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# Caries Research

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# Caries Research

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# 55th ORCA Congress

June 25–28, 2008, Groningen, The Netherlands

## Session 1 Erosion

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### Acid-Mediated Softening of Human and Bovine Enamel at Ultra-Short Exposure Times

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Atomic force microscopy nanoindentation has previously been used to investigate the very early stages of dental erosion, with exposure times as low as 30 s. The aim of this study was to evaluate the feasibility of measuring erosion of human and bovine enamel after even shorter exposure times. 56 human enamel specimens prepared from 21 permanent human molars, and 56 bovine enamel specimens prepared from 10 bovine incisors were embedded in epoxy resin and finely polished. Specimens were exposed to 150 ml 14.4 mmol · l<sup>-1</sup> citric acid, pH 3.20, for 0, 2, 5, 10, 20, 30, or 60 s (n = 8 per group), using a quantitative rotating device providing an equivalent linear speed of 0.25 m · s<sup>-1</sup>. Specimens were rinsed in deionized water and air-dried. Nanoindentation was performed in air using a Hysitron Triboscope. Hardness after each exposure time, H(t), was calculated, and the mean of 5 indentations of each sample was used to perform a non-parametric Kruskal-Wallis analysis. Statistically significant softening of human enamel occurred after the minimum exposure time: H<sub>H</sub>(0 s) = 4.41 (4.18, 4.64) and H<sub>H</sub>(2 s) = 3.82 (3.69, 3.95) GPa; with bovine enamel it occurred after 5 s exposure: H<sub>B</sub>(0 s) = 4.10 (3.90, 4.30) and H<sub>B</sub>(5 s) = 3.34 (3.04, 3.64) GPa (median hardnesses with 95% confidence intervals in brackets). H<sub>H</sub> and H<sub>B</sub> exhibited an approximately linear dependence with time after the initial 5 s exposure; before this time there was an accelerated period of softening (H<sub>H</sub>) or no softening (H<sub>B</sub>). This might reflect the dissolution

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of a thin superficial Bielby-type layer of enamel damaged during specimen preparation. In conclusion, nanoindentation is a very sensitive method capable of measuring enamel surface hardness loss due to acid exposure times as low as 2 s.

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### Surface Roughness of Dental Enamel after in vitro Exposure to Alcopops or Acidic Beverages and Streptococci

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The objective of this in vitro study was to investigate the surface roughness of enamel after exposure to acidic beverages or microbial acids, alone or in combination. 240 slices, cut from 48 dental crowns of impacted wisdom teeth, were fixed in 12-well plates and incubated for 48 h at 37°C with one of two alcopops or one of two acidic soft drinks, or with Schaedler broth, inoculated with *S. mutans* 10449 or *S. oralis* H1. Subsequently the specimens were incubated either first with an acidic beverage (24 h) and then with the streptococcus (24 h) or vice versa. In previous studies, the amounts of released calcium from enamel had been determined. In this study, the roughness (R<sub>a</sub>) of these dental surfaces was measured using an optical profilometric device (perthometer, Mahr, Göttingen, Germany) and compared with the control specimens, incubated in saline for 48 h. 10 measurements of a length of 1.75 mm in randomly chosen areas were performed for each sample and evaluated with MarSurfX20 software. R<sub>a</sub> values (6/group) were compared by Wilcoxon-test (α = 0.05). The specimens were also examined by SEM. Incubation with an acidic beverage led to a significant reduction in R<sub>a</sub> (median 1.94–2.48 μm) compared with the controls (median 3.97 μm) (p = 0.03–0.05). Exposure of the dental slices first to acidic beverages and then to bacteria caused higher R<sub>a</sub> values (median 2.57–3.87 μm) than af-

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(range 23–1,635) and mean LD was 93  $\mu\text{m}$  (range 3–139). After 3 weeks mean IML was 726 vol%  $\cdot \mu\text{m}$  (range 64–2,116) and mean LD was 95  $\mu\text{m}$  (range 9–197). We conclude that the advanced dentine lesions are suitable for studying different oral hygiene protocols on de- and remineralisation.

Supported by GABA.

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### Dentine Regeneration in the Carious Cavity

S.D. Litvinov<sup>a,\*</sup>, G.Y. Nicolau<sup>b</sup>, R.I. Rakhimov<sup>c</sup>, R.R. Demina<sup>d</sup>

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The purpose was to test the possibility of completely regenerating lost dentine in the carious cavity using a polymer-salt-based composite material (LitAr) to restore the mantle and circum-pulpal dentine. A caries treatment method using LitAr was developed [Litvinov et al.: Caries Res 2007;41:272]. LitAr was laid in caries cavities up to the enamel-dentine junction in 25 patients with extensive caries. X-ray examination was conducted after 2 weeks and after 1, 3 and 6 months. After 2 weeks it was possible to detect on the radiographs under the filling material a carious cavity with distinct limits and low X-ray density which differed markedly from the sound dentine. After 1–3 months optical density was diminished and after 6 months the differences in optical density between the carious cavity and the surrounding dentine became more marked. Morphological investigation of the cavity after 6 months revealed for all patients complete biodegradation of the LitAr with the formation of isolated dentine islands surrounded by connective tissue. We could detect no complications, either immediate or long-term. Thus, LitAr seemed to be biodegradable after 6 months with formation of new dentine – this fact was connected with the trend for restoring the cavity up to the enamel-dentine junction. All the data suggest restoration of the physiological processes in the carious cavity.

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### The Influence of Ozone on Cariogenic Bacteria in Deep Carious Lesions ex vivo

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The aim of this study was to evaluate the efficacy of ozone in reducing ex vivo the total bacteria count and the counts of the bacteria *Streptococcus mutans* ATCC 33402 and *Lactobacillus paracasei* ATCC 11974 ex vivo. From 20 patients aged between 7 and 18, during clinical work, samples of cariogenic dentine from deep lesions were taken ex vivo, before and after the treatment with ozone. The samples were placed in Stuart transport

medium and afterwards cultured to ascertain the influence of ozone on the total bacteria count (CFU) and on *S. mutans* and *L. paracasei*. The results showed decrease of the total bacteria count (CFU) by 72.2%. After treatment with ozone, the reduction of *S. mutans* was 71.5%, and of *L. paracasei* 61.4%. All results showed statistically significant difference in the number of bacteria before and after the ozone treatment ( $p < 0.05$ ). Ozone is a very useful disinfectant and it appears that it can successfully eliminate most of the cariogenic bacteria in human dentine samples ex vivo. Because of its antimicrobial properties, its usage is recommendable in the therapy of deep carious lesions as a cavity disinfectant.

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### Immunological Response in the Dental Pulp after Caries Treatment

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The class II major histocompatibility complex (MHC) molecule-expressing cells, termed dendritic cells, and lymphocytes present in human dental pulp, are highly sensitive to exogenous antigenic stimuli. Their drastic changes in number and localization have been induced by dental caries. This study investigated the responses of the immune system under 3 different clinical conditions: shallow and deep cavities and treated caries. Teeth were extracted and immediately cut longitudinally, pulp tissue was extirpated and fixed in formalin for 24 h at 4°C. The specimens were embedded in paraffin, according to standard laboratory procedure, sectioned at 5  $\mu\text{m}$  thickness and stained by the streptavidin-biotin complex immunoperoxidase method. Cells were identified immunohistochemically using the monoclonal antibodies HLA-DR, CD45 and CD20. Initial pulpal response was characterized by a localized accumulation of HLA-DR antibody-positive cells in the pulp tissue beneath the caries lesion. In the pulp of advanced caries, large number of HLA-DR-positive cells were observed with a marked increase of CD45- and CD20-positive cells. This might indicate the occurrence of antigen presentation locally in the pulp tissue which is very important for the immune response. However, six months after treatment, clusters consisting of HLA-DR-positive cells and CD45-positive T lymphocytes were recognized locally in the pulp tissue, regardless of cavity depth. CD20-positive B cells were seen only under the deeper cavities. Present study demonstrated that dental pulps respond to cavity preparation and restoration. Antigen presentation and cellular or humoral immunoresponses persist for many months after caries treatment, which indicates that antigenic substances remain deep in the dentinal tubules.

### A Clinical Trial of Tooth Mousse to Remineralize White Spot Lesions in a Post-Orthodontic Population

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The aim was to investigate the progression and regression of white spot lesions (WSL) in post-orthodontic adolescent subjects using Tooth Mousse in a twelve-week, double-blind, randomized, positive-controlled, parallel-group clinical trial. Subjects, who were recruited from private orthodontic practices, exhibited at least two WSL on the buccal surfaces of teeth 14–24 and 34–44. In the 45 subjects (age 12–18 years) recruited, 408 WSL (mean 9 WSL per subject) were recorded. 23 subjects were randomised into the intervention (Tooth Mousse) group and 22 subjects into the control (placebo cream) group. Subjects were instructed to apply the study product twice daily for 12 weeks after normal oral hygiene procedures (subjects were supplied with toothpaste containing 1,000 ppm F as NaF). Clinical assessments were undertaken by three examiners at baseline (within 7 days of bracket removal), and at weeks 4, 8 and 12. WSL were scored for lesion severity and activity using the ICDAS II criteria. A transition matrix was used to assess changes in severity and activity of a WSL between two examinations. Transitions were coded as either progressing, regressing or stable. Ordinal logistic regression models were used to analyse the transition scores. 92% of WSL were assessed as severity code 2 or 3. At 12 weeks, 31% more of these lesions had regressed with Tooth Mousse than with the placebo control (OR 2.3;  $p = 0.04$ ). Differences in the regression rates between the two treatments were not statistically significant at 4 and 8 weeks. In both treatment groups, active lesions were more likely to regress than inactive lesions (OR 5.07;  $p < 0.001$ ). In conclusion, significantly more post-orthodontic WSL regressed with Tooth Mousse compared to a placebo control over a 12-week period.

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### Antimicrobial Effect of Chlorhexidine Varnish in Orthodontic Patients

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The aim of this study was to evaluate the effect of 1% chlorhexidine-1% thymol varnish (Cervitec, Ivoclar Vivadent, Schaan, Liechtenstein) on mutans streptococci (MS) and *Lactobacillus* spp. (LB) counts in patients with fixed orthodontic appliances. 24 patients were divided into two groups of 12 according to baseline bacterial counts, creating high ( $\geq 10^5$  CFU/ml saliva) and low ( $\leq 10^4$  CFU/ml saliva) bacterial colonization groups. Bacterial analysis was performed using the CTR-bacteria chair-side test (Ivoclar

Vivadent). Patients then went through an intensive mode of application: chlorhexidine varnish was administered three times within one week according to the manufacturer's recommendations. The baseline MS and LB determinations before varnish administration were followed by sampling 1 and 2 months after the period of varnish application. For hypothesis testing,  $\chi^2$  test, Mann-Whitney and Kruskal-Wallis tests were used. One month after administration the group with high colonization levels exhibited a statistically significant reduction of MS and LB counts when compared with baseline ( $p < 0.05$ ). In this group, reduction for MS was from  $10^5$  CFU/ml to slightly below  $10^4$  CFU/ml. For LB, reduction was from more than  $10^5$  CFU/ml to  $10^4$  CFU/ml. The group with low colonization levels exhibited no statistically significant reduction. Two months after treatment a slight growth of MS and LB counts were observed but did not reach the baseline values. This indicated a time period of chlorhexidine efficiency and a necessary schedule for varnish application. In conclusion, for patients with high baseline MS and LB counts, therapy with 1% chlorhexidine-1% thymol varnish every 3 months suppresses salivary MS and LB.

### Effectiveness of Conventional Etch versus Self-Etch Primer in Sealant Application: A Six-Month Clinical Trial

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The purpose of this study was to compare the effectiveness of conventional etch and bond (Excite) versus self etch primer (prompt l-pop) in respect of sealant retention and caries inhibition. 47 6- to 8-year-old children with good cooperation (Frankl rating 3 or 4) were examined before placing sealant, to gain baseline data, and at the end of the study to record dmft according to visual-tactile method. After prophylaxis with a dry brush and irrigation and without any manipulation of the enamel surface, one operator placed pairs of sealant in random order on lower permanent molars on opposite sides of the mouth of each child. Dry field was maintained by cotton roll isolation and saliva ejector. 6-month evaluation was performed after between 6 and 11 months (mean 10.9 months), according to the CCC Sealant Evaluation System criteria [Deery et al.: Community Dent Oral Epidemiol 2001;29:83–91]. Complete retention was recorded in 40.4% of the etch sealants versus 34% in self-etch sealants ( $p = 0.001$ ;  $\chi^2$ ). Total losses were the same in both groups: 4.2%. CCC Score B was found in 38.3% of etch sealants and 46.8%, in self-etch sealants, score C in 17 and 14.9%, respectively. The most common site of loss was the distal portion. Caries prevention, estimated as the mean number of intact surfaces, was found to be better in the Etch group: 76 versus 66% ( $p < 0.001$ ). Considering baseline caries score, those with dmft  $< 3$  at baseline remained more caries-free in the Etch group than in the Self-etch group ( $p = 0.007$ ;  $\chi^2$ ). In conclusion, conventional etch and bond remains the better approach for sealant application at this time.