13TH ANNUAL

PACIFIC
RESEARCH DAY

Wednesday, May 18, 2011

Abstracts

Second-Year Student Research Competition

Senior Research Competition

IDS Student Presentations

Orthodontic Resident Presentations

Dental Hygiene Student Presentations

Stockton Campus Student Presentations

UCSF Invited Presentations

UNIVERSITY OF THE PACIFIC
Arthur A. Dugoni School of Dentistry

EXCELLENCE DAY

advancing research and treatment

13th ANNUAL PACIFIC RESEARCH DAY AND STUDENT RESEARCH COMPETITIONS

ABSTRACTS

WEDNESDAY, MAY 18, 2011

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FACULTY AND STUDENT PRESENTATIONS

THE EFFECT OF THREE DIFFERENT RPM SETTINGS ON TORQUE AND APICAL FORCE WITH VORTEX ROTARY INSTRUMENTS IN VITRO

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OBJECTIVE: Both number of rotations in curved canals and torque relate to fracture resistance of Nickel titanium rotaries, via the distinct mechanisms of brittle and flexural failure. Using rotaries at increased RPM may lead to higher cutting ability and thus overcompensate for increased fatigue. The impact of three different settings for rotational speed (RPM) on peak torque (in Nmm) and apically directed force (in N) during root canal preparation were investigated.

METHODS: Simulated s-shaped canals in plastic blocks (n=12 per group) were instrumented with Vortex rotaries (Dentsply Tulsa Dental) sizes #15-30, .04 taper. A total of 216 preparation procedures were carried out using a custom testing platform. RPM was set at 200, 400 and 600; automated axial feed mirrored clinical handling, resulting in two in-and-out movements to preset insertion depths. Rotaries were used in a sequence recommended by the manufacturer. Torque and apical force were continuously recorded and peak values statistically contrasted using ANOVAs.

RESULTS: File fractures were not observed in any of the three experimental groups. Peak torques and forces varied by instrument size and were highest at 200 RPM for all sizes; torque and force were reduced by 32 and 48%, respectively, at 400 RPM ($p<0.001$). Increase to 600 RPM did not result in further reductions. Moreover, the number of discernible peaks for torque (threshold 0.3Nmm) and force (threshold 0.2Nmm) increased from 200 to 400 RPM and did not increase further with 600 RPM.

CONCLUSION: We conclude that under the present experimental conditions, rotational speed had a significant impact on preparation with Vortex rotaries, with instruments at 400 RPM generating less torque and force compared to 200 RPM.

This work was presented at the 2011 American Association of Endodontists Annual Meeting in San Antonio, Texas, April 13, 2011. It has been accepted for publication in the Journal of Endodontics.

C-REACTIVE PROTEIN IN TYPE 2 DIABETES AND DENTURE STOMATITIS

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OBJECTIVES: *Candida*-associated denture stomatitis (*Ca*DS), is a common disease in complete denture wearers with type 2 diabetes mellitus (T2DM). C-reactive protein (CRP), an acute phase reactant is a sensitive marker of inflammation associated with diabetes. The aim of this study was to determine the concentrations of CRP in patients with T2DM and *Ca*DS, and investigate the relationship between CRP concentrations and glycated hemoglobin A1c (HbA1c) levels, which was used as an indicator of glycemic control.

MATERIALS AND METHODS: The study involved 110 T2DM patients (63 women and 47 men, mean age 63.2±10.5 years) with *Ca*DS, and 20 patients (12 women and 8 men, mean age 65.8±12.9 years), with T2DM and healthy oral mucosa. A group of 20 non-diabetics (11 women and 9 men, mean age 59.2±9.9 years) with healthy oral mucosa served as a control. The yeasts were isolated by the culture method, and identified by microscopic examination and with the test kit, ID 32 C (bioMerieux SA, Marcy-l'Etoile, France). Serum concentrations of CRP were determined by rocket immunoelectrophoresis according to Laurell. Glycemic control was evaluated by measuring HbA1c levels using HPLC together with the Variant Hemoglobin A1c Program (Bio-Rad Laboratories, Hercules CA, USA).

RESULTS: The mean duration of diabetes was 10.6 ± 5.1 years, and the mean HbA1c levels in T2DM subjects were 8.6 ± 1.9%. Patients with T2DM had significantly elevated mean levels of CRP in comparison to patients with normal glucose metabolism (6.12 ± 2.86 *vs* 2.57 ± 0.96 mg/l, $p<0.05$). The highest CRP levels were observed in diabetics with type II (Newton classification) *Ca*DS (8.39 ± 2.70 mg/l). A positive correlation was found between the concentrations of CRP and fasting plasma glucose levels ($r_S=0.517$, $p<0.001$) and HbA1c levels ($r_S=0.572$, $p<0.001$).

CONCLUSIONS: The findings suggest that CRP concentrations can be used as a non-specific indicator of ongoing inflammation in patients with T2DM.

This work was presented at the 89th General Session & Exhibition of the International Association for Dental Research and 40th Annual Meeting of the American Association for Dental Research, March 16-19, 2011, San Diego, CA.
J. Dent. Res. Vol. 90 (Special issue A) Abstract No. 3504, Seq. #389

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

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OBJECTIVES: We are developing efficient vectors to deliver suicide genes to oral squamous cell carcinoma (OSCC) cells. Many oral cancer cells are resistant to lipid-mediated transfection. The cationic polyethylenimine, JetPEI, mediates efficient transfection of cancer cells, but causes cytotoxicity. We examined whether chitosan, a biocompatible polymer with low toxicity, would reduce the toxicity of JetPEI, while maintaining the high transfection capability of JetPEI. We also examined whether complexation of transferrin (Tf) with JetPEI would enhance transfection and reduce toxicity.

METHODS: Varying amounts of chitosan (Sigma) were incubated with 1 µg pCMV.luc plasmid encoding luciferase for 30 min, and then added to 2 µl JetPEI (Polyplus). HSC-3 and H-376 OSCC cells were treated with these complexes, and gene expression was measured 48 h later, using the Luciferase Assay System (Promega) and a luminometer. Tf/JetPEI/DNA complexes (Tf-polyplexes) were prepared in two ways: (1) Tf and JetPEI were incubated for 30 min, and DNA was added. (2) JetPEI and DNA were complexed for 30 min, and Tf was added. The Alamar blue assay was used to determine cytotoxicity.

RESULTS: Chitosan inhibited the 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.

CONCLUSIONS: The chitosan JetPEI complex was not successful in reducing cytotoxicity, contradicting results reported by another laboratory. However, 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.

This work was presented at the 89th General Session of the International Association for Dental Research and 40th Annual Meeting of the American Association for Dental Research, March 16-19, 2011, San Diego, CA.
J. Dent. Res. Vol. 90 (Special issue A) Abstract No. 1359, Seq. #166

ISOLATED HOMINID CRANIAL FRAGMENTS FROM THE OMO RIVER BASIN: 1969-1975 COLLECTIONS

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OBJECTIVES: The hominid-bearing Shungura and Usno formations in the lower Omo Basin date to between ≈3.4-1.4 my. The long temporal span of the Omo stratigraphic sequence provides one of the longest continuous records of hominid evolution. Taxa represented include multiple species of *Australopithecus* and *Homo*. Here we provide the first descriptions and interpretations of fragmentary cranial remains attributable to two hominids from the 1969-1975 collections.

METHODS: Specimen L345-11 is a right parietal fragment that derives from Submember C9 of the Shungura formation (≈2.51 my). Specimen P996-17a-b derives from Shungura Member K (≈1.45 my) and comprises a fragment of frontoparietal, preserving the bregmatic region and portions of the coronal suture, and a fragment of the left pteryonic region. Specimens were CT scanned on a GE Lightspeed VCT scanner and reconstructed with a standard convolution kernel with 0.3 mm voxels. Comparative CT scans were obtained from the Virtual Anthropology laboratory at the University of Vienna. Isosurfaces and triangular meshs were generated for comparative purposes using Amira 3D visualization software (Version 5.3.1).

RESULTS: Specimen L345-11 is a fragmentary right parietal that retains ≈38.5 mm of the coronal and 32.0 mm of the sagittal suture. Cranial thickness at bregma is 7.5 mm but the vault thins to ≈4.0 mm laterally. A low ridge of bone parallels the sagittal suture, indicating the presence of a developing crest. Specimen P996-17a-b comprises a fragment of frontoparietal, preserving the bregmatic region and portions of the coronal suture, and a fragment of the left pteryonic region. Vault thickness at bregma is 11.0 mm and, while thinner at sphenion/krotaphion, it remains as thick as ≈7.5 mm in this region. These specimens are compared to a sample of East African hominid crania.

CONCLUSIONS: While the remains are fragmentary, reconstructions based on CT scans and mirror imaging demonstrates that the L345-11 specimen is most similar to *Australopithecus boisei* females in cresting pattern and vault shape. Given the time period in which the specimen was recovered, it is best classified as aff. *Australopithecus aethiopicus*. The L345-11 specimen represents the first presumed female *A. aethiopicus* recovered. The P996-17a-b compares best with early African *Homo erectus* (KNM-ER 3733 and OH-9). Based on vault contours, brain size, and general robusticity we assign the remains of the P996-17 individual to *Homo erectus*.

Funding provided by a faculty research grant to GDR from the A. A. Dugoni School of Dentistry, University of the Pacific (No. 03-Activity-059) and a grant from the Undergraduate Student Opportunity Fund, University of California, Berkeley to CFH.

GENE DELIVERY TO ORAL CANCER CELLS BY TRANSFERRIN-POLYPLEXES AND TAT-PEPTIDE-LIPOPLEXES

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OBJECTIVES: Many oral squamous cell carcinoma (OSCC) cells are resistant to lipid-mediated transfection. We examined whether complexation of transferrin (Tf) with the cationic polyethylenimine, JetPEI, or the combined use of the HIV-Tat cell penetrating peptide and the cationic lipopolyamine liposome, Metafectene, enhances transfection of OSCC cells.

METHODS: HSC-3, H357 and H376 cells were maintained in DME/10 medium, and H413 cells in DME/10 F12, both with 10% FBS. Cells were seeded in 48-well plates the day before transfection and used at approx. 70% confluency. Tf-polyplexes were prepared in two ways: (1) Tf and JetPEI were incubated for 30 min, and DNA (pCMV.luc plasmid) was added; (2) JetPEI and DNA were complexed for 30 min, and Tf was added. Tat-peptide-Metafectene complexes were prepared in different sequences. The condensation of DNA by the complexes was determined by PicoGreen fluorescence using a Perkin-Elmer LS50B fluorometer. The cells were treated with these complexes for 4 h and gene expression was measured 48 h later, using the Luciferase Assay System (Promega) and a Turner Designs 20/20 Luminometer. The Alamar blue assay was used to determine cytotoxicity, using a Molecular Devices Versamax Microplate Reader

RESULTS: Tf-polyplexes prepared by method (1), but not method (2), increased transfection in both H376 and HSC-3 cells. Tf-polyplexes enhanced gene expression by about 3-fold in HSC-3 cells and lipofection-resistant H376 cells. Tf-polyplexes were slightly less toxic than plain polyplexes, despite the enhancement of transfection. Tat-peptide-lipoplexes produced variable enhancement of gene expression in HSC-3, H357 and H376 cells, but not in H413 cells. These complexes condensed DNA to an equivalent and maximal extent. Tat-peptide-DNA complexes without Metafectene were ineffective in gene delivery, and they did not condense DNA.

CONCLUSIONS: Tf-JetPEI polyplexes and HIV-Tat-Metafectene lipoplexes enhanced gene transfer to a number of OSCC cells. These complexes may be useful in suicide gene therapy oral cancer.

This work is being presented at the 14th Annual Meeting of the American Society of Gene and Cell Therapy, May 18-21, 2011, Seattle, WA.

DISINFECTION OF ROOT CANALS WITH PHOTON INITIATED PHOTOACOUSTIC STREAMING (PIPS)

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OBJECTIVES: This study set out to compare the efficacy of laser-and ultrasonically activated root canal disinfection with conventional irrigation, specifically its ability to remove bacterial film formed on root canal walls.

METHODS: Seventy extracted human premolars were shaped to an apical size #20 .07, sterilized, contaminated *in situ* with oral bacteria for 1 week and incubated for 2 more weeks. Irrigation was done with 6%NaOCl (group 1), NaOCl ultrasonically activated with blunt inserts (group 2) or a pulsed erbium: YAG laser at non-ablative settings (group 3) for a total of 60 s each. Positive and negative controls were also included. Aerobic bacterial sampling was performed and the incidence of positive samples after 24 and 48 hours, as well as bacterial counts (CFU) was determined. Fixed and demineralized sections 1mm and 4mm off the apex were Brown-Brenn stained and assessed for remaining intracanal bacteria/biofilm and dentinal tubule penetration.

RESULTS: All three canal disinfection protocols significantly reduced bacterial counts ($p<0.001$). None of the three techniques predictably generated negative samples, but laser-activated disinfection was superior to the other two techniques in this aspect ($p<0.05$). Histological sections showed variable remaining bacterial presence in dentinal tubules at the 4 mm level and significantly less bacterial biofilm/necrotic tissue remaining at the 1 mm level after laser-activated irrigation ($p<0.05$).

CONCLUSIONS: Under the conditions of this combined *in situ/in vitro* study, activated disinfection did not completely remove bacteria from the apical root canal third and infected dentinal tubules. However, the fact that laser activation generated more negative bacterial samples and left less apical bacteria/biofilm than ultrasonic activation warrants further investigation.

This work was supported by Medical Dental Advance Technologies Group.

OPTIMIZATION OF NON-VIRAL VECTORS FOR GENE THERAPY OF ORAL CANCER

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OBJECTIVES: Our laboratory is developing a gene therapy approach for oral cancer, using non-viral vectors to deliver therapeutic genes to cancer cells. These vectors are less toxic and more cancer cell-specific than viral vectors; but they are less efficient in delivering DNA. To identify the optimal vector for gene delivery, we compared the transfection activities and efficiencies of five different vectors, using the oral squamous cell carcinoma (SCC) cell line, HSC-3. For comparison, we also transfected the cervical carcinoma cell line, HeLa.

METHODS: HSC-3 and HeLa cells were maintained in appropriate media and seeded in 48-well culture plates the day before transfection, and used at approx. 80% confluence. The plasmid, pCMV.Luc, expressing luciferase under the control of the cytomegalovirus promoter was complexed with an optimal volume of the transfection reagents, which were Metafectene, Metafectene Pro, Metafectene Easy (Biontex), Fugene HD (Roche) and Glycofec (Techulon). The cells were lysed with Passive Lysis Buffer (Promega) 48 h after transfection, and the luciferase activity (transfection activity) was assayed using the Luciferase Assay System (Promega). The plasmid pCMV.lacZ expressing β-galactosidase was also complexed with all five transfection reagents to determine the percentage of transfected cells (transfection efficiency). β-galactosidase expression was observed by phase contrast microscopy following the addition of the enzyme substrate (X-gal).

RESULTS: Transfection activity and efficiency of the non-viral vectors in both cell lines decreased in the order Fugene HD, Metafectene Easy, Metafectene Pro, Metafectene and Glycofect. HeLa cells displayed a higher transfection activity and efficiency than HSC-3. The percentage of cells transfected by Glycofect and Metafectene was about the same in both cell lines

CONCLUSIONS: Transfection reagents vary greatly in their ability to transfect oral and cervical cancer cells. Fugene HD and Metafectene Easy may be useful in suicide gene therapy of oral cancer as well as cervical cancer.

RESPONSE OF HUMAN MESENCHYMAL STEM CELLS TO FACTORS RELEASED BY PLATELETS

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INTRODUCTION: Platelet-rich plasma is frequently used by surgeons to improve wound healing. Activated platelets release a mixture of platelet-derived factors (PDF), which contains several growth factors, ADP, Ca2+, serotonin, antitrypsin and other physiologically active factors.

OBJECTIVE: Analyze effects of PDF on cultured human mesenchymal stem cells (hMSC) at the level of gene expression.

METHODS: Platelet fraction was isolated from human adult venous blood by centrifugation and activated by thrombin (Sigma); hMSC (Lonza; passage 2-6) were cultivated in hMSC basal medium (Lonza) with 10% adult human serum with or without PDF. Total RNA was isolated and expression of 84 hMSC-specific genes was evaluated using PCR arrays (SABiosciences Qiagen) in hMSC cultivated in growth medium or differentiation medium, either in absence of PDF (controls, n=6) or in presence of PDF (experiments, n=6). Experimental and control cycle threshold (CT) values were compared using manufacturer's software.

RESULTS: PDF increased expression of genes enhancing cell multiplication when applied to proliferating hMSC. PDF increased expression of osteogenic differentiation markers when applied to differentiating hMSC.

CONCLUSION: This pilot study has shown some effects of PDF on gene expression of hMSC in vitro. The analysis is continued. This study will help to better understand cellular mechanisms, by which PDF in platelet-rich plasma stimulate 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, University of the Pacific, San Francisco, CA.

GARLIC EXTRACT EFFECTS ON SURFACE CHARGE OF SALIVA-COATED HYDROXYAPATITE

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OBJECTIVES: Garlic extract, GE, has been reported to inhibit S. mutans, SM, growth and to inhibit and reverse its binding to hydroxyapatite, HA, to form a single layer, HA-SM. Our goal is to characterize the effects of GE on SM reversible binding to saliva-coated hydroxyapatite, salHA. Our focus is on electrostatic interactions.

HYPOTHESES: 1. SM will not bind GE-treated salHA; 2. GE exposure will cause saliva to dissociate from salHA; 3. Saliva will not bind to GE-treated HA.

METHODS: HA beads are commercial 20 □m spheres. salHA is made by incubating HA in buffer containing paraffin-stimulated human saliva and washing away excess saliva. GE is isolated from fresh white garlic. After exposure, excess GE is removed by washing. SM (UA159) binding is assayed by fluorescence microscopy, after suspension in neutral 20 mM phosphate buffer with salHA and washing to remove free SM. Fluorescence micrographs are obtained at room temperature after staining SM with SYTO-9, or staining saliva with Oregon Green (OG). Zeta potentials, □, are calculated from electrophoretic mobilities measured at 25°C using the Smoluchowski equation, and are taken as estimates of relative surface charge.

RESULTS: Fluorescence micrographs show that the monolayer of SM formed on salHA does not form if salHA is exposed to GE, and is removed if salHA-SM is exposed to GE. GE-treated HA and salHA are somewhat fluorescent in the presence of SYTO-9, but SM is distinct when present. Values of □ for HA, salHA, GE-treated HA and GE-treated salHA are -2.31, -1.04, -1.00, -0.94 mV, respectively. The precision of the □-values is not adequate to distinguish differences due to GE and/or saliva. OG-labeled saliva is not removed from HA by GE, 2.5 M KCl or 1 wt-% sodium dodecyl sulfate, but is removed by 0.5 M phosphate (pH 7).

CONCLUSIONS: Garlic extract inhibits S. mutans binding to saliva-coated hydroxyapatite. Garlic extract exposure reduces the magnitude of the apparent zeta potential of saliva-coated hydroxyapatite, suggesting the binding inhibition is not due to increased electrostatic repulsion. Garlic extract does not cause saliva to dissociate from saliva-coated hydroxyapatite.

EPITHELIAL CELL TYPE AFFECTS INTERLEUKIN-6 RESPONSE TO *PORPHYROMONAS GINGIVALIS*

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OBJECTIVES: Interleukin-6 (IL-6) is a pleiotropic cytokine that may mediate both beneficial and harmful effects in periodontal disease. IL-6 stimulates immunoglobulin secretion and plays an important role in regulating immune responses to periodontal pathogens. Since IL-6 promotes osteoclastogenesis and induces bone resorption, excessive secretion of IL-6 in response to *Porphyromonas gingivalis* (*Pg*) may play a role in inducing alveolar bone loss. Previous studies on the effect of *Pg* on IL-6 production by oral epithelial cells have shown inconsistent results. The objective of the study was to compare IL-6 responses of three human oral epithelial cell lines to virulent and avirulent strains of *Pg*.

METHODS: Two *Pg* strains, avirulent 2561 and highly virulent W83, were sub-cultivated on blood agar plates and suspended in Medium 199 (4 x 10^8 *Pg*/ml). Non-tumor-derived immortalized oral epithelial GMSM-K cells, and HSC-3 and H413 cells, derived from oral squamous cell carcinoma (OSCC) were exposed to live *Pg* at 10^7 bacteria/well, and incubated at 37°C for 6 and 24 h. IL-6 was determined by ELISA.

RESULTS: Control, uninfected H413 cells produced higher levels of IL-6 than HSC-3 and GMSM-K cells. Exposure of HSC-3 and GMSM-K cells to *Pg*-2561 and *Pg*-W83 for 24 h resulted in a 6- and 8-fold increase in IL-6 secretion, respectively. In H413 cells, *Pg*-2561 down-regulated IL-6 by 30%, while *Pg*-W83 up-regulated IL-6 by only 50%.

CONCLUSIONS: The amount of IL-6 secreted by uninfected cells was strongly dependent on the cell type. Both *Pg* strains induced IL-6 secretion at similar levels in HSC-3 and GMSM-K cells. However, H413 cells were not highly responsive to *Pg*. Our results indicate that conclusions on cytokine responses to *Pg* should not be based on studies with a single cell type.

This work was presented at the 89th General Session & Exhibition of the International Association for Dental Research and 40th Annual Meeting of the American Association for Dental Research, March 16-19, 2011, San Diego, CA.
J. Dent. Res. Vol. 90 (Special issue A) Abstract No. 903, Seq. #135

GENDER DIFFERENCES IN MESENTERIC ENDOTHELIAL FUNCTION OF STREPTOZOTOCIN-INDUCED DIABETIC RATS: ROLE OF ENDOTHELIUM-DERIVED RELAXING FACTORS

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OBJECTIVES: To test the hypotheses that: streptozotocin (STZ, 60 mg/kg, iv)-induced diabetes impairs endothelium-dependent relaxation (EDV) in mesenteric artery of rats; there are gender differences in the development of abnormal vascular responses in diabetes; gender alters the relative contributions of endothelium-derived relaxing factors (EDRFs), including prostacyclin (PGI_2), nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) to mesenteric vasodilation in STZ-diabetic rats.

METHODS: EDV to acetylcholine (ACh, 10^{-8} to 10^{-5} M) in mesenteric arteries precontracted with phenylephrine (2 μM) was measured before and after pretreatment with indomethacin (indo, 10 μM), a cyclooxygenase inhibitor, L-NAME (200 μM), a nitric oxide (NO) synthase inhibitor, or barium chloride (100 μM), a Kir channel blocker, and ouabain (10 μM), a Na^+-K^+-ATPase inhibitor for 20 min.

RERULTS: Significant impairment of EDV was observed only in diabetic females. Indo decreased the sensitivity to ACh only in control males. Addition of L-NAME reduced indo-resistant vasodilation in females, but its effect was much greater in diabetic females. In control males, however, L-NAME substantially blocked the remaining relaxation, and this effect was attenuated in diabetic males. Finally, the remaining indo- and L-NAME- resistant vasodilation was abolished by the combination of barium and ouabain in all groups.

CONCLUSIONS: These data suggest that the predominant mediator in mesenteric vasodilation in females is EDHF, whereas in males it is NO. Furthermore, there was a shift in the contributions of EDHF to NO in females and NO to EDHF in males following the induction of diabetes.

This work has been supported by the National Institute of Health (NIDCR, R15 DE016587). This work was presented at the Federation of American Societies of Experimental Biology 2011, April 9-13, 2011, Washington DC

SENIOR RESEARCH COMPETITION PRESENTATIONS

PAX9 GENE POLYMORPHISMS AND MISSING TEETH

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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.

ACKNOWLEDGEMENT: *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

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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 POLYMORPHISM

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INTRODUCTION: The etiology of nonsyndromic cleft lip with or without cleft palate (NCLP) is multifactorial, including genetic and environmental factors. One of the most commonly studied genes is RFC1. The RFC1 (Reduced Folate Carrier 1) gene 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 polymorphisms are associated with NCLP in a sample of Czech families with 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.

ACKNOWLEDGEMENTS: The fieldwork for this study was supported by Rotoplast Intl., Inc.

This study has been accepted for table clinic poster presentation at the CDA Scientific Session in Anaheim, May 13-16.

PORPHYROMONAS GINGIVALIS STIMULATES INTERLEUKIN-8 SECRETION IN HUMAN ORAL EPITHELIAL CELLS

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OBJECTIVES: Infection of epithelial cells with *Porphyromonas gingivalis* (*Pg*) results in production of pro-inflammatory cytokines that are involved in the initiation and progression of periodontal disease. 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 have been reported on *Pg*-induced stimulation of IL-8 in human epithelial cells.

METHODS: Two *Pg* strains, avirulent 2561 and highly virulent W83, were sub-cultivated on blood agar plates and suspended in Medium 199 (4×10^8 *Pg*/ml). HSC-3 and H413 cells, derived from oral squamous cell carcinoma (OSCC), and non-tumor-derived immortalized oral epithelial GMSM-K cells were challenged with live *Pg* at 10^7 bacteria/well, and incubated at 37°C for 6 and 24 h. IL-8 was determined by ELISA.

RESULTS: Control non-infected HSC-3, H413 and GMSM-K cells produced 920, 2624 and 11 pg IL-8/ml, respectively within 6 h, and 2687, 6564, and 22 pg IL-8/ml, within 24 h. Exposure of HSC-3 to *Pg*-2561 and *Pg*-W83 resulted in a 6- and 3-fold increase in IL-8 secretion, respectively. Incubation of GMSM-K cells with these strains increased IL-8 by 21- and 12-fold. In H413 cells, *Pg*-2561 down-regulated IL-8 by 25%, while *Pg*-W83 did not modify IL-8.

CONCLUSIONS: The amount of IL-8 secreted by control cells and their response to *Pg* were strongly dependent on the cell type. GMSM-K cells secreted significantly less IL-8 than OSCC cells. Both *Pg* strains induced IL-8 secretion in HSC-3 and GMSM-K cells. Degradation of IL-8 by *Pg* gingipains may be responsible for the down-regulation of IL-8 observed with H413 cells. The etiology of different epithelial cell responses to *Pg* is not well known.

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HIV-SPECIFIC TRANSGENE EXPRESSION VIA PROGRESSIVELY TRUNCATED TAT-RESPONSIVE LTR PROMOTERS

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OBJECTIVES: Current anti-retroviral therapies against HIV infection are unable to eradicate the chromosomally integrated proviral genome. This study seeks to develop a promoter element that is highly responsive to the HIV transcriptional activator Tat, but minimally responsive to 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 by de-novo synthesis. Five progressively truncated LTR-tar region clones were generated using PCR and inserted into a luciferase-expressing vector. These clones were then co-transfected into HeLa cells with a plasmid expressing the HIV proteins, including Tat (pHXB□bgl). Metafectene alone, PGL-3 basic plasmid and herring sperm DNA were used as negative controls. Luciferase expression driven by the truncated LTR-tar clones allowed us to determine the region of the LTR promoter element most specifically responsive to Tat, and not to other cellular transcription factors.

RESULTS: Luciferase activity in cells transfected with both LTR4 and pHXB□bgl was 12,137 ± 914 RLU/mL, while control cells transfected with LTR4 and PGL-3 basic plasmid (no pHXB□bgl) was 204 ± 4 RLU/mL. This indicates a 60-fold increase in Tat-specific expression. In cells transfected with LTR1, LTR2, or LTR3, luciferase expression was higher in the presence and absence of pHXB□bgl. In cells transfected with LTR5 and LTR6, luciferase expression was lower compared to LTR4 in the presence and absence of pHXB□bgl.

CONCLUSIONS: Higher luciferase expression in cells transfected with LTR1, LTR2, and LTR3 resulted from non-Tat-specific transcriptional activating regions contained on those fragments of the HIV LTR-tar promoter region. LTR4 is the HIV transcriptional activating region most specific to, and yielding the highest gene expression by, Tat activation. Therefore, LTR4 is a candidate for activation of suicide genes in HIV-infected cells.

This work was presented at the 51st Annual ADA/Dentsplay Student Clinician Research Award Program during the 145st American Dental Association Annual Meeting, September 30 - October 3, 2010, Orlando, Fl, and the 89th General Session of the International Association for Dental Research and 40th Annual Meeting of the American Association for Dental Research, March 16-19, San Diego, CA.
This work was supported by Research Pilot Project Awards 03-Activity 071 and 03-Activity 076 from the Arthur A. Dugoni School of Dentistry.

METHYLENETETRAHYDROFOLATE REDUCTASE *C677T* POLYMORPHISM AND NONSYNDROMIC CLEFT LIP AND PALATE

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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.

ACKNOWLEDGEMENTS: *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 presentation at IADR General Session, Barcelona July 14-17, 2010

DENTAL STUDENT PREDICTION OF PEDIATRIC PATIENT ANXIETY

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OBJECTIVES: To investigate the ability of pre-doctoral dental students to predict the anxiety of their pediatric patients, and the variables which could reasonably be expected to influence the accuracy of their predications.

METHODS: Second year dental students from the University of the Pacific School of Dentistry were asked to evaluate the anxiety level of their pediatric dental patients in the school's Pediatric Clinic. Anxiety was assessed in both patients and dental students using a validated facial image scale (FIS). Student participants were also asked to rate their own anxiety around three issues: parent interaction, patient behavior management and technical procedure. Meanwhile, 102 pediatric dental patients (ages 4-9) selected for participation in the study were asked to choose the one emotionally representative face from a set of five using the FIS which represented how they were feeling about their appointment.

RESULTS: Patients reported an average FIS score of 1.69 (scale 1= no anxiety to 5= high anxiety) while students, on average, estimated the FIS score of their patients to be 2.41. Furthermore, just over 25% of students predicted patient anxiety perfectly, while almost 75% of all students were accurate to within 1 rating point of their patient's reported anxiety level. Generally, students tended to over-estimate patient anxiety. Factors which most contributed to a student's incorrect assessment of their patient's anxiety level were (i) the student's own anxiety about the visit and (ii) the student's previous number of negative experiences.

CONCLUSIONS: Students do not always accurately assess their pediatric patients' anxiety levels. Students' errant assessments tend to over-estimate the level of patient anxiety. Two main factors accounted for the disparity; most significantly dental students' personal anxiety level and the number of previous negative experiences regarding behavior management. Therefore, instructor awareness of students' previous patient behavior management experiences, and students' own anxiety about the technical procedure should trigger discussion and instruction around probable patient anxiety levels, and appropriate management of the patient's anxiety.

LIPOSOMAL C6 CERAMIDE IS CYTOTOXIC TO ORAL SQUAMOUS CELL CARCINOMA CELLS AND CAUSES A DECREASE IN SURVIVIN EXPRESSION

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OBJECTIVES: C6-ceramide is a sphingolipid metabolite recognized as an antiproliferative and proapoptotic agent *in vitro* and *in vivo*. However, its use as a therapeutic agent has been limited due to its insolubility. Liposomes are used to solubilize and encapsulate the C6-ceramide to allow uptake into the cell. Survivin is a member of the inhibitor of apoptosis (IAP) family of proteins and is often overexpressed in cancer cells. Earlier studies have shown the down-regulation of survivin in large granular lymphocytic leukemia. We therefore investigated the effect of C6 ceramide in human oral squamous cell carcinoma (OSCC) cell lines, as a potential novel therapeutic agent.

METHODS: HSC-3, human oral squamous cell carcinoma cells were seeded in 48-well culture plates and used at ~80% confluence 24 h later. Palmitoyloleoylphosphatodylcholine (POPC): dioleoylphosphatidylethanolamine (DOPE) or POPC:DOPE:C6 liposomes (Avanti Polar Lipids) were added to the cells in the concentration range 0.1–50 μM of C6. After incubation for 24 h at 37°C, 5% CO_2, cell survival was evaluated by the Alamar Blue assay (Biosource) and the Live/Dead viability assays (Invitrogen). Survivin levels were measured by ELISA (R&D Systems). The morphology of the treated and control cells were examined by scanning and transmission electron microscopy.

RESULTS: Cells treated with the liposomal C6 ceramide resulted in decreased cell viability. The Alamar Blue assay showed a linear reduction with increased concentration of C6 ceramide. The viability with plain POPC:DOPE liposomes was 93±5% of the control. Treatment with 5, 10, and 20 μM liposomal C6 ceramide reduced the viability to 72±3%, 44±0%, and 18±3% of untreated cells. Survivin ELISA results showed a decrease of survivin levels with increasing concentrations of C6 ceramide. Survivin levels in HSC-3 cells were 4462±28 pg/mg protein in untreated cells and 4558±577 pg/mg protein in cells treated with POPC:DOPE liposomes, and decreased to 3099±72 pg/mg protein at 5 μM C6 ceramide, 1574±279 pg/mg protein at 10 μM C6 ceramide, and 707.5±3.5 pg/mg protein at 20 μM C6 ceramide. Electron microscopy indicated deformation of nucleoli by C6 ceramide treatment.

CONCLUSIONS: Liposomal C6 ceramide exerted a desirable effect by reducing cell proliferation, probably because of a decrease in the levels of the anti-apoptotic protein, survivin. Thus, HSC-3 cells are vulnerable to liposomal C6 ceramide in a dose-dependent manner. Further studies will focus on whether liposomal C6 ceramide and the reduced survivin levels will increase the susceptibility of HSC-3 cells to various anti-cancer agents such as doxorubicin and tamoxifen.

2ND YEAR STUDENT RESEARCH COMPETITION

THE EFFECT OF PEPTIC ULCERS ON ORAL HEALTH

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OBJECTIVES: Ten percent of the USA and world population today suffers from peptic ulcers. As a result of its significant prevalence, various research projects have been directed at the bacteria *Helicobacter pylori,* a key factor in the development of peptic ulcers, including its relation to oral health. Since *Helicobacter pylori* has been shown to be present in the oral cavity in both saliva and dental plaque, there is speculation that this bacteria affects the oral health status. The aim of this paper is to present and assess the varying findings of *Helicobacter pylori* and peptic ulcer studies and their implications for oral health and to suggest future research directions.

REVIEW METHODS: Electronic searches were conducted on the topic of *Helicobacter pylori* and their relation to peptic ulcers and oral health. Search parameters included data up to April 2001.

RESULTS: Ten percent of the USA and world population today suffers from peptic ulcers. As a result of its significant prevalence, various research projects have been directed at the bacteria *Helicobacter pylori,* a key factor in the development of peptic ulcers, including its relation to oral health. Since *Helicobacter pylori* has been shown to be present in the oral cavity in both saliva and dental plaque, there is speculation that this bacteria affects the oral health status. Some studies show the importance of proper oral hygiene to the eradication of *Helicobacter pylori,* while other work supports specific eradication therapies when facing inadequate or poor oral hygiene practices. Similarly, some studies demonstrate an increasing prevalence of *Helicobacter pylori* in periodontally involved patients, while other studies show no correlation between periodontitis and *Helicobacter pylori.* Other studies indicate a relationship between *Helicobacter pylori* and apthous ulcers, and another study additionally suggests treating dentists may be affected by this bacterium while still other studies reveal contrasting results.

CONCLUSIONS: Research has given us some understanding of the relationship between peptic ulcers and oral health but questions remain. Although *Helicobacter pylori* has been found in the oral cavity within saliva and dental plaque, its effects on oral health are not well understood at this time. Suggestions for improving oral hygiene to decrease the chances of re-infection by *Helicobacter pylori* remaining within the oral cavity and so decrease the possibility of peptic ulcer formation have been made. *Helicobacter pylori* have also been associated with infection of dentists through aerosols from infected patients and also with recurrent apthous ulcers but there are findings that contradict this association as well. More research must be conducted to better understand and treat the increasing percentages of people suffering from gastrointestinal issues, specifically peptic ulcers, as a result of the *Helicobacter pylori.*

HIV PROMOTER-MEDIATED, SPECIFIC GENE EXPRESSION IN CELLS EXPRESSING THE HIV TRANSACTIVATOR, TAT: A FIRST STEP FOR GENE THERAPY OF HIV/AIDS

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OBJECTIVE: Current anti-HIV medicines can inhibit virus replication, but are unable to eliminate the latent, chromosomally integrated proviral genome. The long term aim of this project is to develop a suicide gene that is activated in HIV infected cells, but not in uninfected cells. We previously constructed plasmids that express the reporter gene, *Photinus pyralis* (firefly) luciferase (*luc*), under the control of the HIV promoter, LTR, and progressively truncated "mutants" of LTR that retained the Tat-responsive element, TAR. Here we compared the ability of these plasmids to express luciferase specifically in Tat-expressing HeLa cells compared to normal HeLa cells.

METHODS: The LTR promoter region of HIV (LTR1) and the progressively mutated LTR2 through LTR6 were generated by *de novo* gene synthesis (Bionexus), and subcloned into the pGL3 basic vector (Promega). Tat-expressing HeLa cells (HeLa-tat-III) were obtained from the NIH AIDS Research and Reference Reagent Program. HeLa cells (ATCC) were used as controls to evaluate non-specific Luc expression. Both cell lines were maintained in DME/10 medium and seeded in 48-well culture plates the day before transfection, and used at approximately 85% confluence. Transfection utilized Metafectene (Biontex) and 1 μg of the pGL3 plasmid containing the LTR constructs. Forty eight hours after transfection, the cells were washed, solubilized with Passive Lysis Buffer (Promega), and centrifuged 15 s to precipitate cellular debris. Luciferase activity in the supernatant, expressed as relative light units (RLU) per mL cell lysate was assayed with the Promega Luciferase Assay System, using a Turner Designs TD 20/20 luminometer.

RESULTS: The LTR constructs had increased transcriptional activation (luciferase activity) in HeLa-tat-III cells compared to HeLa cells not expressing Tat. Luciferase activity obtained with HeLa-tat-III and control HeLa cells transfected with LTR2 was 108,187 ± 2,693 RLU/mL, and 1060 ± 22 RLU/mL, respectively. This is an increase of 102-fold in gene expression in Tat-expressing cells, over control HeLa cells. Transfection of LTR1 and LTR3 resulted in a 51-fold increase, whereas LTR4-LTR6 did not cause significant gene expression. In an independent experiment, LTR2 resulted in a 117-fold enhancement, compared to 113-fold for LTR1 and 56-fold for LTR3.

CONCLUSION: LTR2, extending from bp -105 to bp +338 in the HIV genome, is the HIV transcriptional activating region most specific to and yielding the highest gene expression by Tat activation. Higher luciferase expression compared to LTR-1 was probably caused by the truncation of the modulatory region of LTR, resulting in a starting region of the NH-□B enhancer region in LTR-2. LTR-2 can be used potentially to turn on a suicide gene (Herpes simplex virus thymidine kinase) in a Tat-specific manner in HIV-infected cells. Future studies will explore the effects of the insertion of tandem TAR regions in the LTR, in an attempt to enhance the Tat-specificity of gene expression.

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THE IMPLICATIONS OF BONY AND SOFT TISSUE VARIATION FOR SUCCESS RATES IN MANDIBULAR NERVE (V_3) ANESTHESIA

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OBJECTIVES: Nerve block anesthesia is commonly employed in mandibular dental procedures to serially block the inferior alveolar (IA), mylohyoid (MH), and lingual nerves. Clinically acceptable IA/MH anesthesia can be as low as 80%, due in part to anatomical considerations. In an effort to increase success, clinicians employ modified insertion points or different techniques. Harvey (1970) summarized available methods, finding 11 suggested points of entry, 6 horizontal and 8 vertical bearings, 11 depths of penetration, 9 positions of the foramen, and 12 target areas! The same basic methods are used in subadults and adults. We hypothesize that clarifying the relationships of landmarks and planes employed in block procedures and improving knowledge of bony and soft tissue variation will provide the basis for higher success rates and reduced operator stress levels during IA nerve blocks.

METHODS: We employ two discrete samples to delineate IA-block-related anatomy. First, with reference to literature on craniofacial growth, medial ramal anatomy, and block procedures, we defined 25 measures related to IA blocks. We then acquired 3D landmark data from an ontogenetic series of dry mandibles (n=203). Second, we acquired 2D measurements from cadaver dissections (n=40). Cadaver-based measurements relate to both bony landmarks and the position of the IA neurovascular bundle relative these landmarks. Three-dimensional data were acquired with a Microscribe 3D digitizer, while 2D measures were made with sliding/spreading calipers or rulers, as appropriate.

RESULTS: Based on our dry bone sample, we found: (1) that the vertical height of the mandibular foramen relative to the occlusal plane is variable; (2) that the position of the mandibular foramen is not centered on the ramus (relative to block landmarks it is consistently located anteriorly); (3) that the posterior extent of the lingula can cover the neurovascular bundle medially; and (4) that the mandibular foramen can be 'hidden' beneath a medially expanded crista endocondyloidea. In our cadaver sample we found: (1) that there is substantial variation between the minimum ramus breadth and the distance from the posterior ramal border to the marginal ridge; (2) that the vertical height of the mandibular foramen relative to minimal ramal breadth varies by almost 3.0 cm; (3) that the angulation of the IA nerve between the foramen and its medial curvature varies by 83°; and (4) that the point at which the nerve starts to course medially varies from the level of the foramen to 1.2 cm above it.

CONCLUSIONS: Whereas numerous studies have addressed the extent of variation in mandibular features relative to nerve block procedures, we document a greater range of variation than previously observed. This result is partially driven by our use of block-specific landmarks as data points. Further, by employing two related data sets, dry bone and cadaver dissections, we are able to correlate the bony variations with variations observable in the nerve(s). This methodology expands understanding of the complexity of the bone-nerve relationship. This increased understanding should provide the basis for higher success rates and reduced operator stress in the event of block failure.

FOLATE-RELATED GENES AND OROFACIAL CLEFTS IN SOHAG, EGYPT

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INTRODUCTION: The etiology of nonsyndromic cleft lip with or without cleft palate (N/CLP) is multifactorial (genetic and environmental factors). Among the most commonly studied genes are two genes - MTHFR and/or RFC1 - that are related to folate metabolism. When their function is altered due to mutations, a decreased utilization of folate slows down cell multiplication and it may contribute to orofacial clefting. 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.

OBJECTIVES: The purpose of our study was to determine whether RFC1 A80G polymorphism is associated with NCLP in a sample of patients from Sohag, Egypt and compare it with MTHFR C677T polymorphism in same samples.

MATERIAL AND METHODS: A case-control study design was used. Cases (individuals affected with NCL/P; n=116) and controls (n=104) for this study were identified during Rotaplast medical missions to Sohag, Egypt in 2009. Diagnosis of NCL/P was determined by physical examination of each individual and venous blood and saliva was obtained for DNA analysis. RFC1 A80G genotypes were established by PCR amplification and single nucleotide conformational polymorphism detection using polyacrylamide gel electrophoresis (PAGE).

RESULTS: The difference in distribution of genotypes between cases and controls was statistically significant (p=0.039). Genotypes at nucleotide 80 of the RFC1 gene among 116 cases revealed 27 (23.3%) cleft patients homozygous for the wild-type allele (AA), 51 (44%) heterozygous (AG), and 38 (32.7%) homozygous for the mutation (GG). Even more statistical significant was difference in allele frequencies between cases and controls (p=0.009). Allele frequencies in the cases were 45.3 % for the A allele and 54.7 % for the G allele. Among 104 controls, 39 individuals (37.5%) were homozygous for the wild-type allele (AA), 43 (41.3%) were heterozygous (AG), and 22 (21.2%) were homozygous for the mutation (GG). Allele frequencies in the control group were 58.2% for the A allele and 41.8% for the G allele.

CONCLUSION: Results of this pilot study suggest that the RFC1 A80G polymorphism of may be involved in the etiology of NCL/P in Sohag population.

ACKNOWLEDGEMENTS: Rotaplast International, Inc., funded and supported field work for this study. Pacific Craniofacial Genetics Team and Cleft Prevention Program and Department of Orthodontics funded and supported molecular genetics analysis

This study has been accepted for Table Clinic Students Competition at the CDA Scientific Session in Anaheim, May 12-15, 2011.

IDS STUDENT RESEARCH PRESENTATIONS

ENDO VS IMPLANT – THE STATE OF ART

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OBJECTIVES: One of the major issues confronting the contemporary dental clinician is the treatment decision between extracting a tooth and restoring the area with an implant or preserving the natural tooth by performing a root canal followed by a full coverage crown. The aim of this review is to provide a critical analysis of contemporary dental literature on dental implants and endodontic treatment in the context of identifying factors influencing the treatment decision between them.

METHODS: Our literature search included the following databases: Medline, Ebsco, Ovid, Google Scholar and PubMed for the period from 1981 to 2011. Twenty-two systematic reviews and 115 case studies which compared root canals and implant treatments each using different criteria, were evaluated. Those criteria were mainly clinician and patient related factors and included prosthetic restorability of the tooth, quality of bone, esthetic demands, cost-benefit ratio, systematic factors, potential for adverse effects, as well as the patient's motivation, comfort, perception and preferences. Preconceptions about the success and survival of implants versus endodontically treated teeth were also evaluated. Factors contributing to the failure of endodontically treated teeth such as persistent or secondary intra-radicular infection were examined. Lastly, we analyzed aspects contributing to implant failure such as poor bone quality, chronic periodontitis, systemic diseases, smoking, unresolved caries or infection, advanced age, poor implant location, short implants, inappropriate prosthesis design, eccentric loading and para-functional habits. .

RESULTS: The major studies published to date indicate that there is no difference in the long-term prognosis between single-tooth implants and restored root canal treated teeth. There is a preconception involved in the studies as they interpret the survival of an implant and endodontically treated teeth in different ways. A published study of 1.5 million endodontically treated teeth found a 97% survival rate at 8 years while a prospective clinical study of 635 teeth with an 8-10 year follow up, found root canal treatments of teeth with necrotic pulps to have an 86% success rate. A multi-center implant study of 1022 implants reported a survival rate of 92.2%, and a success rate of 83.4% with an overall implant failure rate of 8.16% in the maxilla and 4.93% in the mandible.

CONCLUSIONS: Implants are not an alternative treatment for endodontics. They are an excellent treatment option for missing, hopeless and questionable teeth. These treatment modalities should be selected based on individual needs and preferences; not based on their survival rates. It can be concluded that endodontic treatment represents a feasible, practical, and economical way to preserve function in a vast array of cases and that dental implants serve as a good alternative in selected situations in which prognosis of the tooth in question is poor.

GOLD VS. PORCELAIN AS RESTORATIVE MATERIAL

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OBJECTIVES: A literature review was done to compare properties of gold alloys and porcelain as restorative dental materials. The following aspects of each restorative material were compared: corrosion, biocompatibility, bacterial adhesion, plaque retention, marginal gap, abrasion to opposing teeth and the effect of ultrasonic scaling instruments on surface smoothness roughness. This literature review seeks to summarize the advantages and disadvantages of each material.

METHODS: The review was conducted on studies done from 1999 to 2010. We reviewed 105 articles published in PubMed and EbscoHost. Gold crowns, gold foils, gold inlays/onlays and porcelain fused to metal crowns were compared. Because of gold's non-esthetic color, most of the articles published on gold were dated prior to 2005. Research articles for PFM crowns were available for the period from 1999 to 2010. Approximately 70% of the studies were in vitro and 30% in vivo.

RESULTS: The downsides of this literature review are:

1- No direct comparison between pure porcelain crowns and gold alloy crowns.
2- There is an extremely broad spectrum of analysis regarding gold or porcelain.
3- Because of a desire for esthetics, there is a shift from use of gold to porcelain (ceramic) material.

In spite of this we were able to conclude the following:

Advantages of Gold: Durable, conservative, kind to the tissue, good closure of margin- hence some PFM's have gold base, standard of excellence, wear and function most resembles enamel.
Disadvantages of Gold: Gold foil placement is technique sensitive, thermal conductivity, galvanic effect when placed near amalgam; cost is much higher than PFM crowns, esthetics.
Advantages of Porcelain: Esthetic, patient acceptance is higher due to superior esthetics to gold, better color adaptation, shape and alignment of your anterior teeth while maintaining esthetics.
Disadvantages of Porcelain: Hardness (abrasive to antagonist), time consuming lab fabrication technique, significant tooth reduction, unforgiving nature of porcelain in regards to low fracture resistance, longevity -lose their superficially applied stains within a few years, and become esthetically less acceptable as gingiva recedes.
CONCLUSION: In the last ten to fifteen years, dental materials have reached a heightened popularity. The most popular esthetic material used today is the porcelain fused to metal (PFM) restoration. According to Dr. Mark Geissberger's book "Esthetic Dentistry in Clinical Practice", "traditional feldspathic porcelains remain the material of choice for mimicking natural tooth structure". Factors that can play a major role in choosing between gold, ceramic and PFM crowns as the restoration of choice are the patient's smile line, patient's desire to match restoration to existing dentition and longevity of restoration. Meeting a patient's esthetic needs or desires has to be one of the first considerations in choosing restorative material along with considering the status of the patient's oral health. In conclusion, the selection of a dental material should be evaluated on a case by case basis and the patient's preferences should be taken into consideration.

SHADE MATCHING TECHNOLOGY

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OBJECTIVE: Color measurement of teeth and restorative materials is an essential part of clinical practice and dental research. Shade matching is a challenging process and is subject to too many variables such as operator's perception of color, ceramist and dental technician's understanding of color and properties of the materials as well as patient's demands. This research project tries to understand the various aspects of shade selection, the currently available technologies and possible future trends in the field of shade matching technology.

METHOD: To understand the basics of color and evolution of shade matching, an online search was done using the CPMC library resources. Articles were obtained from peer-reviewed journals available on PubMed, Ovid online and Ebsco host. Several editions of Dental Digest, JADA, various textbooks and brochures on different shade matching guides were also reviewed. An experiment to evaluate the color acuity of dental students was conducted using students from IDS 2012 and DDS 2012 classes at the University of the Pacific, Arthur A Dugoni School of Dentistry. All students underwent a Munsell color matching test at the X-Rite website within a stipulated amount of time. Equal numbers of female and male students were given the Munsell color matching test and the results were evaluated based on 2 criteria - age and gender of the subjects.
RESULTS: The predominant categories of shade matching technology are the human eye, RGB (Red Green Blue) devices, spectrophotometers and colorimeters. Many challenges are faced by the dental professional while trying to successfully shade match. These include lighting in the dental operatory, optical illusions such as dark colored patient bib or lipstick, color blindness of the clinician, dental experience, medications, caffeine and eye fatigue caused by staring at the same shade for more than 5 seconds. There is not enough literature to support the claim that the upcoming shade matching guides are better than the human eye in accurately determining shade selection. The results of our experiment showed that female students were more accurate than male students and younger students had more accurate color matching skills than older students.

CONCLUSIONS: Review of all the articles which compared different shade matching systems showed that newly introduced systems such as VITA Easyshade compact, X-Rite shade vision system and Olympus "Crystaleye" Dental Color Analysis System are comparable if not better than the currently available methods such as colorimeters, spectrophotometers and digital color analyzers. Professional experience was positively associated with accurate shade selection. Understanding the influence of different variables in shade selection such as operatory lights, and how the eye interprets the tooth's hue, value and chroma can assist the clinician in improving his/her skills in shade selection. The combination of the human eye along with the correct use of current technology appears to give the best shade matching results.

CONVENTIONAL VS DIGITAL IMPRESSIONS

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OBJECTIVES: To evaluate the ideal properties of an impression material and compare digital impressions to conventional impressions. We questioned if digital impression techniques could overcome some of the shortcomings that prove challenging when conventional impression materials are utilized.

MATERIALS AND METHODS: A total of 100 articles were collected from various resources like PubMed, EBSCO Host, Ovid, Google Scholar, Science Direct, Dentistry Today, Dental Product Shopper manufacturer's website and several textbooks. In all, 53 recent articles were selected dating back to 2001 to present. No case studies were selected for this review. All the articles used in this literature review were from peer reviewed journals and all had been published in scientific journals. Digital impression data was scarce to non-existent in the scientific literature, thus manufacturer's data was utilized for the purposes of our comparison.

RESULTS: Both the conventional and digital impressions were found to be clinically acceptable. The disadvantages of using conventional techniques are repeated cost of impression materials, patient discomfort, long chair time, shelf life/storage limitations and dimensional instability. Digital impressions seek to address some of these disadvantages. They are better accepted by patients due to comfort and efficiency, and utilize less time. One of the biggest advantages of digital impressions is the ability to quickly and easily capture missed elements of first impression attempt without putting patients through additional conventional impressions. A published study done at the University of the Pacific Dugoni School of Dentistry showed that crown adjustments took 22% less time chair side before cementation, when the iTero digital impression system was used, compared to the crown adjustment time required at chair side for crowns made from the conventional impression techniques. In spite of having accurate impressions and overcoming some of the drawbacks of conventional impressions, initial cost and dynamic technology are the two limitations for utilizing digital impressions.

CONCLUSIONS: The Dugoni School of Dentistry Study was the one of the few published standardized study available that compared the two modes of impression making. According to the review of recent literature, digital impressions are as reliable as conventional impression, are better accepted by patients and seem to address the issues of accuracy, technique sensitivity, isolation, and clinician / lab communication. The major drawback is the large initial investment involved in purchasing the equipment. In light of the ever-increasing incorporation of computer technology into dentistry, digital impressions will probably become the standard for impression making in the future.

DIAGNOSIS OF CLEIDOCRANIAL DYSPLASIA

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INTRODUCTION: Cleidocranial dysplasia (CCD) is a rare condition (1 per million individuals worldwide) with autosomal dominant inheritance. CCD is characterized by multiple dental and skeletal abnormalities. Individuals with CCD usually have underdeveloped or absent clavicles. As a result, their shoulders are narrow, sloping, and can be brought unusually close together in front of the body. Delayed closing of the fontanels is also characteristic. The common dental abnormalities are failure of primary tooth to exfoliate, supernumerary teeth and malocclusion. Although many cases of CCD may have aberration of chromosome 6p21, still variability exists involving other chromosomes, like 8q22. The shortage of functional RUNX2 protein interferes with normal bone and cartilage development resulting in the signs and symptoms of the cleidocranial dysplasia.

CASE PRESENTATION: We report a 19 year old Asian-Indian female patient who presented to us for dental evaluation. Her medical history was noncontributory. She had a short stature with depressed nasal bridge and hypertelorism. She could bring her shoulders unusually together. On head and neck exam, TMJ has no abnormality detected; left submandibular lymph nodes were palpable. Routine diagnostic tools including clinical examination of head, neck and clavicles revealed hypermobility of shoulders and oral examination helped identify the eruption status of teeth in relation to chronological age. Radiographic examination includes a chest X-ray, lateral cephalogram, hand – wrist radiograph, and panoramic X-ray.
Our case has clinical and radiographic findings corresponding to CCD or Marie-Sainton syndrome: bilateral absence of clavicles, open fontanellae, frontal and parietal bossing, wormian bones, multiple unerupted and impacted permanent and supernumerary teeth, retention of primary teeth, short stature, depressed nasal bridge and hypertelorism.
Cytogenetic analysis of our patient revealed female karyotype with paracentric inversion of chromosome 13q in all the cells analyzed. That breakage has occurred on q12 and q22 in the long arm, and the segment between these break points excluding centromere has been inverted and reunion has taken place.

CONCLUSION: Our patient has classic triad of features of CCD syndrome and presents a variation in the genetic constitution of chromosome 13q paracentric inversion.

This study has been accepted for Table Clinic Students Competition at the CDA Scientific Session in Anaheim, May 12-15, 2011.

DENTAL HYGIENE STUDENT PRESENTATIONS

EFFECTS OF HOOKAH: ARE YOU AWARE?

Miray Gendi

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OBJECTIVES: As many have noticed, Hookah is growing increasingly popular over the years. Many are aware of the consequences associated with smoking cigarettes, cigars, and other tobacco based product, but how many are aware of the oral and systemic effects of Shisha? A survey on Shisha was conducted to determine if the local population using hookah was aware of the risks associated with its use. The sample consisted of 30 people that were conveniently selected. Along with the survey two interviews were conducted.

METHODS: A small survey of thirty people was conducted which contained eight questions. Mena Gendi and Romany Saad were interviewed on their opinion of Hookah smoking, how often they smoke, and why they smoke. It was important to find out if people were aware of the possible health risk associated with smoking the Nargile. It was suspected that many people are not aware of the consequences associated with smoking. Of the thirty people there were two female and twenty eight males. Of the sample group there was only two people who were under the age of eighteen, twenty seven ranged from ages eighteen to thirty and only one was thirty or older. A Convenience sample was taken by making a post on Facebook.com asking people to conduct a small Hookah Survey. Contributing to the survey and interviews, research articles were implemented to better understand the effects of Hookah on the body.

RESULTS: After analyzing the results, the suspected outcomes were incorrect. People who smoke the Nargile are aware that it is harmful. Concerning Hookah's risk factors comparable to smoking cigarettes the study conducted was not able to find out if they thought that it was safer than cigarette smoking. Both interviewees however, did state that they believed that smoking Shisha is much safer than cigarettes. From the small sample that was taken, it seems that many people did not smoke Hookah habitually. It appears that it more of a social past time than a stress reliever like cigarettes are. There were only a few people who smoked it for stress related reasons and it was found that they happen to smoke cigarettes as well. Concerning existing research, Dental articles and journals have shown suspected varies oral and systemic diseases linked with smoking Hookah.

CONCLUSION: Many Dental Journals and articles show that more research needs to be conducted. Researches need to center on the exact link between Hookah and periodontal disease. The study done in Jeddah, Saudi Arabia as many of the researches referenced was extremely beneficially, but one should not rely solely on one research. In order to state that there is direct correlation it is crucial to have multiple studies supporting the same evidence. Also, research needs to be done on whether caries can be transmitted through sharing the Nargile hose. It would be suspected that it does, since there is already supporting evidence of vertical transmission of caries when people share drinks, toothbrushes, pacifiers. On the topic of awareness, the survey conducted revealed that people are aware that smoking Shisha is harmful, but they do not know to what extent. It would be beneficial to have ads and/ or commercials on the health effects of Hookah since it seems to be increasing in popularity.

ORTHODONTICS RESIDENT PRESENTATIONS

POLYMORPHISMS OF THE MTHFR 677CT AND CLEFT LIP AND PALATE IN SOHAG, EYPT – A PILOT STUDY

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INTRODUCTION: Cleft lip and palate anomalies are one of the most common congenital anomalies, affecting 1 in every 500-1000 births (Murray, 1995, Tolarova and Cervenka, 1998). Nonsyndromic cases represent approximately 70% of all clefts and are caused by a combination of genetic and environmental factors.
Among candidate genes for nonsyndromic cleft lip with or without cleft palate (NCL/P), methylene tetrahydrofolate reductate (MTHFR) gene polymorphisms have been identified as possible contributors due to their role in folate metabolism. Point mutations at the 677 position replace thymine (T) for cytosine (C) results in a substitution of valine for alanine. As a result, individuals with homozygous TT have impaired MTHFR enzyme function and a reduced ability to form the methyl form of folate by 35%-50% in comparison with controls (Kang 1991, Frosst 1995).

OBJECTIVE: To determine the role of polymorphism of the MTHFR gene at the 677th nucleotide and its association with NCL/P in Sohag, Egypt.

METHODS: Cases (individuals affected with NCL/P; n=134) and controls (n=106) for this study were identified during Rotaplast medical missions to Sohag, Egypt in 2009. DNA was isolated from blood and saliva and MTHFR 677CT genotypes were established using polyacrylamide gel electrophoresis (PAGE).

RESULTS: Significantly different proportion of genotypes (p=0.018) with higher proportion of TT homozygotes and also significantly higher T allele frequency in cases compared to controls (p=0.011) was found (cases: 40.3% CC, 14.9% TT, 44.8% CT, T allele frequency 0.373; controls: 52.8% CC, 4.7 % TT, 42.5% CT, T allele frequency 0.259).

CONCLUSION: Results of this pilot study suggest that the 677CT variant of MTHFR gene is associated with NCLP in Sohag population. More studies on larger samples and also including other genes and environmental factors are needed to help us understand etiology of NCLP in Egyptian population.

ACKNOWLEDGEMENTS: Rotaplast International, Inc., funded and supported field work for this study. Pacific Craniofacial Genetics Team and Cleft Prevention Program and Department of Orthodontics funded and supported molecular genetics analysis.

Part of this study has been presented at the 111 Annual Session of the American Association of Orthodontists in Chicago, May 13-17, 2011.

STOCKTON CAMPUS STUDENT PRESENTATIONS

EXPRESSION OF SPIDER SILK PROTEINS MaSp1, PySp2,. AND TuSp1 IN THE YEAST *PICHIA PASTORIS*

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OBJECTIVE: *Pichia pastoris* is a methylotrophic yeast that can efficiently express and secrete heterologous proteins. We used *P. pastoris* to try to express segments of the silk proteins encoded by MaSp1, PySp2, and TuSp1 of the black widow spider, *Latrodectus Hesperus,* and to have them successfully secreted out of the cell

METHODS: We transformed MaSp1, PySp2, and TuSp1 into *P. pastoris* and induced expression of these proteins, which was confirmed using a spot western blot

RESULTS: MaSp1 and TuSp1 were successfully expressed and detected in the spot western. PySp2 was not.

CONCLUSIONS: We suspect PySp2 might have been improperly folded and thus not secreted. The expression of MaSp1 and TuSp1 will be further optimized and carried out in larger scale so that significant amounts can be purified for functional analysis.

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ETHICAL DRUG TOXICITY TESTING USING HUMAN STEM CELLS: THE IMPACT OF ANTIEPILEPTIC DRUGS ON NEUROGENESIS

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OBJECTIVE: Embryonic exposure to some antiepileptic drugs (AEDs) can impair cognitive function in later life, perhaps through interference with fetal brain development (*neurogenesis*). Here we test the validity of using human pluripotent stem cells (hPSCs) as a model to study neurogenesis and the neurotoxic effects of two major AEDs, phenobarbital (PHB) and valproic acid (VPA), on stem cell viability, proliferation and neural differentiation.

METHODS: The hPSC line TERA2.cl.SP12 was grown under non-differentiating conditions [DC(-)] or differentiating conditions [DC(+)] toward a neuronal phenotype with the addition of 10μM retinoic acid. The impact of PHB and VPA (10-1000μM) on cell *viability* and cell *death* was quantified after 1, 3 and 7 days exposure using the trypan blue exclusion test and the lactate dehydrogenase assay, respectively. Analysis of the impact of 3 or 7 days exposure to AEDs on cell cycle and proliferation was determined by flow cytometric analysis of DNA content. Pluripotency was assessed by determining the expression of the hPSC marker OCT-4 after 7 days of AED treatment. Neurogenesis was also assessed after a 3 day exposure to the AEDs at the start of differentiation by immunocytochemistry by quantifying the proportion of cells expressing the neuronal marker βIII tubulin at 5, 15 and 25 days.

RESULTS: PHB reduced the number of viable DC(-) and DC(+) cells in a dose- and time-dependent fashion and increased cell death of DC(-) and DC(+) at 1000μM. VPA reduced the number of viable cells regardless of the length of exposure in both DC(-) and DC(+). Cell death decreased after VPA exposure in DC(-) (10-1000μM) and DC(+) (1000μM) conditions. Cell cycle analysis showed a significant inhibition of DC(-) cell proliferation with VPA at all concentrations tested at 3 and 7 days, whereas cell proliferation was only inhibited by PHB at 1000μM. Transient exposure to PHB reducedneurogenesis at 10 and 100μM and completely abolished it at 1000μM. Conversely, transient VPA exposure at lower concentrations (10μM & 100μM) enhanced neurogenesis, consistent with a loss of OCT-4 expression.

CONCLUSION: Our observations are consistent with previous reports that exposure to some AEDs inhibits neurogenesis in rodent brain ***and*** provides a potential mechanism for the cognitive deficits and teratogenicity associated with these AEDs. We show that the use of hPSCs as an *in vitro* model to investigate pharmacological activity on neurodevelopmental processes is a powerful, valid and attractive alternative to *in vivo* methods and has enormous potential in the screening of potential neurotoxic agents and new medicines.

This work was supported by grants from the Center for Alternatives to Animal Testing (CAAT). Some of this work was presented at the GTCbio '4th Advances in Stem Cell Discovery and Development Conference', October 2010, in San Francisco, CA.

EXPRESSION OF THE ECP-2 C-TERMINUS IN *LATRODECTUS HESPERUS*

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OBJECTIVES: Spider silk's high tensile strength and elasticity, as well as its biocompatibility, can potentially be used to revolutionize medicine and technology. Current research is focused on elucidating the silk manufacturing process and determining a means for mass production of the silk. The egg case protein 2 (ECP-2) was recently identified as an important constituent of egg case silk fibers from the black widow spider, *Latrodectus hesperus*. The purpose of this study was to express the C-terminus of ECP-2 in bacteria and investigate its biochemical properties.

METHODS:

- Amplified the ECP-2 cDNA that corresponds to the C-terminus from a library
- Insert the cDNA into a prokaryotic expression vector
- Transform the recombinant expression vector into *E. coli*
- Induce bacteria to express the C-terminal region of ECP-2
- Verify ECP-2 production using Western blot analysis

RESULTS: Lanes 5 through 8 demonstrate that the C-terminal region of ECP-2 can be expressed at high levels in bacteria. Specifically, lanes 5 and 7 contain samples from lysed bacteria after induction with arabinose; these lanes show dark thick bands on the gel. Lanes 6 and 8 contain lysates from non-induced samples.

CONCLUSIONS: Western blot analysis was successfully performed on samples collected from bacteria carrying the expression vector after induction with arabinose. The small-scale expression of the C-terminus of ECP-2 was shown to be possible. Further experimentation will include using the purified protein to spin artificial silk fibers. We plan to make different blends of recombinant expressed silk proteins for the spinning process and test their mechanical properties.

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SEXUAL DIMORPHISM IN AORTIC ENDOTHELIAL FUNCTION OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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OBJECTIVE: To date little is known of the interaction between diabetes and gender in the vasculature. Thus, the objective of this study was to investigate whether there is a gender difference in aortic endothelial cell function in streptozotocin (STZ, 60mg/kg, iv)-induced diabetic rats. The potential roles of superoxide and cyclooxygenase (COX) metabolites in diabetes-induced vascular dysfunction were also studied.

METHODS: Endothelium-dependent vasodilation (EDV) in response to acetylcholine (ACh; 10^{-8} to 10^{-5}M) was measured in aortic rings precontracted with phenylephrine (PE; 2μM) before and after pretreatment with MnTMPyP (25μM), a superoxide scavenger, or indomethacin (indo; 10μM), a COX inhibitor. In addition, the level of endothelial nitric oxide synthase (eNOS) mRNA expression was determined using real time RT-PCR.

RESULTS: STZ-induced diabetes impaired EDV in both genders, but its effect was more pronounced in females. Similarly, eNOS mRNA expression was lower in diabetic animals and the decrease was greater in females than in males. Preincubation with MnTMPyP or indo increased the sensitivity of aortic rings to ACh-induced relaxation only in female diabetic animals.

CONCLUSIONS: These results suggest the predisposition of female rat aorta to vascular injury in diabetes, possibly via altered NO and superoxide production. Furthermore, COX metabolites also play a role in the vascular reactivity in female diabetic rats.

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THE EFFECTS OF VITAMIN D ON DNA REPAIR IN HEAD AND NECK CANCER

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OBJECTIVES: Rad51, a key protein in DNA double-strand break repair, has been implicated in a variety of cancers. Increasing evidence links the rate of DNA repair with levels of Rad51, further contributing to cellular resistance against radiation and chemotherapy. It has been shown that head and neck squamous cell carcinoma (HNSCC) cell lines treated with VD_3 demonstrated a decrease in the Rad51 repair protein, *in vivo* and *in vitro*. We hypothesize that VD_3 impairs the DNA damage response, resulting in increased apoptosis. We further examined the expression of the upstream cell cycle regulator, Checkpoint Kinase 1 (Chk1), as a mechanism through which Rad51 is regulated.

METHODS: For *in vivo* studies, parafinized biopsies from the buccal pouch of DMBA-induced tumors from Golden Syrian hamsters treated or untreated with systemic VD_3 for 14 weeks were examined by immunohistochemical analysis (IHC) of Rad51. Biopsies were also homogenized and quantified for protein analysis. Human biopsies from mild dysplasia to invasive squamous cell carcinoma were evaluated for Rad51 as well. For *in vitro* studies, SCC cells of the tongue and MCF7, a breast cancer cell line, were cultured and treated with increasing amounts of VD_3 at several time points at 70% confluency. Protein expression was quantified then evaluated by western. DNA damage and apoptosis in response to VD_3 over the same time points were evaluated using the Comet Assay. Interaction between Chk1 and Rad51 was evaluated by immunoprecipitation. SCC25 cells were analyzed after transfection with Chk1 siRNA to further test the interaction between Chk1 and Rad51.

RESULTS: Western Blot analysis of lysates from hamsters treated at 6 weeks showed varied Rad51 expression; however, Rad51 expression was consistently reduced in VD_3-treated hamsters sacrificed at 14 weeks in comparison to untreated hamsters. IHC analysis of hamster sections showed similar results. Preliminary studies of Rad51 expression from human biopsies showed variation in expression during carcinogenesis. *In vitro* studies revealed that 100nM VD_3 had the greatest effect on reducing Rad51 expression, and this reduction was more apparent over time. Comet tail length was significantly longer in SCC25 cells treated with 100nM and tail length was apparent as early as 6 hours. Co-IP of SCC25 cells treated with 100nM VD_3 showed an interaction between Chk1 and Rad51. Further investigations will be performed to analyze the effects of Chk1 siRNA on the down regulation of Rad51 in response to VD_3.

CONCLUSIONS: Preliminary *in vivo* findings suggest that the effects of VD_3 on the expression of Rad51 may not be apparent until the 14^{th} week of treatment. This also appears consistent with *in vitro* findings that show a more dramatic decrease in Rad51 expression 48 hours after treatment. Further studies will be necessary to look at the expression of Chk1 over time in response to VD_3, and investigate a mechanism involving the potential of Rad51 in decreasing tumor formation.

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CRACKING THE SHELL

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OBJECTIVE: The black widow spider, *Latrodectus hesperus,* produces seven different silk proteins that can be spun into various kinds of silks. One of these fiber types , called tubuliform silk, as been shown to be composed of at least three different proteins TuSp1, ECP-1 and ECP-2. Tubuliform silks are found in egg sacs and serve to protect spider embryos during development. Analyses of mRNA levels have shown that ECP-2 is expressed at higher levels relative to ECP-1. The ECPs have been hypothesized to constitute the outer layer of the tubuliform silk fibers. To elucidate the structural role of ECP-2, we have attempted to express part of the protein in bacteria. In order to accomplish this task, we amplified a segment of the ECP-2 cDNA coding its N-terminus. The ECP-2 cDNA was amplified using PCR, ligated into a prokaryotic expression vector and transformed into *E. coli.* Following transofrmation, the recombinant protein was induced and its expression level was analyzed by western blot analysis. Here we show that the N-terminus of ECP-2 can be expressed in high levels in bacteria, which should make purification of large amounts of ECP-2 for structural analyses feasible.

METHODS AND MATERIALS:
☐Use a cDNA library prepared from black widow spider silkproducing glands to amplify a segment of the ECP-2 cDNA encoding the N-terminus
☐Amplify the ECP-2 cDNA using PCR
☐Ligate the ECP-2 cDNA into a prokaryotic expression vector
☐Transformation the cloning vector into bacteria
☐Verify the cDNA insert is present within the cloning vector
☐Induce the expression of the cDNA in bacteria
☐Lyse the bacteria and perform a western blot analysis

RESULTS: Once the ECP-2 cDNA was amplified using PCR and ligated into a prokaryotic expression factor, agarose gel electrophoresis was performed. Figure 1 shows the ECP-2 cDNA being released from the prokaryotic expression vector, pBAD-Thio-Topo. Lanes 1 represent the DNA ladder and lanes 2 through 7 represent the cDNA of the ECP-2. Following the validation of the cDNA in the cloning vector, we transformed the cloning vehicle into E. coli. The recombinant protein was induced and the expression of ECP-2 was analyzed using western blot analysis. Figure 2 shows the western blot analysis of extracts collected from bacteria after induction of ECP-2. Lanes 2 and 4 represent the N-terminus of ECP-2 expressed in bacteria with the purified protein in hand, it was run through a computer program and the amino acid sequence was determined. Figure 3 represents the single letter amino acid sequence of the N-terminus of ECP-2. The green highlighted portion on the amino acid sequence is the thioredoxin tag that came from the cloning vector. In the amino acid sequence, there are many cysteine residues; about sixteen in the first one hundred amino acid residues. From the computer program, the isoelectric point of ECP-2 N-terminus was found to be at a pH of 6.15 and the molecular weight is 52 k.

CONCLUSION: For this study, we expressed the N-terminus of the ECP-2 protein. The N- terminus portion of the ECP-2 protein was predicted to be 365 amino acids in length. Western blot analysis reveals that the ECP-2 N-terminus can be expressed in high levels in bacteria. Lanes 2 and 4 shows the induced protein has a molecular mass around 65 kDa. Lane 3 shows cellular extract from cells under non-induced protein conditions and acts as a negative control for our experiment. The western blot analysis was performed in order to determine whether the protein could be successfully expressed in E. coli. The bands in lanes 2 and 4 verify this question. Because our experiments demonstrate that the N-terminus portion of ECP-2 can be expressed in high levels, we plan on expressing ECP-2 on a large scale analysis in the future. Furthermore, in Figure 3, it is shown that many cysteine residues are present within the amino acid sequence. The presence of such high numbers of cysteine residues in the N-terminus in highly unusual. This could imply this region is used in some type of oxidation reduction reaction to create disulfide bonds that link it with other proteins. With the ability to purify large amounts of ECP-2 possible future research can be done to determine its biological function.

ANALYSIS OF THE 5'UNTRANSLATED REGION (5'UTR) OF THE ALCOHOL OXIDASE 1 GENE AS A REGULATOR OF TRANSLATION IN *PICHIA PASTORIS*

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OBJECTIVE: *Pichia pastoris* is a yeast commonly used for foreign protein expression. The coding sequences of foreign proteins are inserted after the AOX1 promoter. The 5' untranslated region (UTR) is part of the mRNA before the coding sequence, which affects the rate of translation (protein production) by ribosomes. We are trying to figure out the correlation between 5' UTR structure and degree of protein expression.

METHODS: Oligonucleotide primers were used in mutagenesis to make deletions in the 5' UTR on a plasmid that contains the beta-galactosidase gene (encoding a reporter protein) as the coding sequence. We used beta-galactosidase assays to measure translation efficiency and thereby protein expression in the yeast.

RESULTS: All deletions caused decreased beta-galactosidase expression in the yeast.

CONCLUSION: Our data suggests that all sequences enhance translation. No negative-acting sequences have ever been found.

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INVESTIGATION OF THE STRUCTURAL ROLE AND FUNCTION OF EGG CASE PROTEIN-2 (ECP-2) IN *LATRODECTUS HESPERUS*

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OBJECTIVES: Due to certain characteristics of spider silk, such as high elasticity and tensile strength, continued research in discovering different spider silk genes can result in many industrial uses. Ultimately, the goal is to be able to produce a spider silk-like protein for artificial fiber spinning. An important gene involved in spider silk formation is ECP-2. Egg case silk, a silk type produced from the tubuliform gland, is identified as a material that is extensible in comparison to dragline silk, which is known to be the strongest fiber made by the black widow spider. Our goal was to overexpress a silk protein in bacteria for potential use in synthetic fiber production.

METHODS: To express the ECP-2 cDNA in bacteria, we amplified the full-length cDNA using PCR from a library using gene-specific primers. The amplified product was ligated into the prokaryotic expression vector pBAD-Thio-TOPO and transformed into bacterial cells. To check for proper insertion of the ECP-2 cDNA, we retrieved plasmid DNA from several different transformants and performed a restriction digestion analysis and agarose gel electrophoresis. After validation of the correct orientation of the cDNA insert, we induced the expression of the cDNA using arabinose, lysed the cells, and performed a western blot analysis using an anti-histidine monoclonal antibody to monitor for expression.

RESULTS: Western blot analysis of bacterial extracts after the induction of ECP-2 indicates that ECP-2 is expressed at fairly high levels in *E. coli.* The recombinant protein was detected near the predicted molecular mass.

CONCLUSIONS: We demonstrate that we can efficiently express full-length ECP-2 in bacteria. The successful expression of ECP-2 indicates that bacteria could be an excellent expression system to produce large amounts of ECP-2 for purification and use for spinning artificial silk fibers for a host of different industrial applications.

This work was supported by a NSF RUI Grant MCB-0950372 entitled Molecular Characterization of Black Widow Spider Silks

EVALUATING DRUG COST AND RESTRICTION PROCESS OF COMMONLY USED PRESCRIPTION DRUGS UNDER EACH 2011 CALIFORNIA STAND-ALONE MEDICARE PART D PLAN.

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OBJECTIVES: Although the Medicare Part D benefit has helped improve prescription medication access and lower out-of-pocket spending for some, variability in drug cost and access restrictions of Part D plans continues to present a challenge for others. The present research sought to examine the reported drug cost and restrictions (Step Therapy (ST), Quantity Limits (QL), Prior Authorization (PA), and Formulary Coverage) associated with each of the 100 (65 generic and 35 brand name) most commonly filled drugs by Medicare beneficiaries under every stand-alone prescription drug plan (PDP) available in California in 2011.

METHODS: The list of the top 100 Drugs by total fills under Part D plans in 2008 was retrieved from the Centers for Medicare & Medicaid Services. Each of these drugs was entered into the Medicare Plan Finder Tool (www.medicare.gov). Data found via use of the Plan Finder Tool revealed the full cost, formulary coverage and restriction processes of every examined drug under each of the 2011 California PDPs for which data were available.

RESULTS: The difference between the lowest and highest plan reported full cost of each generic (brand) drug from all available PDPs ranged from a low of $3.37 ($5.41) to a high of $134.34 ($106.33). In addition, differences were found between generic and brand medications in terms of drug restrictions (3.08% generic vs. 57.1% brand drugs required ST), (58.46% generic vs. 94.3% brand drugs required QL) and (4.62% generic vs. 31.4% brand drugs required PA) across the PDPs. Finally, 13 (20%) generic and 25 (71.4%) brand-name drugs were not covered on one or more PDP formularies.

CONCLUSIONS: Significant differences in medication costs and plan imposed restriction processes exist between the various 2011 California PDPs. Such differences may present additional barriers to Part D plan access and potentially impact health-related outcomes and costs of Medicare beneficiaries.

The authors declare no conflicts of interest, real or apparent, and no financial interests or support for the work mentioned in this abstract. This work will be presented at the International Society of Pharmacoeconomics and Outcomes Research Meeting in May 2011.

EVALUATING THE WILLINGNESS-TO-PAY OF MEDICARE BENEFICIARIES FOR PART D PLAN ASSISTANCE

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OBJECTIVES: Medicare Part D allows each beneficiary the ability to choose and enroll in a privately sponsored Medicare-approved prescription drug plan (PDP). However, with 33 different stand-alone PDPs to choose from in California in 2011 alone, such a choice can be overwhelming. We sought to assist beneficiaries with Part D plan evaluation and quantify their willingness-to-pay (WTP) for such services during the 2011 open enrollment period.

METHODS: Nine outreach events were held in cities across central/northern California during which 395 beneficiaries were assisted with their Medicare Part D plan. During each session, beneficiary-specific information (e.g., prescription medications) was entered into the Medicare Plan Finder Tool (www.medicare.gov) to help facilitate the intervention. Demographic and plan-specific data, along with the results of the intervention, were collected from each assisted beneficiary. At the conclusion of the session, each beneficiary's WTP was elicited.

RESULTS: Of the 329 (83.5%) beneficiaries who answered the question, the median (mean $\pm$ SD) WTP for Part D plan help was \$20 (\$42.34 $\pm$ \$89.82). A significant difference ($p<0.001$) was found in the WTP of beneficiaries as a function of whether or not they received additional governmental assistance (e.g., Medicaid). The median (mean $\pm$ SD) WTP of 81 subsidy-recipients was \$0 (\$14.67 $\pm$ \$25.95) versus \$25 (\$52.23 $\pm$ \$101.62) for 243 non-subsidy recipients. WTP was also dependent ($p<0.01$) on whether or not the beneficiary was enrolled into a new plan during the interventional session. The median (mean $\pm$ SD) WTP of the 120 beneficiaries that were enrolled into a PDP plan onsite was \$25 (\$59.90 $\pm$ \$119.42) versus \$15 (\$33.12 $\pm$ \$67.12) for the 196 beneficiaries who were not. Finally, beneficiaries' WTP was significantly correlated ($r_s = 0.21$; $p<0.001$) with the estimated annual cost savings identified during the intervention.

CONCLUSION: Beneficiaries value Medicare Part D plan assistance and the perceived value varies as a function of certain demographic and interventional characteristics.

The authors declare no conflicts of interest, real or apparent, and no financial interests or support for the work mentioned in this abstract. This work will be presented at the International Society of Pharmacoeconomics and Outcomes Research Meeting in May 2011.

FORMULARY DIFFERENCES ACROSS MEDICARE PART D PRESCRIPTION DRUG PLANS

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BACKGROUND: Provided that certain minimum requirements are met, Medicare Part D plan formularies may differ significantly from one another. We sought to examine the formulary coverage of the most commonly filled medications by Medicare beneficiaries under each stand-alone prescription drug plan (PDP) in the United States in 2011.

METHODS: A list of the top 100 filled prescription medications under Part D plans in 2008 was retrieved from the Centers for Medicare & Medicaid Services. Each of these drugs was entered into the Medicare Plan Finder Tool (www.medicare.gov). Since the same PDPs are available to beneficiaries throughout a region, a different zip code was used to identify available PDPs in each of the 34 Medicare regions. Plan parameter data, along with formulary coverage of each of the top 100 drugs, was retrieved and recorded for all unique 2011 PDPs in the US.

RESULTS: In total, 1,109 PDPs are offered nationwide in 2011. However, only 145 unique PDPs were found to exist in 2011 since many plans with identical formulary coverage and cost-sharing structure are offered in multiple regions. For those unique PDPs, on average, 92 (range: 71-100) of the top 100 drugs were found on PDP plan formularies. A total of 11 (8.3%) PDPs covered $\leq$85 drugs, 39 (26.9%) covered 86-90 drugs, 55 (37.9%) covered between 91-95 drugs and 29 (20.0%) covered >96 drugs. Only 11 (7.6%) PDPs covered all 100 of the most frequently filled medications by Medicare beneficiaries. A significant positive correlation was found between the number of top 100 drugs on a PDP formulary and the average monthly plan premium ($r_s = 0.414$; $p<0.001$).

CONCLUSIONS: PDP formularies vary widely in their coverage of the most commonly filled prescription medications. Furthermore, PDPs with higher monthly premiums are likely to have broader formulary coverage. Beneficiaries should annually reevaluate PDP offerings as plan parameters and formularies can change.

The authors declare no conflicts of interest, real or apparent, and no financial interests or support for the work mentioned in this abstract. This work has not been presented at a scientific meeting.

STEM CELLS IN DRUG DISCOVERY: NEUROPHARMACOLOGICAL PROPERTIES OF NEURONS DERIVED FROM HUMAN STEM CELLS

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OBJECTIVES: Human pluripotent stem cells have enormous potential value in neuropharmacology and drug discovery yet there is little data on the major classes and properties of receptors and ion channels expressed by neurons derived from these stem cells. Recent studies in this lab have therefore used immunocytochemistry and patch-clamp electrophysiology to investigate the cellular and pharmacological properties of the ion channels in neurons derived from the human stem cell (hSC) line, TERA2.cl.SP12.

METHODS: TERA2.cl.SP12 stem cells were grown in cell culture and differentiated using retinoic acid to neural phenotypes and used in electrophysiological experiments 28-50 days after beginning differentiation.

RESULTS: HSC-derived neurons generated large whole cell currents with depolarizing voltage steps (-80 to 30mV) comprised of an inward, rapidly inactivating component and a delayed, slowly deactivating outward component. The fast inward current was blocked by the sodium channel blocker, tetrodotoxin (0.1μM) and the outward currents were significantly reduced by tetraethylammonium (TEA, 5mM) consistent with the presence of functional Na and K ion channels. Application of the inhibitory neurotransmitters GABA (0.1-1000μM) or glycine (0.1-1000μM) evoked concentration dependent currents. The GABA currents were inhibited by the convulsants, picrotoxin (10μM) and bicuculline (3μM), potentiated by the NSAID mefenamic acid (10-100μM), the general anaesthetic pentobarbital (100μM), the neurosteroid allopregnanolone and the anxiolytics chlordiazepoxide (10μM) and diazepam(10μM) all consistent with the expression of $GABA_A$ receptors. Responses to glycine were reversibly blocked by strychnine (10μM) consistent with glycine-gated chloride channels. The excitatory agonists, glutamate (1-1000μM) and NMDA (1-1000μM) activated concentration-dependent responses from hSC-derived neurons. Glutamate currents were inhibited by kynurenic acid (1mM) and NMDA responses were blocked by $MgCl_2$ (2 mM) in a highly voltage-dependent manner.

CONCLUSIONS: Together, these findings show that neurons derived from human stem cells develop an array of functional receptors and ion channels with a pharmacological profile in keeping with that described for native neurons. This study therefore provides support for the hypothesis that stem cells may provide a powerful source of human neurons for future neuropharmacological studie

This work was supported by grants from the Center for Alternatives to Animal Testing (CAAT). This work was presented at the GTCbio '4th Advances in Stem Cell Discovery and Development Conference, October, 2010, in San Francisco and will be published in a special issue of Neurochemistry International (2011).

DIRECT LYSIS OF BACTERIAL CELLS EXPRESSING TAQ POLYMERASE RESULTS IN SUCCESSFUL PCR

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OBJECTIVES: Purified *Taq* polymerase, a crucial component of PCR reactions, is generally purchased from biotech companies, which is costly. It could also be obtained by lab purification, a time consuming process. Our studies were to find a faster, more convenient method to perform PCR without the need to purify *Taq* polymerase from bacteria.

METHODS: *E. coli* cells with a *Taq* polymerase cDNA insert was struck out on LB+Amp plates. A single colony was picked out and inoculated in an LB+Amp broth overnight. Part of the saturated culture was transferred into an LB+Amp solution and placed on an orbital shaker with IPTG induction. The bacterial cells were pelleted and resuspended in water and stored at -80 degrees Celsius. Different volumes of the pellet solution were used to run PCR reactions with target cDNA templates of various lengths. These included the cDNAs corresponding to SCP-1 and the SGSF bHLH transcription factor from the black widow spider cDNA library. Subsequently, gel electrophoresis and UV visualization was performed. This process was repeated to test for *Taq* polymerase stability.

RESULTS: It was discovered that only 5 μL of cell pellets are enough to run the PCR reaction. Therefore, without the protein purification step the enzyme maintained a higher activity level with less *Taq* needed. We also discovered that the bacteria cells could be stored in -80 degrees Celsius and were able to be freeze-thawed multiple times without the loss of significant DNA polymerase activity.

CONCLUSIONS: PCR was successfully performed by the direct usage of live *E. coli* bacteria, which carry the *Taq* polymerase gene expression vector, serving as our *Taq* source. The bacteria pellets can be directly added into PCR reaction tubes without the need of purifying the *Taq* polymerase from *E. coli*. Further research on this economically friendly technique can be applied to various PCR applications. It could also be used for DNA sequencing and on difficult to amplify high GC content DNA strands, and forensic science applications.

This work was supported by a NSF RUI Grant MCB-0950372 entitled Molecular Characterization of Black Widow Spider Silks

SYNTHESIS OF THIAZOLE ORANGE DERIVATIVES AS DNA G-QUADRUPLEX BINDING LIGANDS

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OBJECTIVES: G-quadruplex, a unique DNA secondary structure that inhibits the telomerase activity at the end of the chromosomes, has become a novel target in oncology in recent years. The formation of G-quadruplex structures is facilitated by small molecules (G-quadruplex binding ligands) that contain extended and fused aromatic rings. Thiazole Orange (TO), an example of G-quadruplex binding ligands, is known to bind to both DNA duplex and G-quadruplex. Upon binding, TO fluoresces, which makes it an attractive probe for studying ligand-DNA interactions. However, the selectivity of TO binding to DNA duplex and G-quadruplex is minimal. In the present work, we sought to investigate the feasibility to increase the TO selectivity toward G-quadruplex DNA by introducing side chains to enhance the binding specificity.

METHODS: TO derivatives containing various side chains (diamines, poly diamines, PEG diamines, piperidine/pyrollidine containing amines) were chemically synthesized, and their binding to G-quadruplex DNA was evaluated using UV denaturation. A telomeric DNA fragment was heated at 90 °C for 10 min, slowly cooled down to room temperature, and incubated at 4 °C for 16 h to maximize the G-quadruplex formation. G-quadruplex DNA solutions (1 μM) were further incubated with TO derivatives at different concentrations (0- 15 μM) for 2 h. The UV denaturation of the solutions was monitored at 295 nm using a Cary Bio-100 UV spectometer.

RESULTS: TO derivatives were synthesized by coupling TO acid with side chain moieties using standard peptide chemistry. All of the derivatives were characterized using ^{1}H-NMR and ESI-MS. UV denaturation study showed that TO does not affect the melting temperatures (T_m) of G-quadruplex DNA; however, TO derivatives exert significant stabilization effect. The stabilization effect is dependent on the ligand/DNA ratio. At the ligand/DNA ratio 10:1, the DNA melting temperature increased up to 17 °C as compared to that in the absence of ligands.

CONCLUSIONS: Thiazole Orange derivatives are successfully synthesized and characterized. Preliminary UV denaturation study reveals that the proposed TO derivatives significantly stabilize G-quadruplex DNA. Our work shows that it is a good approach to introduce side chains to Thiazole Orange for developing better G-quadruplex binding ligands. The binding constants and conformation of G-quadruplex DNA will be determined using circular dichroism, fluorescence titration, and gel electrophoresis in due course.

This work was supported by University of the Pacific.

UCSF INVITED PRESENTATIONS

ANALYSIS OF AMELOGENIN ASSEMBLY AT THE OIL-WATER INTERFACE: THE ROLE OF HYDROPHILIC C-TERMINUS

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OBJECTIVES: Self-assembly of amelogenin proteins plays a key role in controlling enamel biomineralization. Amphiphilic nature of the bipolar full-length protein provides the characteristics that allow self-assembly into long nanoribbons in water-in-oil system. The purpose of this study was to elucidate the role of the hydrophilic C-terminus by studying the self-assembly of rH174 and two recombinant MMP-20 proteolytic products, rH163 and rH146 in which the C-terminus is cleavaged.

METHODS: Human recombinant amelogenin protein, rH174, rH163 or rH146 at concentrations between 0.4-3.7 mg/ml was dissolved in calcium and phosphate solutions at acidic conditions and mixed with an oil phase (octanol/ethyl acetate) to form metastable amelogenin water-oil emulsions. Incubation of amelogenin water-oil system for up to 7 days was followed by in situ pH increase to induce apatite formation. The effects on protein self-assembly and crystal formation as a function of calcium and phosphate concentration, pH and incubation time were studied. The gel-matrix was analyzed using Atomic force microscopy, Transmission and Scanning electron microscopy, and Dynamic Light Scattering.

RESULTS: Long nanostrings of amelogenin rH174 self-assembled at the oil-water interface at high calcium and phosphate concentration (>20 mM, Ca/P ratio=1.6), were observed at pH 4.5 after 4-7 days of incubation. A dramatical increase in the number of extended bundles of co-aligned filaments was observed when saturation conditions were introduced in the system by raising the pH to 5.6. rH163, lacking the hydrophilic C-terminus, self assemble into regular nanospheres, which do not vary with pH, ion concentration or time, suggesting a clear role of the C-terminus in stabilization of the water-in-oil emulsion and for the generation of reverse micelles that initiates amelogenin self-assembly into nanoribbons as shown for rH174. Surprisingly, rH146 self-assembly into a mixture of ribbons and helical architectures. Width of rH146 helical structures is similar to untwisted nanoribbons formed by rH174 suggesting that hydrophobic repulsions may be the cause for ribbon twisting and highlight the enhanced contribution of the central domain in amelogenin assembly at the oil water-interface

CONCLUSIONS: Amelogenin assemblies are organized in linear arrays. The formation of ribbons or helices is facilitated in mineralizing conditions, and requires the presence of appropriate Ca/P ratios, degree of saturation and elevated concentrations of amelogenin. However, The presence of a hydrophilic C-terminus is not a prerequisite for nanoribbon formation. This process is speed up in saturation conditions and might provide a suitable model for amelogenin-guided apatite formation.

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3D MICROPATTERNED MEMBRANE FOR REGENERATING THE DENTIN-PULP INTERFACE *IN-VITRO*

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OBJECTIVES: This study focuses in regenerating the dentin-pulp interface *in-vitro*. The microfabrication of a 3D porous micropatterned membrane was developed to create a chemoattractant gradient to induce the formation and the protrusion of odontoblastic processes. The dental pulp cells (DPCs) from the dental papilla are characterized to differentiate into odontoblasts to produce dentin.

METHODS: Dental pulp cells were obtained from adult extracted third molars and cultured until the second passage in growth medium containing 15% fetal bovine serum (FBS). Prior to cell seeding, DPCs were starved overnight in starvation medium containing 0.5% FBS. The DPCs were seeded on cross-sections of 100μm-thick dentin discs and poly-caprolactone (PCL) membrane – a thin, porous micropatterned membrane containing 5 or 10μm diameter pores – in a transwell setting to emulate dentinogenesis. The starvation medium was used in the upper chamber of the transwell and growth medium in the lower chamber, creating a chemoattractant gradient across the disc and membrane. As a control, NIH/3T3 cells were seeded on both disc and membrane, and used in a transwell setting to compare cellular behavior. Confocal microscopy was used to examine and image the stained cells.

RESUTLS: The DPCs and NIH/3T3 cells attached to both 100μm-thick dentin discs and PCL membrane, and exhibited variations of cell processes length in both cell types along the plane of each surface.

CONCLUSIONS: The attachment of DPCs on porous micropatterned membrane with the effects of a chemical diffusion gradient can influence the localization and organization of DPCs. The various lengths of processes observed may include migration of the processes through the porous micropatterned membrane, forming dentin; and as a result, mimicking the dentin-pulp interface *in-vitro*. This study can be used as a model to re-establish the dentin-pulp interface for tooth regeneration and pulp revitalization.

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