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An Intraoral Study of a Bicarbonate Dentifrice. J.S. WEFEL*, K.J. DONLY, M.M. HOGAN, J.D. HARLESS (Dows Institute for Dental Research, College of Dentistry, Univ. of Iowa, Iowa City, IA, USA).

The testing of fluoridated dentifrices in an intraoral model is an accepted procedure to establish a fluoride dose response, as well as an equivalency of the test product. Previous studies have reported on the fluoride dose response of our single-section crown model. The purpose of this study was to test for equivalency of the product compared to a gold standard using the PCK test statistics. Twenty healthy adults' in need of a gold crown on a mandibular molar were recruited for this study. Human consent was obtained prior to the patient's tooth being prepared for a gold crown. A cross-over design was employed, in which one-third of the patients used either the AHDC dentifrice (1100 ppm F^-) a gold standard (1100 ppm F^-) or the 250 ppm F^- dentifrice. These three products are necessary in the PCK test for equivalency or "as least as good as" consideration. The single-sections of enamel and root lesions were analyzed before and after the intraoral exposure using polarized light microscopy. The change in lesion area represents the amount of demineralization observed in these sections and is analyzed as a percent difference. The mean percentage change in enamel lesions for each group with outliers removed was -13.5%, +1.9% and +4.4% for 250 ppm F^-, gold standard and AHDC respetively. Thus, the 1100 ppm F^- dentifrices inhibited lesion progression and on average showed some limited remineralization. Root lesions however showed only demineralization: -62%, -45% and -30% for 250 ppm, Gold and AHDC respectively. The PCK test statistic is calculated by comparing the value of the test product to 1/2 (250 + Gold value). A one-sided t-test may be used to determine if this PCK score is significantly different from zero. When enamel and root percentage differences were used to calculate the PCK score and then tested for significance, the resulting p values were 0.011 and 0.015 respectively. Thus it may be concluded that the AHDC dentifrice is "at least as good as" the gold standard toothpaste. This work was supported in-part by Church and Dwight Co., Inc.

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In Vitro Thin Section Model for Developing Secondary Enamel Caries. K. K. PARK and T.I. KIM* (Indiana University School of Dentistry, Indiana, USA)

Thin sections of extracted teeth have been previously used for studying *in vitro* and *in situ* de- and re-mineralization of enamel and dentin. The objective of this study was to develop an *in vitro* thin, tooth-section model for studying enamel secondary caries. A rectangular box cavity was prepared on the labial surface of an extracted, sound human incisor. Cavities were restored with one of 3 restorative materials (amalgam, composite resin or glass ionomer cement). Eight thin, sections (200-300 μm thickness) were prepared from each restored specimen. Section thickness was manually adjusted (100-120 μm) and all cut-surfaces except the facial-surface around the restoration were sealed using transparent scotch tape and acid-resistant varnish. Specimens were coded and were immersed for 6 days in a solution that was 25 % saturation with calcium and phosphorus relative to hydroxyapatite and contained 0.2 % polyacrylic acid and 0.1 M lactic acid (pH 4.5). Volume % mineral changes and depths of wall and surface lesions were quantitated using contact microradiographs at baseline and at 48-hour intervals during the demineralization period. After all analyses were completed, results were decoded and analyzed using repeated measurement analysis of variance (ANOVA). Data indicated that in specimens restored with resin and amalgam wall lesions became deeper as the depth of surface lesions increased. No wall lesions formed in specimens restored with glass ionomer cements. Delta Z values of resin and amalgam specimens became significantly greater ($p < 0.05$) as demineralization time increased. This *in vitro* thin section model produced typical wall and surface lesions in resin and amalgam specimens and can, therefore, be used to study the chemical dynamics and prevention of enamel secondary caries.

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Characterization of Glucosyltransferase of Human Saliva Adsorbed onto Hydroxyapatite Surfaces. A.M. VACCA-SMITH*, A.R. VENKITARAMAN, K.M. SCHILLING and W. H. BOWEN (Department of Dental Research, University of Rochester, NY, USA).

Streptococcal glucosyltransferase enzyme (GTF) has been identified in salivary pellicle in an active form (Rølla *et al.*, 1983). However, the GTF enzyme in salivary pellicle remains to be characterized. We have explored the activity of the individual GTF enzymes (GTF-S, -SI, -I) of *Streptococcus mutans* GS-5 in solution and on the surface of saliva-coated hydroxyapatite (sHA) and have compared their activities with those of salivary pellicles on hydroxyapatite (HA) surfaces formed from the whole saliva of four donors. Glucan formation was assayed by measuring the incorporation of a ^{14}C-glucose moiety of sucrose into glucan. We examined the effect of starch hydrosylates (STH), when present with sucrose, on the glucan-forming activities of sHA-immobilized GTF enzymes. The glucan-forming activity of GTF-I only was stimulated (by four-fold) in the presence of STH; in contrast, the glucan-forming activities of pellicles of four donors was unaffected by the presence of STH in the reaction mixture. Antiserum, directed against GTF-I immobilized onto HA, did not reduce the glucan-forming activities of GTF-S and GTF-SI, but reduced the activity of sHA-immobilized GTF-I by 55 ± 10% (SD); this antiserum, when coated onto sHA, had no effect on the glucan-forming activities of the pellicles derived from all donors. The glucan-forming activities of GTF-I, -SI and -S were compared in solution and surface phase assays. The glucan-forming activities of both GTF-S and GTF-SI were enhanced on sHA when compared with activities in solution by 96 ± 2% (SD) and 85 ± 3% (SD), respectively; the activity of GTF-I was not enhanced on sHA surfaces. The GTF activity of salivary pellicles of the four donors on sHA was enhanced by 82 ± 18% (SD) when compared with activities in solution assays. The activities of sHA-immobilized GTF-S and GTF-I are dextran-primer-dependent. In contrast, the activity of GTF-SI, when present on sHA surfaces, is independent of the primer dextran. Furthermore, the GTF activity of donor pellicles was independent of a dextran primer. Collectively, these data suggest that GTF activity in human salivary pellicles has primarily GTF-SI characteristics. The bacterial source(s) of the enzyme remains to be identified. This study was supported, in part, by US PHS Grants R37 DE07907, T32 DE07165, and P50 DE07003.

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Defluoridation of Water by Calcium Phosphates. T. JORDAN[1]*, L.C. CHOW[2] and S. TAKAGI[2] ([1]Cornell College, Mt. Vernon, IA; [2]ADAHF Paffenbarger Research Center, NIST, Gaithersburg, MD, USA).

A previous study (Larsen et al. (1993), J Dent Res 72:1519-1525) reported an experimental water defluoridation process based on a reaction of dicalcium phosphate dihydrate (DCPD) and calcium hydroxide with fluoride (F). Calcium hydroxide was used to react with the excess HPO_4^{2-} released from DCPD hydrolysis, but the solution became highly alkaline (pH > 10) at the end. In the present work, the reactions of DCPD and calcium carbonate with F were studied with the use of a constant [F] titration method. Reagent grade DCPD and $CaCO_3$ were ground to a median particle size of 3 μm. Two mixtures of DCPD and $CaCO_3$ were prepared for reactions with F at 1 and 10 ppm in accordance with the anticipated reactions represented by eqns. (1) and (2), respectively.

$$3\ CaHPO_4{\cdot}2H_2O + 2\ CaCO_3 + F^- \longrightarrow Ca_5(PO_4)_3F + 2\ HCO_3^- + H^+ + 6\ H_2O \quad (1)$$

$$3\ CaHPO_4{\cdot}2H_2O + 3\ CaCO_3 + 3\ F^- \longrightarrow Ca_5(PO_4)_3F + CaF_2 + 3\ HCO_3^- + H_2O \quad (2)$$

Results: At 1 ppm F, the reaction followed eqn. (1) except that the apatitic product was $Ca_5(PO_4)_3F_{0.5}(OH)_{0.5}$ instead of $Ca_5(PO_4)_3F$; the pH of the solution was near neutral throughout the reaction because CO_2 escaped from the solution; the mean F uptake rate was 0.95 ± 0.05 mg per hour per gram of mixture. At 10 ppm F, CaF_2 did not form even though the solution was supersaturated with respect to CaF_2; the pH of the solution remained neutral; the mean F uptake rate was 9.6 ± 1.6 mg/hr-g. The results suggest that excess F in drinking water can be effectively removed with the use of DCPD and $CaCO_3$ mixtures. Supported in part by NIH grant DE05354.

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High pressure replica technique for imaging pore morphologies in teeth
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The presence of natural pores in teeth may influence strongly the development of carious defects. These pores are very difficult to detect with common histological methods. Therefore a new technique is developed to obtain three dimensional images of the pore structure in teeth including dentine tubules and the structure of natural or artificial lesions. After cleaning and removing most of the organic substance, the dried teeth were placed as a whole or in parts inside a container with freshly prepared epoxy resin coloured with a fluorescent dye (rhodamin B). The container, usually a flexible silicone hose, is closed tightly and slowly pressurized in an autoclave up to 200 MPa. Using this procedure the coloured epoxy resin is pressed into all pores of the tooth. After curing the epoxy under the high pressure for 24 h at 40 °C, a solid epoxy block containing the tooth is obtained. Because the pressure load is homogeneous there is no mechanical strain on the tooth material.
The blocks are the starting material for further investigations. The present work concentrates on the pore structure of whole teeth by removing the epoxy from the tooth surface mechanically and dissoluting the tooth. The inner pore stucture remains as a three dimensional epoxy matrix. The dentine tubules, for example, show as a hair like structure surrounding the pulp and the root canal. Besides these well known structures, a lot of pores of different size and form appear during the dissolution of the tooth. Typical structures are demonstrated by photographs. It can be concluded that the new method will give a more complete and better overview on teeth morphologies compared to common histological methods.

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Three Dimensional Reconstruction of Initial Caries Lesions in Deciduous Molars.
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The polarized light micromorphology of initial caries lesions is well established. However, little is known about the three dimensional progression of demineralization in dental enamel and early dentin manifestations, especially in deciduous teeth. The aim of this study was therefore the three dimensional reconstruction of demineralization zones in enamel and of early dentin manifestations in deciduous molars, exhibiting clinically detectable initial caries lesions, using polarized light microscopy and specialized 3-D computer aided reconstruction. 10 extracted deciduous molars with approximal caries lesions were fixed in 5% formalin, embedded in Technovit 9100 and serially cut with a saw microtome at 100 μm thickness. At least 20 sections per tooth were investigated by polarizing light microscopy and microphotographed. Different zones of carious demineralization and of early dentin manifestation, as well as the enamel-dentin border and the neonatal line were identified, digitized and subsequently three dimensionally reconstructed using Auto-CAD 12® and 3D Studio® computer programs. Serial sections of the whole tooth represent, despite the loss of hard tissue in between sections, the three dimensional micromorphology of enamel lesions without dentin involvement as well as manifest lesions in enamel and dentin. Whereas conventional polarizing light microscopy clearly distinguishes different demineralization zones, the 3D-reconstruction shows extremly inhomogenic micromorphologic features of zone profiles. Only with the method of 3-D reconstruction it is possible to show the extension of the demineralization zones. It is concluded that this new reconstruction method is able to detect the individual outcome of demineralization and remineralization in deciduous teeth.

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"The overwet phenomenon": A TEM study on possible mechanisms
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This *in vitro* study investigated possible mechanisms involved in the formation of primer globules when All-Bond 2 was applied to total-etched dentin during wet bonding. Twenty-four 1 mm dentin discs were each total etched with 10% H_3PO_4 for 20 seconds and rinsed for 20 seconds. They were divided into 3 groups based upon the status of remaining surface moisture: Group I - surface gently air dried for 3 seconds, Group II - surface blot dried (Kanca technique), Group III - 40 μL of distilled water spread thin on dentin surface after blot drying. Application of All Bond 2 (Bisco) primer was observed under a stereomicroscope prior to the application of bonding resin. Discs in each group were further bonded together to form a disc pair. Bonded specimens were demineralized in EDTA, stained en bloc and post-fixed together with 0.1% ruthenium red and 1% OsO_4, and embedded for TEM examination. Ultrathn sections were observed either unstained or stained with uranyl acetate/lead citrate. A negative staining effect was observed within the meniscus-shaped voids in Group II and III when sections were observed without further staining. Primer globules and the outer primer meniscus were unstained, while the surrounding amorphous zone was highlighted with ruthenium red, having an affinity for tertiary amine. Stained sections revealed the tubules were not sealed within these meniscus-shaped voids, despite the existence of a hybrid layer. Coalescence of small primer globules to form larger globules were often observed. In addition, fine thread-like structures were found arranging in a radial fashion around the primer globules. It was postulated that initial micellar formation of the primers in an aqueous medium provided a favourable environment for subsequent free radical polymerization to proceed within the micellar core. Such a hypothesis was coincident with the concept of emulsion polymerization. (Supported by the CRCG grant, University of Hong Kong)

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"The overwet phenomenon": A SEM study of surface resin globule formation
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Intratubular resin globule formation had been observed when All-Bond 2 was applied to total-etched dentin *in vivo* (Tay *et al.*, 1994). This *in vitro* study investigated the question - how wet should wet bonding be "?", in the absence of a didactic regimen in the manufacturer's instructions. Fifteen dentin discs were each total etched with 10% H_3PO_4 for 20 seconds and rinsed for 20 seconds. They were divided into 3 groups based upon the status of remaining surface moisture: Group I (moisture within dentinal tubules only) - surface gently air dried for 3 seconds, Group II (Kanca technique) - surface blot dried, Group III (excessively moist) - 40 μL of distilled water spread thin on dentin surface after blot drying. All-Bond 2 (Bisco) primer and bonding resin was applied to each conditioned dentin disc and individually light-cured, followed by the application of a layer of hybrid composite (Ælitefil, Bisco). Each disc was sectioned into two halves. Following wet polishing, one half of the restorative interface was acid rinsed with 10% phosphoric acid for 3 seconds and the other half was treated with plasmarized argon for 3 minutes. Specimens were coated with gold and examined without further embedding. Intratubular resin globules were observed beneath short resin cores in Group I. Small meniscus-shaped voids ranging from 80-250 μm in size were observed along the resin-dentin interface in Group II, with much larger voids observed in Group III. These voids were filled with extraneous, large spherical resin globules attached to the hybrid layer underneath. In addition, smaller resin globules were suspended within the voids by an amorphous coat of "partially polymerized" resinous material. It was concluded that the application of All-Bond 2, which already contained 17% water in primer A, could result in an overwet surface phenomenon, in the presence of additional moisture on total-etched dentin. (Supported by the CRCG grant, University of Hong Kong)