



Title	Quantitative evaluation of composite bone graft healing in rabbits
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321 Caries Inhibitory Effect of Fluoride Co-crystallised Sucrose - Establishing Field Trials
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As the caries rate of children in Indonesia increases, a field trial has been established to assess the ability of a 10 ppm fluoride co-crystallised sucrose to inhibit caries development in a group of children whose diet can be strictly controlled. This method based on modifications of those used by Luoma et al (1979) and Bowen and Pearson (1982), is being trialled due to the difficulty of using the more traditional fluoride vehicles in developing countries. Preparatory work involved a detailed analysis of the children's diet, including other sources of fluoride, and "in-vitro" experimentation to demonstrate that fluoride was freely available intra-orally. Repeated urinary analysis of fluoride excretion is being used to monitor total fluoride intake.

It is expected to take three to five years before significant caries inhibition between control and experimental groups might be detected. The trial commenced in mid 1992, prior to which extensive caries recording, including bite-wing radiography, was carried out. Short term effects are being tested using sterilised enamel slates with artificial caries lesions present, which are bonded to selected subject's teeth for three-week periods. Increase in mineral density was evident in those lesions in the test subjects, compared with controls, one year into the trial. This result causes optimism for the longer term ability of fluoride co-crystallised sucrose to provide clinical evidence of caries inhibition and thus it is concluded that this method of caries control should be considered as a useful method in public health prevention of caries in developing countries.

323 An *In Vitro* Study of New Caries Disclosing Dyes.
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The aim of this study was to assess the specificity of three new dyes, to stain carious dentine. Fifteen freshly extracted carious primary and likewise permanent teeth had the caries removed using a slow speed handpiece and a sterile round bur (ISO 014). Caries was removed until the cavity was considered, by clinical means, to be caries free. One of the dyes was then applied to the cavity floor of 5 primary and 5 permanent teeth and removed from only half of the cavity, the other half acting as control. This procedure was repeated until no further staining of the experimental half occurred (range 2-6 times). Ground sections (125µm thick) and demineralised sections (5-7µm thick) were prepared from the treated teeth. The prepared ground sections contained both experimental and control areas. The demineralised sections were stained with haematoxylin and eosin and Van Gieson methods to determine the distribution of micro-organisms in both areas of control and experimental halves of the cavity. Examination of the sections using light microscopic observations confirmed that tissue had been removed unnecessarily due to generalised staining and lack of specificity of the dyes applied. Of the dyes tested, carbolan green at pH of 6.0 and 9.2 was the only one to exhibit differential staining between the experimental and control sites but was not caries specific.

325 Non Invasive Treatment of Occlusal Caries. Results After 2 Years
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The aim of the study was to describe the 2-yr results of a individualized treatment program designed to control occlusal caries in the first permanent molars. The sample consisted of 147 6-8 years old students divided in a control group (n=71) and a test group (n=76). The children of the control group were submitted to a preventive program based on patient education. All test-children received a biannual basic preventive program and a recall system according to individual status of caries and periodontal disease activity. The session consisted of buccal hygiene orientation and fluoride application. The analysis of the baseline, one and two years data, showed a significant reduction in the number of surfaces with active lesions in the test group. In the beginning of the study there were 86 surfaces with active lesion (69 white spots and 17 cavities) and after two years remained only 3 surfaces with the disease. In the control group there were initially 83 active lesions (69 white spots and 14 cavities) and after two years remained 68 surfaces with disease and 23 surfaces had been filled. The children in the test group had 17 active caries lesions at the beginning of the study. From this group only 5 surfaces were filled. The program shows the potential for the control of occlusal caries even over surfaces with cavities. This study was supported by the CNPq, Grant 521007-93.3.

327 Low-power laser irradiation stimulates bone regeneration in a mid-palatal suture.
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In the treatment of orthodontic patients with a narrow maxillary arch, rapid palatal expansion of a mid-palatal suture is generally employed. Herein, we examined the effects of low-power laser irradiation on bone regeneration quantitatively during the expansion of a mid-palatal suture in rats.

The animals were subjected to expansion of mid-palatal suture in which different dosages (18-420J) of a Ga-Al-As diode laser (830nm, 100mW) were irradiated. The animals were administered calcein on days 0, 3 and 6 for triple bone labeling. Bone specimens were stained by the Villanueva bone staining method and embedded in plastic. Then grind sections were made. The evaluation was performed quantitatively by bone histomorphometry.

Newly formed mineralized bone area was significantly increased in an irradiation dose-dependent manner (p<0.05, ANOVA) in the 7 day irradiation group, although it was not increased in the one-shot irradiation group. In the 3 day irradiation group, it was also significantly increased by irradiation on day 0-2 (p<0.01) but not by that on day 4-6.

These findings suggested that a series of low-power laser irradiation accelerated bone regeneration in the suture during rapid palatal expansion, and that laser irradiation in the early stage of the active site was more effective for bone formation.

322 Chemical and Enzymic Disintegration of Carious Dentin. H. NORDBÖ*, R. BELTZ & A.H.L. TJAN (LLU School of Dentistry, Loma Linda, CA, USA).

The tunnel preparation method for the operative treatment of Class II carious lesions complicates the removal of carious dentin, remnants of which may be left in the cavity. Carious dentin consists predominantly of organic material. This investigation aimed at studying the ability of sodium hypochlorite (NaOCl), polyacrylic acid (PAA), and the enzyme Pronase to disintegrate carious dentin in order to reduce the need for drilling. Powdered carious dentin was incubated at +37°C with 5.25% NaOCl or 0.15% buffered Pronase, or with these agents in tandem, and the release of organic (N-containing) components was measured by the Berthelot reaction. Cavity walls cleaned for caries with excavators were also exposed to NaOCl, PAA, Pronase, or combinations of these agents, and studied by stereo microscopy and SEM. It was found that NaOCl extracted 26-29% of the N-containing material of powdered carious dentin and Pronase about 40%. NaOCl and Pronase in tandem was not more efficient than Pronase alone. Cavity walls excavated manually still contained remnants of carious dentin. Pronase contributed substantially to the cleansing of such walls; however, there still remained material scattered over the surface that stained with a caries disclosing dye containing Acid Red. NaOCl and PAA in tandem worked faster than Pronase at removing remnants of carious dentin in cavities. It is concluded that NaOCl may constitute a supplement to the conventional cavity cleansing.

324 Caries Removal in Primary Teeth using Pulsed Nd:YAG Laser.
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The aim of this study was to assess the efficiency of the Nd-YAG laser to remove caries from primary teeth. Twenty freshly extracted primary carious teeth, regardless of the size of the cavities, were treated using the Nd-YAG laser. Half the cavity was laser sealed the other half untreated. The power settings used were: a) 50mJ with 10 pulses per second (pps), b) 80mJ with 20 pps, c) 50mJ with 20 pps, d) 80mJ with 10 pps. A maximum operating time of 2 min was chosen to prevent overheating of the tooth. The laser energy was delivered by an optical fibre held about 1mm above the surface of the carious tissue. Histological sections, both ground and decalcified, showed a caries-free dentine surface without any obvious changes in the underlying structure of the treated area. The caries removal assessment using microradiographs showed a greater opacity of the treated half compared to the control half of the cavity. Microscopic views of ground and decalcified sections of the treated teeth showed that the laser pulses delivered at 80mJ 20 pps were the most efficient and least damaging for caries removal. Haematoxylin and Eosin staining of sections throughout the whole cavity showed similarity in the underlying structure of both treated and untreated halves without any sign of damage from the laser. Microradiographs of the prepared sections of treated teeth indicated that the degree of mineralisation of the cavity floor after laser application was similar to that of the sound dentine. The remaining dentine was shown to be both clinically and histologically caries free.

326 Intravenous Administration of Neurogenic Peptides Enhances Bone Formation. C. SHIH and H.Y. YEH* (Department of Biology and Anatomy, National Defense Medical Center, Taipei, Republic of China).

Neurogenic peptides—calcitonin gene-related peptide (CGRP), substance P (SP) and vasoactive intestinal peptide (VIP)—immunoreactive nerve fibers are co-existed in epiphyseal plate, periosteum, bone and bone marrow. Recent studies showed that CGRP plays a role in dentin mineralization and has osteogenic stimulating effects both in vitro and in vivo. In addition, SP and VIP could enhance bone formation in vitro. The purpose of this study was to investigate the osteogenic potential of SP and VIP after intravenous administration. To this end, intravenous injection of 0, 0.04, 0.4, 4, 40 or 400 µg of SP or VIP per kg of body weight through tail vein of 8-week-old Sprague-Dawley male rat was performed two hours before surgery. By using Ficoll-Paque density gradient separation method, light density (LD) bone marrow white cells were harvested and seeded onto a previously prepared feeder layer of fibroblasts in Petri dishes. Ten days after adding LD white cells, in the controls (without intravenous injection of SP or VIP), SP(0.04, 0.4 or 4 µg)-injected group and VIP(all the doses except 400 µg)-injected group there were 2 bone colonies; with 40 and 400 µg of SP there were 3 and 5 colonies, respectively (p<0.01 and p<0.001); with 400 µg of VIP there were 4 colonies (p<0.005). In addition, the size of bone colonies in the SP or VIP (40 or 400 µg)-injected group was significantly (p<0.01) increased as compared to the controls. The results of this study indicated that SP and VIP could enhance bone formation by stimulating the bone stem cell mitosis, osteoprogenitor cell differentiation and/or osteoblastic activity. This study was supported by National Science Council Grant NSC 83-0412-B016-010.

328 Quantitative Evaluation of Composite Bone Graft Healing in Rabbits.
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Previous studies have qualitatively evaluated the improved induction and integration of composite bone grafts (bone with added demineralised bone matrix powder, DBM) using histological methods.

Aim: To quantitatively evaluate the healing capacity of composite intramembranous (IM), and of composite endochondral (EC) bone grafts, in rabbits, in the presence of DBM.

Methods: Surgical defects were created in the skulls of 12 rabbits. Intramembranous grafts alone (3 rabbits), endochondral grafts alone (3 rabbits), and composite grafts of bone with demineralised cortical bone powder (IM + DBM, 3 rabbits and EC + DBM, 3 rabbits) were fixated into the defects. Healing was evaluated, 2 weeks later, by image analysis of stained histological sections. New bone stained deeper red than old bone with HPS stain.

Results: The defects with the composite bone grafts showed rapid osseous healing throughout the whole width and depth of the graft. The EC grafts alone showed 11% (by area) of new bone compared to 19% for the IM graft alone. For the composite grafts, the EC + DBM showed 25% new bone whereas the IM + DBM showed 32% new bone. The method error using the image analyser was 6%.

Conclusions: 1) Image analysis is a reliable method for the quantitative measurement of bone induction. 2) Fixated composite bone grafts (with added DBM) showed more new bone formation than grafts of bone alone. 3) IM grafts showed more new bone formation than EC grafts. 4) Composite bone grafts merit further clinical evaluation.

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