15TH ANNUAL
PACIFIC
RESEARCH DAY

Wednesday, May 29, 2013

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FACULTY
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PRESENTATIONS
Preliminary data comparing the effect of Birex® disinfectant wipes on *Staphylococcus aureus* in suspension and on nutrient agar plates

Jennifer Cheung¹*, Senait Gebremedhin¹, Krystyna Konopka¹ and Nejat Düzgünes¹

¹Department of Biomedical Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

**OBJECTIVES:** Wipes soaked in Birex® disinfectant are claimed to be tuberculocidal, virucidal, bactericidal, and fungicidal; effective against *Mycobacterium tuberculosis*, HIV-1, *Staphylococcus*, *Salmonella*, *Pseudomonas*, *Streptococcus*, MRSA (Methicillin-resistant *Staphylococcus aureus*), and H1N1 (swine flu). Although the effectiveness of the wipes has never been tested, they are used in many different settings, including offices, laboratories, hospitals, and medical and dental environments. This study aims to test the effectiveness of Birex® against *Staphylococcus aureus* (ATCC 14775) in suspension and on nutrient agar plates, as well as on plates with streaked saliva samples.

**METHODS:** *S. aureus* were grown in nutrient broth for 48 h at 37°C. The density of *S. aureus* was determined using McFarland Standards and serial dilutions were made to obtain the desired cell density. 10 μl of 9.0 x 10⁹ colony forming units (CFU)/ml of *S. aureus* was inoculated into 1 ml of solution extracted from the Birex® wipes, and incubated for 5, 10, and 20 min to test the effect of Birex on *S. aureus* in suspension. After incubation with Birex, 10⁴, 10⁵, and 10⁶ dilutions were made to neutralize the Birex solution. These dilutions were plated in duplicate on nutrient agar plates and allowed to grow for 48 h at 37°C. Controls of untreated *S. aureus* were plated at 10³, 10⁴, and 10⁵ CFU/ml. Whatman filter discs with Birex, Lysol, and 10% Bleach solution were tested in triplicate on nutrient agar plates with *S. aureus* and zones of inhibition were measured. A disc with distilled H2O was used as a control. In addition, 10 saliva samples were collected, streaked on blood agar plates, and treated with three different disinfectants: Lysol, Sanitex, and Birex.

**RESULTS:** Controls plated at 10³, 10⁴, and 10⁵ CFU/ml of *S. aureus* had an average of 5.0, 61.5, and 597.0 CFU, respectively. Plates with Birex-treated *S. aureus* showed an average of 11.5, 6.5, and 3.0 CFU at 10⁶ CFU of *S. aureus/ml* treated with Birex for 5, 10, and 20 minutes, respectively. Birex effectively inhibited *S. aureus* growth in suspension. On the nutrient agar plates with *S. aureus*, Birex, Lysol and 10% bleach produced zones of inhibition of 8.3 mm, 14.3 mm and 29.7 mm, respectively. The control disc showed no zone of inhibition. On the blood agar plates streaked with saliva samples, the inhibition zones for Lysol, Sanitex and Birex were 10.4 mm, 6.1 mm and 1.3 mm, respectively.

**CONCLUSIONS:** Birex® disinfectant wipes are effective in eliminating *Staphylococcus aureus* in suspension, killing over 99.77% of *S. aureus* bacteria within 5 minutes of inoculation with Birex, 99.87% within 10 minutes, and 99.94% within 20 minutes. While discs with Birex solution did show some effect on inhibiting the growth of *S. aureus*, it was over 70% less effective compared to discs with 10% bleach and 42% less effective than discs with Lysol. With the saliva samples, Birex was 8 times less effective than Lysol, and over 4.5 times less effective than Sanitex in inhibiting microbial growth.
HIV-specific promoters to eliminate HIV-infected cells by gene therapy

Nejat Düzgüneş*, Senait Gebremedhin, Amy Au, Matthew Milnes and Krystyna Konopka

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

OBJECTIVES: Current antiretroviral therapies against HIV infection are unable eradicate the chromosomally integrated proviral genome. We are developing an HIV-specific promoter to drive the expression of suicide genes that would induce cell death in HIV-infected cells, but not in uninfected cells. Here we examined the expression of luciferase driven by the HIV promoter, LTR, and 5 mutant LTRs. Our aim is to design a promoter that is responsive to the HIV transcriptional activator, Tat, but not to cellular transcription factors in uninfected cells.

METHODS: The full-length LTR and five progressively truncated versions of the promoter were generated using PCR-based cloning techniques, and designated LTR1-LTR6 (Bionexus). These promoters were inserted into the pGL3 Basic Vector encoding luciferase (Promega). These plasmids were transfected into HeLa cells, and HeLa-tat-III cells that constitutively express Tat, using the transfection reagent Metafectene (Bionex). Luciferase activity (relative light units (RLU)/ml cell lysate) was measured 48 h later, using the Luciferase Assay System (Promega).

RESULTS: Luciferase expression from LTR1 containing the wild type HIV promoter, was 595 RLU/ml in HeLa cells, and 30,373 in HeLa-tat-III cells, showing the specific activation by Tat. In LTR2, the LTR modulatory region was truncated, but the NF-κB binding region was maintained. Luciferase expression from the LTR2 construct increased from 1,060 RLU/ml in HeLa cells to 108,187 RLU/ml in HeLa-tat-III cells, a 102-fold increase. In LTR3 without the NF-κB binding region the corresponding values were 498 and 25,387 RLU/ml. The other constructs resulted in much lower gene expression in both cell types.

CONCLUSIONS: HIV-specific cell killing may be possible by generating a suicide gene construct driven by the LTR2 promoter. It is expected that the incorporation of LTR2, or an improved construct with higher Tat-specificity, into a lentiviral vector may lead to the therapeutic transduction of all HIV-harborining cells.

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Characteristics</th>
<th>Fold Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTR1</td>
<td>Wild type</td>
<td>51</td>
</tr>
<tr>
<td>LTR2</td>
<td>LTR minus modulatory region</td>
<td>102</td>
</tr>
<tr>
<td>LTR3</td>
<td>LTR2 minus NF-κB binding region</td>
<td>51</td>
</tr>
<tr>
<td>LTR4</td>
<td>LTR3 minus SP-1 binding region</td>
<td>5</td>
</tr>
<tr>
<td>LTR5</td>
<td>LTR4 with AP1 binding site knockout mutation</td>
<td>8</td>
</tr>
<tr>
<td>LTR6</td>
<td>LTR core promoter and TAR region only</td>
<td>8</td>
</tr>
</tbody>
</table>

Supported by Research Awards 03-Activity-071 and 03-Activity-076 from the Arthur A. Dugoni School of Dentistry. Presented at the 26th International Conference on Antiviral Research, May 11-15, 2013, San Francisco.
Variations on an African theme: comparison of the Olive Baboon and Black and White Colobus Monkey in relation to cranial and dental evolution

Hesaneh Tabatabaifar¹ and Dorothy Dechant²*

¹Department of Biological Sciences, ²Institute of Dental History and Craniofacial Study, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVE: As Old World Catarrhine Primates, the Colobine and Cercopithecine monkeys share some basic anatomical, behavioral and genetic traits, but they have been evolving in separate lineages for around 15 million years, and exhibit many different adaptations. The Olive Baboon (Papio anubis) and Black and White Colobus (Colobus guereza) are two species of Old World Cercopithecine and Colobine monkey respectively. This study attempts to determine whether the cranial and dental morphology of these two species reflect their dietary preferences and adaptations.

METHODS: This study describes two male skulls, one of the Olive Baboon, Papio anubis (specimen CV-95) and one of the Black and White Colobus Monkey, Colobus guereza (specimen CV-91). These specimens are found in the P&S Comparative Anatomy Collection of Institute of Dental History and Craniofacial Study at University of the Pacific Dugoni School of Dentistry. Measurements were taken using a metric plastic dial caliper, to avoid damaging specimens, and a metal sliding caliper. Photographs were taken using a Canon digital ELPH (12.1 mega pixels).

RESULTS: The Olive Baboon male has a cranium over twice the length of the male Colobus Monkey, and a much longer, broader palate supporting parallel tooth rows. Relative to the Colobus Monkey, the Olive Baboon cranium has a larger area of temporalis origin (side of cranium) but smaller area of insertion (coronoid process of mandible), and a smaller area of masseter origin (length of zygomatic arch) but similar area of insertion (ramus of mandible). Both monkeys exhibit large, long canines relative to their adjacent incisors. The molars of both species are bilophodont in shape, with those of the baboon having more rounded cusps (bunodont) and those of the Colobus having more crescent-shaped cusps.

CONCLUSIONS: The Olive Baboon is a large terrestrial primate adapted to an omnivorous diet, while the Black and White Colobus Monkey is a smaller arboreal primate adapted to a leaf-eating diet. Both monkeys consume considerable amounts of course vegetation of somewhat different consistencies. In adaptation to its diverse diet, the Olive Baboon has evolved incisors for cutting and scraping, and bunodont molars (low bulbous cusps) in a bilophodont shape mainly for crushing foodstuffs. In adaptation to its folivorous diet, the Colobus Monkey has evolved incisors for gripping and tearing leaves, and selenodont molars (prominent crescent-shaped cusps) in a bilophodont shape for slicing leaves. As terrestrial primates living in large social groups, male Olive Baboons have evolved long, stout upper canines for predator deterrence and to maintain social order within the male hierarchy. Large canines with significantly long roots anchored in the maxilla and large cheek teeth have led to a parallel shaped dental arcade, unlike the U-shaped dental arcade of the Colobus Monkey.
Improving biofilm formation and photodynamic therapy of Candida biofilms

Senait Gebremedhin*, Hardev Dhillon, Nejat Düzcüneş and Krystyna Konopka

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

OBJECTIVES: The formation of Candida biofilms contributes to the failure of antifungal therapy and the high recurrence rates associated with denture stomatitis. Here we examined the photoactivity of the porphyrin-based photosensitizer TMP-1363 towards Candida biofilms formed on denture acrylic, in the absence and presence of the azole antifungal, miconazole. Photodynamic therapy (PDT) utilizes light to activate a photosensitizing agent (photosensitizer) in the presence of oxygen. The exposure of the photosensitizer to light results in the formation of toxic oxygen species, causing localized photo-damage and cell death. To improve biofilm formation we used different pre-coating agents to develop a standardized Candida biofilm on denture acrylic discs.

METHODS: The effect of TMP-PDT ± miconazole on Candida biofilms was determined for three Candida strains obtained from ATCC: C. albicans GDH18 (ATCC MYA-274), C. albicans UTR-14 (ATCC MYA-2732) and C. glabrata GDH1407 (ATCC MYA-275). Poly(methyl methacrylate) (PMMA) discs were soaked in 70% ethanol and dried for 15 min at room temperature. The discs were then pre-coated with 100% or 50% FBS, and 100% or 50% saliva. Saliva was centrifuged at 3000 rpm for 30 min at 1771g and the supernatant was filtered using a 0.22 μm syringe-driven filter unit. The effect of pre-coating was evaluated by crystal violet staining. Discs were submerged in the standardized Candida suspensions and incubated for 90 min at 37°C (adherence phase). After removal of non-adherent cells, discs were submerged in YNB/100 mM glucose and incubated for 48 h at 37°C (biofilm formation phase). Candida biofilms were incubated with miconazole (25 μg/ml) for 2 h at 37°C, followed by treatment with TMP-1363 (10 μg/ml) for 30 min at 37°C. The plates were exposed to broadband visible light (350-800 nm) from a light bulb at a distance of 10 cm from the bulb to the plate, for 30 min. The irradiance at the surface of the plate was 32.5 mW/cm². Infrared radiation was minimized using a 1 cm-thick water filter. The metabolic activity of the biofilms was measured by the XTT assay.

RESULTS: The most mature Candida biofilm was developed on discs pre-coated with 100% FBS. TMP alone did not reduce the metabolic activity of Candida biofilms. Miconazole alone reduced metabolic activity of biofilms by 47%, 4% and 26% for C. glabrata MYA-275, C. albicans MYA-274 and C. albicans MYA-2732, respectively. Treatment with miconazole + TMP-PDT was more effective, reducing metabolic activity by 58%, 46% and 60%, for C. glabrata MYA-275, C. albicans MYA-274 and C. albicans MYA-2732, respectively.

CONCLUSIONS: A significant synergistic effect was observed for C. albicans MYA-274 biofilms with combined treatment with miconazole + TMP-PDT.

This work was supported by Research Pilot Project Award Activity 085 from the Arthur A. Dugoni School of Dentistry.
Autophagy and inflammation in the ultrastructure of C6-ceramide-treated HSC-3 oral cancer cells

Barbara Plowman*, Michael Yee and Nejat Düzgün

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

OBJECTIVES: In the disease condition, autophagy may be involved in cancer, neurodegenerative diseases as well as immunity and inflammation. These stress conditions may be controlled by a homeostatic process whereby the mechanism of autophagy breaks down and recycles the organelles and proteins in both beneficial and detrimental ways. We have shown the ultrastructure involved in autophagy of HSC-3 oral squamous cancer cells when treated with C6-ceramide, a sphingolipid that is antiproliferative and proapoptotic. The C6 ceramide-treated cells show cytotoxicity, inflammation and decreased metabolic activity.

METHODS: HSC-3 cells were plated at a density of 1.5 \times 10^6 in 6-well plates. Following treatments, they were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.3, with 30 mg calcium chloride. They were post-fixed in 1% osmium tetroxide in 0.1M cacodylate buffer, treated with 1% tannic acid in 0.1 M sodium sulfate buffer, and rinsed in 0.05M sodium sulfate buffer and en bloc stained in 3% uranyl acetate. Cells were dehydrated in a series of graded alcohol, infiltrated in Spurr resin, polymerized at 60 °C and sectioned by ultramicrotomy. Sections were post-stained in uranyl acetate and lead citrate and viewed with a Philips 201 transmission electron microscope.

RESULTS: Control cells showed different densities, but typical ultrastructure with nuclei and mitochondria. C6-ceramide treated cells showed different densities as well as variations in the overall appearance of the cells. Endoplasmic reticulum and the centrioles indicate actively dividing cells. The ultrastructure of Fig. 5 correlates well with the 5-step model of the autophagosome developing into the autolysosome. The omegasome is characteristically cup-shaped in Fig. 5 and 8 while apoptosis and cytotoxicity are shown in Fig. 8. Inflammation is shown as micropinocytotic vesicles at both high and low magnifications in Fig. 8 and Fig. 9. The more ceramide one adds, the more the metabolic activity is decreased. The ceramide kills the cells or stops growth. Inflammation is seen as micropinocytotic vesicles within the cells of higher density.

CONCLUSIONS: The ultrastructure shown in this study follows the model of autophagy. C6 ceramide promotes decreased cell growth and apoptosis in oral cancer cells. The ultrastructure shows the omegasome, the autolysosome and lysosomes occurring in the cells. C6 ceramide may facilitate cell death by causing more cytotoxicity with increased micropinocytotic vesicles as inflammation, while autophagy proceeds to clean up the cells and recycle cell components.
Evaluating the use of CAMBRA (caries management by risk assessment) in community based oral health programs

Paul Subar*

Special Care Clinic/Hospital Dentistry Program, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: Caries Management by Risk Assessment (CAMBRA) is an assessment tool that utilizes a medical-model approach to identifying and treating the underlying bacterial cause of dental caries. Adding CAMBRA and evaluating it as part of the distance collaboration project known as the Virtual Dental Home allows the correlation between a patient’s caries risk status, salivary pH, and bacterial ATP activity to be established and assists in providing a baseline from which to gauge future intervention.

METHODS: Using a modified caries risk assessment instrument, RDHAP’s (Registered Dental Hygienists in Advanced Practice) practitioners assessed and uploaded data to a cloud-based record management system. They then tested pH, and bacterial ATP activity. Based on the assessment, appropriate protocols were followed. Data were reviewed and preventive products recommended. At regular intervals, salivary pH and bacterial activity were tested to assess efficacy of the CAMBRA regimen prescribed. Salivary pH was assessed from the maxillary anterior vestibule and sublingual areas in order to establish a stimulated and resting pH for the patient. Bacterial ATP activity was established via the CariFree Meter which uses an established ATP bioluminescent luciferin-luciferase method. Dry swabs are applied to the lingual surfaces of lower anterior teeth, saturated in the attached solution, and placed into a calibrated CariFree Meter. Patients were re-evaluated in 3 month intervals in the same fashion after using the prescribed products, based on their risk factor.

RESULTS: Evidence strongly suggests that the use of CAMBRA for patients with special needs improves caries risk assessment and prevention outcomes. The positive impact that the customized interventions have offered to the initialized population is supported by the concluding data analysis done for this project. From the date of the Research Enhancement Award approval through February 2013, a total of 1,301 patients had at least one initial CAMBRA evaluation using the modified assessment tool. The patients were categorized into the following three caries risk categories:

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Patient Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Risk:</td>
<td>449</td>
</tr>
<tr>
<td>Medium Risk:</td>
<td>376</td>
</tr>
<tr>
<td>High Risk:</td>
<td>476</td>
</tr>
</tbody>
</table>

363 patients had subsequent assessments and treatment. The change to their initial caries risk level were as follows:

<table>
<thead>
<tr>
<th>Risk Change</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower risk from initial assessment</td>
<td>33</td>
</tr>
<tr>
<td>Increased risk from initial assessment</td>
<td>56</td>
</tr>
<tr>
<td>No Risk Change from initial assessment</td>
<td>274</td>
</tr>
</tbody>
</table>

In collaboration with the Pacific Center for Special Care, we look forward to continuing to grow this assessment tool in the oral health community. This could be done in a variety of ways including inviting new populations to implement this tool as well as extending the project to practitioners to utilize this tool within various models of care. The recent hiring of a practice management software specialist who has assisted in the extraction of the detailed caries risk data is a further example of the viability of this project as a future standard of care.

This work was supported by Research Enhancement Award (REA) - Activity 077 from the Arthur A. Dugoni School of Dentistry.
Siblings or strangers? A cranial and dental comparison between *Alouatta seniculus* and *Ateles geoffroyi*

Suho Bae¹ and Dorothy Dechant²*

¹Department of Biological Sciences, University of the Pacific, Stockton, CA and ²Institute of Dental History and Craniofacial Study, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: In this study, two New World monkeys, *Alouatta seniculus* (the Red Howler Monkey) and *Ateles geoffroyi* (the Black-Handed Spider Monkey) are analyzed. Literature searches were conducted to gather information on the diet, anatomy, habitat, behavior and locomotion of the two species. One skull of each species was observed and measured for cranial and dental traits to address the possibility that features of the teeth and crania (form) reflect the contrasting dietary preferences and possible behavioral differences (function) in these two monkeys.

METHODS: Two skulls, one of *Alouatta seniculus* (specimen CV-65) and one of *Ateles geoffroyi* (specimen M-3) were studied. Measurements were taken using a plastic metric dial caliper, to avoid damaging specimens. The caliper had uncertainty of +/- 0.05mm. Photographs were taken with a Nikon Coolpix Digital Camera. These specimens are found in the P&S Comparative Anatomy collection of the Institute of Dental History and Craniofacial Study, located in San Francisco at the University of the Pacific’s Arthur A. Dugoni School of Dentistry.

RESULTS: *Alouatta seniculus*, the Howler monkey, has a shearing and bilophodont molar form, while *Ateles geoffroyi*, the Spider monkey, has a bunodont and brachydont molar form. The two monkeys show similar wear on the upper incisors, but the Howler monkey has an underbite. The Howler monkey has a greater area of the cranium dedicated to temporalis origin, a more robust zygomatic for masseter origin, and a more massive ramus for masseter insertion than does the Spider monkey. The Spider monkey has a higher, more rounded braincase than the Howler, which has a low, oblong-shaped braincase. The foramen magnum is located in a posterior position on the basicranium of the Howler monkey, but is located almost directly beneath the braincase of the Spider monkey.

CONCLUSIONS: Field observations indicate that *A. seniculus* eats fruits and leaves in equal proportions while *A. geoffroyi* eats more fruits than any other foodstuffs. The bilophodont molar form of *A. seniculus*, and its underbite, correlate with adaptation to eating large quantities of leaves. The Howler’s large temporalis and masseter muscles indicate a coarser daily diet than that of the Spider monkey. Contrast in location of the foramen magnum may correlate with consistent quadrupedalism in the Howler versus quadrupedalism coupled with occasional vertical posture and locomotion in the Spider monkey. The Spider monkey tends to have a bigger braincase possibly due to its need to remember where food sources are located and because the howling adaptation of the Howler monkey limits the size of the braincase due to its enlarged larynx.
Non-viral gene delivery to oral squamous cell carcinoma and cervical cancer cells, and suicide gene therapy

Nejat Düzgünes¹, Aruna Singh²*, Senait Gebremedhin¹ and Krystyna Konopka¹

¹Department of Biomedical Sciences, and ²International Dental Studies Program, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA

OBJECTIVES: Suicide gene therapy of oral squamous cell carcinoma (OSCC) may be a viable approach to the treatment of this cancer. However, human OSCC cells are relatively resistant to efficient transfection by non-viral vectors. To identify an optimal vector for gene delivery, we compared the transfection activities and efficiencies of Glycofect, Metafectene, Metafectene Pro, Metafectene Easy and Fugene HD, using the OSCC cell line, HSC-3, and the cervical carcinoma cell line, HeLa.

METHODS: The cells were seeded at a density of 1.5 x 10⁵ in 1 ml of appropriate media in 48-well culture plates one day before transfection, and used at approximately 80% confluence. Complexes were prepared at the optimal ratio of reagent:DNA with the plasmid pCMV.Luc (VR-1216), added in serum-free DMEM, and incubated with the cells for 4 h at 37 °C. Serum-containing medium was added, and the cells were incubated for 48 h. Transfection activity was assayed using the Luciferase Assay System (Promega). To evaluate transfection efficiency, pCMV.lacZ was used in a similar manner, and X-gal was added as a substrate for β-galactosidase. For gene therapy experiments, HSV- tk was delivered to the cells, and the cells were incubated with 20 ug/ml ganciclovir for 3, 6 and 9 days. Cell viability was assessed by the Alamar blue assay.

RESULTS: Metafectene Easy and Fugene HD mediated the highest transfection activity and efficiency in both cell lines [figure1]; the activity was higher in HeLa cells than in HSC-3 cells. With suicide gene therapy, HeLa cell viability was 22±3% of controls by 9 days with Fugene HD and 26±3% with Metafectene Easy. With HSC-3 cells, cell viability was 42 ± 25% with Fugene HD, and 58 ± 28% with Metafectene Easy. The reduction in viability was statistically significant in both cases (p ≤ 0.005; average of 3 independent experiments), although there was considerable variability between experiments with HSC-3 cells.

CONCLUSIONS: Metafectene Easy and Fugene HD may be useful in gene therapy of OSCC and cervical carcinoma. The reasons for the differential susceptibility of these cells to transfection by non-viral vectors are not known.

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Traditional v/s digital impressions in fixed and implant dentistry

Aanchal Sekhon1*, Aruna Singh1*, Rashmi Kurian1*, Mazher Syed1*, Deeptha Surampudi1* and Bina Surti2

1International Dental Studies Program and 2Department of Integrated Reconstructive Dental Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVE: Technical advances in dentistry have changed the way that dental offices treat and communicate with patients. The traditional workflow involves an intra-oral impression poured in dental stone which has proven itself in clinical practice though there are some disadvantages of traditional impression materials. Today, intra-oral scanning is one of the most exciting areas in dentistry where three-dimensional scanning of the mouth is done in a large number of procedures such as Restorative dentistry, Orthodontics and Implant dentistry. Many intra-oral scanning devices have been developed all over the world which are based on several non-contact optical technologies and principles. The aims of the present literature review are:

1) Comparison of traditional and digital impressions in Fixed and Implant dentistry.
2) To provide an extensive review of the existing intraoral scanning systems for Fixed and Implant dentistry with attention to the working principles, clinical application and benefits of current technology.

METHODS: To compare the traditional impressions with digital impressions, an online search was done using the CPMC library resources. Articles were obtained from peer reviewed journals available on Pubmed, Ovid online, Ebsco host. Various textbooks, brochures and product websites on different digital impression machines were reviewed. Numerous in-vitro studies comparing efficiency, internal fit, marginal accuracy, occlusion, working & retake time, ease of use and patient comfort of digital impression with conventional impressions were analysed. Seven Intraoral scanning systems (CEREC, Lava COS, E4D, Trios, iTero, IOS Fastscan, True definition scanner) were analysed and compared with six conventional impression materials. Traditional impression materials reviewed were Reversible & Irreversible Hydrocolloid, Polyethers, PVS, Condensation & Addition silicones.

RESULTS: Tissue management and gingival retraction are pivotal for both conventional and digital impressions. Within the limits of in-vitro studies, accuracy of digital impression is similar to conventional impression. Crowns from digital impressions revealed significantly better marginal and interproximal fit than crowns from traditional impressions. Marginal discrepancies in both groups were within the limits of clinical acceptability. In digital impressions, learning curve and cost of the scanning systems are the challenges faced by the dentists. However, the taste and presence of metallic powder in the mouth was unappealing to some patients. This has been overcome to a great extent by the advent of powderless scanning systems.

CONCLUSION: Based on our review of various Intraoral scanning systems available, it can be concluded that the digital technology is making its way into the dental profession and the proliferation is being accepted with open arms by dentists and patients. It is also noted that if backed by strong scientific research, dentists are willing to embrace the latest standards of care.
Veneers in dentistry

Jignesh Parmar*, Kapil Grewal*, Harsh Kalyani* and Murtaja Kamal Aldeen*

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

OBJECTIVES: Veneers are steadily increasing in popularity among today’s dental practitioners for conservative restoration of the teeth. Since its first use in 1938, it has been continuously going through the evolution both in the material as well as the techniques. The main purpose of this research project was to shed light on the process of evolution and the current state of veneers in dental field. With the advancement in dental bonding system, the application of veneer is ever evolving. As with any new procedure, in vitro and in vivo investigations are required to assess the ultimate clinical efficacy of these restorations. We reviewed the current literature for indications, contraindications, proper selection of the veneers cases and factors to be considered in Treatment planning of this esthetic material. Also, long term success of different veneer types and failures were reviewed.

METHODS: Laminates/Veneers have been a conservative treatment approach since its introduction. The continued development of dental ceramics offers clinicians many options for creating highly aesthetic and functional porcelain veneers. A comparison of all the studies evaluating the success rates have affirmed its clinical applications. The review goes over the different materials and techniques which include the direct/indirect composites and ceramics and overview the advantages and disadvantages of each. There have been studies comparing the effectiveness of direct and indirect veneers, however very little reliable evidence is present to show the benefit of one over other. The poster sheds light on some of the important steps involved in treatment planning and design considerations including different types of veneers preparations. The success of esthetic dentistry depends on matching the patients expectations and understand the science behind the materials and techniques to achieve the desired outcome.

RESULTS: A total of 19 papers evaluating the long term success of veneers were included in the review and it was found that the success rate ranged anywhere between 53% to 100% with the average being 87%. Bonding to enamel is considered the gold standard. Dentin has a lower modulus of elasticity than enamel and it flexes more under load subjecting the veneered porcelain to shear and tensile. The failures of veneers have mainly been attributed to fractures. Failures could occur due to lack of attention to details during treatment planning the case, improper selection of materials and technique and if the preparation is not conservative and extends too deep into the dentine. Most common type of failure is attributed to that due to fractures. Bulky appearance, failures in shade selection, debonding and marginal leakage are some of the other reasons leading to failed veneer restorations. In case of failures, existing veneers can either be repaired or re-done. With newer technology using Er:YAG lasers veneers can now be removed more efficiently without damaging the underlying tooth structure.

CONCLUSION: Veneers offers many wonderful advantages when they are carefully planned and when cases are properly selected. Over the period of time many types of veneers have evolved. Today veneers can be either in composite or porcelain. Composite material selected for veneers can be Microfilled, Hybrid or Nano Hybrid. Similarly, Porcelain veneers are available as glass based, alumina based and zirconia based. Porcelains have evolved from feldspathic to IPS Empress to IPS Emax to Lava Plus. Although, there is no reliable evidence proving that one type of veneers is better than the other types but some studies have proved that there is long term survival rates for feldspathic porcelain and glass infiltrated ceramics. Thin ceramic veneers bonded to acid-etched enamel have been suggested as the most acceptable and predictable type of veneers. In the clinical practice the selection for the type of veneer material should be done after critically evaluating each case for patient's expectations, Midline position, Lip fullness, incisal edge position, occlusion, shapes of teeth and desired color change. The input and the expectations of patients are the primary determinants for a proper selection of veneering materials as well as correct Treatment planning which will result in a successful veneering treatment.
Prevalence of congenitally missing teeth among dental students

Zhanna Konovalenko1*, Arash Abolfazlian2, Mirek Tolar3, Tarek Abousheta4, Taran Cheema4, Lateefa Al-Kharafi4, Shweta Deshmukh4, Alanoud Alotaibi4, and Marie M. Tolarova4

1International Dental Studies Program, 2Graduate Program in Orthodontics, 3Pacific Regenerative Dentistry Laboratory and 4Pacific Craniofacial Genetics Laboratory, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: Congenitally missing teeth (CMT) are the most common dental developmental anomalies in humans. Based on number of missing teeth, three major subgroups are recognized: hypodontia (agenesis of one or more, but less than 6 teeth), oligodontia (congenitally missing 6 or more teeth), and anodontia (an extremely rare condition when all primary and permanent teeth are missing). CMT can occur either as an isolated anomaly or as a part of a syndrome. A polygenic multifactorial model of etiology best explains etiology of nonsyndromic CMT. Maxillary 3rd molars are the most commonly missing teeth regardless of ethnic origin. Prevalence of agenesis of other permanent teeth, excluding third molars, ranges from 1.6 to 9.6%, depending on a population studied. Primary dentition may also be affected, but with a lower prevalence (from 0.5 to 0.9%). Excluding 3rd molars, more recent studies indicate that tooth agenesis is statistically significantly higher in females.

OBJECTIVES: The purpose of our study was to ascertain the prevalence of CMT among dental students, obtain information about family history, and prepare background data for heritability estimate, recurrence risk calculation, and molecular genetic analysis.

RESULTS: Our sample comprised 1067 students (552 males, 515 females) of the Dugoni School of Dentistry. Out of this, 187 (17.5%) students had one or more missing teeth (probands). There was a small difference between genders: 18.3% of males and 16.7% of females were missing teeth. The most common missing tooth type was the 3rd molar, which was reported in 72.2% of students with missing teeth. Excluding 3rd molars, 52 individuals (4.4%) were missing other teeth. Lateral incisors were the second most common missing tooth (11.2%), followed by 2nd premolars (3.2%). It was much more likely to have missing teeth on both sides (60.9%), than either on the left (16.0%) or right (18.7%) side. We observed almost the same number of CMT in upper (35.3%) and lower (37.4%) arch. A genetic component is a likely etiologic factor. Twenty percent (20.9%) of probands with missing teeth had a 1st degree relative with CMT, 7.5% had a 2nd degree relative missing a tooth, and 32.6% had both. No family history in 1st and/or 2nd degree relatives was indicated in 36.9% of probands.

CONCLUSION: Our results are not based on general population data, however, the sample is large enough to provide a valuable information in respect to the type of missing teeth and family history of CMT. The study is continued focusing on association of mutations in PAX9 gene with specific types and patterns of CMT in probands and on occurrence of PAX9 mutations in relatives of probands.
Minimally invasive dentistry

Zhanna Konovalenko\textsuperscript{1,*}, Ae Ran Ku\textsuperscript{1,*}, Shelja Bhatia\textsuperscript{1,*}, Angela Laithangbam\textsuperscript{1,*} and Priya Prasannakumar\textsuperscript{2}

\textsuperscript{1}International Dental Studies Program and \textsuperscript{2}Department of Integrated Reconstructive Dental Sciences, University of Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: Minimally invasive dentistry adopts a philosophy that integrates prevention, remineralization and minimal intervention for the placement and replacement of restorations. It incorporates the concept of removal of the minimal amount of healthy tissues. MID as you know encompasses the whole spectrum of detecting, diagnosing, intercepting and treating the disease of dental caries in a whole new light and is an evidence based philosophy.

OBJECTIVES: Learning about the diagnosis and prevention of dental decay from the standpoint of minimally invasive dentistry. Acquiring knowledge about state of the art techniques and innovative tools as well as dental materials used in minimally invasive dentistry.

METHODS: Review of available literature on the topic. Total of over 48 articles were used for this presentation. Articles were researched using PubMed, Medline and Ovid databases. Articles were chosen based on the relevance to 4 key areas that we focused in minimally invasive dentistry: diagnosis, prevention, new methods and materials used in the field. The keywords used for the search were the following: minimally invasive dentistry, CAMBRA, caries detection, xylitol, MI paste, baking soda toothpaste, prevention in dentistry, diagnodent, FOTI, hard tissue laser, dental decay.

CONCLUSIONS: Studies show indubitable evidence of the use of tools such as diagnodent, FOTI and others in caries detection. Promising information is available the use of hard tissue laser as one of the conservative ways of treatment. Repair of existing restorations is advocated to conventional method of replacement. Literature review supported used of preventive measures such as mouth rinses, baking soda, MI paste, Xylitol gum and other products for prevention of dental decay. Adhesive restorative materials are the materials of choice in minimally invasive dentistry. This transforms conventional G.V.Black's concept of "extension for prevention" into "prevention for extension."
Material selection: PFM vs e.max vs Lava vs Procera

Yaming Hu*, Ardalan Keshtkar*, June Pang*, Dong Yan* and Jufen Zhou*

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

OBJECTIVES: The increase in crown restorative options has produced questions about materials behind each brand name, best material choices for each clinical situation, which crown restoration has less complications and which crown choice will meet the esthetic and longevity expectations of the modern, esthetically-driven patient.

1. Describe the structural characteristic, mechanic properties, and clinical applications of PFM, Procera, Lava and E.max.
2. Compare the strength and esthetics
3. Investigate survival rate and clinical complications
4. An easy user guide in material selection and clinical applications

METHODS: Literature review of the publications in the most recent 10 years was performed. The search was conducted using key words PFM, Procera, LAVA, E.max, crown, PFM, strength, esthetics, survival, and complications from PubMed.

RESULTS: After comparing the translucency, strength and fracture toughness of all materials, we found there is no material with satisfaction in both strength and esthetics. The translucency of all-ceramic is superior to PFM overall. E.max press or LAVA Ceram is more translucent than Procera, or Zirconia materials. By contrast, flexural strength is high in materials where translucency is low, thus PFM, Procera and Zirconia have higher strength than E.max press or Lava Ceram.

As for the survival rate and complications, the 5-year survival rate of E.max single crown (97.4%) is higher than PFM (95.6%), and the survival rate of Procera and LAVA is slightly lower than PFM. In 3-unit FPD application, the survival rate of PFM is still superior to all-ceramic materials, but E.max (93% at 8-year) and LAVA (92.9% at 4-year) are comparable to PFM (94.4% at 5-year) with appropriate case selection. In general, all-ceramic materials have higher clinical complications, the largest complication being ceramic chipping and fracture.

CONCLUSIONS: All ceramic restorations are more esthetic than PFM restorations. With appropriate case selection and clinical application, all ceramic offers a predictable and highly successful restoration. The clinical decision to place a ceramic or PFM restoration should be based on individual case scenarios. For an anterior restoration in a patient with high esthetic expectations, an all-ceramic restoration is probably more suitable than a PFM. For a posterior FPD in a case with heavy occlusal force, PFM is probably a more reasonable choice than all-ceramic materials.
Tooth whitening: An overview

Dilpreet Cheema*, Cristina Hernandez*, Rachana Jha* and Sukhmani Marwaha*

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

OBJECTIVES: Tooth whitening has been a hallmark of beauty since ages. Whitening agents are one of the most in demand products in Dentistry today. These agents are used to whiten the discolored/ stained teeth, which may be a result of many extrinsic and intrinsic factors. This research focuses on the evolution of whitening agents, Methods and products used in office and At home whitening procedures, advantages & disadvantages and some drawbacks of whitening procedure.

METHODS: In the search about in office bleaching agents, it was seen that there are bleaching methods involving use of lights and lasers. The most common bleaching agents used in clinics today are Carbamide peroxide, since it is more stable that hydrogen peroxide. Taking into consideration various concentrations that are used, it was seen that more than the concentration, the time for which the bleaching agent is in contact with the teeth has more effect on whitening effect. Also, the use of various lights and lasers was debatable.

At home bleaching systems and over the counter bleaching products are cost effective and less invasive alternatives to whiten discolored teeth without dentist’s supervision. The take home bleaching systems dispensed by the dentist has shown the same long-term results as in office bleaching with or without light. Different over the counter products such as OCT whitening trays, whitening strips, toothpastes, rinses, chewing gums, dental floss are available in the market. Toothpastes, chewing gums, and dental floss have abrasive action to remove superficial stains where as rinses and paint-on brushes with low levels of hydrogen peroxide have some whitening effect, but without clinical relevance. Various studies comparing these products have shown that OTC whitening gels trays and whitening strips with carbamide peroxide have similar results followed by whitening toothpastes and paint on gels.

RESULTS: Tooth whitening has been in the dental market since ages and has been of great success. Although there are obvious esthetic advantages of this system, there are also some disadvantages of relapse, resulting tooth sensitivity and changes in enamel structure. Many studies and research papers support this.

CONCLUSION: There are numerous materials and products available in the market for tooth whitening and it is important to understand their differences and indications to make appropriate recommendations to our patients.
Cytokine induction by *Porphyromonas gingivalis* depends on epithelial cell type

Pushpinder Sethi\(^1\), Michael Yee\(^2\), Nejat Düüzünes\(^2\) and Krystyna Konopka\(^2\)

\(^1\)International Dental Studies Program and \(^2\)Department of Biomedical Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: Infection of epithelial cells with *Porphyromonas gingivalis* results in the production of pro-inflammatory cytokines involved in the initiation and progression of periodontal disease. Interleukin-6 (IL-6) is a pleiotropic cytokine that stimulates immunoglobulin secretion and plays an important role in regulating immune responses to periodontal pathogens. Interleukin-8 (IL-8) is a potent chemoattractant inducing the influx of neutrophils into periodontal lesions. The objective of the study was to compare IL-6 and IL-8 responses of three human oral epithelial cell lines to two *P. gingivalis* strains.

METHODS: *P. gingivalis* strains, 2561 and W83, were sub-cultivated on blood agar plates and suspended in Medium 199. Non-tumor-derived immortalized oral epithelial GMSM-K cells, and oral squamous cell carcinoma, HSC-3 and H413 cells, were exposed to live and heat-inactivated *P. gingivalis* at 10\(^8\) bacteria/well (inoculation ratio ~500 bacteria per cell), and incubated at 37°C for 6 and 24 h. IL-6 and IL-8 were determined by ELISA.

RESULTS: The levels of IL-8 produced by GMSM-K cells were much lower than that produced by HSC-3 and H413 cells. Live *P. gingivalis* induced significant IL-6 and IL-8 secretion in GMSM-K and HSC-3 cells. Heat-inactivation of *P. gingivalis* enhanced greatly IL-6 and IL-8 stimulation in these cells. Uninfected H413 cells produced higher levels of IL-6 and IL-8 than HSC-3 and GMSM-K cells, but these cells were not responsive to live *P. gingivalis*. However, heat-inactivated *P. gingivalis*-2561 and *P. gingivalis*-W83 resulted in a 2- to 4-fold increase in IL-6 and IL-8 secretion in H413 cells.

CONCLUSIONS: The amount of IL-6 and IL-8 secreted by control cells and their response to *P. gingivalis* was strongly dependent on the cell type; thus, conclusions on cytokine responses to *P. gingivalis* should not be based on studies with a single cell type. Heat-inactivated *P. gingivalis* strains stimulated higher IL-6 and IL-8 secretion, suggesting an effect of *P. gingivalis* cysteine proteinases (gingipains) on both cytokines.

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Cracking the code of life: DNA sequencing from ambition to reality

Tarek Abousheta¹*, Miroslav Tolar², Lateefa Alkharafi¹, Shweta Deshmukh¹, Alanoud Alotaibii¹, Fateh Arslan¹ and Marie M. Tolarová¹

¹Pacific Craniofacial Genetics Laboratory, Department of Orthodontics and ²Pacific Regenerative Dentistry Laboratory, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: The advent of DNA sequencing has significantly broadened and accelerated biological research and discovery. Rapid speed of sequencing attained with modern DNA sequencing technology has been instrumental in sequencing of the human genome in the Human Genome Project. Related projects, often supported by scientific collaborations across continents, have generated complete DNA sequences of many animal, plant, and microbial genomes.

HISTORY: Early DNA sequencing techniques included the Plus and minus method by Sanger and Coulson, 1975, then the method of Maxam and Gilbert, 1977, followed by the Chain-terminator method by Sanger, Nicklen and Coulson, 1977, that paved the road for modern sequencing methods used today. The dye-terminator sequencing is the first true automated DNA sequencing method (Applied Biosystems, 1986). Since 2005, Next Generation Sequencing methods have been emerging that challenged a supremacy of the Chain-terminator method.

THE HUMAN GENOME PROJECT: The goals of the US Human Genome Project were mapping and sequencing of human genome and of several model organisms. The project was completed on June 25, 2000.

APPLICATIONS: Genomic medicine: sequencing of the human genome and of all major pathogens is beginning to have a major impact on diagnosis, treatment and prevention of diseases. Metagenomics: understanding of genetic diversity to be found on earth. In the Pacific Craniofacial Genetics Laboratory, the use of DNA sequencing methods has been instrumental in identifying Single Nucleotide Polymorphisms (SNP) in candidate genes (MTHFR, RFC1, MSX1, PAX9, TGFB3, TAS2R38 and TAS1R2) and their associations with craniofacial and dental anomalies and diseases.

WHAT IS NEXT: DNA sequencing is still costly. A goal has been set to lower that cost significantly. The X Prize Foundation has established the $10 million Archon X Prize for Genomics that will be awarded to the first team submitting 100 human genome sequences in 30 days or less at a maximum cost of $1,000 per genome sequence. Next step in scientific and medical research will be to capture human genomic diversity. An international team plans to create a massive human genome catalog that would serve as a new gold-standard reference for analysis of human genetic variations. At present, a sizable bioinformatics core is an essential part of each sequencing center. A currently popular vision that an investigator with a single benchtop machine could replace a large sequencing center can only be realized, if productivity of computers and bioinformatics software would increase even more dramatically than that expected for sequencers.
Orofacial clefts in Kuwait and genotypes of TGFβ3 polymorphism rs2300607 AT: A pilot study

Lateefa Alkharafi¹*, Miroslav Tolar², Tarek Abousheta¹, Shweta Deshmukh¹, Hisham Burezq³, Dalal Alhajer³, Fatema Alrayes³ and Marie M. Tolarová¹

¹Pacific Craniofacial Genetics Laboratory and ²Pacific Regenerative Dentistry Laboratory, Department of Orthodontics, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco; ³Ministry of Health, Kuwait

INTRODUCTION: Nonsyndromic cleft lip and palate (NCLP) anomalies belong to the most common craniofacial anomalies with the occurrence of 1.48/1000 live births in Kuwait. Etiology of NCLP is multifactorial, with genetic and environmental components. The transforming growth factor beta 3 (TGFβ3) is required for the normal adhesion of the medial edge epithelium of the palatal shelves leading to their fusion. Previous experimental studies in mice suggested an association of TGFβ3 mutations with developmental defects of the secondary palate.

OBJECTIVES: The purpose of this study was to study association of TGFβ3 rs2300607 AT polymorphism genotypes with NCLP in Kuwait by analyzing this polymorphism in patients with NCLP, in their parents and in control individuals.

MATERIAL AND METHODS: Saliva and blood specimens were collected from Kuwaiti patients affected with NCLP and their parents (sample of cases) and from unaffected individuals from the same location (control sample of the same size). Following a finger prick with a disposable lancet, six drops of blood were collected on a filter paper and dried. Saliva specimens were also collected and transferred to a filter paper by a sterile disposable pipette and dried. Analysis of specimens was conducted at the Pacific Craniofacial Genetics Laboratory, Arthur A. Dugoni School of Dentistry in San Francisco, CA. It included DNA extraction from dry blood or saliva spots, polymerase chain reaction (PCR), and polyacrylamide gel electrophoresis (PAGE). Proportions of the TGFβ3 rs2300607 AT genotypes (AA, AT, TT) as well as allele frequencies were calculated and means for case and control samples were compared.

RESULTS: When analyzing TGFβ3 rs2300607 AT genotypes in cases, wild type AA was observed in 38.7%, AT heterozygote in 45.2%, and mutated homozygote TT in 16.1% in cases. In controls, AA genotype was observed in 50%, AT heterozygotes in 39.3% and TT homozygotes in 10.7%. Mutated T allele frequency was 0.387 in cases and 0.304 in controls. Although mutated allele T frequency and frequencies of AT and TT genotypes were higher in cases, the difference between cases and controls was not statistically significant.

CONCLUSION: Results of this pilot study suggest that TGFβ3 rs2300607 AT polymorphism may not be associated with NCLP in Kuwait. The study continues by genotyping of other TGFβ3 polymorphisms and polymorphisms of other NCLP candidate genes. Contributing environmental factors are also studied in order to better understand the etiology of NCLP in Kuwait.

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Cleft lip and palate in India

Shweta Deshmukh1*, Marie M Tolarova1, Tarek Abousheta1, Lateefa Alkharafi1, Alanoud Alotaibi1, Fateh Arslan1 and Miroslav Tolar 2

1Craniofacial Genetics Laboratory and 2Pacific Regenerative Dentistry Laboratory, Department of Orthodontics, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA

INTRODUCTION: India is one of the most populous countries in the world. The estimated population is 1.1 billion. As many as 1 in 1000 births to 1 in 500-550 births are affected with cleft lip and/or palate in India. This variation in prevalence is attributed to a varying quality of collected data. Unrepaired cleft lip and palate cases have accumulated every year making this a significant healthcare problem in India. Approximately 70% of orofacial clefts (OFC) are non-syndromic, with multifactorial etiology that includes genetic as well as environmental factors.

PREVALENCE AND CHARACTERISTICS OF OROFACIAL CLEFTS IN INDIA: Reddy et al, 2010, conducted a study in Andhra Pradesh, South India. Three districts (Cuddapah, Medak and Krishna) were chosen because of diversity of their conditions. A strong correlation was found between illiteracy and OFC occurrence. Poor rural areas are characterized by low literacy and high malnutrition. In addition, illiteracy and consanguinity are strongly correlated. Consanguinity can further contribute to the higher incidence of OFC. The study showed male predominance in cleft lip and palate, predominance of left affected side (64%) and of unilateral cases (79%). More females than males were affected with cleft palate. Another study focused on cultural and religious beliefs and social status. The children affected with OFC and their families showed strained relationships, poor exposure and limited social interactions. Attending school and interaction with peers was difficult for affected children. It is believed that corrective surgery of children affected with OFC could improve their self-esteem and confidence, lead to a better social acceptance in school and later in life.

INITIATIVES TO ADDRESS CHALLENGES AND BURDEN OF ORAL CARE: Enormous backlog is estimated to 1 million of non-operated patients with OFC. Limited local resources generated interest of non-governmental organizations and professional associations which led to establishment of the INDIACRAN (Indian Collaboration on Craniofacial Anomalies) aiming to address challenges in quality of cleft care and in research on etiological factors. The main goal is to adopt a comprehensive multi-disciplinary approach and address quality of care through inter-center collaborations, registry and collection of data for future research. Early surgical interventions are becoming more generally available in India, although still efforts of non-profit organizations like Smile Train and Transforming Faces Worldwide are important.
Association of TGFβ3 polymorphism rs2300607AT and nonsyndromic cleft lip and palate in Philippines

Kenny Liu, Kavitha Ravi, Mirek Tolar, Tarek Abousheta, Walied Touni, Alanoud Alotaibi, Shweta Deshmukh, Lateefa Alkharafi and Marie Tolarova

1Graduate Program in Orthodontics, 2Pacific Craniofacial Genetics Laboratory and 3Pacific Regenerative Dentistry Laboratory, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: Nonsyndromic cleft lip with or without cleft palate (NCLP) is a serious malformation of orofacial region with the highest prevalence in Asian populations. The etiology of NCLP is multifactorial involving multiple genetic and environmental factors. Transforming growth factor beta 3 (tgfb3) has an important role in morphogenesis of lip and palate. Recent case-control, family-based and GWAS studies have supported a role TGFB3 in etiology of NCLP. Two single nucleotide polymorphisms (SNP's) of the TGFB3 gene were found associated with NCLP in the Japanese population. We studied the rs2300607AT SNP located in the intron 1 of the TGFB3 gene in Philippines. The Philippine population includes a high proportion of indigenous people of Asian origin.

MATERIAL AND METHODS: Blood specimens of 168 cases with NCLP and 113 controls were collected during Rotaplast cleft medical missions to Cebu City, Philippines, in 2003, 2005 and 2007. The genotypes were established using polyacrylamide gel electrophoresis of rs2300607-specific DNA fragments amplified by PCR.

RESULTS: Proportions of genotypes were 39.88% AA, 50.00% AT, 10.12% TT in cases and 42.48% AA, 42.48% AT, 15.04% TT in controls. The A allele frequency was 0.65 for cases and 0.64 for controls. The T allele frequency was 0.35 for cases and 0.36 for controls. The differences between cases and controls were not statistically significant.

CONCLUSION: Our results suggest that TGFB3 rs2300067AT polymorphism is not associated with NCLP in population from Cebu City, Philippines.

The fieldwork for this study was supported by Rotaplast International, Inc.
Application of mechanical force to PDL stem cells in vitro

Nadim Guirguis¹*, Natalie Yang², HeeSoo Oh³ and Miroslav Tolar⁴

¹Graduate Program in Orthodontics, ²Doctor of Dental Surgery Program, ³Craniofacial Research Instrumentation Laboratory and ⁴Pacific Regenerative Dentistry Laboratory, Department of Orthodontics, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: During the process of orthodontic tooth movement, complex and dynamic interactions occur between cells of the periodontal ligament (PDL) and the surrounding tooth and bone tissues. The PDL cells are first to be exposed to a mechanical force creating their extension or relaxation and to hypoxia or hyperoxia that is caused by compression or dilatation of periodontal blood vessels, respectively. PDL cells respond by secretion of mediators targeting inflammatory and bone cells.

OBJECTIVES: Design and construct a device suitable for cultivation of PDL stem cells in vitro and for their quantifiable extension (stretch) and relaxation (collapse). It should also allow for a simultaneous exposure of PDL stem cells to a low or high oxygen atmosphere. Changes of gene expression will be detected by real time polymerase chain reaction (RT PCR) in the primed PDL stem cells.

MATERIALS AND METHODS: The human PDL stem cells were isolated from the extracted third molars (IRB approval 09-99.1). They are cultivated on a strip of elastic StageFlexer membrane coated with collagen I (FlexCell International). We have modified a Rose chamber assembly to hold the strip. Using a screw mechanism and a microscope grid for optical follow-up, the strip can be stretched unidirectionally with precision of 0.05 mm. The strip is pre-stretched by 0.6 mm. Then PDL stem cells are seeded on the strip. In the stretch experiment, the strip is further extended by 0.5 mm. In the relaxation experiment, the strip is relaxed by 0.5 mm. The pre-stretched strip with seeded cells serves as a control. The experiments are done in a low (5%) or high (21%) oxygen atmosphere that is maintained using equipment manufactured by Biospherix, Inc.

RESULTS: The functionality of our in vitro model has been tested. The RT PCR determinations are still to be performed.

CONCLUSION: We have designed, constructed and tested an in vitro model of mechanotransduction in human PDL cells. A study of reactions of isolated PDL cells to application of force in an in vitro model may bring insights into the biological mechanisms of orthodontic tooth movement that cannot be obtained in the in vivo settings.

This work was supported by the Pacific Regenerative Dentistry Laboratory, Department of Orthodontics, Arthur A. Dugoni School of Dentistry.
Regenerative treatment of dental pulpitis?

Kavitha Ravi¹, Ove Peters² and Mirek Tolar³

¹Research Fellowship Program, Department of Orthodontics, ²Department of Endodontics and ³Regenerative Dentistry Laboratory, Department of Orthodontics, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: A major sign of irreversible pulpitis is an intense pain, which often leads to emergency treatment. The root canal system is cleaned and shaped to the fullest extent possible to remove inflamed pulp tissue and then it is filled. If all pulp tissue is removed, how can be dental pulp regenerated?

A REVASCULARIZATION TECHNIQUE was introduced lately. It led to renewed presence of vital pulp tissue in some patients, but sometimes, the pulp space remained empty. Is it a right direction towards pulp regeneration?

REGENERATION is a process leading to a complete restoration of tissue’s function. Tertiary dentin is not identical with primary or secondary dentin. Is dental pulp capable of regeneration?

EXPERIMENTAL MODELS showed some features of pulp regeneration. For example, dental pulp stem cells were isolated from human extracted teeth and induced to proliferate and differentiate in culture. Tooth slice models reproduced new dentin formation in vitro or reaction of pulp cells to infection. In an animal model of dental pulpitis, pulp regeneration from cultured dental pulp stem cells was documented and that procedure might be already available to patients in Japan.

CONCLUSION: Preparation of stem cells requires some work and time. It will be indicated to support a better outcome of large surgeries. In dentistry, the majority of therapeutic procedures are relatively small. It is possible to regenerate pulp-like tissue using canal disinfection with triple antibiotic paste (metronidazole, minocycline and ciprofloxacin), followed by introduction of a blood clot. Future development directions are modulation of pulpal inflammation to retain pulpal health (vital pulp therapy) and cell based methods such as the placement of cell-scaffold constructs into empty pulp spaces. It is hoped that future biologic-based pulp therapies will be predictable and enhance structural strength so that the natural dentition can be retained longer.
Differential diagnosis of lichenoid oral lesions

Blake Isaacson1*, Elham Mahdavi2 and Nasser Said-Al-Naief3

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

INTRODUCTION: Lichen planus (LP) is a chronic inflammatory disease affecting cutaneous and mucosal tissues. Oral lichen planus (OLP) affects approximately 2% of the population between the ages of 30-60. Several entities may present clinically and histologically with a lichen planus like pattern and may be difficult to differentiate from true lichen planus. It is important to accurately diagnose these lesions because treatment and prognosis may vary.

CASE REPORT: A 63-year-old Asian male presents to UOP dental clinic with a 3-month history of “pain on both cheeks.” Medical history is significant for hepatitis B, hypertension, and type II diabetes mellitus, taking multiple medications for diabetes, cholesterol, hypertension, and pain. Oral exam reveals bilateral erythroplakia, left mucosa pattern appearing consistent with reticular lichen planus and right buccal mucosa showing a pattern consistent with erosive lichen planus. Excisional biopsy of right buccal mucosa was performed and histologic report confirmed initial diagnosis of erosive lichen planus; no malignancy identified with follow-up recommended. The Patient was prescribed Kenalog/ORABASE 5g to apply topically, with reevaluation in 3 months.

LITERATURE REVIEW: Literature discussing diagnostic strategies of OLL’s was reviewed. There is a general agreement that distinguishing lichen planus from other lichenoid lesions may be at times difficult without clinical correlation. An experienced clinician and pathologist can recognize key points to distinguish. Classic OLP is an idiopathic autoimmune disease. The differential diagnosis include lichenoid reactions associated with: drugs, amalgam or other dental restorations, cinnamon reaction and others. Identical clinical presentation is seen in graft versus host disease, lupus erythematosus, erythema multiforme, and oral lichenoid dysplasia. A proper clinical history accompanied by histopathologic and systemic assessments and correlation are keys for diagnosis of any OLL.

CONCLUSION AND DISCUSSION: Typically, true OLP presents with reticular pattern (Wickham’s Striae) with bilateral symmetrical oral mucosa involvement. Other OLL’s may be distinguished by collecting a thorough history, accompanied, by most of the situations, with a unilateral or systemic presentation. Ultimately, histopathological and clinical correlation is needed to accurately diagnose and to rule out any potential malignant changes. The clinical and histological examination of our patient points toward a diagnosis of erosive lichen planus. The possibility of an antihypertensive drug related lichenoid reaction was also entertained in this patient, but the time those eruptions evolved favored otherwise. Nevertheless, communication with our patient was difficult due to language barriers and we were unable to obtain a complete drug history. The significance of obtaining a complete drug history in addition to a thorough clinical history via leading patients’ questioner is also emphasized.

REFERENCES:
Broadly neutralizing anti-HIV antibodies PG6 and PG16 do not inhibit HIV-1 envelope protein (Env)-mediated cell-cell fusion

Michael Yee1*, Deborah Chau2*, Krystyna Konopka1 and Nejat Düzgünes1

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

OBJECTIVES: An effective HIV vaccine must be able to block infection by a wide range viral isolates. PG9 and PG16 antibodies were identified from culture media of activated memory B cells of an infected African donor, and shown to neutralize a large variety of HIV strains (Walker et al. Science 326, 285; 2009). They recognize conserved epitopes on the variable V2 and V3 loops of the viral envelope protein, gp120. Since HIV-1 infection of CD4+ lymphocytes or macrophages occurs via both free virions and cell-cell fusion, we examined the effect of PG9 and PG16 antibodies on HIV Env-mediated cell-cell fusion.

METHODS: We used the HIV fusion assay developed previously in our laboratory (Yee et al. Open Virol J 5, 12; 2011). Clone69TRevEnv cells (NIH AIDS Reagent Program) were maintained in DMEM supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), L-glutamine, geneticin, hygromycin B and tetracycline. Tetracycline was removed from the medium to induce Env expression (“HIV-Env cells”). Cells were plated at 2.0 x 10^5 cells/ml in 48-well plates for 24 h and labeled with 1-2 µM Calcein-AM Green (Invitrogen), for 30 min at 37°C. Highly CD4+ SupT1 cells (maintained in RPMI 1640 medium with 10% FBS, penicillin, streptomycin and L-glutamine) were labeled with CellTrace™ Calcein red-orange (Invitrogen) for 30 min at 37°C, and 2.0 x 10^5 cells were incubated with the adherent HIV-Env cells, with or without antibodies for 3 h. PG9 and PG16 antibodies were obtained from the International AIDS Vaccine Initiative. 2G12 was purchased from Polymune Scientific. Syncytia were observed under a Nikon Diaphot inverted fluorescence microscope with a Jenoptik digital camera.

RESULTS: Monoclonal antibodies PG9, PG16, and 2G12 (up to 100 µg/ml), all of which are reported to inhibit HIV-1 infection, had little or no inhibitory effect on fusion between HIV-Env and SupT1 cells, even at high concentrations. By contrast, Hippeastrum hybrid agglutinin at 1 µg/ml completely inhibited fusion.

CONCLUSIONS: Antibodies PG9 and PG16 are ineffective against cell-cell transmission of the virus or viral genetic material. The search for immunogens to elicit antibodies like PG9 and PG16 that are broadly neutralizing must be reconsidered to include viral antigens that also induce antibodies that inhibit cell-cell fusion.
Perception of California medical students with respect to oral health

Michael Suh¹* and Allen Wong²

¹Doctor of Dental Surgery Program, and ²Advanced Education in General Dentistry Program, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA; and the ²Union City Dental Care Center, and the ²Hospital Dentistry Program, Union City, CA

OBJECTIVES: When it comes to addressing the dental needs of children, California has been identified as being “off track,” as warned by the Pew’s Children’s Dental Campaign. Thus, there is a call for medical physicians, specifically in California to possess the fundamental knowledge of oral health in order to effectively treat patients as people are more likely to pursue pain treatment from general physicians in emergency rooms. Surveying California medical school students will suggest their comfort level with treating oral disease, previous dental experiences which may affect the way they address oral health, and attitude in terms of learning more about oral health education.

METHODS: A brief survey was conducted and sent via email to medical students in California. The group targeted included 3rd and 4th year medical students that extensive had clinical experiences. 100 responses from the following five medical schools were received: University of California, Davis; University of California, Irvine; University of California Los Angeles; University of California, San Francisco; Loma Linda University School of Medicine

RESULTS: Results illustrate students’ own dental experiences, which may influence their way of addressing oral health. Majority of students have dental experiences, which suggest positive outlook in that students will more likely understand the importance of maintaining good oral hygiene. Results demonstrate most medical students in California are not receiving enough education on oral health, and clearly, medical students want to learn more about oral health and desire a teaching method consisting of both clinical and didactic components.

CONCLUSIONS: The need for collaborative care between the fields of dentistry and medicine is clearly evident. In this study, a medical school student’s perception of oral health was addressed through a brief survey consisting of various questions. It was found that a majority of medical school students do not feel comfortable in treating oral disease. These students desire more knowledge in order to effectively treat patients, and it is critical that the dental community be active to help medical students learn more about oral health.

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Curing HIV: Targeting lectin-coupled liposomes to the highly mannosylated HIV envelope protein

Deborah Chau\textsuperscript{1*}, Senait Gebremedhin\textsuperscript{2}, Takahiro Chino\textsuperscript{2} and Nejat Düzgüneş\textsuperscript{2}

\textsuperscript{1}Doctor of Dental Surgery Program and \textsuperscript{2}Department of Biomedical Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: HIV-infected cells that are producing virions express the HIV envelope protein (Env) on their surface, thereby exposing their identity. Our long term goal is to exploit this fact to target cytotoxic liposomes to HIV-infected cells by attaching carbohydrate binding proteins (lectins) that recognize the highly mannosylated Env. The lectins \textit{Hippeastrum} hybrid agglutinin (HHA) and \textit{Galanthus nivalis} agglutinin (GNA) bind specifically to Env, and inhibit HIV infection and Env-mediated cell-cell fusion. We coupled HHA to liposomes, and evaluated whether these liposomes could bind specifically to Env-expressing cells.

METHODS: Fluorescein-labeled HHA (EY Laboratories) was combined with N-hydroxy succinamide-palmitic acid (Sigma) at a mole ratio of 5 palmitic acid:1 lectin molecule for 24 h. The palmitoylated lectin was added to 100 nm-diameter rhodamine-labeled liposomes at a ratio of 40 lectins per liposome along with deoxycholate for 24 h. The lectin-coupled liposomes were dialyzed using dialysis tubing in Hepes-buffered saline (HBS) over 24 h. The lectin-coupled liposomes were passed through a Sepharose CL-4B column equilibrated with HBS and 2 ml fractions collected. The fractions were tested for protein using the Bio-Rad assay and for fluorescence using a Perkin-Elmer LS-50B fluorometer.

Clone69TRevEnv cells (NIH AIDS Reagent Program) were maintained in DMEM supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), L-glutamine, geneticin, hygromycin B and tetracycline. Tetracycline was removed from the medium to induce Env expression ("HIV-Env cells"). Cells were plated at 2.0 \times 10^5 cells/ml in 48-well plates for 24 h, incubated with 1% albumin for 1 h at 6°C, and then incubated with 1 μg/ml lectin (free or coupled to liposomes) for 3 h on ice. Cells were observed under a Nikon Diaphot inverted fluorescence microscope with a Jenoptik digital camera. Fluorescence of the cells was also quantified using a Guava flow cytometer.

RESULTS: The fractions of lectin-coupled liposomes tested in the binding assay bound to both Env-expressing and non Env-expressing cells. The control fluorescein-coupled lectin as well as the control rhodamine-coupled lectin tested in the binding assay bound lightly to both Env-expressing and non Env-expressing cells. The control liposomes tested in the binding assay did not bind to Env-expressing or non Env-expressing cells.

CONCLUSIONS: The lectins must be examined further for Env-binding specificity. Whether synthetic lectins that inhibit HIV infection at very low concentrations bind specifically to Env-expressing cells will also be investigated.

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Treatment of *Porphyromonas gingivalis* with azithromycin encapsulated in cationic liposomes

Jenna Gaw1*, Michael Yee2, Tamer Alpagot3 and Nejat Düüzgûnes2

1Doctor of Dental Surgery Program, and Departments of 2Biomedical Sciences and 3Periodontics, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: *Porphyromonas gingivalis* is one of the most significant periodontal pathogens. Although debridement is the first course of action in the therapy of periodontal disease, the use of antibiotics may be necessary in severe forms of periodontitis. The localized delivery of antibiotics into the periodontal pocket and to the surface of microorganisms would have several advantages over systemic administration: (i) A high concentration of the drug at the pathogen membrane; (ii) preventing antibacterial effects on normal protective flora elsewhere in the body; and (iii) minimizing the potential for the development and transfer of drug resistance in intestinal flora. Liposomes can encapsulate antibiotics either in the aqueous interior or the lipid bilayer, and may be a potential delivery vehicle to localize the antibiotics at the surface of periodontal bacteria. Previous studies in our laboratory showed that fluorescently labeled cationic liposomes bind quantitatively to *P. gingivalis*. Azithromycin is effective against *P. gingivalis* (MIC90 = 0.38 μg/ml; MIC50 = 0.032 μg/ml). We therefore examined the effect of azithromycin encapsulated in liposomes and as a free drug for its effect on *P. gingivalis*.

METHODS: *P. gingivalis* strain 2561 was incubated on blood agar plates and then in medium 199 under anaerobic conditions. Liposomes were composed of palmitoyloleoylphosphatidylcholine (POPC), dioleoyltrimethylammoniumpropane (DOTAP) (Avanti Polar Lipids, Alabaster, AL), and azithromycin (5:5:2 molar ratio). Control liposomes contained no antibiotic. The liposomes and free azithromycin dihydrate (Sigma, St. Louis, MO) (stock dissolved in dimethyl sulfoxide (DMSO)) were added to *P. gingivalis* in phosphate-buffered saline at 1 μg/ml. After incubation for 48 h at 37°C under anaerobic conditions, *P. gingivalis* were plated on blood agar plates and incubated for 48 h at 37°C under anaerobic conditions. Colonies were counted after this period.

RESULTS: DOTAP:POPC:azithromycin liposomes at 1 μg/ml azithromycin reduced *P. gingivalis* colonies by ~40%, based on two experiments. Control DOTAP:POPC liposomes had no effect on *P. gingivalis* colony formation. DMSO alone (<0.5%) added to *P. gingivalis* reduced colonies by ~40%. Although free azithromycin at 1 μg/ml reduced *P. gingivalis* colonies by ~40%, based on two experiments, this could be attributed to the carrier, DMSO.

CONCLUSIONS: The solvent, DMSO, even at the dilution used, is toxic to *P. gingivalis*. Thus, the effect of free azithromycin cannot be determined because of DMSO toxicity. However, liposome-encapsulated azithromycin may be effective in the treatment of *P. gingivalis* infections.

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Liposomal delivery enhances photodynamic therapy of oral squamous cell carcinoma with Zn- and Al-phthalocyanine used as photosensitizers

Laura (Hayoung) Kim 1 *, Michael Yee 2, Paulina Skupin-Mrugalska 3 and Nejat Düzeğen 1

1 Doctor of Dental Surgery Program and 2 Department of Biomedical Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA; and 3 Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poznan, Poland

OBJECTIVES: Photodynamic therapy (PDT) utilizes specific visible light to activate photosensitizers, which then generate reactive oxygen species (ROS) that cause cytotoxicity. PDT has been studied as a promising method to eliminate cancer cells. As a potential method of specific drug delivery, liposomes are used to encapsulate photosensitizers, and they are endocytosed by the target cells until sensitization by light. We focused our study on oral squamous carcinoma cells as the target for PDT. We investigated the effectiveness of the liposome-encapsulated photosensitizers, zinc phthalocyanine (ZnPc) and aluminum phthalocyanine chloride (AlPc), on oral squamous carcinoma cells.

METHODS: Liposomes were composed of palmitoyloleophosphatidylcholine (POPC): phosphatidylglycerol (PG), and contained either zinc phthalocyanine or aluminum phthalocyanine chloride. Free or liposome-encapsulated ZnPc and AlPc were added to HSC-3 cells in the concentration range 0.1-11 M, and incubated for 24 h at 37°C. The cells were then exposed to light (690 nm) from a High Power LED Multi Chip Emitter (9.8 V; Roithner Lasertechnik, Vienna, Austria) for 20 minutes. The light intensity at the surface of the plate was 3.0 mW/cm 2 according to Thorlabs TM100A Optical Power Meter (Thorlabs Inc. Newton, NJ). The total light dose was 3.6 J/cm 2 . Cytotoxicity was evaluated by the Alamar Blue assay that measures metabolic activity, using a Molecular Devices (Mountain View, CA) Versamax microplate reader.

RESULTS: Cells treated with ZnPc and AlPc encapsulated in liposomes resulted in further decrease in cell viability when compared to cells treated with free ZnPc and APC. The Alamar Blue assay showed a linear reduction with increased concentrations of ZnPc and AlPc in both free and liposomal form. For 0.1, 0.5, 1 M liposomal ZnPc, the viability was reduced to 82.4±9.3%, 39.2±9.4%, and 0±0%, respectively. With free ZnPc, the values were 114±11.1%, 83.5±11.3%, and 51.7±12.8%, respectively. For 0.1, 0.5, 1 M liposomal AlPc, the viability was reduced to 50.0±7.2%, 5.0±3.0%, and 6.7±1.3%, respectively. With free AlPc, the viabilities were 104±5.6%, 70.5±8.7%, and 39.7±2.9%, respectively.

CONCLUSIONS: HSC-3 cells are vulnerable to liposomal ZnPc and AlPc in a dose-dependent manner, following light activation. Liposomal ZnPc and AlPc both reduced cell metabolic activity more effectively than the free ZnPc and AlPc. Our studies indicate that liposomal delivery of ZnPc and AlPc results in a more efficient elimination of oral squamous cell carcinoma.

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TGFβ3 rs2268625 CT polymorphism and its association with orofacial clefting

Nick Leon-Guerrero¹*, Casey Luu¹*, Mirek Tolar², Tarek Abousheta¹³, Lateefa Al-Kharafi³; Shweta Deshmukh³, Alanoud Alotaibi³ and Marie M. Tolarova³

¹Doctor of Dental Surgery Program, ²Pacific Regenerative Dentistry Laboratory and ³Pacific Craniofacial Genetics Laboratory, Department of Orthodontics, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: Nonsyndromic cleft lip with or without cleft palate (NCL/P) is a common orofacial anomaly with a multifactorial etiology. The genetic component of NCL/P etiology is complex with multiple genes involved. The transforming growth factor beta 3 (TGFβ3) produces a cytokine that is involved in cell differentiation and embryonic development. It is among the strong susceptibility genes that have been shown to be involved in morphogenesis of lip and palate.

OBJECTIVES: To study association of TGFβ3 rs2268625 CT single nucleotide polymorphism (SNP) with NCLP in a sample from Cebu City (Philippines) and compare with results of a similar study in Guatemala.

MATERIAL AND METHODS: Case-control study design was used. Cases (n=189) were patients affected with NCLP identified during Rotaplast cleft medical missions in Cebu City, Philippines. Controls (n=53) were individuals with no family history of NCLP from the same hospital. Venous blood was drawn from both patients and controls and spotted on filter papers. Dry specimens on filter papers were shipped to the Craniofacial Genetics Laboratory for molecular genetic analysis that included DNA isolation, PCR amplification, and determination of genotypes for TGFβ3 rs2268625 CT SNP by sequencing.

RESULTS: For rs2268625 CT polymorphism in Cebu City sample, a significantly lower proportion of wild type CC homoyzgotes (6.9% vs 18.9%), and higher proportions of CT heterozygotes (43.4% vs 35.9%) and also TT homozygotes (49.7% vs 45.3%) were observed in cases compared to controls. Frequency of mutated T allele was 0.714 in cases and 0.632 in controls. Previous study on the same polymorphism in Guatemala that involved 236 NCLP cases and 168 controls showed similar results: a significantly higher proportion of TT homozygotes among cases than among controls (p=0.041; 54.0% vs 48.98%). Frequency of mutated T allele was 0.744 in cases and 0.694 in controls.

CONCLUSIONS: Our findings suggest that rs2268625 CT mutation in the TGFβ3 gene is associated with NCLP in samples from both Cebu City, Philippines, and Guatemala.

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Dental management and proposed therapies for patients at-risk for bisphosphonate-related Osteonecrosis of the jaw (BRONJ)

Melody Pongmanopap1*, Homayan Asadi2 and Nasser Said-Al-Naief3

1Doctor of Dental Surgery Program, 2Department of Biomedical Sciences and 3Department of Dental Practice, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: Bisphosphonates are frequently prescribed for patients with multiple myeloma, Paget’s disease, and metastatic cancers. Their mechanism of inhibiting osteoclast action to reduce bone turnover is highly effective in reducing the devastating effects of the mentioned diseases. However, this same mechanism reduces the vascularization of bone and thus its ability to heal. Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a destructive condition in which an avascular region of bone is present in the maxillofacial region for more than eight weeks, and is often precipitated by dental procedures such as surgical extractions. The goal of this literature research is to find updated information on how to manage patients who have already begun bisphosphonate therapy and are in urgent need of invasive dental procedures, in addition to finding possible therapeutic solutions if BRONJ should develop.

METHODS: A literature review was performed using the PubMed database to find recent studies that suggested precautionary steps to be taken for patients with BP therapy and possible treatments for patients who have developed BRONJ.

RESULTS: One study followed sixty-eight patients who underwent necessary invasive surgical procedures and took several precautions to lower their incidence of BRONJ. Sixty-five patients healed from the surgery with no complications. Another study focused on implants and their influence on BRONJ. This study suggested that the accumulation of microfractures in the bone during normal implant function increased the occurrence of bacterial infection and development of BRONJ. Two preliminary studies were conducted on ozone therapy and plasma rich in growth factors (PRGF). Both of these therapies showed positive results on patients with BRONJ.

CONCLUSIONS: As more studies are being conducted in understanding the multiple factors that cause BRONJ, dental providers can make more informed decisions regarding how to treat their patients with BP therapy. Patients may or may not complete their necessary dental treatment prior to beginning therapy, so a provider should take precautions when performing invasive procedures. Primary wound closure, antibiotics, strict smoothening of bone, and avoidance of dentures are a few precautionary steps. In addition, patients who develop bony sequestra from BRONJ may undergo ozone therapy or PRGF showed improved healing after curettage. With these findings, the incidence of BRONJ can be minimized as much as possible.
Roles of the TAS2R38 and TAS1R2 taste receptor genes in dental caries status

Cory Wood\textsuperscript{1*}, Shana Vohra\textsuperscript{1*}, Mirek Tolar\textsuperscript{2}, Tarek Abousheta\textsuperscript{3}, Lateefa Al-Kharafi\textsuperscript{3}, Shweta Deshmukh\textsuperscript{3} and Marie M. Tolarova\textsuperscript{3}

\textsuperscript{1}Doctor of Dental Surgery Program, \textsuperscript{2}Pacific Regenerative Dentistry Laboratory and \textsuperscript{3}Pacific Craniofacial Genetics Laboratory, Department of Orthodontics, University of the Pacific Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: High incidence of dental caries worldwide is a major public health concern. The high incidence is due to many factors influencing the disease and its progression. Past studies have established that there is a genetic component influencing development of caries in an individual. This study focused on two genes, TAS2R38 and TAS1R2. TAS2R38 is a bitter taste perception gene, which encodes for a G protein-coupled receptor. TAS1R2 gene is a sweet taste perception gene, which also encodes for a G protein-coupled receptor. Our study examined the single nucleotide polymorphisms (SNP) of TAS2R38 and TAS1R2 taste genes and their influence on dental caries occurrence in a sample of dental students at the Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA.

OBJECTIVES: Understanding influence of these genes on dental caries is important for caries prevention and for patient education. If high-risk patients are identified, prevention strategies can be implemented early to compensate for the genetic predisposition to dental caries.

MATERIAL AND METHODS: The sample consists of 20 students who volunteered to participate in our study. In each participant, caries status was determined by charting their current carious lesions, restorations, and missing teeth. From this information, the DMFT (decayed missing and filled teeth) and DMFS (decayed missing and filled surfaces) scores were determined. Each individual also provided a 24-hour food recall and background health and oral hygiene information. Saliva specimens were collected from all individuals and spotted on filter paper. DNA was extracted from saliva and analyzed using RTPCR for 5 SNPs: TAS2R38-SNP1 rs713598 C/G, TAS2R38-SNP2 rs1726866 G/A, TAS2R38-SNP3 rs10246939 C/T, TAS1R2-SNP1 rs4920566 G/A, TAS1R2-SNP2 rs9701796 G/C.

RESULTS: The research is still in progress and results have not yet been finalized.

CONCLUSIONS: The research is still in progress and the conclusion has not yet been reached. The study hopes to find a link between the TAS2R38 and TAS1R2 taste receptor gene polymorphisms and dental caries development that could be used for risk assessment and prevention of dental caries in the future.

ACKNOWLEDGMENT: This work was supported by Craniofacial Genetics Laboratory, Department of Orthodontics, Dugoni School of Dentistry.
Recovery kinetics of xylitol-inhibited *Streptococcus mutans*

Zach Worsley*1, Lily Kim1, Jonathan Kim1, Katerina Polosukhina2 and Stefan Highsmith2

1Doctor of Dental Surgery and 2Department of Biomedical Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

INTRODUCTION: *Streptococcus mutans* is the most common bacteria found in plaques that causes caries. Xylitol has been shown to inhibit *S. mutans*’ growth, but not kill the bacteria.

GOAL: This study aims to measure the rate of recovery for xylitol-inhibited plaktonic *S. mutans*. This information should be useful in determining the appropriate frequency to administer xylitol clinically.

HYPOTHESES: Our hypotheses are,

1) *S. mutans* will increasingly regain its ability to grow at increasing times after excess xylitol is removed, and

2) We can determine the rate constant for the recovery from inhibition by analyzing the increasing rates of exponential growth at increasing times after xylitol removal.

METHODS: Planktonic *S. mutans* is incubated in growth medium containing 10 wt% xylitol, which completely inhibits growth. After one hour the inhibited *S. mutans* is diluted into xylitol-free medium, reducing [xylitol] to ~2 wt%, which does not inhibit growth. Aliquots are taken at increasing times, added to medium containing glucose, and the rate of exponential growth is determined from the increase in absorbance at 650 nm. The increase in growth rate from 0 to 100% over time is analyzed to obtain a rate constant for recovery.

RESULTS: Activity returns to 100% of control after about 60 minutes. The rate constant for recovery is .057/min ($\approx$18 m). The results are confounded by the time required to measure growth being slow compared to the recovery time, making the measured rate constant an upper limit of the true value.

CONCLUSION: The results suggest that inhibition of *S. mutans* growth can be maintained by administration of xylitol seventeen minutes after inhibitory levels of xylitol are depleted in the oral cavity.
Growth recovery of xylitol-inhibited *Streptococcus mutans* at pH 5 and pH 8

Jonathan Kim¹*, Lily Kim¹, Zach Worsley¹, Katerina Polosukhina² and Stefan Highsmith²

¹Doctor of Dental Surgery and ²Department of Biomedical Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

**INTRODUCTION:** *Streptococcus mutans* is the most common bacteria in oral cavity that contributes to tooth decay. The dental community is trying out many materials to resolve the issue of tooth decay. Xylitol is one of the materials used to inhibit the growth of *S. mutans* and biofilm formation in the oral cavity. The pH of saliva is above 7, while that in a caries-inducing biofilm is below 5.

**GOAL:** This study investigates the rate of growth of xylitol-inhibited planktonic *S. mutans* at increasing times after xylitol removal in media at pH 5 and pH 8.

**HYPOTHESES:** There are two hypotheses. The first hypothesis is that exposure of *S. mutans* to Xylitol will inhibit its growth at both pHs. The second hypothesis is that *S. mutans* recovers better in solution at pH 8 than at pH 5.

**METHODS:** *S. mutans* is suspended in pH 5 and pH 8 phosphate buffer in the presence of 10 wt% xylitol to inhibit its growth. After one hour, it is diluted in media with the same pH to low [xylitol] that does not inhibit growth, and growth rates are determined by adding glucose to aliquots that are taken at increasing times.

**RESULTS:** In the absence of xylitol, *S. mutans* grows much faster at pH 8 than at pH 5. 10wt% xylitol completely inhibits growth at either pH. The recovery of growth after xylitol removal is faster at pH 5.

**CONCLUSIONS:** Xylitol inhibits *S. mutans* growth at pHs associated with saliva and plaque. When xylitol is removed, the ability to grow is recovered more quickly in the lower pH medium.
Photodynamic therapy of Candida biofilms

Austin Davies1*, Michael Yee2, Senait Gebremedhin2, Nejat Düzgün2 and Krystyna Konopka2

1Doctor of Dental Studies Program and 2Department of Biomedical Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: Photodynamic therapy (PDT) utilizes light to activate a photosensitizing agent (photosensitizer) in the presence of oxygen. The exposure of the photosensitizer to light results in the formation of toxic oxygen species causing localized photo-damage and cell death. Candida-associated denture stomatitis is a common recurrent disease in denture wearers. The formation of C. albicans biofilms contributes to the failure of antifungal therapy and to recurrent infections. In this study, the photodynamic activity of the porphyrin-based photosensitizer TMP-1363 towards Candida biofilms, formed on denture acrylic discs, and planktonic Candida was investigated in the absence and presence of the azole antifungal, miconazole.

METHODS: The effect of TMP-PDT ± miconazole on Candida biofilms was determined for two clinical isolates, C. albicans 6122/06 and C. tropicalis 8122/06. Biofilms were developed on poly(methyl methacrylate) discs over a 2-day period. Candida biofilms were incubated with miconazole for 2 h at 37°C, following by treatment with TMP-1363 (10 µg/ml) for 30 min at 37°C. The plates were exposed to broadband visible light (350-800 nm) from a light bulb at a distance of 10 cm from the light bulb to the plate, for 30 min. The irradiance at the surface of the plate was 32.5 mW/cm². Infrared radiation was minimized using a 1 cm water filter. The metabolic activity of the biofilms was measured by the XTT assay. The suspension of planktonic Candida was added to wells of 96-well plates, and the cells were treated and irradiated as described for Candida biofilms. The effect of PDT against planktonic Candida was tested by the microdilution plate assay.

RESULTS: The photodynamic activity of TMP-1363 alone towards Candida biofilms reduced metabolic activity of biofilms by ~40%, while miconazole alone reduced metabolic activity by ~30%. Combined treatment with miconazole + TMP-PDT was more effective, reducing metabolic activity by 55% and 73% for C. tropicalis and C. albicans biofilms, respectively. The photodynamic activity of TMP-1363 alone towards both planktonic Candida species reduced colony forming units (CFU) by ~72%. Treatment with miconazole + TMP-PDT and miconazole alone was slightly less effective for planktonic C. tropicalis, while C. albicans was not sensitive to this treatment.

CONCLUSIONS: The biofilms formed by C. tropicalis and C. albicans on denture acrylic discs were susceptible to the photodynamic activity of the porphyrin-based photosensitizer TMP-1363. Miconazole increased the sensitivity of biofilms to TMP-PDT. Planktonic Candida was sensitive to TMP-PDT, but miconazole did not enhance the efficacy of TMP-PDT.

Presented at the Hinman Student Research Symposium, October 26-28, Memphis, TN, and the 91st General Session of IADR and 42nd Annual Meeting of the AADR, March 20-23, 2013, Seattle, WA.
Photodynamic therapy of Porphyromonas gingivalis via liposome-encapsulated photosensitizers

Alex Ko*1, Michael Yee2, Paulina Skupin-Mrugalska3 and Nejat Düzmüneş2

1Doctor of Dental Surgery Program and 2Department of Biomedical Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA; and 3Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poznan, Poland

OBJECTIVES: Photodynamic therapy (PDT) exploits visible light and photosensitizers to inactivate cells. Porphyromonas gingivalis is one of the most significant periodontal pathogens. The use of PDT may be useful in eliminating oral pathogenic bacteria. Liposomes can encapsulate photosensitizers and may be a delivery vehicle to target periodontal bacteria. Using fluorescence spectroscopy, we investigated the binding specificity of liposomes with varying charge to P. gingivalis at different lipid concentrations. We then examined the effect on P. gingivalis of the photosensitizer, zinc phthalocyanine (ZnPC), either free or encapsulated in liposomes (“L-ZnPC”).

METHODS: Liposomes were composed of palmitoyloleoylphosphatidylcholine (POPC):phosphatidylglycerol (PG), pure POPC, or dioleoyltrimethylammoniumpropane (DOTAP):POPC, and contained the fluorescent probe rhodamine-phosphatidylethanolamine. The liposomes were added to P. gingivalis in suspension in the concentration range 10-100 μM. After incubation for 30 min at room temperature, P. gingivalis were centrifuged at 14,000 rpm for 10 min. Fluorescence levels in the supernatant and pellets were measured using a Perkin-Elmer Luminescence Spectrometer. ZnPC, or L-ZnPC (1 mole% of lipids), were incubated with P. gingivalis for 2 h, the bacteria were irradiated with red light for 20 min and grown on blood agar plates for 48 h under anaerobic conditions.

RESULTS: Positively charged DOTAP:POPC liposomes at 100 μM were bound quantitatively (~100%) to P. gingivalis, whereas ~17% of negatively charged POPC:PG liposomes and ~23% of neutral POPC liposomes were bound. Following light treatment, free ZnPC and (DOTAP:POPC) L-ZnPC at 5 μg/ml, reduced the CFU to 71% and 37% of untreated controls, respectively. At 10 μg/ml, the CFU were reduced to 64% and 23% of controls, respectively.

CONCLUSIONS: Positively charged liposomes exhibit the best binding specificity to P. gingivalis. Localization of the ZnPC at the surface of P. gingivalis via liposome binding can enhance the photodynamic antibacterial cytotoxicity of ZnPC.

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Simplifying the dental implant: Review of new technologies applied to root analogue implants

Ivan Chicchon¹*, Gary Richards², Anders Nattestad³ and Mirek Tolar⁴

¹Doctor of Dental Surgery Program, ²Department of Biomedical Sciences, ³Department of Oral and Maxillofacial Surgery and ⁴Pacific Regenerative Dentistry Laboratory, Arthur A Dugoni School of Dentistry, University of the Pacific, San Francisco, CA

INTRODUCTION: Immediate placement of dental implants (IP) is the technique, by which implants are surgically inserted immediately following the extraction of the tooth, which they will replace. IP is beneficial, because it can significantly shorten the time of treatment, decrease the number of surgical appointments, and provide the option of immediate esthetics, while maintaining success rates comparable to those in delayed placement.

The surgical technique involved in IP can be challenging because of the incongruence between the standard dental implant and the extraction socket. Due to the challenging nature of this technique, success in using IP may vary among different practitioners. Others still may be discouraged from incorporating this technique into their clinical practice. Therefore, many patients may be unable to receive the benefits of immediate implant placement.

PROPOSAL: The surgical technique of IP can be greatly simplified by using an implant that perfectly matches the morphology of the extraction socket, known as a Root Analogue Implant (RAI). While early clinical research of RAIs yielded mixed results, RAIs can now be used more predictably. Recent technological advances can make treatment with RAIs a practical, simple, and successful option for immediate implant placement.

OBJECTIVES: The aim of this review is to provide an overview of the developments in this treatment modality as well as describe protocols and directions for further improvement.
Sex differences in aortic endothelial function of streptozotocin-induced diabetic rats: A possible role of superoxide production

Xiaoyuan Han¹*, Rui Zhang¹, Leigh Anderson³ and Roshanak Rahimian²

¹Doctor of Pharmaceutical and Chemical Sciences Program, and ²Department of Physiology and Pharmacology, University of the Pacific Thomas J. Long School of Pharmacy and Health Sciences, Stockton, CA and ³Department of Biomedical Sciences, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA

OBJECTIVES: Little is known of the interaction between diabetes and gender in the vasculature. This study was designed to investigate 1) whether there were gender differences in rat aortic endothelial dependent vasodilation (EDV) in streptozotocin (STZ, 60 mg/kg, iv)-induced diabetic rats at early stage of the disease (1 wk), and 2) the potential role of superoxide in diabetes-induced vascular dysfunction.

METHODS: EDV to acetylcholine (ACh; 10⁻⁸ to 10⁻⁵ M) was measured in aortic rings precontracted with phenylephrine (2 μM) before and after pretreatment with MnTMPyP (10 mM), a superoxide scavenger. In addition, the level of endothelial nitric oxide synthase (eNOS) and NADPH oxidase (Nox, a potent sources of superoxide) 1, 2 and 4 mRNA expression were determined using real-time RT-PCR.

RESULTS: ACh-induced relaxations were significantly impaired in aortic rings from both male and female diabetic rats. However, the extent of impairment was significantly greater in diabetic females than diabetic males. Preincubation with MnTMPyP increased EDV only in diabetic female group. Accordingly, our data showed that in females, the level of Nox4 mRNA expression was substantially enhanced, whereas the level of eNOS mRNA expression was decreased at 1 wk after the induction of diabetes.

CONCLUSIONS: These data suggest that the predisposition of female rat aorta to vascular injury in diabetes is possibly due to the superoxide production.

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Keep calm and chew on: a comparison of dietary adaptations in *Indri indri* and *Loris tardigradus*

Anmol Bhangu\(^1\)* and Dorothy Dechant\(^2\)

\(^1\)Department of Biological Sciences, University of the Pacific, Stockton, CA and \(^2\)Institute of Dental History and Craniofacial Study, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA

OBJECTIVES: The purpose of this study is to compare two prosimians in the families Lorisidea and Indridae. The species under comparison are the Indri (*Indri indri*) of Madagascar and the Red Slender Loris (*Loris tardigradus*) of Sri Lanka and southern India. Emphasis is placed on how the cranial and dental anatomies of *I. indri* and *L. tardigradus* reflect their dietary preferences and behavior. *I. indri* is a large, diurnal animal that relies heavily on young foliage for sustenance. In contrast, *L. tardigradus* is one of the smallest of the lorisines and is primarily nocturnal and insectivorous.

METHODS: Cranial and dental measurements were taken of two Prosimian species, *I. indri* and *L. tardigradus*. The specimens are found in the P&S Comparative Anatomy Collection of the Institute of Dental History and Craniofacial Study at the University of the Pacific’s Arthur A. Dugoni School of Dentistry in San Francisco. One specimen, consisting of the cranium and mandible, was studied for each species. The specimen number of the *I. indri* skull was CV-51 and that of the *L. tardigradus* skull was CV-54. The various tooth shapes and types were observed. All measurements were taken using a General brand plastic dial caliper (±.005mm). Photographs were taken using a Sony Cybershot (8.1 MP) camera.

RESULTS: The skull of *I. indri* (the Indri) is larger, with its maximum cranial length and breadth dimensions being almost twice the size of *L. tardigradus* (the Loris). Relative to *L. tardigradus*, the cranium of *I. indri* accommodates a larger temporalis muscle and its longer zygomatic arch indicates a larger massester muscle. However, relative to the Indri, the mandible of the Loris shows a higher coronoid process and more cylindrical condyle, indicating distinctive temporalis attachment and condylar shape possibly adapted to reducing lateral movement of the jaw. The orbitals of *L. tardigradus* are considerably larger than those of *I. indri*, relative to their respective body sizes. The large wedge-shaped upper incisors of the Indri are much larger than the reduced peg-like upper incisors of the Loris, though the Loris’ canine is relatively more pronounced. The molars are similar in shape between the two species, both showing brachydont (low in height) and somewhat bilophodont (double section to tooth, especially in lower molars) shape, though the cusps are relatively more pronounced in the Loris.

CONCLUSIONS: The data and observations reflect the folivorous diet of *I. indri* and the insectivorous diet of *L. tardigradus*. *I. indri* has dentition designed for crushing and grinding while *L. tardigradus* has dentition designed to puncture and pierce the exoskeletons of insects. These species also have digestive systems, temporalis muscle configurations, and facial features that reflect their different adaptations.
Vesicular coat proteins in enamel maturation

Kei Katsura¹*, Jeremy Horst², Yan Zhang³, Yukiko Nakano³, Orapin Horst⁴, Thuan Le² and Pamela Den Besten³

¹Doctor of Dental Surgery and Philosophy Degree Dual Program, ²Department of Pediatric Dentistry Residency Program, ³Department of Oral and Craniofacial Sciences and ⁴Department of Endodontics, University of California, San Francisco, CA

OBJECTIVES: This study aimed to identify the causal genetic mutation in an affected family with autosomal recessive Amelogenesis Imperfecta (AI) and to determine the structure, function, and interactions of the encoded causal protein during enamel development.

METHODS: PCR and sequencing of candidate genes (Amelx, Enam, Ambn, Amtn, Klk4, Mmp20, and Wdr72) were performed to detect mutation(s) responsible for the AI phenotype. Immunostaining localized the WDR72 protein in mouse mandibles and a human ameloblast-lineage cell line. Affymetrix gene microarrays of microdissected mouse secretory and maturation-stage ameloblasts were analyzed for mRNA expressions. Protein structural predictions were performed with I-TASSER, MODELLER, SMURF, and HHPRED.

RESULTS: Identification of a 5-basepair deletion in Wdr72 segregated with affected patients marked the seventh mutation associated with hypomature autosomal recessive AI (p.G255fsX293). Bioinformatics analyses of WDR72 uniformly predicted β-propeller and α-solenoid domains, a pattern specific to membrane-bending proteins that has only been known to interact with others bearing β-propeller and/or α-solenoid folds. Sequence and structural homology of WDR72 to β’-COP, a critical subunit in vesicle coatamer formation and trafficking from the Golgi to the endoplasmic reticulum, was supported by in vivo and in vitro localization of WDR72 to intracellular vesicle-like structures. Accordingly, WDR72 co-localized with IVNS1ABP in vitro, the only other up-regulated protein in maturation-stage ameloblasts predicted to contain the β-propeller/α-solenoid iteration. Consistent with our findings, the p.G255fsX293 mutation appears to truncate α-solenoid interaction regions that would be crucial to ameloblast vesicle formation during enamel formation.

CONCLUSIONS: WDR72 is essential for normal enamel development and is predicted to play a role in vesicle formation during maturation. Microarray, informatics, and localization studies suggest WDR72 and IVNS1ABP as potential binding partners forming an intracellular coatamer complex to potentially establish a new family of vesicle-forming proteins critical for enamel development.

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