

1681 Tissue Engineering and Oral Health. A.I. SPIELMAN (New York Univ.), Y. KUBOKI (Hokkaido Univ.), (Organizers), U. WIKESJÖ (Temple Univ.), M. UEDA (Nagoya Univ.), B. BAUM (NIH), L. TERRACIO (New York Univ.).

Tissue engineering recently emerged as a novel field dealing with the repair of damaged tissue and the creation of bioartificial organs. The present symposium covers four areas essential for the field of oral health: repair of bone, soft tissue, salivary glands and muscle. The first speaker, Dr. Ulf Wikesjö, will address the role of Bone Morphogenetic Protein (BMPs) in periodontal and alveolar reconstruction. The presentation will compare and contrast the native regenerative potential of dento-alveolar structures to that of various BMP technologies, using new personal and recently published data. The second speaker, Dr. Minoru Ueda, will cover the latest developments for application of tissue engineering in mucosal and skin reconstruction in oral and maxillofacial surgery. The third speaker, Dr. Bruce Baum will discuss salivary tissue re-engineering. His presentation will review the progress made in using gene transfer and tissue engineering approaches to repair or replace damaged salivary glands. Further, the potential clinical applications of salivary glands for gene therapeutics, i.e., as a gene-based drug delivery site, will also be described. In addition, there will be a critical discussion of the problems currently limiting clinical use of these novel treatments. The final speaker, Dr. Louis Terracio, will present a rarely covered topic in the field of Oral Health: tissue engineering of artificial muscle. The presentation will include three areas of the author's work: 1. Studies using a defined aligned collagen substrate to grow muscle with an *in vivo*-like phenotype, 2. A "Proof of Concept" work on the Space Shuttle, and 3. Current work on a ground-based bioreactor and on engineering of a collagen substrate with sufficient mechanical properties to hold sutures and have altered biodegradability. These presentations should open a forum for discussion on tissue engineering and future treatment options for oral health practitioners. Sponsored by AAOB/AOB - American and the Japanese Associations of Oral Biologists; Mineralized Tissue; Oral and Maxillofacial Surgery; and Implantology Research Groups.

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Secondary White-Spots Lesions Affect *In Vitro* Composite Marginal Adaptation. GL. GALVANI, S. CHERSONJI*, M. ZAMARINI, R. MONGIORGI, L. MONTEBUGNOLI, C. PRATI. School of Dentistry and Dept. Environmental Sciences, University of Bologna, ITALY.

Introduction: The development of secondary caries around composite margin has not been completely documented. It is still unclear if enamel fracture caused by composite shrinkage may affect perimarginal enamel and are responsible for the formation of secondary demineralization (defined as secondary white-spots). **Purpose:** 20 class V and 12 Class II MOD restorations were placed *in vitro* in third molars. Each sample was immersed in demineralizing solution to induce demineralization. **Methods:** Scotchbond I plus Filtek Flow (group A) and plus F 2000 (group B) and LPT I plus Espe experimental Flowable composite (group C) were used in the study. Each sample was stored in demineralizing solution (lactic acid, pH 4.5) at 37° for 2 or 4 weeks. Solution was changed every week. SEM analysis was performed using a JEOL 5400 microscope. **Results:** perimarginal enamel showed many fractures around margins and numerous porosities, voids and pits. Perimarginal prismatic enamel was completely removed, while interprismatic enamel was still in place and only partially dissolved after 4 weeks. Enamel not in relationship with composite margins (1-1.5 mm from composite margin) showed only few alterations and less degree of demineralization. **Conclusions:** enamel surface around margin of restorations is more affected by demineralization than sound enamel surface. Enamel fractures caused by composite shrinkage and pre-bond etching procedures increase the number of porosities and pits and strongly contribute to the initial demineralization (secondary white spots) that affect the clinical life of restorations.

1682 Special needs dentistry within the dental curriculum. R. BEDI*¹, D. O'DONNELL*², M. ALSARHEED*¹ (¹Eastman Dental Institute, University College London; ²University of Hong Kong).

Special needs dentistry has been incorporated into most undergraduate curricula. However, the content and course length varies. The evidence from the literature indicates that introduction of the subject to undergraduates can have both positive and negative effects. The aim of the workshop is to review the changes in the dental curriculum of special needs dentistry. A special focus on the educational courses in the United Kingdom and Hong Kong will be considered. The areas for discussion in the workshop will be the advantages and disadvantages of introducing this subject into the undergraduate teaching. The research findings of the impact of undergraduate courses will be discussed. Variations in undergraduate curricula are clearly apparent, and methodological tools available to evaluate curricula will be discussed. In addition, new teaching methods - problem based learning outcome studies on the impact of teaching special needs within the dental curriculum will be included. There will also be opportunities to develop research collaborations between different institutions and research groups and to develop a network of interested groups/individuals who may wish to submit a proposal for a symposium on the subject of a future IADR meeting.

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Fluoride concentration in normal and mouth rinsed plaque *in vivo*. S. TSUBOI*, S. R. WOOD, B. NATTRESS, H. NAKAGAKI, J. KIRKHAM, C. ROBINSON (Aichi-gakuin Univ., Japan and Univ. of Leeds, UK)

A dental plaque is a very important factor as a cause of the decayed tooth. However, fluoride (F-) in dental plaque is considered to be one of the factors that affect the quality of the tooth. It is not clear which part of the dental plaque contained F- although F-concentration in the marginal area increases with age as dental plaque sticks to the marginal area easily. The aim of this present study was to determine the effect of mouth rinse on the site-specific F- concentration and distribution in the supra adjacent plaque biofilms compared with normal dental plaque surfaces. *In situ* two devices containing human enamel were fitted to the upper molars of 6 consented volunteers. After 7d, one device was first removed. The other device was removed after mouth rinsed by distilled water. These devices were immediately frozen in liquid N₂, freeze-dried, embedded in methylmethacrylate and serially sectioned. F- conc. in the sections were determined using an ion-selective electrode following dissolution of the plastic using chloroform, extraction in perchloric acid and the addition of acetate buffer. F- conc. in normal plaque biofilms tended to be highest at the plaque-saliva interface, decreasing towards the enamel surface. F- conc. in mouth rinsed plaque looks like lower than normal plaque. However, there is no significant difference of F- conc. between normal plaque and mouth rinsed plaque. We suggest that fluoride accumulate in the plaque bio-mass.

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Microorganisms in mañoco that inhibit *S. mutans* growth. RA GONCALVES, A. MARTINEZ, AM ACEVEDO*. Faculty of Science and Institute of Dental Research, Central University of Venezuela, Caracas, Venezuela

Mañoco derived from yucca (*Manihot Esculentia Crantz*) is a typical component of the diet of Venezuelan and Brazilian Indian communities. Three different species of mañoco from the Venezuelan Amazon showed presence of *L.lactis* 2 and *L.fermentum* and yet had cariostatic potential (Goncalves et al., 2000). A possible mode of activity is the ability to inhibit growth of *S.mutans*. Mañoco samples were collected from three Indian communities (Magua, Maroa and Makiritare) and their lactic acid microorganisms were grown in a specific aciduric medium and identified using the API-50CH system. Inhibition of *S. mutans* by *L.lactis* 2 and *L.fermentum* was assessed by growing these bacteria alone and together in TSB medium and using a cross plate technique and filter paper disks impregnated with media containing these microorganisms. The disks were placed on petri dishes containing TSB medium previously inoculated with *S.mutans* and incubated at 37°C for 24 h before measuring the size of any inhibition halo. Also tested by the differential plate method was a filter sterilized cell free supernatant (CFS) prepared from the media in which *L.lactis* 2 and *L.fermentum* were cultured. *S. mutans* incubated alone or in the presence of *L. lactis* 2 or *L.fermentum* showed constant growth; however, inhibition halos of 4 and 3 mm were observed with *L.lactis* 2 and *L.fermentum*, respectively, when media or CFS impregnated disks were used. Inhibition halos with CFS were slightly larger. Heat treatment of CFS at 45, 65, 80 and 100 °C significantly reduced its inhibiting potential. It appears that CFS contains a soluble and thermosensitive molecule that can inhibit *S. mutans* growth and perhaps be used in caries prevention.

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Aciduric microflora in dentine caries lesions. A. LAGER*, G. SVENSÄTER and D. ERICSON (Faculty of Dentistry, Malmö University, Malmö, Sweden).

Deep dentine caries comprises an ecosystem where aciduric and acidogenic microorganisms are prevalent. To isolate such microorganisms, a pH-selective agar based on Todd-Hewitt broth was tested. Dentine caries was removed with conventional drilling (3 lesions) or the chemo-mechanical Carisolv method (3 lesions). The carious lesions were sampled under aseptic conditions using sterile rose-burs. Samples were taken superficially in the lesion, in the centre and in the clinically caries-free dentine after completed excavation, transferred to reduced transport medium and incubated anaerobically on blood agar and on pH selective agar plates (pH 4.0, 4.5, 5.0, 5.5). Colony forming units (CFUs) were counted, characterised morphologically, isolated to blood agar plates and re-incubated anaerobically. Cultures were gram-stained and pure cultures frozen in skim milk. The total number of CFUs decreased with lesion depth and was the same in caries-free dentine for both excavation methods. The percentage CFUs (median) on the pH 5.0 plates (of total median CFUs on blood) was 6.4% superficially, 18% in the centre and 6% in the bottom of the lesion. Gram+ cocci and rods dominated. Bacterial numbers decreased with lesion depth and bacterial growth decreased with decreasing pH. Aciduric bacteria were found in all layers of carious dentine. The centre of the carious lesions harboured a mixed flora with high numbers of streptococci (50-100% of total CFUs), but also lactobacilli, gram+ rods, gram- cocci and rods, and anaerobic-cocci in various combinations. Drilling and Carisolv seem equally effective in reducing the number of bacteria in the cavity floor and pH-selective agar plates may be used for isolation of microorganisms in carious dentine.

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Attenuation of 1310-nm and 1550-nm Laser Light through Dental Enamel. R. S. JONES*, D. FRIED (Univ. of California, San Francisco, San Francisco, CA).

Inexpensive laser diodes and fiber-optic technology have revived optical transillumination as a promising diagnostic method for the early detection of dental caries. The principal factor limiting transillumination through dental hard tissue is light scattering in the normal enamel and dentin. Previous studies have shown that the scattering coefficient decreases with increasing wavelength. Therefore, the near-IR region is likely to be well suited for fiber optic transillumination. The objective of this study was to measure the optical attenuation of near-IR light through dental enamel at 1310 and 1550-nm. These laser wavelengths are readily available due to their use in fiber optic communication. In this study, the collimated transmission (I_t/I_0) of laser light through polished thin sections of dental enamel for various thickness from 0.1 to 2.5 mm was measured in cuvettes of index matching fluid with $n=1.63$. Linear regression of Beer-Lambert plots of $\ln(I_t/I_0)$ vs. thickness show that the attenuation coefficients are 3.3 cm^{-1} ($r=0.97$) and 3.0 cm^{-1} ($r=0.99$) for 1310 and 1550-nm, respectively. This study indicates that near-IR laser wavelengths are well suited for the transillumination of dental enamel for caries detection since the attenuation through normal tissue is more than an order of magnitude less than in the visible. Supported by NIH/NIDCR ST32DE07306.

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Novel and high performance mutanase from *Bacillus circulans* RM1 I. SHIMOTSUURA*, T.ONO, H.KIGAWA, M.OHDERA, K.TAKADA (Lion Corporation, Kanagawa, Japan)

Glucan molecules in dental plaque are mainly consisted of α -1,6 glucosidic links and α -1,3 glucosidic links in a complicated manner. To remove dental plaque effectively by enzymes, both α -1,6 glucanase (dextranase) and α -1,3 glucanase (mutanase) are required. The aim of this study is to isolate high performance mutanase and to characterize its enzymatic properties. Mutanase-producing microbes were isolated from soil. Plaque removal efficacy of their mutanases in combination with dextranase was evaluated through *in vitro* and animal tests using *Streptococcus mutans* strain 10449. The mutanase that showed the highest performance was originated from *Bacillus circulans* RM1 and was a typical endo-type enzyme. This mutanase was purified by column chromatographies and appeared as a single band in SDS-PAGE with a molecular weight of 150kDa. The isoelectric point was about 4.7. The optimum pH and temperature were around pH4, and 60 °C, respectively. These properties of this mutanase were different from other mutanases that had been reported. The nucleotide sequence of this mutanase was determined and the deduced amino acid sequence was not homologous to other proteins. In conclusion, the novel and high performance mutanase capable of effectively removing dental plaque was isolated. A dentifrice formulated with this mutanase together with dextranase is expected to be very useful. isaosimo@lion.co.jp