases and 122 controls. Distribution of cases during the year differed significantly from controls (p=0.050). Analysis in respect to the type of cleft revealed that this difference was due to highly significantly different distribution of CL cases (p=0.0015), while other analyzed cleft types (CLP and CP) did not show any significant difference from controls. The highest proportion of CL cases was born in January, February and March. The time of this critical period for cleft lip (5-6 weeks of prenatal development, i.e. 32-33 weeks before birth) fell to May, June and July. Some environmental factors occurring during those months could contribute to causes of cleft lip anomalies. Cleft lip anomalies are considered to have a higher proportion of environmental factors in their etiology than CLP and CP. Environmental causes that might cause an irregular seasonal distribution are: infectious agents, a cyclic variation in diet, seasonal change in nutrition, air pollution, purrity of water sources, amount of activity and exercise and changes in occupation throughout the year. A coincidence of the critical period for development of CL with the rainy season (May – July) seems to be interesting. Further studies are needed to determine what specific factors are involved in the increased seasonal prevalence of children born with CL in Barquisimeto, Venezuela.

The field work for this study was supported by funding from Rotaplast International, Inc. Processing and analysis of the data was supported by the Department of Orthodontics, University of the Pacific School of Dentistry.

(Presented at the CDA Scientific Session, Anaheim, April, 2003)

THE EFFICACY OF ECOTRU AND TRICIDE DISINFECTANTS ON HARD SURFACES OF DENTAL UNITS IN A LARGE DENTAL SCHOOL CLINIC

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Infection control has been one of the most important advances in dentistry in the past thirty years. There are many disinfectants available to dental practitioners to maintain the current standards of infection control. Several studies have examined the efficacy of disinfectants on various hard surfaces. We examined the efficacy of two disinfectants (Ecotru and Tricide) on three hard surfaces of a dental unit in a large dental school clinic. These three surfaces include a bench top adjacent to the patient dental chair, a plastic computer screen used to shield notebook computers from contamination, and the shoulder of the patient dental chair. Bacterial samples were obtained from three surfaces of ten dental units after patients had been dismissed from treatment. These surfaces were then disinfected with either Ecotru or Tricide using the spray-wipe-spray method. Bacterial samples were then taken again. The culture media used were blood agar plates and Hycheck slides containing a tryptic soy agar medium and a DE neutralizing agar medium. These samples were then incubated for 48 hours at 36°C. Bacterial colonies were quantified by placing the colony counts in one of five categories of bacterial density: no colonies, very low, low, medium, and high. Although both disinfectants were effective against bacteria on the dental unit hard surfaces, Ecotru appeared to be a more effective disinfectant than Tricide.

EFFECTS OF NITRIC OXIDE AND PROSTAGLANDIN INHIBITION ON PARASYMPATHETIC VASODILATATION IN THE RAT SUBMANDIBULAR GLAND

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OBJECTIVES: Endothelium-dependent mechanisms play a crucial role in the neural regulation of blood flow in rat submandibular glands through the production and release of vasodilatory and vasoconstricting factors. Chemical mediators, such as nitric oxide and prostaglandins, function in vasodilatation and thereby increase in microvascular blood flow. We hypothesized that inhibition of endothelial cell nitric oxide and prostaglandin synthesis would result in an inhibition of parasympathetically-mediated increases in blood flow within the rat submandibular gland (SMG). METHODS: A total of 11 male Sprague-Dawley rats (340-360 grams) were used. Parasympathetic stimulation was delivered via the chorda-lingual nerve at frequencies of 2, 5, and 10 Hz (6v, 2 ms). Laser Doppler flowmetry was used to measure relative blood flow in perfusion units (p.u.) through the SMG with and without inhibitors. After initial blood flow determinations, L-NAME was administered by continuous infusion (300µg/min, i.v.) to inhibit nitric oxide production. Indomethacin (1 mg/ml) was then administered i.p. to inhibit prostaglandin synthesis. Data were analyzed for statistical significance using one-way repeated analysis of variance followed by comparison of individual means (Bonferroni's t-test). RESULTS: In the absence of inhibitors, SMG blood flow calculated as the integrated area of perfusion values increased with increasing stimulation frequency (see Table). The administration of L-NAME partially blocked parasympathetic vasodilatation, and a further decrease in perfusion was seen after the addition of indomethacin.

-	2 Hz	5 Hz	10 Hz
Control	5,180 ± 4,530*	$14,340 \pm 6,830^{a}$	$20,710 \pm 7,6606^{a,b}$
L-NAME	$3,690 \pm 3,560^{\circ}$	$11,460 \pm 7,410^{c}$	$16,570 \pm 7,640^{\circ}$
L-NAME + Indomethacin	$2,640 \pm 2,880^{\circ}$	$9,520 \pm 6,760^{\circ}$	$15,440 \pm 7,580^{\circ}$
*min x p.u., mean ± standard devia	tion		

^a p<0.01 (5Hz vs 2 Hz); ^b p<0.01(10Hz vs 5Hz); ^c p<0.05 (treatment vs control)

CONCLUSION: Parasympathetic stimulation causes an increase in blood flow in the submandibular gland, and the magnitude of that increase was lessened by inhibiting nitric oxide synthesis. Vasodilatation was further diminished by the inhibition of prostaglandin production. Thus, both nitric oxide and prostaglandins play a role in parasympathetic regulation of blood flow in the rat submandibular gland.

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YOUNG OR OLD, WHEN TO HAVE KIDS? ASSOCIATION BETWEEN MATERNAL AGE AND NON-SYNDROMIC CLEFT LIP AND PALATE ANOMALIES

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Several studies suggested association existed between maternal age and congenital birth defects. Our study sample consisted of 201 cases, 107 males and 94 females, and 88 controls, 32 males and 56 females. The mean maternal age of the all cases was 25.8 years old (SD = 7.1). The youngest age was 11 years old and the oldest age was 54 years old. The mean maternal age of the controls was 27.05 years old (SD = 5.99). The youngest age was 14 years old. The oldest age was 40 years old. A very significant difference existed between the mean maternal ages of a child affected with cleft lip and/or palate and of controls (p = 0.032). An even more significant difference existed between CLP cases and controls (p = 0.016). Our analysis of maternal age by subgroups revealed that mothers 20 years old or younger were at an increased risk for having a child with orofacial cleft, specifically with CLP significantly (p=0.01, Odds ratio 2.32). The risk for having a child with CL was also increased for this age group. Mothers 40 years old and older had an increased risk for having a child affected with cleft lip and/or palate. Our results have shown that maternal age does play an important role in non-syndromic cleft lip and/or palate. Parental age at the time of conception, the health of the mother and the care given during the pregnancy may play huge roles in the causes of congenital abnormalities like this.

(Presented at the CDA Scientific Session, Anaheim, April, 2003)

IS THE FIRST ALWAYS BEST? A STUDY OF THE ASSOCIATION OF INCREASED BIRTH ORDER WITH **INCREASED INCIDENCE OF CLEFT LIP AND PALATE**

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Cleft lip and palate are among the most common congenital anomalies. Late birth order is associated with several complex disorders as well as birth defects. Among these defects is cleft lip and palate. Data from the sample of cleft patients in Barquisimeto, Venezuela supports the research linking cleft lip and palate incidence to late birth order. A comparison of 214 patients diagnosed with cleft lip (CL), cleft palate (CP) or cleft lip and palate (CLP) with 57 control participants with no history of cleft was statistically significant with a p-value of 0.04. This data also supports previous evidence of patients with clefts occurring with greater frequency children with birth rank of greater than 3 - 32% of cleft patients were of birth order greater than three compared to 19% of controls.

Several studies have found an increased association with the incidence of cleft lip and palate with increased maternal age. The association with increased birth order may be as a result of the increasing age of the mother with subsequent children. However, some studies have shown a negative association between maternal age and oral clefts with a positive association with cleft lip and palate - indicating that increasing birth order alone may increase the risk of a child being born with a cleft lip and/or palate.

(Presented at the CDA Scientific Session, Anaheim, April, 2003)

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PROTAMINE ENHANCES TRANSFERRIN-LIPOPLEX-MEDIATED GENE DELIVERY AND HSV-tk/GANCICLOVIR-MEDIATED CYTOTOXICITY IN ORAL CANCER CELLS

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Oral squamous cell carcinoma (OSCC) is the sixth most common cancer in the United States. Gene therapy approaches are being used in preclinical studies for the treatment of OSCC. Our laboratory is working on methods to enhance gene delivery to OSCC cells in culture as a first step toward the delivery of the Herpes Simplex Virus thymidine kinase (HSV-*tk*) "suicide gene" in an animal model of the disease. Cationic liposome-DNA complexes (lipoplexes) are a promising non-viral vector for the gene therapy of oral cancer. During gene delivery, it is important to protect DNA from nucleases and to condense it to form a compact complex with cationic liposomes. Protamine is an arginine-rich, natural cationic peptide of MW 4000-4250 that condenses DNA. Transferrin-complexed lipoplexes have been shown to enhance gene delivery to a variety of cells, including HSC-3 human squamous cell carcinoma cells, possibly via binding to transferrin receptors.

We examined whether protamine could enhance gene delivery to HSC-3 cells by Transferrin-lipoplexes. We also investigated whether HSV-*tk*/ganciclovir mediated cytotoxicity in these cells. Lipoplexes were prepared by the complexation of transferrin and protamine with DOTAP/Cholesterol liposomes followed by association of plasmid DNA (pCMVluc) encoding the marker enzyme luciferase. The presence of protamine in the range 0.5-2 μ g per μ g DNA enhanced luciferase expression 3-4 fold, depending on the DOTAP/DNA (+/-) charge ratio. HSV-tk/ganciclovir-mediated cytotoxicity in HSC-3 cells was also enhanced by the association of protamine with transferrin-lipoplexes, increasing the percentage of cells killed from 62% to 100% by the 9th day of the experiment. These results suggest that condensation of DNA by protamine can be useful in both gene delivery and gene therapy applications.

A COMPARISON OF FIVE MOTORIZED FILING SYSTEMS INVESTIGATING APICAL TRANSPORTATION WHEN TAKEN BEYOND THE APEX IN STRAIGHT AND CURVED CANALS

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Motorized filing systems are becoming more and more popular in the practice of endodontics. In this preliminary study, we investigated the action of five motorized filing systems when they are taken beyond the apex. The five systems used were AETTM (Ultradent), GT TM (Tulsa Dentsply), K3TM (Sybron Dental), ProTaperTM (Tulsa Dentsply), and RaCETM (Brassler). Canal curvatures were determined by taking radiographs of single-rooted anterior teeth from a facial to lingual and mesial to distal aspects. Schneider angle determinations were used to classify the canals as straight or curved. The over-instrumentation of the teeth was digitally recorded from an apical aspect. The ranges of over instrumentation were from one to three millimeters past the apex. Digital video images of pre and post instrumentation apical positions were superimposed digitally to determine the extent of apical transportation' with each of the five instrument systems. The extent of apical transportation was negligible with all of the systems except the AET system.

DIMENSIONAL ACCURACY OF SEVEN DIE MATERIALS

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Purpose: The purpose of this study was to compare 1.) the dimensional accuracy and 2) the handling characteristics of seven die materials.

Materials and methods: A master die was machined from surgical stainless steel in the shape of the frustum of a cone, and three measurements (1: vertical; 2 and 3: horizontal) were made from three scribed reference lines. Individual polyvinyl siloxane impressions were made (n=10) for each of the specimens. The fabricated dies were measured (50X) to the nearest .0001 mm. Data was subject to ANOVA/ Duncan tests at significance level 0.05 and pairwise comparisons. Results: Type IV resin-impregnated dental stone and copper-electroplated dies most closely approximated the dimensions of the master die, and were not significantly different from each other in any of the pairwise comparisons. Epoxy, polyurethane, and bis-acryl dies had excessive shrinkage or expansion in at least one dimension. Conventional Type IV and Type V dental stone dies exhibited setting expansion within the range appropriate for a Type V gypsum. Conclusions: Type IV resin-impregnated dental stone and copper-electroplated dies were more accurate than the other die materials tested.

SEARCHING FOR ENVIRONMENTAL FACTORS CAUSING CLEFT LIP AND PALATE IN VALDIVIA, CHILE

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The UOP School of Dentistry faculty and students have participated in sixteen Rotaplast medical missions to developing countries to provide free cleft lip and palate reconstructive surgeries and to collect data.

The case-control sample of orofacial cleft cases collected in Valdivia, Chile (2000), included 114 individuals with a non-syndromic cleft lip and/or palate and 55 control individuals.

The epidemiological factors of diagnosis, sex ratio, birth weight, birth length, birth order, and the age of parents were evaluated. Our study suggests that epidemiological characteristics such as parental age, birth length, and birth order, may contribute to the causes of cleft lip and palate in Valdivia, Chile. Results showed that the paternal age of cases was significantly higher (p=0.012), cleft children had lower birth length (p=0.01), and they were much more likely to be born in a higher birth order (p=0.01) compared to controls. The statistical differences in these variables may reflect a different lifestyle, diet, and socioeconomic status in specific age groups. Further studies are needed to confirm and extend these findings on a larger sample.

This is a joint effort of the UOP School of Dentistry and Rotaplast International, Inc.

* Presenter

MANAGEMENT OF A MUCUS EXTRAVASATION PHENOMENON (MUCOCELE) IN A PEDIATRIC PATIENT: CASE REPORT[§]

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This article reports on a ten year, four month old girl who presented to the University of the Pacific School of Dentistry Pediatric Clinic with an asymptomatic, swelling in the floor of her mouth. The patient had been aware of the swelling for approximately one week. Her medical history was non-contributory and there were apparently no traumatic injuries to this area of the mouth. After clinical and radiographic examination, and consultation with an oral pathologist, surgical excision was recommended. An excisional biopsy was performed which resulted in a diagnosis of mucus retention phenomenon (mucus extravasation phenomenon), as confirmed by histological findings.

ABSTRACTS

SENIOR RESEARCH COMPETITION

[§]2nd Year Student Presentation not participating in the ADA/Dentsply Competition

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 postero-superiorly and anteroinferiorly from this point. We recorded this fusion sequence in 55.0, 46.2, and 75.0% of infants aged as late fetal/new born, 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.

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*Co-presenters

GENE DELIVERY TO ORAL CANCER CELLS BY LIPID-DNA COMPLEXES **CONTAINING HUMAN SERUM ALBUMIN**

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Objectives: Oral squamous cell carcinoma (OSCC) is the most prevalent cancer involving the oral cavity and oropharynx. Our long-term goal is to deliver therapeutic genes for the treatment of OSCC. Cationic lipid-DNA complexes (lipoplexes) are used as vectors for gene delivery both in vitro and in vivo. Since serum inhibits gene transfer by lipoplexes, we examined whether DNA complexed with the cationic lipid reagent Escort and human serum albumin could transfect human SCC cells (HSC-3) in the presence of fetal bovine serum (FBS), as observed for other types of cells. Methods: Cells were transfected with the luciferase-expressing plasmid, pCMVluc, complexed with either DOTAP/DOPE (Escort) or Escort + Albumin ("EA") in the presence of increasing concentrations of FBS. Efficiency of transfection was determined as luciferase activity, expressed as relative light units (RLU)/ml of cell lysate. Cells transfected with the pCMV.HSV-tk plasmid expressing Herpes Simplex Virus thymidine kinase were treated with ganciclovir. Cell viability was quantified by the Alamar Blue assay. Results: Albumin enhanced Escort-mediated transfection. EA-mediated transfection was inhibited by serum in a dose-dependent manner. Lipoplexes with a 2:1 (+/-) charge ratio were more resistant to the effect of serum than those with either a 1:1 or 4:1 (+/-) charge ratio. In the absence of serum, the dclivery of the HSV-tk gene to HSC-3 cells by plain Escort and EA lipoplexes, followed by ganciclovir treatment for 7 days, resulted in >70% and >90% cytotoxicity, respectively. The cytotoxic effect of HSV-tk plasmid was completely abolished when 10% FBS was present during Escort- or EA-mediated transfection. Conclusions: (i) Escort-mediated transfection can be enhanced by albumin; (ii) complexation of Escort with albumin does not overcome the inhibitory effects of serum on HSV-tk-mediated cytotoxicity in HSC-3 cells; and (iii) additional non-viral vectors should be tested for resistance to the inhibitory effect of serum.

(Presented at the 32nd Annual Meeting of the AADR, San Antonio, TX, March 12-15, 2003)

TRANSCRIPTION FACTOR NF-KB BINDING SITES IN THE CMV PROMOTER AS AN INHIBITOR OF HIV REPLICATION

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Objectives: The cellular transcription factor NF-kB is a major regulator of transcriptional activity at the human immunodeficiency virus (HIV) 5'- long terminal repeat promoter (LTR). This suggests NF-KB as a potential target for anti-HIV therapy. We are exploring the use of the Cytomegalovirus (CMV) promoter element as a DNA decoy for cellular transcription factors including NF-KB. Structural analysis of the CMV promoter element using the transfac database demonstrates the presence of three NF-KB-binding consensus sequences, suggesting that the CMV promoter may sequester sufficient NF-KB to interfere with transcriptional activation at the HIV LTR promoter. Methods: To evaluate the possibility of NF-KB-specific competition between the CMV and LTR promoter elements, we co-transfected HeLa cells with the HIV proviral clone HXBABgl, and effector plasmids containing: 1) CMV promoter, 2) RSV promoter, 3) SV-40 promoter, SV-40 enhancer, and SV-40 promoter/enhancer complex, 4) anti-NF-KB ribozyme under the transcriptional regulation of the CMV promoter. p24 viral protein concentration in the supernatant was assayed using ELISA to determine expression levels of HXB∆Bgl. We also evaluated these effectors using an LTR-luciferase reporter plasmid, to validate that their effect was mediated at the LTR promoter, rather than by post-transcriptional regulation of p24. Results: CMV-promoter containing plasmids reduced LTR-mediated expression of p24 and luciferase by 10-fold and 5-fold respectively, when compared to an RSV promoter-containing plasmid. Whether the anti-NF-kB ribozyme generated an additive effect beyond the reduction in HIV replication mediated by the CMV promoter was not evident. Conclusions: Inhibition of HIV replication by the CMV promoter is mediated at the HIV LTR. Studies on the generation of a mutant plasmid lacking the NF-DB consensus sequence in the CMV promoter, and an anti-NF-kB ribozyme expressing plasmid under the transcriptional regulation of the RSV promoter, are in progress.

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FRACTURE STRENGTH OF EMPRESS CERAMIC INLAYS UNDER THREE **CONDITIONS**

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Objectives: The fracture strength of all-ceramic restorations has been arbitrarily thought to be inadequate to allow occlusal adjustment prior to cementation. A recent study (Geissberger et al, JPD, 4/2002) described how a Teflon tape liner facilitated the handling and removal of inlays/onlays during the try-in procedure. The purpose of the present study was to determine what effect a Teflon tape liner had on the fracture strength of Empress ceramic compared to unlined-uncemented as well as cemented inlays. Methods: Thirty 15 x 15 mm squares of ceramic were fabricated from 2 mm thick Biostar hard stent acrylic. Ten squares were then assigned to one of three groups: uncemented without liner; uncemented with Teflon tape liner; and, cemented. The intaglio surfaces of all specimens were acid-etched with HF. A single low-fusing metal mold was fabricated to sequentially hold both groups of uncemented specimens. Individual metal molds were made and air-abraded for each cemented inlay to simulate the effect of in vivo etched dentin, and the inlays were cemented with Variolink II. All specimens were then fractured using a custom-made rounded probe in an Instron universal testing machine using a crosshead speed of .5 mm min-1. Results were recorded in Newtons, and statistically analyzed using oneway analysis of variance and Tukey test. Results: No liner - 244.40, SD 37.95; Teflon tape liner - 290.62, SD 44.96; cemented 1882.50, SD 111.69. Cemented ceramic specimens had significantly higher fracture strengths than either of the uncemented groups (p < 0.001). The two uncemented groups did not have significantly different fracture strengths (p = 0.346). Conclusions: Cemented ceramic inlays had more than six times the fracture strength of two subgroups of uncemented in lays. Recommendations for deferring occlusal adjustment of allceramic restorations until after cementation appear to be warranted.

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GLIAL CELLS AS POTENTIAL THERAPEUTIC TARGETS IN OROFACIAL NEUROPATHIC PAIN

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OBJECTIVES: Several studies have implicated microglial cells in the development of chronic pain associated with spinal nerve injury (sciatic nerve). In the trigeminal system, neuropathic pain behaviors are preceded by a marked increase in the synthesis and release of IL-6 in the brainstem following infraorbital nerve constriction. The most likely source of Il-6 and other inflammatory mediators in the brainstem are activated microglial cells. Thus, the purpose of this study was to assess microglial cell activation following trigeminal nerve injury using immunocytochemical methods. METHODS: Male, Sprague-Dawley rats (n=15) were divided into two 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. The animals 6. RT1a IMMUNOREACTIVITY (ACTIVATED MICROGLIA) 3 AND 7 were sacrificed on days 3, 7 and 21, and the effects of DAYS AFTER CCI nerve injury were measured using ant-rat RT1a, which is specifically expressed by activated microglial cells and not Nucleus Caudalis other glial cells (astrocytes, oligodendrocytes). Right Immunostaining was quantified using NIH Image analysis
 565*
 156

 1024
 451

 14
 2
3 Days 7 Days software (Scion Corp.). RESULTS: Image analysis demonstrated an increase in microglial-associated RT1a Nucleus Interpolaris activity in all ipsilateral trigeminal sensory subnuclei on days 3 and 7 (see Figure). Diffuse, nonspecific activity 3 Days 7 Days Sham
 171
 28

 680
 299

 13
 28
was observed on the contralateral side of the brainstem, and no up-regulation of RT1a occurred in sham-injury Nucleus rats. RT1a immunoreactivity was no longer undetectable Oralis at 21 days after injury.



CONCLUSIONS: The results of this study demonstrate that microglial activation occurs in the brainstem following trigeminal nerve injury, thus providing further support for the hypothesis that microglia may play an important role in the development of orofacial neuropathic pain. In addition, our data suggest that microglial activation may be a therapeutic target, specifically as an early point of clinical intervention in the development of persistent pain conditions.

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INFLAMMATORY HYPERSENSITIVITY IN A RAT MODEL OF OROFACIAL NEUROPATHIC PAIN IS SYMPATHETICALLY DEPENDENT

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OBJECTIVES: Injury to a peripheral nerve can results in the development of neuropathic pain, which in some cases is exacerbated by peripheral sympathetic activity. The purpose of this study was to test the hypothesis that the development and maintenance of inflammatory hypersensitivity in a rat model of trigeminal neuropathic pain is dependent on an interaction between nociceptive fibers and post-ganglionic sympathetic nerves. METHODS: Male Sprague-Dawley rats (n=8) were anesthetized (pentobarbital, 50 mg/kg i.p.). The right IoN was exposed and single 5-0 chromic gut ligature was placed loosely around the branches of the IoN (CCI). Another 8 rats served as sham-injury controls. In a second group of 6 CCI and 3 sham-injury rats, unilateral sympathectomy was performed by avulsion of the right cervical sympathetic ganglion. Finally, the effect of a-adrenergic blockade, dihydroergotamine (1 mg/kg i.p., n=3) or phentolamine (1 mg/kg i.p., n=5), immediately before formalin challenge was also determined. Twenty-one days after injury, behavioral responses to and injection of 2.5% formalin into the right upper lip were monitored continuously for 45 minutes using a computer program. Behaviors were scored as Normal, Directed Grooming, or Other (flinching, etc.). RESULTS: As observed in previous studies, CCI and sham-injury rats demonstrated an initial nociceptive response to formalin that involved both rubbing and flinching behaviors. This was followed by a quiescent period, and then by a prolonged period of rubbing and in the case of CCI rats, flinching and other pain related behaviors. Sham-injury rats rarely exhibited pain behaviors

in the Other category. The total time recorded for all pain-related behaviors was significantly greater in CCI rats than in sham-injury animals (p<0.001). Sympathectomy at the time of IoN injury led to the abolition the enhanced nocifensive behaviors, and consequently the behavior of CCI rats was indistinguishable from that of sham-injury animals. Similarly, dihydroergotamine (partial α -antagonist and 5HT agonist) and phentolamine (α -adrenergic antagonist) both appeared to reverse the effects of CCI on pain behaviors after formalin challenge. CONCLUSIONS: The data demonstrate that the development of inflammatory hypersensitivity after constriction of the IoN depends on sympathetic activity. Further, α-adrenergic blockade (phentolamine) diminishes the inflammatory hypersensitivity. Finally, dihydroergotamine, either via α-adrenergic or more likely through central 5HT receptor mechanisms, also abolished the inflammatory hypersensitivity observed in CCI rats.

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DIFFERENCES IN THE REPRESENTATION OF MESIODISTAL ROOT ALIGNMENT BETWEEN PERIAPICAL AND PANORAMIC X-RAYS

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The purpose of this study was to determine how much difference there was between periapical and panoramic x-rays in their representation of mesiodistal root alignment. Fortyseven post-orthodontic pairs of periapical or panoramic x-rays were used. Each x-ray pair was taken on the same day by the same technician. The long axis of each tooth from first molar to first molar was located by three judges working independently. The averaged results were used for all measurements. All differences were statistically significant except for one maxillary tooth pair. The greatest average difference was 7.0 degrees in the maxilla and 7.8 degrees in the mandible. Overall, differences were greater in the mandible and greater for anterior tooth pairs. These data suggest that the differences in the representation of mesiodistal root alignment between periapical and panoramic x-rays are relatively small. However, it is recommended that panoramic x-rays be supplemented with anterior periapical films in order to best assess the alignment of neighboring roots.