

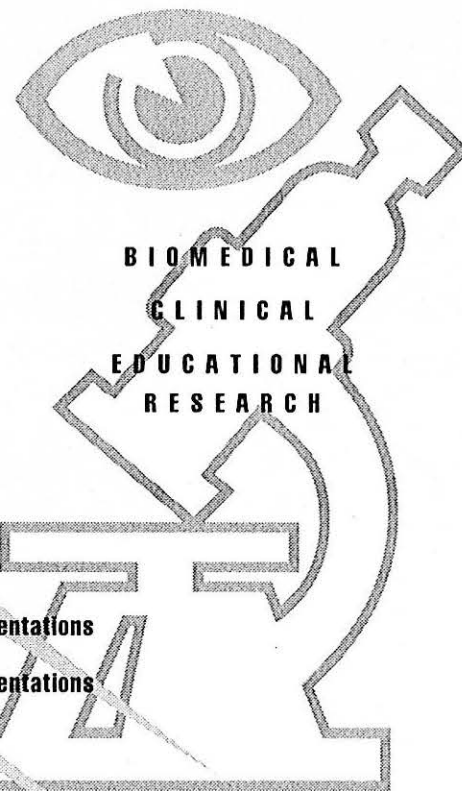
2ND ANNUAL

**UOP RESEARCH DAY &
SENIOR RESEARCH
COMPETITION**

tuesday, may 30, 2000



**ortho resident presentations
faculty & student presentations**



**BIOMEDICAL
CLINICAL
EDUCATIONAL
RESEARCH**

NOON PROGRAM

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ABSTRACTS

THE DEVELOPMENT OF LIPOSOMES FOR LOCAL DRUG DELIVERY: LONG TERM STABILITY STUDIES

Matthew D. Swatman

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The goal of our laboratory is to develop liposomes that will effectively deliver therapeutic agents to specific targets within the oral cavity. The purpose of this investigation is to determine the binding stability of polyethylene glycol (PEG) coated anionic (-A) or cationic (-C) liposomes to target molecules. We compared the binding to fibronectin and collagen, known to exist in the periodontal pocket, and the binding to plastic or glass surfaces used in in vitro experiments. Observations were continued for up to 7 days. All of the liposomes contained 35 mole % of cholesterol for stability and 0.4 mole % rhodamine-PE, a fluorescent label that permitted us to determine the amount of liposomes bound to the target. PEG-A and PEG-C bound to plastic, fibronectin-coated glass, collagen-coated glass and glass in the order of decreasing affinity. Curve peeling of the data describing the Ln of the % of the initial rhodamine signal against time showed that after 1 week: collagen still bound 18% and 15% and fibronectin bound 30% and 42% of the initial bound PEG-A and PEG-C, respectively. We conclude that: (1) plastic should be avoided for in vitro binding experiments; (2) PEG-C and PEG-A would bind to fibronectin over collagen; (3) both PEG-C and PEG-A will bind sufficiently to warrant further study as components of an oral drug delivery system.

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DEVELOPMENT OF LIPOSOMES AS A VEHICLE FOR LOCAL DRUG DELIVERY IN TREATMENT OF PERIODONTITIS: LIPOSOME BINDING AFFINITY TO HUMAN ROOT DENTIN

Gerald Kim

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The purpose of this research program was to test the feasibility of using liposomes (bacterial-sized vesicles of phospholipid) to station antibiotics on the surface of root dentin, to keep periodontal-diseased pockets free from bacteria. The goal of this study was to determine the ideal liposome that could bind to root dentin, by altering the liposome surface charge and composition, along with different root surface conditions. Sixty premolars extracted for orthodontic reasons were divided into three root surface groups: non-treated, root planed, and root planed with 10 minutes of 25% citric acid treatment. Each of these three groups was exposed to four different types of liposomes: anionic polyethyleneglycol-coated (an-PEG), cationic polyethyleneglycol-coated (cat-PEG), anionic non-coated (an-nonPEG), and cationic non-coated (cat-nonPEG). Each type of liposome was labeled with rhodamine to quantify the amount of liposome binding per unit root-surface area under the various conditions. Zeta potential measurements were used to identify the surface charge of each type of liposome. It was found that all four types of liposomes bound poorly to untreated dentin, root planed dentin, and root planed with citric acid-treated dentin, regardless of the class of charge or the presence of PEG coating. In addition, all four liposome species bound to less than 1% of the total root surface. These results suggest that: 1) all of the variables studied contributed little to the amount of binding of liposomes per unit area of root surface; 2) the use of liposomes by themselves, without suspension or gel media, is inefficient since the small amount of binding per cm^2 of root dentin compounded with the small volume of drugs contained in each liposome results in insufficient therapeutic concentrations at the target location.

CREVICULAR FLUID ELASTASE LEVELS IN RELATION TO PERIODONTITIS AND METABOLIC CONTROL OF DIABETES

T. Alpagot^{1,2}, S. Silverman², W. Lundergan¹, C. Bell¹ and D.W. Chambers¹

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The purpose of this study was to investigate the associations between gingival crevicular fluid (GCF) elastase levels, clinical measures of periodontal status, and metabolic control of diabetes. Forty patients were recruited from diabetes Center at University of California in San Francisco. Twenty five subjects were type 1 diabetics and 25 subjects were type 2 diabetics. Metabolic control was evaluated by glycosylated hemoglobin (HbA1c) levels. Control group was consisted of 35 medically healthy individuals. Clinical measurements (gingival index, probing depth and attachment level) and GCF samples were taken from the mesio-buccal surfaces of 2 premolars and 2 molars from the most diseased sextant. GCF elastase was determined by measurement of p-Nitroanalide resulting from hydrolysis of elastase specific peptide. The mean amounts of elastase in both type 1 and type 2 diabetics were significantly higher than the mean amounts of elastase in control group ($p < 0.001$). Crevicular fluid elastase levels were significantly correlated with probing depth and attachment level in all groups ($0.01 < p < 0.001$). HbA1c levels were not correlated with elastase, probing depth and attachment level. The results suggest that GCF elastase is a risk indicator for periodontitis in patients with diabetes mellitus, and periodontal disease is not associated with the level of diabetic control

This study was supported by the Pacific Dental Research Foundation, Grant # PDRF 508.

LONGITUDINAL EVALUATION OF GCF MMP-3 AND TIMP-1 LEVELS AS PROGNOSTIC FACTORS FOR PROGRESSION OF PERIODONTITIS

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To determine whether Matrix metalloproteinase-3 (MMP-3) and Tissue Inhibitor of Metalloproteases-1 (TIMP-1) in gingival crevicular fluid (GCF) could serve as prognostic factors for the progression of periodontitis, we monitored GCF MMP-3 and TIMP-1 and periodontal status of selected sites in 40 medically healthy subjects over a 6-month period. Clinical measurements (gingival index, plaque index, bleeding on probing, suppuration, probing depth and attachment level) and GCF samples were taken from 2 healthy and 2 periodontitis sites of each patient at baseline, 3-month and 6-month visits by means of sterile paper strips. GCF levels of MMP-3 and TIMP-1 were determined by sandwich ELISA assays. The mean amounts of MMP-3 and TIMP-1 in diseased sites were significantly higher than the mean amounts of these enzymes in healthy sites ($p < 0.001$). Significantly higher GCF levels of MMP-3 and TIMP-1 were found at progressing sites than in nonprogressing periodontitis sites ($0.001 < p < 0.01$). GCF levels of MMP-3 were highly correlated with clinical measurements taken at baseline, 3-month and 6-month visits ($p < 0.001$). TIMP-1 levels were only moderately correlated with probing depth and attachment level ($0.01 < p < 0.05$). These data indicate that sites with high GCF levels of MMP-3 and TIMP-1 are at significantly greater risk for progression of periodontitis.

This study was supported by the Pacific Dental Research Foundation, Grant # PDRF 511.

EVALUATION OF THE SENSITIVITY OF TWO DENTAL EXPLORERS

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Twelve experienced instructors from the fixed prosthodontics and the restorative dentistry departments at the University of the Pacific School of Dentistry evaluated a crown margin of known opening using both a #3 and a #17 explorer. The margin opening was controlled by use of a newly developed measuring instrument. This instrument simulates a perfectly seated clinical crown which can be continuously unseated and the micron gap measured. Each instructor evaluated the margin by tactile feel only. The evaluator was randomly given either the #3 or the #17 explorer first. The marginal opening was set at 300 microns and the evaluator was asked whether they would cement a crown with this marginal opening. The gap was then closed in 12.5mm increments until the evaluator stated that it was acceptable for cementation. The same procedure was repeated with the other explorer. The results of the study were as follows: Ten evaluators would accept a larger marginal opening with a #3 explorer compared to a #17 explorer. One evaluator would accept a larger marginal opening with a #17 explorer compared to a #3 explorer. One evaluator found no difference between the #3 and the #17 explorers.

BINDING OF SECRETORY LEUKOCYTE PROTEASE INHIBITOR (SLPI) TO HUMAN CELLS IS NOT AFFECTED BY TREATMENT WITH HEPARINASE

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It has been reported that secretory leukocyte protease inhibitor (SLPI), a potent serine protease inhibitor, may be a major anti-HIV factor present in whole saliva. The anti-HIV effect of SLPI does not depend on its anti-protease activity and is most likely due to the interaction of SLPI with cell surface molecules other than the primary HIV-1 receptor, CD4. Previously we observed that binding of recombinant (r) SLPI to human cells did not correlate with the expression of surface proteins CD4, CD26 and CCR5. Recently, it has been reported that the cell surface glycosaminoglycan, heparan sulfate (HS), may participate in HIV-cell attachment and virus entry. Cationic rSLPI forms a tight complex with the negatively-charged polysulfated glycosaminoglycan, heparin. Thus, it can be hypothesized that binding of rSLPI to HS expressed on the cell surface may affect HIV interaction(s) with its receptors and inhibit infection. In this study we examined if treatment with heparinase I, the enzyme used in the HIV infection studies, affects the binding of rSLPI to human cells. Differentiation of monocytic THP-1 cells into adherent macrophage-like cells leads to a marked increase of cell surface HS. Cells were pretreated with heparinase for 1 h at 37°C, washed and incubated with rSLPI. The amount of SLPI in cell lysates was measured using a SLPI ELISA assay. The binding of SLPI was cell type-dependent. For example, after incubation with rSLPI at 10 µg/ml, the amount of cell-associated SLPI was 41, 258 and 235 ng/ml/million cells, for non-adherent THP-1, differentiated THP-1 and HeLa cells, respectively. Treatment with heparinase did not reduce SLPI binding. Under the same conditions, HIV-1 infection of HeLa-CD4 cells after heparinase treatment was reduced by approximately 70%. Based on these findings, we propose that binding of rSLPI to human cells, and hence its HIV inhibitory activity does not involve cell surface HS.

This work was supported by the Pacific Dental Research Foundation, Grant 516.

INTRACELLULAR DELIVERY OF ANTI-HIV AGENTS VIA pH-SENSITIVE LIPOSOMES AND TRANSFERRIN-LIPOPLEXES

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Intracellular delivery of novel macromolecular drugs HIV-1 may be achieved by encapsulation in or association with certain types of liposomes. Liposomes may also protect these drugs against nucleases. Low molecular weight charged antiviral drugs may also be delivered more efficiently via liposomes. An HIV-1 protease inhibitor encapsulated in conventional negatively charged multilamellar liposomes was about 10-fold more effective and had a lower EC₉₀ than the free drug in inhibiting HIV-1 production in human macrophages. The EC₅₀ of the reverse transcriptase inhibitor PMEA was reduced by an order of magnitude when delivered to HIV-1-infected macrophages in pH-sensitive liposomes. A 15-mer antisense oligodeoxynucleotide against the Rev response element was ineffective in free form against HIV-1 replication in macrophages, while delivery of the oligonucleotide in pH-sensitive liposomes inhibited virus replication. The oligodeoxynucleotide encapsulated in sterically stabilized pH-sensitive liposomes with prolonged circulation *in vivo*, was also highly effective. A ribozyme complementary to HIV-1 5'-LTR delivered in pH-sensitive liposomes inhibited virus production by 90%, while the free ribozyme caused only a slight inhibition. Co-transfection of a gene encoding a Rev-binding aptamer and a proviral HIV clone into HeLa cells via transferrin-associated cationic liposomes inhibited virus production. Delivery of plasmids containing the CMV promoter via transferrin-lipoplexes was also highly effective in inhibiting HIV production from the proviral clone. Thus, liposomes can be used to enhance the therapeutic effect of certain anti-HIV agents. This may be advantageous in the development of novel macromolecular drugs which may combat virus strains resistant to the currently available drugs.

STORED MECHANICAL ENERGY IN MOLECULAR MOTORS

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We have correlated force production by muscle fibers with substrate-induced structural changes of the myosin motor domain. The myosin motor domain structure includes a movable segment called the lever arm. We compared ATP and GTP as substrates. Both ATP and GTP are hydrolyzed by muscle actin-myosin, but others have shown that GTP produces much less force. It is hypothesized that when ATP is hydrolyzed by myosin, the lever arm rotates making the motor domain more compact, which enables it to store mechanical energy. In solution, more compact structures have smaller hydrodynamic size, and therefore have smaller rotational decay times, τ . In the absence of actin, we measured τ for the myosin motor domain at 20°C in the presence of ATP and GTP. The results confirm that when ATP is the substrate, the lever arm rotates (τ is smaller). On the other hand, when GTP is the substrate, it does not. The data suggest that the lever arm must be rotated to create stored mechanical energy in order for force to be produced.

Supported by NIH grant AR42895.

| SUBSTRATE | ISOMETRIC FORCE, % | Rate of Hydrolysis at 25°C, 1/s | τ without substrate, ns | τ with substrate, ns |
|-----------|--------------------|---------------------------------|------------------------------|---------------------------|
| ATP | 100 | 0.071 | 252.9 \pm 1.4 | 241.5 \pm 0.9 |
| GTP | 10 | 0.093 | 248.9 \pm 2.2 | 251.1 \pm 1.3 |

NEURAL REGULATION OF BLOOD FLOW IN THE RAT SUBMANDIBULAR GLAND

L.C. Anderson and J.R. Garrett

Blood flow in salivary glands is regulated mainly by sympathetic and parasympathetic nerve activity. This study was carried out to determine the relative contributions of cholinergic, adrenergic and peptidergic neurotransmitters to the control of submandibular gland blood flow in the rat using laser-Doppler flowmetry. Parasympathetic impulses caused a rapid atropine-sensitive vasodilation followed by a maintained increase in blood flow, a portion of which remained in the presence of both atropine and the nitric oxide synthase (NOS) inhibitor, L-NAME. In contrast, continuous sympathetic stimulation caused an intense vasoconstriction that was followed by a prolonged after-vasodilation. The same number of impulses delivered in bursts, however, resulted in a cyclic vasoconstriction followed by a rapid vasodilation (a net vasodilation). Alpha-adrenoceptor blockade largely abolished the vasoconstriction, and the duration and magnitude of the after-vasodilation were reduced. Inhibition of NOS by L-NAME reduced the vasodilation. The addition of a β -adrenoceptor antagonist eliminated the sympathetic vasodilator response, but in the presence of complete α - and β -adrenoceptor blockade and L-NAME a small vasoconstrictor response remained. We conclude from these data that the vasoconstrictor effects of sympathetic stimulation are due to α -adrenoceptor activation and probably also NPY, whereas the vasodilator effects are due to nitric oxide (NO) and β -adrenoceptor activity. Parasympathetic vasodilation was due both to NO-independent mechanisms mediated by acetylcholine and substance P, and to NO-dependent mechanisms mediated by VIP.

Supported by the University of Washington Graduate School Fund (LCA) and the Wellcome Trust (JRG).

HYALURONAN, CD44S, AND CD44V7-8 EXPRESSION IN ORAL CANCER AND PRECANCER

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Altered expression of hyaluronan (HA), CD44s, and CD44 splice variants have been associated with invasion and metastasis of variety of malignancies. The aim of this study was to examine the expression of HA, CD44s, and CD44v7-8 in progressive grades of dysplasia and oral squamous cell carcinomas (SCC). Biopsies of 19 dysplasias and 14 squamous cell carcinoma from oral mucous membranes were selected for study because they were either part of a series of biopsies from a single patient or because they included normal appearing epithelium (internal controls). Biopsies of normal mucosa (10) were also included for comparison. Formalin-fixed, paraffin-embedded sections were immunohistochemically stained utilizing a standard overnight incubation protocol. HA was identified with biotinylated HA binding-protein, and staining specificity was controlled by predigestion with streptomyces hyaluronidase. Antigen retrieval (microwave-citrate buffer) was used for CD44 stains. A semiquantitative scale was used in assessing the stained sections with emphasis on expression at the epithelial-connective tissue interface. HA was expressed by keratinocytes (membrane & cytoplasmic) in at least the lower $\frac{1}{2}$ of epithelium of all specimens; HA was frequently underexpressed by keratinocytes at the connective tissue interface in progressive lesions and in SCCs. In all cases CD44s was moderately expressed (membrane) in the lower half of the epithelium and occasionally underexpressed by tumor basal cells. CD44v7-8 membrane staining was present in the lower $\frac{1}{2}$ to $\frac{3}{4}$ of the epithelium; variable reduced basal cell staining was evident in neoplasia. Results suggest reduced expression (heterogeneous pattern) of HA, CD44s and CD44v7-8 occurs in the process of oral SCC invasion

Supported by NIDCR/NCI grant P50 DE 11912.

STEALTH LIPOSOMES THAT BIND TO COLLAGEN AND FIBRONECTIN FOR 1 WEEK COULD BE USED FOR DRUG DELIVERY

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The goal of our laboratory is to develop liposomes that will effectively deliver therapeutic agents to specific targets within the oral cavity. The purpose of this investigation is to determine the binding stability of polyethylene glycol (PEG) coated anionic (-A) or cationic (-C) liposomes to target molecules. We compared the binding to fibronectin and collagen, known to exist in the periodontal pocket, and the binding to plastic or glass surfaces used in *in vitro* experiments. Observations were continued for up to 7 days. All of the liposomes contained 35 mole % of cholesterol for stability and 0.4 mole % rhodamine-PE, a fluorescent label that permitted us to determine the amount of liposomes bound to the target. PEG-A and PEG-C bound to plastic, fibronectin-coated glass, collagen-coated glass and glass in the order of decreasing affinity. Curve peeling of the data describing the Ln of the % of the initial rhodamine signal against time showed that after 1 week: collagen still bound 18% and 15% and fibronectin bound 30% and 42% of the initial bound PEG-A and PEG-C, respectively. We conclude that: (1) plastic should be avoided for *in vitro* binding experiments; (2) PEG-C and PEG-A would bind to fibronectin over collagen; (3) both PEG-C and PEG-A will bind sufficiently to warrant further study as components of an oral drug delivery system.

Supported by Grants from the Pacific Dental Research Foundation and American Association of Orthodontists Foundation.

IONIC STRENGTH DEPENDENT ELECTRO-OSMOTIC FLOW IN GRAFTED POLYMER LAYERS ON LIPOSOME SURFACES: APPLICATIONS TO INTRAVENOUS DRUG DELIVERY

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Liposomes decorated with surface-grafted neutral polymers, particularly poly(ethylene glycol) (PEG), are pharmaceutically useful as drug delivery vehicles because of their low immunogenicity. Protection of a liposome surface from immune-system recognition is achieved by end-grafting sufficiently long PEG polymers at sufficiently high densities. The degree of surface coverage and the hydrodynamic thickness of the surface polymer layer are therapeutically important and can be studied by electrophoretic measurement of polymer-induced hydrodynamic drag. From the measured liposome electrophoretic mobility, the standard Smoluchowski equation yields the location of the liposome "shear surface", which is related to the polymer-layer hydrodynamic thickness. Since PEG is a completely neutral polymer, the coat thickness is expected to be nearly independent of ionic strength. We measured electrophoretic mobilities of liposomes containing 10% negatively-charged PEG-grafted lipids, with PEG ranging from 350 to 5000 daltons, in NaCl ranging from 0.5 to 100 mM. Surprisingly, a *strong* ionic-strength dependence of the coat thickness was found, with the thickness decreasing as ionic strength increases. To analyze this effect, we solved the Navier-Stokes equation for electro-osmotic flow in the polymer layer assuming a rectangular polymer-segment density profile and Stokes friction between polymer and water. It was shown that the Smoluchowski equation gives the correct steric coat thickness *only* for very low ionic strengths and/or high hydrodynamic friction in the polymer layer. With increasing ionic strength and/or decreasing friction, the apparent thickness of the coat decreases. Comparison of this model with experiment shows the correct qualitative behavior of the apparent coat thickness on ionic strength. We have achieved a new understanding of the use of particle electrophoresis to determine polymer coat thicknesses on liposomes and have established new criteria for reliable electrophoretic measurements of steric coat thicknesses for liposome-polymer formulations used in intravenous drug delivery.

THERAPY FOR ORAL SQUAMOUS CELL CARCINOMA USING CATIONIC LIPID-DNA COMPLEXES FOR GENE DELIVERY

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Cationic lipids have been shown to be efficient vectors for the delivery of therapeutic genes to various epithelial cell types. Oral Squamous Cell Carcinoma (OSCC) is a well-diagnosed epithelial malady with a high rate of morbidity due to insufficient early diagnosis and treatment. A number of gene therapy strategies using viral and non-viral vectors for the treatment of OSCC are being explored. Our long-term goal is to deliver the plasmid-based HSV-tk "suicide gene" to OSCC cells using cationic lipid-DNA complexes. In this system gene expression in a large percentage of cells is a key factor in achieving a therapeutic effect, i.e. ganciclovir-mediated cytotoxicity. Thus, transfection efficiency was optimized first in HeLa cells and then in the OSCC cell line, SCC-9, using a variety of transfection reagents and the plasmid pCMV.SPORT- gal expressing β -galactosidase (β -gal). Forty eight hours after transfection, the cultures were fixed in paraformaldehyde and the number of cells visibly expressing β -gal, as demonstrated by conversion of the substrate (X-gal) to a blue product, were determined. Diffuse and nuclear expression of β -gal activity in the cells demonstrated effective intracellular delivery of the vector and expression of β -gal. Four cationic lipid formulations were used: Lipofectin (GIBCO-BRL), Lipofectamine (GIBCO-BRL), Fugene (Boehringer Mannheim) and Escort (Sigma). Of these lipid reagents, Fugene provided the highest efficiency of transfection. Approximately 30% of HeLa and 10% of SCC-9 cells were positive for β -gal staining. We conclude that cationic lipid-DNA complexes may be used for suicide gene therapy of OSCC, considering the likelihood that phosphorylated ganciclovir may diffuse into neighboring cells via gap junctions (the "bystander effect").

This work was supported by an AADR Student Research Fellowship.

COMPARISON OF FOUR INTERMEDIATE MATERIALS FOR USE WITH CONDENSABLE COMPOSITES WITH MARGINS ON DENTIN

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This in vitro study examines the marginal sealing ability of four different intermediate materials applied before placement of a condensable composite. class ii preparations were made with gingival margins 1 mm. apical to the cej of 60 extracted teeth, randomly assigned to groups of 12. following restoration, teeth were thermocycled, soaked in .5% basic fuchsin, then sectioned longitudinally. the resin-modified glass ionomer cement demonstrated significantly less microleakage than the use of a dentin bonding agent alone or in combination with flowable composite, flowable compomer, or autopolymerizing composite ($p < .05$, Dunn's test). this study supports the use of the glass ionomer open sandwich technique in deep class ii direct composite restorations.

INTRODUCTION OF EXOGENOUS GENES IN CARDIAC MUSCLE CELLS FOR OVEREXPRESSION OF Ca²⁺ ATPase AND MODIFICATION OF CELL FUNCTION

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Using recombinant adenovirus vectors we are able to introduce cDNA encoding Ca²⁺ transport ATPase isoforms in cardiac muscle cells in culture, thereby increasing by three fold the cellular content of ATPase. The recombinant ATPase is functional and exhibits kinetics compatible with the specific isoform used, i.e. the skeletal muscle ATPase is faster than the cardiac ATPase even when expressed in cardiac cells. An increased Ca²⁺ transport by the overexpressed ATPase results in faster relaxation of cardiac beats, an effect that is believed to be beneficial in cardiac failure.

ASSOCIATIONS OF TOOTH LOSS AND EDENTULISM WITH ESTROGEN REPLACEMENT THERAPY AND FEMORAL BONE MINERAL DENSITY: CROSS-SECTIONAL EVALUATION OF U.S. ADULTS FROM NHANES III

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The objectives of this study were to evaluate the possible associations of tooth loss and edentulism with 1) femoral bone mineral density (BMD) and 2) history of estrogen use in a sample of U.S. adults 40 years or older (N=8,621). The number of missing teeth per person and edentulism were the outcome variables. Based the total BMD of the proximal femur, according to the diagnostic criteria established by the World Health Organization, subjects were classified as having osteoporosis, osteopenia and normal BMD. Multivariate analysis conducted in the whole sample revealed that, after adjusting for confounders, there was a significant association between edentulism and BMD. However, among dentate individuals, there was not a significant association between missing teeth and BMD. The adjusted odds ratios (95% CI) for edentulism were 1.56 (1.24, 1.96) and 1.19 (1.00, 1.41) for osteoporotic and osteopenic persons, respectively (in reference to persons with normal BMD). Postmenopausal women who reported having used estrogen supplementation in the past, presented significantly less missing teeth and were less frequently edentulous than those who never used estrogen ($P<0.005$). The data also suggests that this association between estrogen and missing teeth is greatly influenced by the protective effect of estrogen against periodontal loss of attachment.

ASSOCIATIONS OF PERIODONTAL DISEASE WITH FEMORAL BONE MINERAL DENSITY AND ESTROGEN REPLACEMENT THERAPY: CROSS-SECTIONAL EVALUATION OF U.S. ADULTS FROM NHANES III

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The objectives of this study were to evaluate the possible association of periodontal disease with 1) femoral bone mineral density (BMD), and 2) estrogen replacement therapy in a large sample of U.S. adults (N=11,655). The mean clinical attachment loss (CAL) per person was the main outcome variable. Based on the total BMD of the proximal femur and using the WHO diagnostic criteria, subjects were classified as having osteoporosis, osteopenia, or normal BMD. After adjusting for confounders, females with high calculus scores and low BMD had significantly more CAL than females with normal BMD and similar calculus scores ($P<0.0001$). No association was observed among women with low and intermediate levels of calculus. The greater CAL present among women with low BMD was associated with gingival recession. Patterns of findings were similar but equivocal among men, of whom only 66 were osteoporotic. After adjustment for possible confounders, postmenopausal women who reported having used estrogen replacement therapy presented significantly less mean CAL than those who never used estrogen. These findings indicate that in the presence of high calculus scores, females with osteoporosis are at increased risk for attachment loss and that this risk may be attenuated by the use of estrogen replacement therapy.

ORTHODONTIC TREATMENT OUTCOMES

D. Poulton and S. Baumrind

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This study includes samples of treated cases, using study casts taken before and after full fixed appliance orthodontic treatment. Samples were evaluated from offices representing three modes of practice: traditional private practice, (3 offices, 83 cases), dental corporation (2 offices, 53 cases), and a dental management service organization (1 office, 35 cases). Orthodontic specialists provided the treatment in all cases. Within each office, lists were produced which documented sequentially all orthodontic cases started after the date selected, generally about 3 years prior to the date of listing. From these lists, at least 25 cases were selected serially which fit the study criteria. The reasons for not including listed cases in the study were noted in each instance. The casts were duplicated and coded so that those doing measurements did not know their origin. The PAR index and the HLD index were applied to all casts by four evaluators who were calibrated and had extensive experience in the use of one of the indices. In addition, subjective evaluation of the difficulty of the cases and the amount of improvement achieved was performed by experienced orthodontists. It is not possible to generalize about the modes of treatment because of the limited number of offices sampled, but some interesting differences were observed.

COMPETENCY-BASED EVALUATION AS PREDICTION RATHER THAN DESCRIPTION

Colin Bell and David W. Chambers

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

The transition to competency-based education requires rethinking evaluation. Traditionally students' eligibility for graduation is inferred from an average of grades describing work in various disciplines. In competency-based evaluation, student ability is projected from information (a portfolio) that includes more than discipline descriptions. This study was undertaken to test the hypothesis that competency can be projected more accurately using general evaluations than from discipline-specific descriptions. The eight clinical competency ratings for 146 students in the last two, clinical years of their education were used. The averages of from 3 to 15 faculty ratings were collected in 5 disciplines (endodontics, fixed and removable prosthodontics, operative dentistry and periodontics). Discipline/discipline projections were calculated for each department. First clinical quarter ratings were used to predict graduation competency, second quarter predicting graduation, etc. R-values for the predictions 6 or 7 quarters in advance of graduation were all close to .100; 1 or 2 quarter projections were between .350 and .400. Projections were recalculated with a different set of predictors. The discipline projected was removed and all other disciplines, plus diagnosis and judgment and patient management ratings were added. Predicting competency in a specific discipline using ratings other than the predicted discipline 6 or 7 quarters out resulted in r-values of .350; 1 or 2 quarters in advance $r = .450$. Of the 27 comparisons for which full data exist, disciplines predict the same disciplines better than general ratings predict these disciplines in only 1 case. The average discipline/discipline correlation was .229; the average other/discipline correlation was .407.

ABLATION AND CARIES INHIBITION OF OCCLUSAL SURFACES BY IR LASERS

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Recent studies have indicated that IR lasers designed specifically for hard tissues ablate enamel at markedly different temperature thresholds based on whether the principal absorber is water or mineral. The objective of this study was to test the hypothesis that specific IR laser irradiation used for ablation of the occlusal pits and fissures also inhibits caries progression. The pit and fissure areas of extracted human teeth were ablated with Er:YAG ($\lambda=2.94 \mu\text{m}$, 200 μs pulse duration), CO₂ ($\lambda=9.6 \mu\text{m}$, 5 μs pd), or Er:YSGG ($\lambda=2.79 \mu\text{m}$, 200 μs pd) laser irradiation (n=10 per group). As a control, "enamelplasty" was performed on non-irradiated pits and fissures using a high-speed 1/4 round carbide bur. The teeth were then subjected to pH cycling to create artificial caries-like lesions. Thin (80 μm) sections were subjected to polarized light microscopy and transverse microradiography (TMR). The ΔZ (relative mineral loss, vol. % x μm) values(SD) were 2074(703), 1053(787), 1047(416), and 583(261) for bur, Er:YAG, CO₂, and Er:YSGG respectively. The % inhibition of caries progression was 50% for both CO₂ and Er:YAG and 72% for Er:YSGG. Statistically, all laser groups were significantly superior ($P<0.005$) in caries inhibition compared to the control (bur) group. The results indicate that these lasers are well suited for removal of hard tissue on pits and fissures and, in addition, have marked caries preventive effects.

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