UNIVERSITY OF THE PACIFIC
Arthur A. Dugoni School of Dentistry

EXCELLENCE DAY

advancing research and treatment

12TH ANNUAL PACIFIC RESEARCH DAY

Abstracts

Faculty and Student Presentations

Senior Research Competition

Second-Year Student Research Competition

Wednesday, May 19, 2010

12th ANNUAL PACIFIC RESEARCH DAY AND STUDENT RESEARCH COMPETITIONS

ABSTRACTS

WEDNESDAY, MAY 19, 2010

SPONSORED BY GRANTS FROM
WESTERN DENTAL SERVICES
PROCTER & GAMBLE ORAL HEALTH
JOHNSON & JOHNSON

TABLE OF CONTENTS

Faculty and Student Abstracts

Senior Research Competition

2nd Year Student Research Competition

IDS Student Presentations

FACULTY AND STUDENT PRESENTATIONS

EFFECT OF PLATELET-RICH PLASMA ON HUMAN MESENCHYMAL STEM CELLS – ANALYSIS OF GENE EXPRESSION

Manal Abu Al-Melh, Waleed Soliman, Abir Balghonaim and Mirek Tolar

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

OBJECTIVES: Platelet-rich plasma (PRP) has been frequently used for enhancement of regeneration and healing of injured tissues. A little is known about cellular and molecular mechanisms of its effects. We have shown that PRP stimulates proliferation and osteogenic differentiation of human mesenchymal stem cells (hMSC) in vitro. The goal of this study is to analyze effects of PRP on hMSC at the level of gene expression.

METHODS: Platelet fraction was isolated from human adult venous blood by centrifugation and platelet factors were released by activation of platelets with recombinant thrombin (Sigma); hMSC (Lonza; passage 2-6) were cultivated in hMSC basal medium (Lonza) with 10% adult human serum with or without PRP factors. Total RNA was isolated and expression of 84 hMSC-specific genes was evaluated using PCR arrays (Qiagen) on StepOnePlus RT PCR apparatus (Applied Biosystems). The analysis was done for hMSC cultivated in growth medium or differentiation medium, either in absence of PRP factors (controls, n=6) or in presence of PRP factors (experiments, n=6). Experimental and control cycle threshold (CT) values were compared using manufacturer's software.

RESULTS: PRP factors increased expression of genes regulating cell division in hMSC cultivated in growth medium. PRP factors increased expression of osteogenic differentiation marker genes when a differentiation medium was used.

CONCLUSIONS: This pilot study showed effects of PRP on gene expression of hMSC in a model system in vitro. The analysis is continued. Identification of hMSC genes whose expression is affected by PRP factors will help to better understand mechanisms, by which PRP stimulates healing and regeneration of damaged tissues.

This work was supported by the Research Pilot Project Award 03-Activity 065 from the Arthur A. Dugoni School of Dentistry, San Francisco, CA.

BMP4 AND ITS ROLE IN ETIOLOGY OF OROFACIAL CLEFTS

S. Arhamsadr*[1], J. Feusier[1], M. Tolar[2] and M. Tolarová[3]

[1]Doctor of Dental Surgery Program; [2]Pacific Tissue Engineering Laboratory; [3]Pacific Craniofacial Team and Cleft Prevention program, Department of Orthodontics, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: Orofacial clefts (OFC) are one of the most common, serious birth malformations in humans. Cleft lip develops due to a failure of medial nasal prominences, lateral nasal prominences, and maxillary prominences to come into contact and fuse correctly in the upper embryonic face. Cleft palate is result of a failure of fusion of palatal shelves. Etiology of OFC is very complex and still mostly unknown, however, it is generally understood that the multifactorial model – involving both genetic and environmental factors - fits best to nonsyndromic cleft lip and palate (NCL/P). Over the past decade, research has focused on identification of genes that contribute to the etiology of NCL/P. Recently, mutations of bone morphogenetic protein 4 (BMP4) have also been suggested, however, no consistency exists in the published reports.

Bone morphogenetic proteins (BMPs) are multi-functional growth factors that belong to the transforming growth factor beta (TGFbeta) superfamily. Roles of BMPs in embryonic development and in cellular functions of postnatal and adult animals have been extensively studied recently. Bone morphogenetic protein 4 gene (BMP4) is located in 14q22-q23.

BMP4 and OFC: Previous work has suggested that cleft lip with or without cleft palate is genetically distinct from isolated cleft secondary palate. Liu et al (2005) suggested, however, that abnormal Bmp signaling was implicated in both anomalies. Associations of NCLP with BMP4 polymorphisms were suggested (Jianyan et al. 2009, Suzuki et al. 2009), but no data are available for nonsyndromic cleft palate.

OBJECTIVES: To analyze association of BMP4 and cleft palate anomalies by comparing genotypes of patients affected with cleft palate with those affected with other types of cleft and with controls.

MATERIAL AND METHODS: We have identified 50 patients affected with cleft palate, and 60 controls, performed DNA isolations, learned PCR and how to prepare specimens for sequencing.

CONCLUSION: The study is in progress and we will discuss our preliminary results.

The fieldwork for this study was supported by Rotaplast International, Inc.

IS TGFB3 GENE ASSOCIATED WITH NONSYNDROMIC CLEFT LIP AND PALATE IN SAN SALVADOR?

B. Behdad*[1], M. Ajami[1], M. Tolar[2], A. Balghonaim[3], A. Tarifard[3], K. Liu[3], H. Berdichevsky[3], J. Leach[3], M. Christie[3] and M. Tolarova[5]

[1]Doctor of Dental Surgery Program; [2]Pacific Tissue Engineering Laboratory; [3]Pacific Craniofacial Team and Cleft Prevention Program, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: Nonsyndromic cleft lip with or without cleft palate belongs to common malformation with a multifactorial etiology. Genetic factors and environmental factors and many gene-gene, gene-environmental, and environmental-environmental interactions are involved. There are more than thirty potential loci and genes that are associated with orofacial clefts and among them TGFB3 is considered one of the strong candidates in humans. Previous study done in the Pacific Craniofacial Genetics Laboratory showed significantly higher proportion of rs2268625 C/T and rs2300607 A/T mutations of the TGFB3 gene in Guatemala population (Al-Jabeiti,et al, 2008).

OBJECTIVES: We would like to find out whether polymorphism of TGFB3 is associated with nonsyndromic cleft lip and palate (NCLP) in San Salvador. Our hypothesis is that similar etiological factors – including genetic factors – are involved in etiology of NCLP El Salvador that is neighboring country.

MATERIAL AND METHODS: Blood specimens of 71 cases with NCLP and 52 controls were collected during Rotaplast medical missions to San Salvador, El Salvador. We started isolation of DNA for these specimens, followed by PCR and establishing genotypes for TGFB3 rs2300607 A/T mutation using polyacrylamide gel electrophoresis.

RESULTS: We identified specimens from 24 patients (20 males and 4 females) affected with bilateral complete cleft lip and palate - the most severe type of NCLP. To this date, 14 specimens were analyzed. Ten had wild type genotype AA and four were homozygotes for mutated allele T. There was no heterozygote found among specimens of these 14 patients.

CONCLUSIONS: The study continues with analysis of remaining 10 patients affected with bilateral clefts and specimens from patients with other type of clefts and controls.

The fieldwork for this study was supported by Rotaplast International, Inc.

PERCEPTION OF EDUCATION AND PRACTICE PATTERNS IN SPECIAL CARE DENTISTRY

Elisa M. Chávez, Paul Subar, Jeffrey Miles, Allen Wong, Eugene LaBarre and Paul Glassman.

University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: To compare the perception of dental education in the care and management of patients with complex needs to alumni practice patterns.

METHODS: Over the past ten years, there has been an evolution of didactic and clinical experiences provided to students, in the care of patients with complex needs. Alumni who graduated from 1997 to 2007 were surveyed regarding practice patterns and their perceptions of their pre-doctoral education in the care of patients categorized and defined as medically compromised, frail elders and developmentally disabled. Subjects rated perceptions on a Likert scale. Of 1200 surveyed, 375 responded.

RESULTS: Regression analyses showed a positive relationship between perceptions, as students, of the training they received to the number of medically compromised patients they currently treat ($p<0.05$). Those who completed AEGD/GPR programs thought, as pre-dental students, that opportunities to treat these patients were more valuable than did those who did not pursue post-doctoral general dentistry programs ($p=<0.05$). A significant relationship emerged between dentists' positive assessment, after entering practice, of the training that they received compared to the number of patients they treat who are medically compromised and developmentally disabled ($p<0.05$). After practice experience, recent graduates reported significantly higher value of their education in the care of these patients compared to earlier graduates ($p=0.036$).

CONCLUSION: Pacific alumni who reported pre-doctoral educational experiences as more valuable, treat more patients with complex needs compared to those who thought it less valuable. We did not assess if there was a preexisting bias about the value of the coursework that lead to their perceptions. Interestingly, recent graduates perceived their education in this area to be more valuable after they were in practice than during school. Student perceptions may shape practice patterns but even positive perceptions may underestimate the value of educational experiences as they will relate to future practice.

LECTINS AND T-20, BUT NOT NEUTRALIZING ANTIBODIES, INHIBIT HIV-1 ENV-MEDIATED SYNCYTIUM FORMATION BETWEEN Clone69T1RevEnv AND SupT1 CELLS MONITORED BY FLUORESCENCE MICROSCOPY

Nejat Düzgüneş[1]*, Michael Yee[1], Jan Balzarini[2] and Krystyna Konopka[1]

[1]Department of Microbiology, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA, [2]Rega Institute for Medical Research, Catholic University of Leuven, Leuven, Belgium

OBJECTIVES: The HIV-1 envelope protein Env (gp120/gp41) mediates the fusion of the viral envelope with the host cell membrane. We developed an HIV fusion assay, using fluorescent Clone69T1RevEnv cells expressing Env, and highly CD4+ SupT1 cells. We examined whether previously established inhibitors of HIV-1 infection, including a peptide, lectins, and neutralizing antibodies, inhibit Env-mediated syncytium formation.

METHODS: Clone69T1RevEnv cells were induced to express Env by removing tetracycline from the medium. The cells were labeled with Calcein-AM Green, incubated for 3 h with SupT1 cells labeled with CellTrace™ Calcein red-orange, with or without the inhibitors, and observed under a Nikon inverted fluorescence microscope. Co-localization of the two fluorescent probes following syncytium formation resulted in orange fluorescence. Antibodies were obtained from the NIH AIDS Research & Reference Reagent Program, Polymun (2G12) and D. Dimitrov (m14; NIH). T-20 was from the AIDS Reagent Program.

RESULTS: The lectins *Hippeastrum hybrid* agglutinin (HHA) and *Galanthus nivalis* agglutinin (GNA), and the peptide T-20, inhibited syncytium formation at 1 μg/ml. Antibodies to gp120 (IgG1B12, m14 IgG, F105 and 2G12), and gp41 (2F5 and 4E10) that inhibit HIV-1 infection had little or no inhibitory effect on syncytium formation.

CONCLUSIONS: The observation that antibodies that inhibit HIV infection are not effective against syncytium formation, suggests that the mechanisms of interaction of Env with cell membrane CD4 and co-receptors may be different in cell-cell and virus-cell membrane fusion, as suggested previously (J Gen Virol 76, 669-679; 1995). These results also indicate that "neutralizing" antibodies may not be able to inhibit the spread of viral genetic material from infected cells to uninfected cells. This fluorescence assay can be adapted to screen novel inhibitors of membrane fusion in high-throughput assays.

Parts of this work were presented at the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Sept 12-15, 2009; Biophysical Society 54th Annual Meeting, San Francisco, February 20-24, 2010; and the 23rd International Conference on Antiviral Research, San Francisco, April 25-28, 2010. This work was supported by Research Pilot Project Award 03-Activity 076 from the Arthur A. Dugoni School of Dentistry.

EFFICIENT GENE DELIVERY TO ORAL CANCER CELLS BY POLYETHYLENIMINE-DNA COMPLEXES

Senait Gebremedhin[1], Freddie Martinez[2], Jen Fountain[2], Krystyna Konopka[1] and Nejat Düzgüneş[1]

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

OBJECTIVES: One of the problems in the use of cationic-lipid-DNA complexes (lipoplexes) for gene therapy of oral squamous cell carcinoma (OSCC) is the low efficiency of transfection of OSCC cell, and its variability from one cell type to another. Cationic polymer-DNA complexes (polyplexes) are internalized via caveolae, instead of coated pits as in the case with lipoplexes. We therefore tested whether polyplexes mediate efficient gene delivery to various human OSCC cells.

METHODS: HSC-3, H413 and H357 cells were seeded the day before transfection, and used at 85% or 50% confluence. The plasmid pCMV.luc (1 μg DNA) expressing luciferase under the control of the cytomegalovirus promoter was complexed with 1, 2 or 4 μl of the cationic polyethylenimine, jetPEI (Polyplus Transfection). The plasmid was also complexed with a cationic lipid transfection reagent, Metafectene (Biontex) (1 μg DNA/2 μl Metafectene). Luciferase expression was assayed 48 h after transfection.

RESULTS: The efficiency of transfection with jetPEI polyplexes was much higher than that obtained with lipoplexes, and higher gene expression was observed at 50% confluence compared to that at 85% confluence with all three cell lines. For example, in lipofection-resitant H357 cells at 50% confluence, the use of 1, 2 or 4 μl jetPEI resulted in a 922-, 1081- and 1440-fold increase, respectively, in luciferase activity over that obtained with Metafectene. At 85% confluence, polyplexes enhanced gene expression by 149-, 252- and 545-fold at jetPEI volumes of 1, 2 and 4 μl, respectively.

CONCLUSIONS: Cell density is a significant variable in efficient transfection of OSCC cells. The use of polyplexes instead of lipoplexes can drastically increase transfection efficiency in OSCC cells. Our laboratory is currently investigating whether jetPEI-DNA complexes are internalized by caveolae.

This work was presented at the 39th Annual Meeting of the American Association for Dental Research, March 3-6, 2010, Washington, DC. This project was funded by an AADR Student Research Fellowship, and a Research Pilot Project Award 03 Activity 072 from the Dugoni School of Dentistry (JF).

THE ALL-POWERFUL UNIVERSAL SUPER-SECRETOR: DOES IT REALLY EXIST?

Seth R. Gomez*, Namphuong T. Tran*, Geoff P. Lin-Cereghino and Joan Lin-Cereghino

Department of Biological Sciences, College of the Pacific, University of the Pacific, Stockton, California

INTRODUCTION: *Pichia pastoris* is a species of yeast that is used to make mass quantities of vital human proteins such as insulin. However, *P. pastoris*' secretory machinery limits its ability to secrete all types of heterologous proteins in substantial amounts. We desired to isolate mutant strains that had higher levels of secretion efficiency than wild type yeast.

OBJECTIVE: We have already isolated mutants that are able to super-secrete the protein β-galactosidase. Our objective is to see if these same mutants have the potential to become universal secretors, meaning that they can secrete high levels of other heterologous proteins.

METHODS: Using mutants LL1 and AH1, transformed with plasmids for expression of HRP (horseradish peroxidase) or SLPI (secretory leukocyte protease inhibitor), we induced expression of these proteins and analyzed their secretion levels using spot western blots and an HRP assay.

RESULTS/CONCLUSION: Our data suggests that HRP and SLPI are able to be secreted in our mutants. So far, LL1 shows potential to secrete more than the wild-type strains.

VITAMIN-D MEDIATED INHIBITION OF RAD51 EXPRESSION IN A HAMSTER BUCCAL POUCH MODEL

Rhea P. Hautea*, Karim Shallwani, Eric Thomas and Joanna S. Albala
Department of Biological Sciences, University of the Pacific, Stockton, CA

INTRODUCTION: Excessive alcohol and tobacco use have the potential to lead to multiple genetic mutations that can result in abnormal cell growth in the upper aerodigestive tract leading to head and neck tumors. Vitamin D3 (VD3) has been shown to suppress the growth of head and neck squamous cell carcinomas (HNSCC); however, the molecular mechanisms underlying this process are not clearly understood. In a histological study, increased Rad51, a vital protein involved in double-stranded DNA repair, was found to correlate with human malignancies in cancer patients. Preliminary studies by the Albala lab have shown that VD3 can inhibit tumor formation in a hamster buccal pouch model. The present study aims to extend preliminary *in vivo* findings by identifying the expression of Rad51 in sectioned hamster tissues after treatment with VD3.

METHODS: Paraffinized tissues samples from 40 hamsters sacrificed at 2, 6, and 14 weeks were obtained from the Department of Otolaryngology-Head and Neck Surgery, University of California Davis School of Medicine. The right buccal pouch of each hamster was painted with 7,12-dimethylbenz[α]anthracene (DMBA). Twenty of the forty hamsters received an intraperitoneal injection of VD3. At sacrifice, buccal pouch and tumor samples were collected and fixed in formalin.To prepare for immunohistochemistry, paraffin-embedded samples were cut into 4 micrometer thick sections. Immunocytochemical analysis of the Rad51 protein was performed using an avidin-biotin peroxidase complex. Sections were also stained with Hematoxylin and Eosin for histological examination of tumors and buccal pouch morphology. Protein analysis of the tissue was performed through Western Blotting. A portion of the collected tissue samples were flash frozen and stored at -80°C. Hamster tissues were homogenized in 1% Triton-X lysis buffer. Lysate concentrations were determined using the Bradford Protein Assay, loaded into wells at an equal concentration, and subjected to routine polyacrylamide gel electrophoresis (SDS-PAGE). Following electrophoresis, the resolved polypeptides were transferred to a polyvinylidene difluoride (PVDF) membrane and incubated with an anti-Rad51 antibody (Santa Cruz Biologicals). Protein expression was visualized by enhanced chemiluminescence (ECL).

RESULTS: Our preliminary immunohistochemical analysis suggests a decrease in Rad51 expression in response to VD3 treatment. Initial protein examination of the hamster lysates supports the association between VD3-treatment with a down-regulation of Rad51 protein. Furthermore, histological staining reveals a VD3-associated delay in dysplasia in VD3-treated cells from 2 to 14 weeks.

CONCLUSION: We have examined the effect of Vitamin D3 treatment on Rad51 expression in head and neck squamous cell carcinoma. Our results suggest that the application of VD3 may impair the DNA damage response in tumor-induced tissue. Further investigations may support the benefit of VD3 treatment in preventing the onset of carcinogenesis or progression of disease.

This work was supported by funds from the University of the Pacific.

SUSCEPTIBILITY OF CANDIDA BIOFILMS TO HISTATIN-5 AND FLUCONAZOLE

Krystyna Konopka[1]*, Barbara Dorocka-Bobkowska[2], Senait Gebremedhin[1], Julian Wilson[1] and Nejat Düzgüneş[1]

[1]Department of Microbiology, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA, [2]Department of Prosthodontics, University of Medical Sciences, Poznan, Poland

OBJECTIVES: The high recurrence rate of *Candida*-associated denture stomatitis may be explained by the greater antibiotic resistance of *Candida* biofilms formed on denture acrylic, compared to planktonic yeasts. Histatins, a family of basic peptides secreted by the major salivary glands in humans, especially histatin 5, possess significant antifungal properties. We examined antifungal activities of Hst-5 against planktonic or biofilm *C. albicans* and *C. glabrata*, and the effect of fluconazole on *C. albicans* and *C. glabrata* biofilms

METHODS: Three *C. albicans* isolates, GDH18, UTR-14, and 6122/06 (clinical), and two *C. glabrata* isolates, GDH1407 and 6115/06 (clinical) were used. Biofilms were developed on poly(methyl methacrylate) discs and incubated with Hst-5 (0.01-100 μM) for 1 h at 37°C. Fluconazole (1-200 μM) was added in YNB medium and incubated for 24 h at 37°C. The metabolic activity of the biofilms was measured by the XTT assay. The concentration of Hst-5 causing a 50% reduction in the metabolic activity of biofilms (50% RMA) was obtained from the generated dose-response curves. The fungicidal activity of Hst-5 against planktonic *Candida* was tested by microdilution plate assay. Colonies were counted after a 48 h incubation at 37°C. The concentrations of peptide giving 50% reduction in viable counts (IC_{50}) were determined.

RESULTS: Both biofilm and planktonic *C. albicans* GDH18, UTR-14 and 6122/06 were highly susceptible to Hst-5, with 50% RMA (biofilm) of 4.6 ± 2.2, 6.9 ± 3.7 and 1.7 ± 1.5 μM, and IC_{50} (planktonic cells) of 3.0 ± 0.5, 2.6 ± 0.1 and 4.8 ± 0.5 μM, respectively. Biofilms of *C. glabrata* GDH1407 and 611/06 were less susceptible to Hst-5, with 50% RMA of 31.2 ± 4.8 and 62.5 μM, respectively. Planktonic *C. glabrata* GDH1407 and 611/06 were insensitive to Hst-5 (IC_{50} >100 μM). Biofilm-associated *Candida* cells were highly resistant to fluconazole in the range 1-200 μM, e.g. at 100 μM only ~20% inhibition was observed for *C. albicans*, and ~30% inhibition for *C. glabrata*.

CONCLUSIONS: Hst-5 exhibits antifungal activity against biofilms of *C. albicans* and *C. glabrata* developed on denture acrylic. Biofilms of *C. glabrata* are significantly less sensitive to Hst-5 than biofilms of *C. albicans*. Our results demonstrate the therapeutic potential of Hst-5 against *Candida* biofilms that are usually resistant to conventional antifungal agents.

This work was supported by Research Pilot Project Award 03-Activity 054 from the Arthur A. Dugoni School of Dentistry (K. Konopka), it was presented at the 39th Annual Meeting of AADR, March 3-6, 2010, Washington, DC. J. Dent. Res. Vol. 89 (Special issue A) Abstract No. 1461, Seq. #184, and published by K. Konopka, B. Dorocka-Bobkowska, S. Gebremedhin and N. Düzgüneş, in Antonie van Leeuwenhoek 97: 413-417, 2010.

METRIC, MORPHOLOGIC, AND FUNCTIONAL ANALYSIS OF FRONTAL BONE ONTOGENY IN HOMO SAPIENS.

Caitlin L. Ibarra[1], Gary D. Richards[1,2]*, Rebecca S. Jabbour[1,3] and Caroline F. Horton[1]

[1]Human Evolution Research Center, University of California, Berkeley, [2]Department of Physiological Sciences, Arthur A. Dugoni School of Dentistry, University of the Pacific, [3]Department of Biology, Saint Mary's College of California.

OBJECTIVES: Whereas bones cannot be equated with functional skeletal units, understanding their ontogeny provides a basis for delineating such units. To initiate the process of defining functional units in the frontofacial region, we made a broadly based metric and morphological assessment of frontal bone ontogeny.

METHODS: Our sample (*n*= 510) comprises modern and archaeological crania (7th fetal month-adult). We measured 16 dimensions of the frontal bone and nasal capsule and recorded observations on the developmental course of the *sutura frontalis*, frontoparietal fontanel, superior sagittal sinus, and frontal crest. We also examined the relationship of the supraorbital region and nasal capsule to the frontal squama, including tuberosity positioning.

RESULTS: We found increases in interorbital and orbital breadths to diminish early in postnatal growth. This growth pattern reflects the early development of the nasal capsule and eyes. This accelerated growth of the basal frontal buffers the impact of early *sutura frontalis* closure on frontal squama morphology. Further, this growth pattern occurs in conjunction with minimum and maximum frontal breadths that are increasing equally, but at a faster rate relative to the orbitonasal region.

CONCLUSIONS: Growth of the frontal squama, therefore, appears to occur by circumferentially directed expansion of the superolateral regions via high appositional and sutural growth rates. Biomechanical results of this developmental pattern appear to lead to the development of frontal tuberosities, internal crests, and suture-fontanel closure. Knowledge of the mosaic pattern of growth in a bone, when coupled with the morphological results of that growth mosaic, provides insights into the location of functional cranial matrices.

Funding provided by a Research Pilot Project Award from the Arthur A. Dugoni School of Dentistry, University of the Pacific to GDR. This abstract is published in the American Journal of Physical Anthropology 2010, 41:134-135 and was presented at the Annual Meeting of the American Association of Physical Anthropologists, Albuquerque, New Mexico, April 17th, 2010.

DETECTION OF LOW & HIGH RISK HPV SUBTYPES IN OROPHARYNGEAL CARCINOMA AND PRECANCEROUS CONDITIONS BY REALTIME PCR.

Nasser Said-Al-Naief, Peter Kulesza, William Carroll and Joseph Sebastian

University of the Pacific, San Francisco, CA, Northwestern University, Chicago ILL; University of Alabama Birmingham, Birmingham AL; and LCA, NC

INTRODUCTION: The management of advanced oropharyngeal SCC is challenging. It is essential to understand the factors that influence the tumor response to treatment in order to identify new therapeutic regimens and improve treatment outcomes. Although tobacco and alcohol consumption is responsible for the majority of head and neck squamous cell carcinoma (HNSCC), human papilloma virus (HPV) infection has been recently identified as an important etiologic agent with important treatment and prognostic implications since HPV + tumors affect a younger non smoking population and have a distinctly better survival after treatment than the HPV-negative cohort.

MATERIALS & METHODS: The identification and genotyping of high risk (HPV 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 59 and 70) and low risk (HPV6 &, 11) subtypes was investigated in 18 H&N neoplasms and pre-cancerous lesions from 13 males and 3 female (age ranged from 40 to 74 yo). The study included 8 invasive tonsilar SCC (5 moderately differentiated SCC {MDSCC} & 3 poorly differentiated tumors {PDSCC}, 5 laryngeal tumors (4 Squamous Papillomas, one of which exhibited moderate dysplasia and one MDSCC), 2 well-differentiated squamous cell carcinomas {WDSCC) involving the L.arytenoid and fosa of Rossenmuller respectively and one invasive PDSCC of the soft tissues of the neck of unknown primary. 2 invasive WDSCC involving dorsal tongue and floor of mouth respectively and another involving lateral tongue showed keratosis with moderate dysplasia were also included.

Results 61.1% (11/18) of all lesions were HPV 16+ and none demonstrated low risk HPV subtypes. 5/6 MDSCC were HPV 16+ and one tumor was intermediate-inconclusive. Similarly, 3/4 PDSCC demonstrated HPV 16+ subtype. Neither the oral lesions nor the tumors involving the L. aryt. & fossa of Ross. demonstrated HPV presence. All 3 laryngeal Papillomas were + for HPV16.

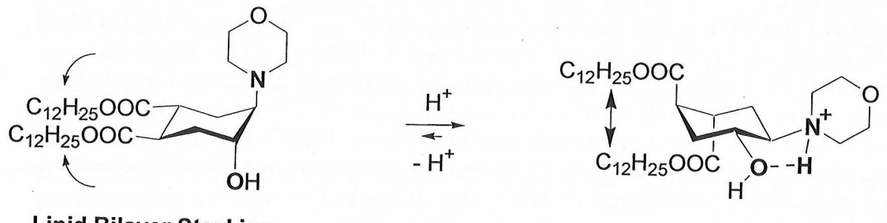
CONCLUSIONS: Despite the small sample size, the present study further confirms the detection of high risk HPV and the role of the virus in the development of oropharyngeal carcinoma and laryngeal papilloma & the absence of HPV in oral neoplastic and precancerous lesions which may also reflect the limited potential of the virus in the development of these lesions. Detection of high-risk HPV may be also beneficial in confirming cytological or histological diagnosis of infection, identify subclinical infection, or determine type-specific risk of precancerous epithelial lesions witch intern leads to improving the prognostic outcomes and significant reduction of morbidity of oropharyngeal carcinoma. The study also demonstrated the validity of utilizing this technique for possible prediction of transformation of dysplastic and pre-neoplastic lesions of the oropharynx to invasive carcinomoma which would also significantly reduce morbidly and improve survival rate. Larger studies comparing different grades of dysplasia with long tem clinical follow-up may further support these findings which should also prompt the clinicians to investigate the presence of HPV in MDSCC and PDSCC with lesser emphasis on WDSCC via Real Time PCR.

TRANS-2-AMINOCYCLOHEXANOL-BASED LIPIDS AS PH-SENSITIVE CONFORMATIONAL SWITCHES FOR THE PEG-GRAFTED LIPOSOMES

Nataliya M. Samoshina[1,2]*, Xin Guo[1], Barbora Brazdova[2], Yu Zheng[2], Xin Liu[2], Andreas H. Franz[2] and Vyacheslav V. Samoshin[2]

[1]Thomas J. Long School of Pharmacy and Health Sciences, University of the Pacific;
[2]Department of Chemistry, University of the Pacific, 3601 Pacific Ave., Stockton, CA 95211

Recently we described a novel strategy to render pH-sensitive lipid amphiphiles and their colloids: a protonation-induced conformational change of the built-in *trans*-2-aminocyclohexanol moiety. In liposomes, this ring flip loosens packing of the attached lipid tails, leading to contents leakage. Herein we report our latest studies on the pH-sensitivity of various PEG-grafted (sterically hindered) liposomes comprising *trans*-2-aminocyclohexanol-based lipids. These liposomes are stable at pH 7.4 and yet release their content in a few seconds at pH 5.3-5.5.



Lipid Bilayer Stacking **Lipid Bilayer Permeation**

This work is supported by the ACS TEVA US Scholar Grant (2009) and NSF MRI Grant (2007).

ALUMNI PRACTICE PATTERNS FOR THE DENTAL CARE OF SPECIAL POPULATIONS

Paul Subar, Elisa M. Chávez, Jeffrey Miles Eugene LaBarre, Allen Wong and Paul Glassman

University of the Pacific, Eberhardt School of Business, Stockton, CA and University of the Pacific, Arthur A. Dugoni School of Dentistry. San Francisco, CA

OBJECTIVES: To determine if advanced education in general dentistry or a general practice residency resulted in practice patterns in the care of patients with complex needs different from Pacific alumni who did not seek such training.
Methods: We surveyed Pacific alumni who graduated from 1997 to 2007 regarding their post-doctoral education and their practice patterns for the care of patients categorized as medically compromised, frail elders and developmentally disabled. Definitions for each patient category were provided. Alumni were asked about their practice setting and postdoctoral education. Approximately 31% of those surveyed responded.

RESULTS: Regression analyses showed respondents not in private practice were more likely to have taken an Advanced Education in General Dentistry or General Practice Residency after dental school compared to respondents in private practice ($p < .001$).
Respondents in private practice treated significantly more patients with developmental disabilities, or who were medically compromised, in the 65 and older category compared to respondents not in private practice ($p < .01$). Across two age categories (age 17 and under, and age 18-64), respondents not in private practice treated more medically compromised patients compared to respondents in private practice ($p < .01$ in both cases). Across all age groups, respondents not in private practice treated significantly more patients with developmental disabilities, or medical compromise, than those in private practices ($p < 0.001$).

CONCLUSIONS: Pacific alumni who completed post-doctoral training in general dentistry, practice more often in non- private practice settings. Alumni in settings other than private practice treat a higher percentage of medically compromised patients below age 65. Other settings included community clinics, military, hospital, Veteran's Administration, long term care and academics among others. Interestingly, alumni in private practice report treating a significantly higher proportion of patients over 65 with developmental disabilities or medical compromise than those in alternative settings.

EXPRESSION OF CYCLOOXYGENASE 2 AND LIPOXYGENASE 12 AND 15 GENES IN HUMAN PULPITIS – A PILOT STUDY

Mirek Tolar[1], Claudia Boessler[2], Jeff Miles[3] and Ove A. Peters[2]

Departments of [1]Orthodontics, [2]Endodontics and [3]Restorative Dentistry, Arthur A Dugoni School of Dentistry, University of the Pacific, San Francisco, CA

OBJECTIVES: Acute pulpitis arises as a response to contact with Gram+ and in particular Gram- bacteria. Early, pro-inflammatory factors prevail. Later, anti-inflammatory and pro-healing factors are upregulated. Bacterial challenge leads to prostaglandin production via cyclooxygenases (COX 1 and 2; PTGS 1 and 2 genes) in the early phase. Lipoxygenases 12 and 15 (ALOX 12 and 15 genes) catalyze synthesis of resolvins and protectins in the later phase. The aim of this pilot study was to compare gene expression for these enzymes in normal and inflamed human pulp. We also wanted to determine, if the quantity of total RNA isolated from a single tooth pulp was sufficient for analysis of gene expression by real time polymerase chain reaction (RT-PCR).

METHODS: Normal pulp was collected from a wisdom tooth. The inflamed pulp was collected from a tooth with a diagnosis of irreversible pulpitis that was extracted due to reasons unrelated to this study. The RNeasy kit (Qiagen) was used for isolation of total RNA. The First Strand kit for synthesis of complementary DNA, primers and SYBR green master mix for RTPCR were obtained from Qiagen. Real time RT-PCR was performed using a StepOnePlus apparatus (Applied Biosciences).

RESULTS: The yield of RNA from the inflamed canine was 18.1 ng/μL. The yield of RNA from the normal wisdom tooth was 47.0 ng/μL. Equal quantities of RNA (18.1 ng/μL) were used for RT PCR. Gene expression in the inflamed pulp was compared to the normal pulp. In pulpitis, expression of COX2 was elevated 22.3 times, expression of lipoxygenase 12 was elevated 2.1 times and expression of lipoxygenase 15 was elevated 8.5 times. These results, together with the macroscopic appearance of the pulp may indicate that pulpitis in the canine was in a later phase of inflammatory reaction. The quantity of total RNA isolated from one tooth pulp was sufficient for a gene expression assay.

CONCLUSIONS: Pulpitis is one of the most painful dental diseases. It may lead to pulp necrosis and ultimately tooth loss. A biological-based therapy for pulpitis may be capable of preserving a viable pulp and of promoting regeneration of damaged tissues. To this end, we will further examine biochemical data from pulps and experimental data from a cell-culture based *in vitro* model of early-phase pulpitis.

PORPHYROMONAS GINGIVALIS STIMULATES IL-18 SECRETION IN MONOCYTIC THP-1 CELLS

Michael Yee[1*], Andy Kim[1], Nejat Düzgüneş[1], Tamer Alpagot[2] and Krystyna Konopka[1]

[1]Department of Microbiology, and [2]Department of Periodontics, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: *Porphyromonas gingivalis* (*Pg*) is one of the most important bacteria that contribute to the pathogenesis of chronic periodontitis. Interleukin-18 (IL-18), a potent pro-inflammatory cytokine that mediates both Th1- and Th2-driven immune responses, is considered to be a key factor in the initiation and progression of periodontal disease. We examined the IL-18 levels secreted by differentiated human macrophage-like THP-1 cells after exposure to live and heat-inactivated *Pg*, and to lipopolysaccharide (LPS) from *Pg* and *E. coli*.

METHODS: THP-1 cells were differentiated with 1.6 nM phorbol 12-myristate 13-acetate (PMA) for 48 h at 37°C. Two *Pg* strains, the avirulent 2561 (ATCC 33277) and highly virulent W83 (ATCC BAA-308) were sub-cultivated on blood agar plates and suspended in Medium 199 (4×10^8 *Pg*/ml). The bacterial suspension was mixed 1:4 with RPMI/10% FBS and added to differentiated THP-1 cells, at ratios of 2-100 bacteria/cell, and incubated at 37°C for 24 h. LPS from *E. coli* and *Pg* were also tested at 0.1 and 5 μg/ml. IL-18 was determined by ELISA. The cytotoxic effect of live and heat-inactivated *Pg* was evaluated by Trypan blue exclusion. Supernatants obtained from differentiated THP-1 cells stimulated with 100 live *Pg*/cell, were also screened for multiple cytokines using a Multi-Analyte Profiler ELISArray. This kit determines the levels of the inflammatory cytokines IL-1A, IL-1β, IL- 2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17A, IFN-γ, tumor necrosis factor (TNF)-α, and granulocyte-macrophage-colony-stimulating factor (GM-CSF).

RESULTS: Exposure to live *Pg* induced the expression of IL-18 in differentiated THP-1 cells. For example, treatment with live *Pg* 2561 and W83 at 100 bacteria/THP-1 cell produced 388.3 ± 19.3 and 255.4 ± 76.1 pg IL-18/ml, respectively. IL-18 up-regulation was strongly reduced by heat-inactivation. Exposure to 100 heat-inactivated *Pg* 2561 and W83 produced 52.2 ± 13.6 and 51.1 ± 8.9 pg IL-18/ml, respectively. The cytotoxic effect of live *Pg* 2561 was higher than that of the W83 isolate, while the cytotoxicities of both heat-inactivated *Pg* isolates were similar. Treatment with LPS from *Pg* and *E. coli*, at 5 μg/ml, resulted in the production of 30.8 ± 5.8 and 39.2 ± 7.6 pg IL-18/ml, respectively. Analysis by the Multi-Analyte Profiler ELISA indicated the stimulation of IL-1β, IL-8, and TNF-γ.

CONCLUSIONS: Live *Pg* stimulates IL-18 secretion by differentiated THP-1 macrophage-like cells, however, it appears that the virulence of *Pg* does not have a significant effect on the level of IL-18 production. The cytotoxic effect of live *Pg* appears to be related to the level of IL-18 secretion. The levels of IL-18 induction by both heat-inactivated *Pg* isolates are similar, and are probably caused by LPS.

This work was presented at the 39th Annual Meeting of AADR, March 3-6, 2010, Washington, DC. J. Dent. Res. Vol. 89 (Special issue A) Abstract No. 379, Seq. #60.

SENIOR RESEARCH COMPETITION PRESENTATIONS

DENTAL CARE AND CHILDREN WITH CLEFT LIP AND PALATE IN CHILE

Alejandro Assatourians*[1], Stephen Arnason[1], Javier Quilodran[2], Marie M. Tolarova[3]

[1]Doctor of Dental Surgery Program; [2]Operation Tooth Fairy, La Serena, Chile; [3]Pacific Craniofacial Team and Cleft Prevention Program, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA.

INTRODUCTION: Cleft lip with or without cleft palate and cleft palate alone are common and serious birth defects that affect about one in every 350-1,000 newborn infants worldwide. Comprehensive treatment typically requires a collaboration of numerous disciplines, including surgical intervention, speech therapy, orthodontics, dental rehabilitation, and nutritional and psychological counseling. Many regions of the world do not have the available resources to provide treatment for patients affected with cleft lip and palate.

Every year, 235,000 babies are born with cleft lip/palate worldwide. In addition to disfigurement of the face, which is a source of serious psychological, adaptive, and physical difficulties for the affected children and their families, the treatment is lengthy and costly and many individuals born with a cleft in developing countries don't have a chance for a treatment, not even for surgical repair. In addition to the cleft lesion, these children often have dental anomalies and increased prevalence of oral disease.

DENTAL HEALTH OF CHILDREN WITH OROFACIAL CLEFTS: Many authors have studied the incidence of altered tooth form and composition in the population of cleft patients. Hypodontia, hyperplasia (supernumerary or excess tooth structure), and hypoplasia or dysplasia in this population are well documented. The upper incisors are the most commonly affected teeth, though there are often anomalies outside of the cleft region as well. Malocclusion and maxillomandibular disharmony are common.

Increased incidence of dental caries and gingivitis is also very common. As in most cases, dental disease in patients affected with orofacial clefts should be preventable. In order to make this possible, we must understand the nutritional, developmental, demographic, and dental status of this specific population.

OPERATION TOOTH FAIRY: Operation Tooth Fairy is American foundation aimed at helping patients with cleft lip and palate by specifically addressing the tremendous need for dental treatment. A recent trip to La Serena, Chile, has provided a prime example of the need for dental care of patients affected with cleft lip and palate.

RESULTS: In three days, twenty five patients, in the age from 6 to 46 years, were treated comprehensively, including a periodontal, restorative, and oral surgery treatments (extractions), and preventative procedures (sealants, OHI, and nutritional counseling). Patients with cleft lip/palate were treated at the "Universidad Del Mar" in La Serena, Chile, where a treatment room with 2 dental operatories was provided for Operation Tooth Fairy. Fifty two operative surfaces were completed. Patients were also seen by a psychologist, and speech therapist.

We thank Universidad Del Mar in La Serena, Malvina Araya, Operation Tooth Fairy, La Serena, Chile; Pablo Castro, Operation Tooth Fairy, Kentfield, CA.

A CLINICAL STUDY: DIGITAL VERSUS CONVENTIONAL IMPRESSION

Sofia Fontecilla[1], Karen A. Schulze[2], Parag Kachalia[2], Warden Noble[2] and Marc Geissberger[2]

[1]Dental Surgery Program Class of 2010, [2]Department of Restorative Dentistry, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA

OBJECTIVES: The purpose of this clinical study was to evaluate the time needed for adjustments of single unit posterior crowns prior to cementation whether they were fabricated on a model of a conventional impression versus a model of a digital impression.

MATERIAL AND METHODS: A total of 93 crowns were evaluated for this study. 50 conventional and 43 digital impressions were taken for single unit posterior crowns. The tooth must have opposing contact and at least one proximal contact. The digital impression was performed using the iTero 3D intraoral scanner from Cadent Inc. The conventional impressions were done using Aquasil (Dentsply) or Impregum (3M ESPE) impression material on a disposable tray. Regular stone models were fabricated from the conventional impressions while the models from the digital impressions were milled from a plastic block. The plastic blocks were manufactured at the manufacturing site of Cadent Inc. in New Jersey. All crowns for these models were fabricated in the dental laboratory California Dental Arts, Cupertino, CA. Prior to cementation of these crowns a protocol of criteria for seating a crown had to be followed. The student dentist checked for proximal contacts, internal aspects of the crown, margins, occlusion and esthetics. The time was monitored for the adjustments on these crowns. For analysis of statistical differences the student t-test was applied.

RESULTS: The average times for conventional and digital impressions were 72 (±38) min and 58 (±30) min, respectively. Statistical analysis revealed a significant difference between the two average adjustment times with $p = 0.043$. From the conventional crowns seven crowns could not be cemented but had to be returned to the lab for adjustments or redo. From the digital impressions only five crowns could not be cemented. All other crowns were successfully adjusted and cemented on the same day.

CONCLUSIONS: A time saving of about 20% on crown adjustments prior to cementation can be achieved when using the digital impression system iTero versus the conventional impression method.

CELL-PENETRATING PEPTIDES ENHANCE GENE DELIVERY BY LIPID-DNA COMPLEXES

Jen Fountain[1], Senait Gebremedhin[2], Krystyna Konopka[2] and Nejat Düzgüneş[2]

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

OBJECTIVES: (i) To test the hypothesis that cell-penetrating peptides (HIV-Tat-peptide) and microtubule disrupting agents (vinblastine) can facilitate cytoplasmic delivery and nuclear entry of DNA, and (ii) to observe the intracellular localization of cationic liposome-DNA complexes (lipoplexes) in HSC-3 and H413 human oral squamous cell carcinoma (OSCC) cells.

METHODS: HSC-3 and H413 cells were maintained in DME and DME/F12 media with 10% FBS. Cells were seeded in 48-well plates the day before transfection and used at approx. 70% confluency. The plasmid pCMV.luc expressing luciferase under the control of the cytomegalovirus promoter was complexed with optimal volumes Metafectene, with or without the HIV-Tat-peptide, and incubated with the cells for 4 h. Transgene expression was assayed 48 h after transfection, using the Promega Luciferase Assay System. For fluorescence microscopy, cells were seeded in fibronectin-coated Lab Tek II chambered cover glasses. Cells were transfected with rhodamine-labeled Fluo-Metafectene-DNA plasmid complexes. Lysosensor and Hoechst dye were used to stain the lysosomes and the nucleus, respectively. Fluorescence was observed 2, 4 and 48 h after transfection using a Zeiss LSM-510 Confocal Fluorescence Microscope.

RESULTS: HIV-Tat-peptide, at 1 and 5 μg, mixed with 1 μg DNA, and then complexed with Metafectene, enhanced gene expression in HSC-3 cells by 4- and 5-fold, respectively. Treatment of HSC-3 cells with 2-4 μg/ml vinblastine also enhanced gene expression 2-fold. HSC-3 cells displayed a higher level of cell-associated rhodamine fluorescence compared to H413 cells. Metafectene-DNA complexes co-localized with lysosomes in the perinuclear region. Vinblastine treatment enhanced perinuclear localization of lipoplexes.

CONCLUSIONS: HSC-3 cells internalize Fluo-Metafectene more efficiently than H413 cells, that are resistant to transfection. Cell penetrating peptides and microtubule-disrupting agents may be useful for gene delivery to OSCC cells.

This project was funded by an AADR Student Research Fellowship, and a Research Pilot Project Award 03 Activity 072 from the Dugoni School of Dentistry (JF). This work was presented at the 39th Annual Meeting of the American Association for Dental Research, March 3-6, 2010, Washington, DC.

SUBMANDIBULAR VASCULAR AND SECRETORY RESPONSES IN TYPE 2 DIABETIC RATS

Chris Galea[1], Kiri Herchold[1], Brent DeLong[1], Robinson Sabino da Silva[4], Roshanak Rahimian[3] and Leigh Anderson[2]

[1]Dental Program and Departments of Physiological Sciences (School of Dentistry)[2] and Physiology and Pharmacology (School of Pharmacy)[3], University of the Pacific and [4]Institute of Biomedical Sciences, Sao Paolo, Brazil

INTRODUCTION: Type 2 diabetes represents a major public health problem, and recent studies suggest that xerostomia is a common oral complication inpatients with type 2 diabetes. Therefore, the purpose of this study was to examine submandibular vascular and secretory responses in a rat model of type 2 diabetes.

METHODS: Male Zucker fatty diabetic (ZDF, n=8) and Zucker lean (control, n=9) rats were anesthetized and vascular reactivity and salivary flows were measured in response to sympathetic stimulation (2 and 4 Hz continuously, or 20 Hz and 40 Hz in bursts of 1s every 10s). Glandular perfusion was monitored using laser-Doppler flowmetry. For the measurement of salivary flow rates, the submandibular duct was cannulated and the saliva was collected and weighed.

RESULTS: ZDF rats demonstrated a significant increase serum glucose levels compared with controls (497 ± 28 mg/dl vs 138 ± 12 mg/dl). Mean submandibular salivary flow rates (ml/min/g) were significantly reduced in ZDF compared to control rats at frequencies. In control rats, continuous sympathetic stimulation resulted in a net vasoconstriction (-12.6 ± 3.2 % and -27.5 ± 4.6 %) at 2 Hz and 4 Hz, but a net vasodilatation (36.2 ± 5.1 % and 9.3 ± 2.3 %), at 20 and 40 Hz. However, in ZDF rats burst stimulation resulted in a net vasoconstriction (-7.4 ± 8.9 % and 17.0 ± 7.4 %). In addition, the net vasoconstriction induced by 2 Hz continuous stimulation was greater in ZDF compared with control animals ($p<0.05$).

CONCLUSIONS: From these data we conclude 1) that salivary responses to sympathetic stimulation are diminished in type 2 diabetes and 2) that there is an associated decrease in the submandibular vasodilatory response to sympathetic stimulation.

Supported by a grant from the NIDCR (R15 DE016587)

CATIONIC LIPOSOMES ENHANCE ADENOVIRAL GENE DELIVERY TO ORAL SQUAMOUS CELL CARCINOMA CELLS

Grace Lim[1], Senait Gebremedhin[2], Jason Ouellette[1], Ilya Ulasov[3], Maciej Lesniak[3],Krystyna Konopka[2] and Nejat Düzgüneş[2]

[1]Doctor of Dental Surgery Program, and [2]Department of Microbiology, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA; and [3]Division of Neurosurgery, Department of Surgery, University of Chicago, Chicago, IL

OBJECTIVES: To test the hypotheses that (i) adenoviral vectors will efficiently deliver the tumor-specific, survivin-driven luciferase gene to oral squamous cell carcinoma (OSCC) cells, and (ii) cationic liposomes will enhance adenoviral transduction.

METHODS: Two recombinant adenoviral vectors that encode luciferase were used: Ad-Sur-luc and Ad-CMV.luc with the human survivin and cytomegalovirus promoters, respectively. A recombinant adenovirus with polylysine-modified fiber knobs (Ad-pK7-CMV.luc) was also tested. The viruses were incubated with HSC-3, H413, and H357 human OSCC cells at different MOI, and luciferase expression was determined after 48 h. The viruses were also mixed with Metafectene or Metafectene-Pro (Biontex), before incubation with the cells to assess the effect of cationic liposomes on transduction.

RESULTS: Representative luciferase activities with Ad-CMV.luc (MOI 100) in HSC-3, H413 and H357 cells were 30460±1510, 10305±529 and 30907±1015 RLU/ml, respectively. Luciferase activities obtained with Ad-Sur.luc were 200±36, 97±12 and 100±8 RLU/ml, respectively. Metafectene Pro complexed with Ad-CMV.luc increased gene expression in all cell lines by 4 to 9-fold. Metafectene Pro complexed with Ad-Sur.luc enhanced luciferase expression by 13 to 17 fold. Ad-pK7-CMV.luc incubation with HSC-3, H413 and H357 cells resulted in luciferase activities of 15081±1694, 14427±3099 and 24187±272 RLU/ml, which decreased to 4037±446, 3359±206 and 8017±254 RLU/ml, respectively, when the virus was pre-incubated with Metafectene-Pro.

CONCLUSIONS: Transduction of OSCC cells with adenoviral vectors alone did not result in high levels of reporter gene expression that were expected. However, complexing cationic liposomes with the viral vectors greatly improved gene expression. The use of viral vectors in conjunction with cationic liposomes can be a promising tool to help achieve the delivery of suicide genes in the therapy of OSCC.

This project was supported by a Research Pilot Project Award (DRES03-Activity 062) from the University of the Pacific, Arthur A. Dugoni School of Dentistry (JO). This work was presented at the 39th Annual Meeting of the American Association for Dental Research, March 3-6, 2010, Washington, DC.

STUDY OF ASSOCIATION OF NONSYNDROMIC CLEFT LIP AND PALATE IN PHILIPPINES AND RFC1 GENE

A. Tahbaz[1], M. Tolar[2], A. Balghonaim[3], K. Liu[3], H. Berdichevsky[3], A. Tarifard[3], A. Sethu Madhavan[3], J. Leach[3], M. Christie[3], M. Petrovska[3], V. Lee[3], N. Chandwani[4] and Marie M. Tolarová[3]

[1]Doctor of Dental Surgery Program, [2]Pacific Regenerative Dentistry Laboratory, [3]Pacific Craniofacial Team and Cleft Prevention Program, [4]Department of Pediatric Dentistry, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: The etiology of the nonsyndromic cleft lip with or without cleft palate (NCLP) involves interactions of environmental and genetic factors. Several authors and also our studies suggested that A80G polymorphism of the reduced folate carrier 1 (RFC1) gene, which is involved in the transport of folate across the cell surface membrane, was associated with NCLP. Specifically, the mutated G allele was found significantly more frequently in NCLP. Our pilot study of Philippine population that we presented last year, did not confirm association of the RFC1 A80G polymorphism with NCLP in population of Cebu City.

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

METHODS: Individuals affected with NCLP (n=105) and unaffected individuals (n=80) were identified during Rotaplast medical missions to Cebu City, Philippines in 2003, 2005, and 2007. RFC1 A80G genotypes were established by PCR amplification followed by detection of single-nucleotide conformational polymorphism using polyacrylamide gel electrophoresis.

RESULTS: In cases, 33.3% of individuals had A80/A80 genotype, 24.8% had G80/G80 genotype, and 41.9% were heterozygotes (A80/G80). Proportions of genotypes in controls were 35.0% A80/A80, 21.3% G80/G80, and 43.7% A80/G80. The A allele frequency was 0.543 for cases and 0.569 for controls, while the G allele frequency was 0.447 for cases and 0.431 for controls. There was no difference found between neither genotypes distribution nor allele frequency between cases and controls.

CONCLUSION: Results confirm our findings from the pilot study suggesting that polymorphism of the RFC1 A80G may not be involved in the etiology of NCLP in population of Cebu City, Philippines. A strong association of RFC1 gene with NCLP found in Guatemala and San Salvador (Costanzo et al, 2004; Kai et al, 2009, DeLurgio et al 2009) and present findings support hypothesis that different genetic factors forming a genetic susceptibility to NCLP exists in different populations (Tolarova and Mosby, 2007.

The fieldwork for this study was supported by Rotaplast Intl.

2ND YEAR STUDENT RESEARCH COMPETITION

PAX9 GENE POLYMORPHISMS AND MISSING TEETH

A. Abolfazlian*[1], M. Tolar[2], K. Heetland[3], A. Chin[1], A. Balghonaim[4], K. Liu[4], J. Zhu[4], A. Tarifard[4], M. Christie[4], H. Berdichevsky[4], J. Leach[4], M. Petrovska[4], V. Lee[4] and M. Tolarova[4]

[1]Doctor of Dental Surgery Program; [2]Pacific Regenerative Dentistry Laboratory; [3] Graduate Program in Orthodontics; [4]Pacific Craniofacial Team and Cleft Prevention Program, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: It was shown in both mouse and human tooth development that PAX9 and MSX1 are the most important genes regulating progression through early stages of tooth development. These genes are encoding transcription factors involved in epithelial/mesenchymal interactions. Their key function seems to be maintenance and regulation of Bmp4 expression in dental mesenchyme. If the functions of PAX9 and MSX1 are disturbed, the tooth will not develop.

OBJECTIVE: To study one mutation in Exon1 and seven mutations in Exon2 of PAX9 gene in the sample of individuals with missing teeth (probands) and their parents and siblings. Probands as well as their respective family members were classified by the type of missing teeth and family history of hypodontia.

METHODS: Our sample consisted of 43 individuals with congenitally missing teeth; 21 were dental student volunteers and 22 were patients from orthodontic clinic. Altogether, 82 saliva specimens were collected from cases and their immediate family members. Majority of specimens were collected using our own protocol: participants were asked to rinse their mouth with 0.5 oz of Listerine® for 30 seconds followed by a rinse with water for 30 seconds in order to get rid of food particles. They were then asked to spit into a 50 mL Falcon tube until 3-5 mL of saliva was collected. The Falcon tubes were transferred to the Craniofacial genetics laboratory where drops of saliva were spotted on filter paper and allowed to dry. Modified Chelex method was used to extract DNA. A smaller number of specimens were collected using Oragene saliva kit. Following DNA isolation, PCR was done using specific primers for each polymorphism, agarose electrophoresis followed to confirm PCR product, which was then purified and sent to sequencing laboratory. The sequenced specimens were analyzed for PAX9 genotypes.

RESULTS: Out of forty genetically examined individuals with hypodontia, nine were positive for some of the PAX9 polymorphisms. All nine were heterozygotes. Seven individuals had a PAX9 mutation in the DNA-binding region. The numbers are not definitive, because the study is still in progress.

CONCLUSIONS: Results of this pilot study indicate a rather strong genetic component associated with PAX9 gene mutations in individuals with hypodontia. Evaluation of a larger sample will enable us to draw more definitive conclusions regarding the inheritance of hypodontia related to PAX9 gene mutations.

We thank the staff and volunteers of The Pacific Craniofacial Team and Cleft Prevention Program. This study was supported in part by Research Pilot Project Award 03-Activity 073 from the University of the Pacific, Arthur A. Dugoni School of Dentistry.

PLATELET-RICH FIBRIN – TECHNICAL ASPECTS OF PREPARATION

Eric Baker[1], Timothy Betita[1], Preston Hansen[1], Anders Nattestad[2] and Mirek Tolar[3]

[1]Doctor of Dental Surgery Program, [2]Department of Oral and Maxillofacial Surgery, and [3]Department of Orthodontics, Arthur A Dugoni School of Dentistry, University of the Pacific, San Francisco, CA

OBJECTIVES: Preparation of platelet-rich fibrin (PRF) is simple. Freshly collected blood is immediately spun down. Fibrin clot is formed during centrifugation and then is taken out of the tube and applied. Our aim was to examine effects of variations in technical parameters of the procedure on the fibrin clot size and quality.

METHODS: Pairs of vacutainers for preparation of serum (Beckton Dickinson) are filled with 3 ml of freshly collected human venous blood and immediately (within 2 minutes) spun in different speeds: 500, 1000, 2000, and 3000 rpm for 30 minutes on a laboratory centrifuge at room temperature. Fibrin clots are pulled out, washed with saline, weighed and measured (length, width, height).

RESULTS: The fibrin clot is pale, practically devoid of red blood cells. The lower centrifugation speeds are preferable, because the clot collects more platelets and white blood cells and only a small amount of red blood cells.
This work is in progress. Detailed results will be shown on the poster.

CONCLUSIONS: PRF is called second-generation platelet-rich plasma concentrate. It is completely natural - no additives are needed for its preparation. A finer tuning of the procedure may be beneficial for specific surgical procedures.

CLEFT LIP AND PALATE AND RFC1 A80G GENE POLYMORPHISMS

Saoly Benson[1], L. McCullough[1], M. Tolar[2], T. Mosby[3], P. Calda[4], H. Berdichevsky[5], Jamie Leach[5], A. Balghonaim[5], A. Tarifard[5], A. Sethu Madhavan[5], K. Liu[5], Mark Christie[5], and M. Tolarova[5]

[1]Doctor of Dental Surgery Program; [2]Pacific Tissue Engineering Laboratory; [3]St. Jude Children's Research Hospital, Memphis, TN; [4]Charles University in Prague, First Faculty of Medicine, Prague, Czech Republic;[5]Pacific Craniofacial Team and Cleft Prevention Program, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: The etiology of nonsyndromic cleft lip with or without cleft palate (NCLP) is multifactorial, including genetic and environmental factors. Among the most commonly studied genes is RFC1 (Reduced Folate Carrier 1) gene that encodes a cell membrane protein essential for internalizing folate bound to a folate-binding protein from circulating blood into cells. The active form of folate is essential for multiplication, differentiation, and maintenance of cells. Thus, insufficient folate levels in the body have been shown to contribute to various congenital anomalies, including neural tube defects and orofacial clefts.

OBJECTIVES: The purpose of our study was to determine whether RFC1 A80G polymorphism is associated with NCLP in a sample of Czech families of patients affected with NCLP.

MATERIAL AND METHODS: A case-control study design was used. Our samples were comprised of 194 individuals affected with NCLP (cases) and 45 unaffected individuals NCLP (controls). DNA was isolated from dry blood spots on filter paper. RFC1 A80G genotypes were amplified by PCR and genotypes were established using polyacrylamide gel electrophroesis (PAGE).

RESULTS: Cases, in comparison with controls, presented a significantly higher proportion of GG homozygotes ($p=0.038$) and a significantly higher G allele frequency ($p=0.033$).

CONCLUSIONS: Results of this pilot study suggest that the RFC1 A80G polymorphism may participate in the etiology of NCLP in the Czech population. Evaluation of a larger sample will be needed to draw a more definitive conclusion about the role of G allele of RFC1 A80G in the etiology of NCLP in the Czech Republic.

The collection of specimens and data for this study was supported by Department of Obstetrics and Gynecology, First Faculty of Medicine, Charles University in Prague, Czech Republic. This study has been accepted for table clinic poster presentation at the CDA Scientific Session in Anaheim, May 13-16.

EPIDEMIOLOGICAL STUDY OF HYPODONTIA

Allison Chin*[1], Arash Abolfazlian[1], Kelly R. Heetland[2] and Marie M. Tolarová[3]

[1]Doctor of Dental Surgery Program; [2]Graduate Program in Orthodontics; [3]Pacific Craniofacial Team and Cleft Prevention program, Department of Orthodontics, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: The purpose of this study was to ascertain the prevalence of hypodontia among dental students.

METHODS: From the 615 probands, (335 males, 280 females), consisting of DDS and IDS students from the University of the Pacific Arthur A. Dugoni School of Dentistry. Descriptive epidemiology data were collected from 2004 to 2010, and pedigrees were constructed and analyzed. Two forms of data collection were used: (1) A structured questionnaire and (2) a family pedigree drawn by students for recording occurrence of congenitally missing teeth and diastema in the first, second, and third generation relatives. Descriptive epidemiology data were analyzed according to ethnicity, tooth type, gender and family history.

RESULTS: When 3rd molars were included, 122 probands (19.8%) presented with one or more missing teeth. When 3rd molars were excluded, 6.3% (n=39) had one or more missing teeth.

Occurrence of congenitally missing teeth

Number of missing teeth	males		females		total	
	n	%	n	%	n	%
0	271	80.9	222	79.3	493	80.2
1	24	7.2	22	7.9	46	7.5
2	20	6.0	18	6.4	38	6.2
3	8	2.4	6	2.1	14	2.3
4	11	3.3	11	3.9	22	3.6
8	1	0.3	1	0.4	2	0.3
total	**335**	**100.0**	**280**	**100.0**	**615**	**100.0**

Third molars were the most common type of tooth missing and were reported in 75.4% of students with missing teeth, while 15.6% of students were missing other teeth. Maxillary lateral incisors were the second most common missing tooth type, which occurred in 3.3% of probands, followed by second premolars (2.5%). It was much more likely to be missing teeth from both arches (37.9%) than either the upper or the lower arch. It was at least three times more likely to be missing teeth from both the right and left or right side (20.2%)

Our data suggest a strong family history of missing teeth side (64.0%) than solely from the left (15.8%). In 60.7% (n=74) cases, probands had one or more relatives with congenitally missing teeth. Nearly eighteen percent of probands with missing teeth had a first-degree relative also missing a tooth, 7.4% had a second-degree relative missing a tooth, 35.2% had both. No family history was indicated in 36.9% of probands.

CONCLUSIONS: Our results revealed that prevalence of hypodontia among Pacific dental students is 198.05/1000. When missing 3rd molars were excluded, it equaled 63.31/1000. A positive family history for missing teeth was observed in 17.3% of our probands, suggesting that a genetic component is a likely etiological factor, which is consistent with other genetic studies.

We thank the staff and volunteers of the Pacific Craniofacial Team and Cleft Prevention Program, and all the students, patients, and their families who participated in this study. This study has been accepted for table clinic poster presentation at the CDA Scientific Session in Anaheim, May 13-16.

PRENATAL CLEFT LIP AND PALATE DETECTION AND COUNSELING

Lisa Marie DiPasquale[1] and Marie M. Tolarová[2]

[1]Doctor of Dental Surgery Program; [2]Pacific Craniofacial Team and Cleft Prevention Program, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA.

INTRODUCTION: Cleft lip and palate is the second most common congenital anomaly and the most common and most serious anomaly of the orofacial region. The prevalence rates vary between 0.5 and 3 per 1000 births with considerable variations between populations, genders and geographic regions. About 660 children are born with a cleft in the world every day, over 235,000 children every year, bringing many social, psychological and economical difficulties both to the children born with a cleft and to their families. A prenatal detection of cleft lip and palate has allowed parents to learn about treatment that will be needed as well as about networking with other parents having a child affected with a cleft.

PRESENT STATUS OF PRENATAL DIAGNOSIS OF OROFACIAL CLEFTS: Ultrasound is the most common method of prenatal detection. Generally, this is done at 18-20 weeks *in utero*. Currently, 14%-25% cases of cleft lip with or without cleft palate (CL ± P) are detected prenatally in the US and developed countries. Often, CL ± P does not occur as isolated event. Nearly 12% of detected "isolated clefts" are actually associated with other abnormalities. Because of a wide variety of chromosomally and/or genetically determined malformations associated with CL±P, it has been advised to do karyotyping, which can asses chromosomal abnormalities. It should be offered in all cases with an identifiable cleft. Three- dimensional (3D) imaging is the newest and most sensitive technology that is able to diagnose CL ± P with an increased acuity. It was reported that a routine ultrasound screening detected only 20% of CL ± P actually present. Chorionic villus sampling, amniocentesis, and alpha feto-protein testing are other methods of prenatal examination, however, they may not be useful for diagnosis of CL±P.

CONCLUSIONS AND FUTURE DIRECTION: While there is an exciting frontier of improved technical possibilities, a prenatal diagnosis represents a confirmation of an abnormal development and is marked with emotional turbulence and distress. Often viewed as the first glimpse into the identity of their unborn child, prenatal ultrasound diagnosis of a cleft can be very challenging for the new parents, who are grieving a loss of normality of their infant. There is no doubt that the ultimate goal is prevention of cleft lip and palate anomalies. While a "magical bullet" is very unlikely, exciting advancements are being made on the research front in offering insights into the genetic and nutritional components involved in etiology of orofacial clefts. We believe that, with a help of a preconceptional screening in a near future, genetic factors could be identified and environmental factors involved in etiology of CL ± P could be modified or excluded. Then, a prenatal ultrasound could be used to confirm that a fetal face had developed normally. I have had the opportunity to participate in the research conducted in the Pacific Craniofacial Genetics Laboratory, where the roles of several genes implicated in the formation of a cleft are assessed and nutrients ("candidate nutrients"), whose deficiency or excess may be associated with CL ± P, are identified. All this research brings us closer to days when a significant proportion of orofacial clefts will be prevented.

CYTOKINE RESPONSES OF ORAL EPITHELIAL CELLS EXPOSED TO *PORPHYROMONAS GINGIVALIS*

Shawn Kim[1]*, Michael Yee[2], Tamer Alpagot[3], Nejat Düzgüneş[2] and Krystyna Konopka[2]

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

OBJECTIVES: The periodontopathogen *Porphyromonas gingivalis* (*Pg*) adheres to, invades and replicates within human oral epithelial cells. The pro-inflammatory cytokine interleukin-8 (IL-8) is a potent chemoattractant inducing the influx of neutrophils into periodontal lesions. IL-8 is the primary focus of this project, since conflicting results are reported on (i) the *Pg*-induced stimulation of IL-8 in human epithelial cells and (ii) the effects of cysteine proteinases (gingipains) produced by *Pg* on IL-8 secretion. We first screened for multiple cytokines secreted by human oral squamous HSC-3 cells after exposure to live *Pg* and lipopolysaccharide (LPS) from *Pg*. After confirming IL-8 production, we quantified the IL-8 secreted by HSC-3 cells after exposure to live and heat-inactivated *Pg*, and to LPS from *Pg* and *E. coli*.

METHODS: HSC-3 cells were seeded in 48-well plates one day before the experiment and used at 80-90% confluence. The *Pg* strain 2561 was sub-cultivated on blood agar plates and suspended in Medium 199 (4×10^8 *Pg*/ml). HSC-3 cells were challenged with live or heat-killed *Pg* at 10^7 or 10^8 bacteria/well, and incubated at 37°C for 6, 24 and 48 h. LPS from *E. coli* and *Pg* were tested at 5 μg/ml. The Multi-Analyte Profiler ELISArray kit was used to profile pro-inflammatory cytokines and chemokines. The IL-8 level was determined by ELISA.

RESULTS: Experiment #1: Exposure of HSC-3 cells to live *Pg* induced lower levels of IL-8 than treatment with heat-inactivated *Pg*. For example, treatment with 10^8 live and heat-inactivated *Pg* produced 9.6 ± 2.3 and 18.0 ± 1.9 ng IL-8/ml, respectively. Experiment #2: Exposure of HSC-3 cells to live *Pg* induced higher levels of IL-8 than heat-inactivated *Pg*. For example, treatment with 10^8 live and heat-inactivated *Pg* produced 25.4 ± 5.7 and 4.2 ± 0.8 ng IL-8/ml, respectively. Treatment with LPS from *Pg* at 5 μg/ml for 6 h resulted in the production of 3.4 ± 0.2 ng IL-8/ml, while *E. coli* LPS did not stimulate IL-8 secretion. Analysis by the Profiler ELISA indicated the stimulation of IL-6 and IL-8 production by HSC-3 cells stimulated with *Pg* for 6 or 24 h. *Pg* LPS induced the production of TNF-α in addition to IL-6 and IL-8.

CONCLUSIONS: *Pg* induced significant IL-8 secretion in HSC-3 cells. Degradation of IL-8 by cysteine proteinases (gingipains) produced by live *Pg* may be responsible for the higher levels of secreted IL-8 observed with heat-killed bacteria (Experiment #1). Results from Experiment #2 suggest that other factors besides the gingipains may influence the level of IL-8 secretion by HSC-3 cells exposed to *Pg*. Further experiments are needed to elucidate the mechanisms of *Pg*-induced cytokine production in oral epithelial cells and the effects of gingipains.

This work was supported by Research Pilot Project Award 03-Activity 054 from the Arthur A. Dugoni School of Dentistry (K. Konopka) and was presented at the 39th Annual Meeting of AADR, March 3-6, 2010, Washington, DC. J. Dent. Res. Vol. 89 (Special issue A) Abstract No. 381, Seq. #60.

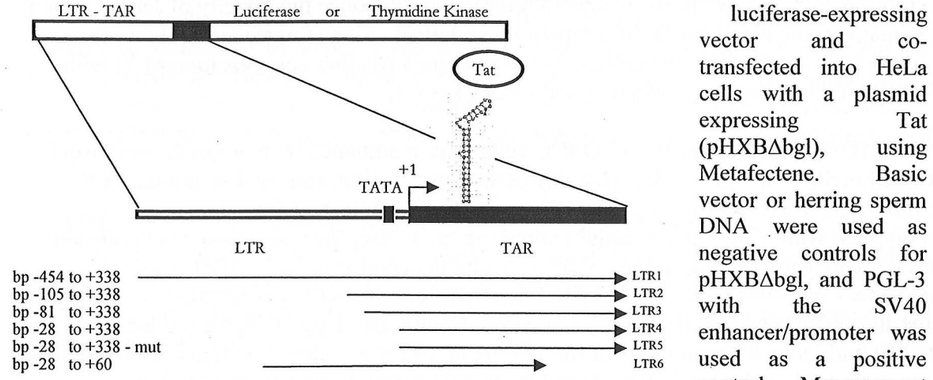
GENE EXPRESSION MEDIATED BY PROGRESSIVELY TRUNCATED HIV-LTR PROMOTERS AND HIV-TAT: HOW TO KILL HIV-INFECTEDCELLS

Joseph King[1], Senait Gebremedhin[2], Krystyna Konopka[2], Nejat Düzgüneş[2] and Matthew Milnes[2]

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

OBJECTIVE: Current anti-retroviral therapies against HIV infection are unable to eradicate the chromosomally integrated pro-viral genome. This study seeks to develop a promoter element that is responsive to the HIV transcriptional activator TAT, but not cellular transcription factors. This HIV-specific promoter may be used to drive the expression of suicide genes that would induce cell death specifically in HIV-infected cells.

METHODS: The full-length HIV-LTR-*tar* (promoter) region was generated using *de novo* gene synthesis. Five truncated clones were generated using PCR. The clones were placed into a luciferase-expressing vector and co-transfected into HeLa cells with a plasmid expressing Tat (pHXBΔbgl), using Metafectene. Basic vector or herring sperm DNA were used as negative controls for pHXBΔbgl, and PGL-3 with the SV40 enhancer/promoter was used as a positive control. Measurement of luciferase in the presence or absence of pHXBΔbgl allowed us to evaluate the effectiveness of the progressively truncated HIV LTR-Tar region in limiting the transcriptional activation to cells expressing Tat. This also allowed us to determine the region of the LTR promoter most specifically responsive to Tat, and not to other cellular transcription factors.



RESULTS: Luciferase activity in cells transfected with both LTR4 and pHXBΔbgl was 12,137 ± 914 RLU/mL, while that in control cells transfected with LTR4 and basic vector (no pHXBΔbgl) was 204 ± 4 RLU/mL. In cells transfected with LTR1, LTR2, or LTR3, luciferase expression was relatively higher in the presence and absence of pHXBΔbgl. In cells containing LTR5 and LTR6 luciferase expression was generally lower compared to LTR4 in the presence and absence of pHXBΔbgl. This indicates a 60-fold increase in Tat-specific expression.

CONCLUSION: LTR4, extending from bp -28 to bp +338, is the HIV transcriptional activating region most specific to and yielding the highest gene expression by Tat activation. Higher luciferase expression in cells containing LTR1, LTR2, and LTR3 probably resulted from non-Tat-specific transcriptional activating regions contained on those fragments of the HIV LTR-*tar* promoter region. LTR4 is a candidate for specific activation of suicide genes in HIV-infected cells.

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

CATIONIC POLYMER-MEDIATED GENE DELIVERY TO ORAL CANCER CELLS: EFFECTS OF TRANSFERRIN AND CHITOSAN

Freddie Martinez[1], Senait Gebremedhin[2], Krystyna Konopka[2] and Nejat Düzgüneş[2]

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

OBJECTIVE: Our laboratory is developing methods to introduce suicide genes into human oral squamous cell carcinoma (OSCC) cells as a therapy for oral cancer. JetPEI, a cationic polyethylenimine, mediates efficient transfection of cancer cells, but causes cytotoxicity. Our aim is to develop a gene carrier that can emulate or surpass the transfection efficiency of JetPEI, while lowering its toxicity. The natural cationic polymer, chitosan, is known for its biocompatibility and low toxicity, but its transfection activity is low. We examined whether chitosan would reduce the toxicity of JetPEI, while maintaining the high transfection capability of JetPEI. Since transferrin (Tf) receptors are overexpressed on cancer cells, we also examined whether complexation of Tf with JetPEI would enhance transfection and reduce toxicity.

METHODS: HSC-3 and H-376 OSCC cells were maintained in appropriate media and seeded in 48-well culture plates the day before transfection, and used at approx. 50% confluence. The cells were treated with complexes of chitosan (Sigma) and JetPEI (Polyplus) with pCMV.luc plasmid expressing luciferase. Varying amounts of chitosan were incubated with 1 μg DNA for 30 min, and then added to 2 μl JetPEI. Two sequences were used in forming Tf/JetPEI/DNA complexes (Tf-polyplexes): (1) Tf (32 μg) and JetPEI (1 or 2 μl) were incubated for 30 min, and 1 μg DNA was added. (2) JetPEI and DNA were complexed for 30 min, and Tf was added. Luciferase expression was measured by the Luciferase Assay System (Promega) and a Turner Designs luminometer. The data were expressed as relative light units (RLU) per ml of cell lysate. The Alamar blue assay was used to determine cytotoxicity, using a Molecular Devices microplate reader.

RESULTS: Chitosan inhibited transfection of HSC-3 cells in a dose-dependent manner. Tf-polyplexes prepared by method (1), but not method (2), increased transfection in both H376 and HSC-3 cells. In lipofection-resistant H-376 cells, the presence of Tf enhanced gene expression by 3.2-fold (1 μl JetPEI) and 2.5-fold (2 μl JetPEI). In HSC-3 cells, a 3.4-fold enhancement by Tf was observed with 2 μl JetPEI. Tf-polyplexes were slightly less toxic, despite the enhancement of transfection.

CONCLUSION: The chitosan JetPEI complex was not successful in reducing cytotoxicity, contradicting results reported by another laboratory. Complexation of Tf with JetPEI enhanced transfection activity in HSC-3 and H376 cells. This is the first report of the enhancement of cationic polymer-mediated transfection by complexation of Tf to polyethyleneimine polymers.

METHYLENETETRAHYDROFOLATE REDUCTASE C677T POLYMORPHISM AND NONSYNDROMIC CLEFT LIP AND PALATE

L. McCullough*[1], S. Benson[1], M. Tolar[2], T. Mosby[3], P. Calda[4], H. Berdichevsky[5], A.Balghonaim[5], A. Tarifard[5], A. Sethu Madhavan[5], K. Liu[5], S. Prasad[6], and M. Tolarova[5]

[1]Doctor of Dental Surgery Program; [2]Pacific Tissue Engineering Laboratory; [3]St. Jude Children's Research Hospital, Memphis, TN; [4]Charles University in Prague, First Faculty of Medicine, Prague, Czech Republic;[5]Pacific Craniofacial Team and Cleft Prevention Program, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA; [6]Goverment Dental College and Research Institute, Bangalore, India

BACKGROUND AND PURPOSE: The etiology of nonsyndromic cleft lip with or without cleft palate (NCLP) is multifactorial, including genetic and environmental factors. Methylenetetrahydrofolate reductase (MTHFR) and folate intake are among those factors intensively studied recently. When MTHFR function is altered due to mutations, a decreased utilization of folate slows down cell replication and it may contribute to orofacial clefting. However, there is no consistency of results from studies of the MTHFR gene. The purpose of our study was to determine whether MTHFR C667T polymorphism is associated with NCLP in Czech families with patients affected with NCLP.

METHODS: Case-control study design was used in our study. Our samples comprise of 119 individuals affected with NCLP (cases), 135 unaffected family members, and 86 unrelated unaffected individuals. DNA was isolated from venous blood. MTHFR 677CT genotypes were established by PCR amplification and SNPs using polyacrylamide gel electrophoresis (PAGE).

RESULTS: Significantly different proportion of genotypes (p=.01) with higher proportion of TT homozygous and also significantly higher T allele frequency in cases compared to controls (p=.005) was found (cases 31.93% CC, 17.65 TT, 50.24%CT, T allele frequency 0.4286; controls 47.68% CC, 5.81% TT, 46.51% CT, T allele frequency 0.2907). There was also higher proportion of TT homozygotes found in subgroup of family members compared to controls (13.33% vs. 5.81%), however the difference found in proportion of genotypes and difference in allele frequencies were not found to be statistically significant.

CONCLUSION: Results of this pilot study suggest that the C667T variant of MTHFR gene is associated with NCLP in Czech population. More studies on larger samples and also including other genes and environmental factors are needed to help us understand the etiology of NCLP in Czech population.

The collection of specimens and data for this study was supported by the Department of Obstetrics and Gynecology, First Faculty of Medicine, Charles University in Prague, Czech Republic. This study has been accepted for presentation at IADR General Session, Barcelona July 14-17, 2010

USE OF ANIMAL MODELS IN STUDIES OF HUMAN CRANIOFACIAL MORPHOGENESIS

R. Mehregan*[1] and Marie M. Tolarová[2]

[1]Doctor of Dental Surgery Program; [2]Pacific Craniofacial Team and Cleft Prevention Program, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA.

INTRODUCTION: There are obvious limitations to how much information can be obtained from studying craniofacial development and defects in human. Studying patients and individuals affected with congenital anomalies only allows looking at end product of the complex and intricate process of craniofacial development; whereas use of animals such as quail, duck, chimeras, finches, and mice as study models has proven helpful in understanding the intricate relationships and processes involved in craniofacial development.

ANIMAL MODELS: (1) Duck-Quail Chimeras. Duck and quail have both a specific shape of beak and rate of maturation induced by neural-crest-derived mesenchyme thus Quail-Duck Chimeras have been used to study the patterns of morphology in these birds. "Quck" (duck embryo with a quail-like beak) and "Duail" (quail embryo with a duck-like beak) studies point to the dominant regulatory role of neural-crest-derived mesenchyme in establishing species-specific beak morphology. (2) Darwin's finches. By studying geological distribution of finch species Darwin discovered that ground finches have deep and wide beaks for crushing seeds, whereas cactus finches have long and pointed beaks for reaching into cactus flowers. (3) Chicken. BMP signaling is required to stimulate mesenchymal cell proliferation and directed outgrowth of the facial prominences. Decreased BMP activity in the mesenchyme regulates cell survival in the epithelium and increases epithelial thickness. Noggin-induced clefts in birds are a reasonable phenocopy of human cleft lip and palate since only the side of the beak is affected and all the midline structures derive from the frontonasal mass are still formed. (4) Mouse. Amino acid alteration in BMP4 result in delayed lip closure in mice. Msx1 and Msx2 genes are part of this pathway and their expression is induced at higher level of BMP receptor signaling. Msx1 knockout mice have cleft secondary palate and tooth agenesis. Double knockout of Msx1 and Msx2 have more severe phenotypes including bilateral cleft lip and palate. (5) Drosophila. BMP signaling is involved in dorsal closure of Drosophila, a model system for wound healing. A conserved genetic pathway seems to be involved in both wound healing and cleft lip and palate.

CONCLUSION: Birds offer advantages for studying craniofacial morphology that complement the strengths of other vertebrate models such as mice. Bird embryos can be manipulated in many ways to alter gene expression and the results can be applied to other vertebrates. However, despite all the similarities of development to humans, the avian system is not a perfect study model for human development and has its limitations.

The authors thank R.A. Schneider for generously providing help and support with the topic of avian craniofacial development.

ISOLATION, EVACUATION, ILLUMINATION SYSTEM: AN ALTERNATIVE TO RUBBER DAM?

Amanda Pinder[1], Vikram Tiku[1], Foroud Hakim and Jessie Vallee

[1]Doctor of Dental Surgery Program, and [2]Department of Restorative Dentistry, University of the Pacific Arthur A. Dugoni School of Dentistry

INTRODUCTION: Currently there are Isolation, Evacuation, Illumination systems (IEIS) available in the dental marketplace. The IEIS retracts the tongue and cheeks, provides continuous suction, illuminates the oral field, protects the airway and esophagus, and includes a bite block for sustainable opening by the patient.

OBJECTIVE: The goal of this study is to compare the relative intraoral humidity control attained by an IEIS to the gold standard, rubber dam. A secondary goal is to measure the consistency of humidity control throughout a restorative procedure.

METHOD: During routine clinic hours, humidity readings are recorded during procedures with an IEIS or rubber dam.

RESULTS: Early results show equivocal humidity control by the IEIS compared to the rubber dam, but more data is required.

CONCLUSION: Depending on the outcome of the research, the efficacy and applicability of an IEIS can be further evaluated for use by the dental practice community as well as dental education.

We would like to acknowledge the Restorative Department at the University of the Pacific Arthur A. Dugoni School of Dentistry for their support and guidance.

SEQUELAE AND BENEFITS OF INCREASED VERTICAL DIMENSION OF OCCLUSIONA

Jordan Priestley[1]* and Gabriela Pitigoi-Aron[2]

[1]Doctor of Dental Surgery Program, and [2]Department of Restorative Dentistry, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: Clinically there are a number of reasons in which increasing the vertical dimension of occlusion would be both useful and desirable. However, doing so has traditionally been stigmatized. Many reports indicate that adverse sequelae may outweigh the clinical benefits of opening vertical dimension. To confirm or refute this sentiment a literature review of articles published in peer reviewed journals was conducted.

PURPOSE: To search existing published literature for indications and outcomes following increased vertical dimension. Particular emphasis was placed on the physiological and clinical impacts following such procedures.

METHODS: Peer reviewed journal articles were selected and studied. The information gathered was compiled into a literature review paper for presentation.

RESULTS: A negative sentiment regarding increased vertical dimension has waxed and waned throughout the history of modern dentistry. Currently, studies show that there are indications and positive clinical results following such procedures. Contrary to past belief, vertical dimension is a dynamic, adaptive range. Histological studies confirm that masticatory myofibers actually have the capacity to remodel over a period of time. Initial discomfort is a frequent complaint, however the vast majority of symptoms resolve within one week. Increased EMG activity and temporomandibular disorder are in fact not sequalae of increased vertical dimension, despite anecdotal accounts.

CONCLUSION: Increasing the vertical dimension of occlusion is a viable option to satisfy functional and aesthetic demands. Initial symptoms resolve relatively quickly and a new vertical dimension of occlusion is achieved and maintained.

THE INFLUENCE OF siRNA ON GENE EXPRESSION AND CELL PROLIFERATION IN CANCER CELLS

Scott Sutter[1]*, Michael Yee[2], Nasser Said-Al-Naief [3], Krystyna Konopka[2] and Nejat Düzgüneş[2]

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

OBJECTIVES: Small interfering RNA (siRNA) is a group of predefined gene inhibitors that are capable of aborting cell proliferation via selective targeted gene suppression. siRNAs may prove to be very valuable in targeting cancer cell proliferation, that in turn, would help in the development of new targeted gene therapies. We investigated the influence of siRNAs targeting two proteins involved in cell division, LDHA and Stat3, on the survival and proliferation of cancer cells. To assess the efficacy of siRNA delivery, we investigated the inhibition of luciferase (luc) expression by anti-luc siRNA in cells co-transfected with plasmids expressing this enzyme under the control of human survivin (SRVN), cytomegalovirus (CMV) and Simian virus 40 (SV40) promoters.

METHODS: HeLa human cervical carcinoma cells were seeded in 48-well culture plates and used at ~80% confluence. The plasmids {pSRVN.Luc-1430, pCMV.Luc (VR-1216) and pSV40.Luc pGL3-Control Vector (CV)} expressing luciferase were complexed with DharmaFECT DUO and anti-luc or negative siRNA control (Dharmacon). Transfection activity (relative light units (RLU)/ml of cell lysate) was assessed by luciferase expression assayed 48 h following transfection, using the Promega Luciferase Assay System and a luminometer. In addition, DharmaFECT was complexed with anti-LDHA, anti-Stat3 or negative siRNA control (25 nM) and added to the cells. To quantify cell proliferation, cells were trypsinized at 24, 48, and 72 h following siRNA treatment, Trypan blue was added, and the cells were counted on a Countess automated cell counter.

RESULTS: Challenging the cell cultures with 25 nM siRNA against Stat3 and LDHA did not cause any inhibition of cell proliferation. Luciferase activity expressed by pCMV.Luc (221,513$\pm$ 25,911 RLU/ml) was diminished greatly by specific siRNA (6,693$\pm$1,364 RLU/ml). Luciferase expression from pGL3-CV (8345$\pm$1007 RLU/ml) was also inhibited significantly by siRNA (115$\pm$18 RLU/ml). Control, non-target siRNA also had some inhibitory activity (197533$\pm$ 28433 RLU/ml and 2349$\pm$ 486 RLU/ml).

CONCLUSIONS: The current study confirms the applicability of siRNA and the transfection reagent DharmaFECT DUO in assessing the capability of inhibiting target gene expression in cancer cells. The lack of antiproliferative activity of a siRNA against Stat3 and LDHA may be attributed to the potentially low transfection efficiency of DharmaFECT in this system. It is also possible that the extent of target gene knockdown was not sufficient to affect the cell division machinery of these cells. Future studies will explore siRNA against other target genes, and evaluate the extent of Stat3 and LDHA knockdown in these cells. These siRNAs will also be tested in the proliferation of oral squamous cell carcinoma cells.

GARLIC EXTRACT INHIBITS S. MUTANS BINDING TO HYDROXYAPATITE

Diane Vo [1], K. Polosukhina[2] and S. Highsmith[2]

[1]Doctor of Dental Surgery Program, [2]Department of Physiological Sciences, University the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: Garlic extract (GE) has been reported to inhibit S. mutans (SM) growth and cause cell cytotoxicity. SM is a significant causative factor in oral biofilm and caries formation. Our goal is to characterize the effects and extent of GE on SM viability, on SM reversible binding in a single layer on hydroxyapatite (HA), and on biofilm formation on HA beads. We are particularly interested in the effects of GE on electrostatic interactions.

HYPOTHESES: (1) SM viability is reversibly inhibited by treatment with GE. Viability will be restored with removal of GE. (2) GE will reduce repulsive negative surface charge of SM and HA, which will increase binding of SM to HA.

METHODS: SM is suspended in neutral phosphate buffer for planktonic preparations, incubated for 1 hour with 20μm HA beads for a single layer of SM, or incubated for 48 hour with HA beads in the presence of sucrose for biofilms. White garlic is pressed, and the extract is diluted with an equal volume of water to obtain GE. After incubation, free GE is removed by washing with phosphate buffer. SM viability is determined by fluorescence spectrophotometry using SYTO9/propidium iodide to obtain live/dead ratios. Surface charge is estimated by zeta potentials (ζ), calculated from electrophoretic mobilities using the Smoluchowski equation. Planktonic growth is determined from increasing turbidity at 600 nm. Fluorescence micrographs are obtained after staining with SYTO9 alone or with propidium bromide.

RESULTS: Live/dead ratios indicated planktonic SM treated with GE are 43% dead. Washing with buffer had no significant restorative effect. Growth rates of SM after GE exposure confirmed that 40% of SM is killed. ζ for HA, SM, and single layer HA-SM were –2.61, –0.51, and –1.10 mV, respectively. Treatment with GE did not change ζ for SM, but ζ for HA and HA-SM was -1.55 mV, consistent with no SM binding to HA. Adding GE to single layer HA-SM layer caused SM to dissociate of SM from HA beads.

CONCLUSIONS: Washing/removal of GE did not restore SM viability: it is irreversibly killed. GE exposure results in SM dissociating from HA beads, perhaps by decreasing surface charges.

IDS STUDENT RESEARCH PRESENTATIONS

TEETH WHITENING: A REVIEW OF LITERATURE

Masooma Cheema, Catherine Legaspi, Veronica Montgomery, Navneet Sahota, Fabiola Palacios-Barrionuevo, Richard White and Noelle Santucci

Department of Restorative Dentistry, International Dental Studies, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA

INTRODUCTION: There is a steady rise in demand by the general public to have whiter teeth. Because Teeth Whitening has come more popular, various whitening products and systems have been introduced in the market. In addition to the in-office light-activated system, take home professionally dispensed kits, over-the-counter products have made it convenient for people to achieve adequate results.

OBJECTIVE: The purpose of this poster was to review the literature regarding Teeth Whitening Systems, look at the historical prospective, types of systems and products available, mechanism of action, side-effects and their management.

METHOD: We searched various journals in pub-med and product review journals using the keywords "teeth whitening", and "products for teeth whitening". Reviewed 100 article and journals.

RESULTS: Much literature has been published in regards to the tooth whitening procedures and products. Recent studies explain that no exact mechanism of tooth bleaching has been established regarding the reaction that occurs on enamel and dentin. But, it has been suggested that diffusion of peroxide into dental structures reacts with the organic colored material within and leads to lightening of the shade. Bleaching procedures has inherent side effects on both hard and soft tissues in the oral cavity, although when they are performed properly, their effects are generally mild and transient. Therefore, teeth whitening is deemed a safe, effective and convenient procedure. In-office light activated whitening offers immediate results with mild rebound and take home touch up kits are required. Whitening trays and strips offer similar results in terms of whitening when compared with In-office systems, but require longer time. Trays can be used for all teeth whereas strips are usually limited to anteriors. Lasers are being used for teeth whitening but do not show superior results when compared to the conventional methods of teeth whitening.

CONCLUSION: Teeth whitening has been one of the major accomplishments in modern cosmetic dentistry. Proper diagnosis, selection of whitening materials, placement techniques, and an understanding of the biologic interaction with soft and hard tissue are all factors that determine not only immediate success but also long-term success, safety, and patient satisfaction as well. It is also one of the most simple esthetic treatment a dentist can offer to his patients.

LASERS IN DENTISTRY

***Meenakshi Dewan, *Jigna Khetani, *Prerna Kultham, Armand Lugassy, Laura Reid, Noelle Santucci and Richard Lubman**

International Dental Studies Program, Department of Restorative Dentistry, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: A laser is a device for generating a high-intensity, parallel beam of monochromatic (single wavelength) electromagnetic radiation. The first commercial dental laser was introduced in 1990. In the last two decades, advances in lasers have led to their increased use in medicine and dentistry. Both soft and hard tissue applications including esthetic contouring of the gingiva, cavity preparation etc. have gained momentum in the dental field.

OBJECTIVES: The objective of this literature review was to understand the mechanism of lasers, the various types available, their uses in dentistry and safety.

METHODS: Resources from various search engines such as Pubmed, Google Scholar and Library chronicles were used with key words such as lasers, hard lasers and soft laser and their safety.

RESULTS: The laser can interact with the tissue in various ways. Their effects on hard and soft tissues depend on the wavelength, power density and energy density. Desired effects such as photothermal, photochemical or photomechanical effects may be produced by altering such parameters. Lasers are being used for procedures like caries detection(DIAGNODENT), cavity preparation, curing resins, gingival troughing, gingivectomy, sulcular debridement, soft tissue curettage, bone contouring and endodontic procedures.

CONCLUSIONS: Lasers are excellent tools, more precise and may eliminate the need of local anesthesia in restorative procedures and provide excellent hemostasis when used in surgeries. But they also bear a very high risk for severe injury and damage. It is therefore imperative to use strict protective measures and recommended energy parameters while using this technology.

SINGLE TOOTH IMPLANT REPLACEMENT AS A TREATMENT MODALITY IN RESTORATIVE DENTISTRY

Mohammad Faizan Ali, Aouse Khalil, Naira Tariq Kraipak and Nazish Mir.Advisors-Gitta Radjaeipour, Larry Gardner, Noelle Santucci, Richard Lubman and Robert Ahlstrom

Department of Restorative Dentistry, International Dental Studies Program, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: Implant dentistry is the art and science of bioartificial restoration of lost dental structure that can adequately fulfill the esthetic, functional and phonetics requirements of an individual. Leventhal introduced the concept in bone surgery in 1951nand then Brenmark in 1969 introduced the concept in dentistry using commercially pure titanium, which is the current standard of care. The introduction of Implants in Dentistry has had a tremendous impact and implants have emerged as a viable treatment option in various clinical scenarios.

OBJECTIVES: The purpose of this review was to explore the various Implant systems that are available and to explore the rationale for single tooth implants as a treatment modality. We studied the various factors that a clinician needs to consider prior to case selection and also compared single tooth implant replacement with conventional endodontic therapy. In order to understand the technique, The Delayed loading and Immediate Loading Protocols were studied. A review of various Abutments as well as latest developments in CAD CAM for single tooth implant restorations was reviewed.

METHODS: Literature for this review was obtained from Peer Reviewed Articles through PUB MED database. Various commercially available Implant systems, which include Nobel Biocare, Astra Tech and Biomet 3i were reviewed.

RESULTS: A review of literature suggests that the Implant survival rate was 95% in comparison to 94% to endodontic therapy. One study indicates that most implant systems exceed the 5-year survival rate easily however; it is vital for clinicians to understand that studies carried out on implant and endodontic therapies are measuring different parameters. Implant studies look at implant survival rate whereas endodontic studies look at success of endodontic therapy hence it is difficult to compare the two. Therefore decisions should be based on criteria rather than success rates. Various studies indicate single tooth Implant replacement is a viable treatment option and has a high success rate of 99% in the mandible and 97% in the maxilla. It should be pursued when conventional treatment modalities such as endodontic therapy are not possible or have a poor prognosis. Delayed placement and loading protocol has higher success rates in comparison to immediate loading protocol. The Immediate Loading protocol requires precise case selection and it is challenging for clinicians to manage esthetic complications. Papillary contracture is the most common esthetic complication associated with maxillary anterior implants.

CONCLUSION: Implant Dentistry is an ever changing and evolving field and it is important for clinicians to be aware of the various new technological advancements. Implant success depends on proper case selection and operator technique. It is vital for clinicians to understand the various clinical considerations involved in treating patients by using single tooth implants. The ability to identify potentials for failure and to modify these factors to enhance success is valuable.

DENTIN BONDING AGENT APPLICATORS: EFFECT ON SHEAR BOND STRENGTH OF DENTAL COMPOSITE TO DENTIN

Kunal Karan[1]*, Karen Schulze[2] and Lawrence Gardner[2]

[1]International Dental Studies, [2]Department of Restorative Dentistry, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA

INTRODUCTION: Properties of dental composite materials have improved to a great extent and this has made dental composite a viable and reliable treatment option. Over the years, use of composite materials has grown quite significantly (also as an esthetic alternative to amalgam), but bonding to dentin remains an area of skepticism. It is well documented that adequate bonding to dentin is of paramount importance to produce more consistent long term clinical results. The growing demand from both patients and dentists has resulted in a market that offers a plethora of composite materials and other aids for restorative dentistry. The responsibility to make every possible effort to ensure highest clinical success lies with the dental care provider.

OBJECTIVES: The purpose of this laboratory based experimental study was to compare the shear bond strength achieved by using three different applicators while all other variables (bonding agent, application protocol, composite material) were kept the same. This would help us determine if a particular type of applicator had a distinct advantage over other applicators used in this experiment.

METHODS: Extracted human posterior teeth were used for this study. The teeth were first sectioned to remove the apical 2/3rd of the root and then in a longitudinal plane to generate sections with exposed dentin. The specimens were then secured on a mounting jig using mylar strips and then reinforced using mounting stone. The two halves of the jigs were then attached using screws. Following the same steps, a total of 18 specimens were prepared and then randomly distributed in three groups. The exposed dentin sections were acid etched (Ultra-Etch, Ultradent) for 10 seconds followed by a 10 second water rinse. Dentin bonding agent (Peak LC Bond, Ultradent) was applied using a different applicator for each group (Group 1- Benda Brush, Centrix; Group 2- Kerr Applicator, Kerr; Group 3- Peak LC Bond applicator tip, Ultradent). After the bonding agent was applied and cured for 20 seconds, resin composite (Permaflo, Ultradent) was placed over all specimens and light cured for 40 seconds. The specimens were then stored overnight at 37°C and 100% humidity. The following day all specimens were tested until fracture using the universal testing machine Instron 1011. Student t-test was applied for statistical evaluation.

RESULTS: The average bond strength for Benda Brush, Kerr Applicator Brush and Peak LC Bond applicator were 16.7 (±2.7), 20.0 (±7.6) and 16.4 (±3.3) respectively. The shear bond strength was highest in the Micro Brush group, but the difference was not found to be statistically significant.

CONCLUSIONS:

1) Based on the results of this study we did not find any statistically significant difference in the shear bond strength achieved with the three different applicators tested.
2) Until studies find significant advantage associated with a particular applicator, it might be in the best interest of both dentists and patients to use applicators based on personal preference and cost of applicators.

THE EVOLUTION OF ALL-CERAMIC RESTORATIONS: MATERIAL & TECHNOLOGY

Kunal Karan*, Michael Tiplea, Saritha Ketepalle, Nayereh Rashidan, Robert Christoffersen, Wei-Shao Lin, Noelle Santucci and Richard Lubman

Department of Restorative Dentistry, International Dental Studies, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, California.

INTRODUCTION: The scope of use of all-ceramic restorations has increased dramatically over the last 40 years. Over this period of time much of the materials research and development has been directed towards producing stronger, reinforced restorations with improved physical and esthetic properties. The evolution of materials has left dental practitioners with a wide range of ceramic materials to choose from but this also necessitates that they have a good knowledge of the physical properties of materials being used today. Therefore, precise attention to detail with regard to material selection is of paramount importance for long term clinical success.

OBJECTIVES: The purpose of this literature review was to summarize the landmarks in development of all-ceramic restoration materials, in terms of chemistry, properties and processing; and the impact these factors have on case based material selection. This information will enable dentists to make better clinical decisions and choice of materials for use in particular situations.

METHODS: A search for current and past literature was done for systematic reviews, clinical studies and laboratory based experiential studies on all-ceramic materials. Textbooks were also used as references for this review and relevant information was gathered and summarized.

RESULTS: Since the 1960s, when all ceramic restorations were first used as Porcelain Jacket Crowns there have been innovative developments in this field, both in material science and laboratory processing. The 1980s saw the inception of CAD/CAM technology. New heat pressed materials like IPS Empress I&II were introduced in 1990s and the first decade of 21st century brought along the development of Zirconia based materials. All along the way manufacturers have tried to provide dentists with materials with the best possible combination of high strength and excellent esthetics. While this has provided the impetus for some great innovations, development of "the ideal material" remains an ongoing pursuit. Currently some products are marketed as universal ceramic materials, but there is not a single material or system that meets the requirements for all clinical situations. Until this is done and affirmative results are produced, the onus of achieving high clinical success is on the dentist and this requires careful choice of materials based on scientific data.

CONCLUSIONS:

1) No evidence to advocate the universal application of a single ceramic material or system for all clinical situations because properties of available products do not meet the requirements for clinical success in all situations.
2) More longitudinal clinical studies conducted over adequate study periods are required to analyze the durability of these materials prior to making definitive clinical recommendations.

MINIMALLY INVASIVE DENTISTRY: A LITERATURE REVIEW

Krishna Neelagiri*, Priya Prasannakumar, Shinil Thomas, Sireesha Moola, Avery Lieberman, Noelle Santucci and Richard Lubman

Department of Restorative Dentistry, International Dental Studies, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA

INTRODUCTION: Minimally Invasive Dentistry (MID), a modern, evidence-based approach to caries management in dentate patients, uses a medical model whereby disease is controlled by the "oral physician" and an affiliated dental team. The "minimally invasive" approach to treating dental caries incorporates the dental science of detecting, diagnosing, intercepting and treating dental caries on the microscopic level. This approach to treating dental caries includes many non-surgical modalities, as well as the key concept that dental caries should be treated as an infectious disease.
OBJECTIVES: The objective of this poster encompasses a review of literature of several published articles that include the assessment of caries risk to reinforce patient self-help and early detection of the disease with the help of QLF, digital radiography and DIFOTI. This poster also reviews the several conservative methods and techniques available in the market today including but not limited to ART, air abrasion, Caridex, laser and ozone. The biomimetic restorative materials available presently such as glass ionomers and composite resins are also discussed with respect to their advantages in restoring conservative preparations.
METHOD: We searched the medical journal database for research articles and review articles using the keywords "Minimally Invasive Dentistry" and reviewed approximately 100 articles and journals.
RESULTS: Caries Management by Risk Assessment (CAMBRA) helps in modification of oral flora to promote oral health, patient education and participation and remineralization of non cavitated lesions. Early detection using newer technologies like QLF and DIFOTI have more specificity and sensitivity over explorers which are only usually 17-40% correct. Methods such as air abrasion, chemomechanical caries removal and laser have the advantages of requiring minimum anesthesia in addition to more precise and conservative removal of tooth structure during cavity preparations. Fluoride in any form has been shown to reduce caries incidence by 25%. Xylitol and baking soda increase the pH of the oral cavity and help neutralize the acid produced after sucrose intake. The adhesive properties of biomimetic restorative materials such as Glass Ionomers and Composite resins have helped in the restoration of conservative preparations.
CONCLUSIONS: Minimally Invasive Dentistry is the way to future. Minimal Intervention is not just a technique, but a philosophy. Scientific developments in cariology, dental materials and diagnostic systems have changed dentistry's approach to diagnosis and management of dental caries. This conservative approach minimizes the restoration/re-restoration cycle, thus benefiting the patient over a lifetime.

ACKNOWLEDGMENTS

We would like to highlight the following individuals, groups and organizations in appreciation of their interest, time, efforts, support and generosity in ensuring that Pacific Research Day continues to be a showcase for the achievements of Students, Staff and Faculty at Pacific, as well as a forum for discussion of research results and for establishing collaborations. Mr. Eric Bertumen of the Basic Sciences Department deserves special recognition for his work on the organization of Pacific Research Day.

Western Dental Services
Procter & Gamble Oral Health
Johnson & Johnson

Dr. Patrick Ferrillo, Dean

Basic Sciences Department
- Barbara Chacon
- Nancy Bellaci
- Malou Ruperto
- Emilia Segura

Evaluation Committees
Senior Research Competition
- Dr. Stefan Highsmith
- Dr. Bill Lundergan
- Dr. Benjamin Zeitlin

Second Year Student Research Competition
- Dr. Joel Cohen
- Dr. Stephen Koons
- Dr. Dottie Burk
- Dr. Ove Peters

Photo & Design
- Joan Yokom
- Brian Blanchard
- Sandra Shuhert
- Devon Bailey
- Jon Draper

Student Store
-Maria Murtagh
-Matthew Clerici
-Nell Herbert
-Freedom Santos
-Norma Gallegos

Research and Graduate Studies
-Dr. Carol Brodie