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 (Rₐ) 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. Rₐ values (6/group) were compared by Wilcoxon-test (p = 0.03–0.05). The specimens were also examined by SEM. Incubation with an acidic beverage led to a significant reduction in Rₐ (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 Rₐ values (median 2.57–3.87 μm) than after incubation with only one of the beverages.

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Abrasion, respectively. We concluded the method was suitable for before softening, 0.020 mm. Measurements on the same indentation did not differ more than 0.012 mm. Enamel loss was measured after every 30 single brushing strokes up to 390 and then after every 100 strokes. When there was no increase in surface loss, it was assumed that the softened enamel was brushed away. Enamel loss was calculated from the change in indentation depth of the same indent before and after abrasion. To report the measurement error the repeatability coefficient was calculated. Within- and between-group comparisons were performed by t-test.

The aims of the study were to assess the thickness of softened enamel removed by tooth brushing and to analyse the measurement error. 30 human enamel specimens were indented with a Knoop diamond. Surface softening was performed with citric acid (50 ml; pH 3.6) for 3 min. Specimens were brushed with 590 single strokes in an automatic brushing machine with a manual soft toothbrush in toothpaste slurry or in artificial saliva under a load of 150 g. The slurry or artificial saliva was renewed every 120 single brushing strokes. Treatment groups were: 1 softening, brushing with toothpaste; 2 softening, brushing without toothpaste; 3 no softening, brushing with toothpaste (n = 10 in each group). Enamel loss was measured after every 30 single brushing strokes up to 390 and then after every 100 strokes. When there was no increase in surface loss, it was assumed that the softened enamel was brushed away. Enamel loss was calculated from the change in indentation depth of the same indent before and after abrasion. To report the measurement error the repeatability coefficient was calculated. Within- and between-group comparisons were performed by t-test. Mean enamel losses in the treatment groups were: (1) 0.339 μm; (2) 0.016 μm; (3) 0.028 μm. The calculated thickness of the softened enamel amounted to 0.311–0.323 μm. The two measurements on the same indentation did not differ more than 0.012 μm before softening, 0.020 μm after softening and 0.055 μm after abrasion, respectively. We concluded the method was suitable for enamel loss assessment.

**Impact of Toothpaste Abrasivity and Toothbrush Filament Stiffness on Abrasion of Eroded Enamel**

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Toothbrushing abrasion plays a significant role in the development of tooth wear, particularly when combined with erosion. This in vitro study aimed to evaluate the impact of toothpaste slurry abrasivity and toothbrush filament stiffness on abrasion of eroded enamel. Eroded enamel samples (HCl, pH 2.6, 15 s) were brushed with 40 strokes in an automatic brushing machine using manual toothbrushes with different filament stiffness (filament diameter 0.15, 0.20 or 0.25 mm). The toothbrushes were applied with a paste-free control (REA 2) or toothpaste slurries with different abrasivities (REA 6 or 9). Erosion and abrasion were followed by storage of the enamel samples in artificial saliva [Klimmek et al.: Caries Res 1982;16:156–161] for 3 h. After every 4 cycles, the samples were stored in artificial saliva for 15 h. After 60 cycles, enamel loss was measured by profilometry and statistically analysed by two-way and one-way ANOVA and Bonferroni/Dunn post-hoc tests. Mean loss of enamel was mainly influenced by the abrasivity of the slurry and increased along with REA value (REA 2, 0.0–0.2 μm; REA 6, 2.1–3.3 μm; REA 9, 2.9–3.7 μm). Abrasion of eroded enamel was also affected by the filament stiffness of the toothbrush, but only groups brushed with REA 6 toothpaste slurry showed significant differences between the different toothbrushes: in this group toothbrushes with 0.2 mm filament diameter caused higher enamel loss than brushes with 0.15- and 0.25-mm filaments. Toothbrushing abrasion of eroded enamel is mainly influenced by the abrasivity of the toothpaste slurry, but is also modified by the toothbrush filament stiffness.

**Effects of Toothbrushing with Different Forces on Eroded Dentine**

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The organic dentine matrix, increasingly exposed with continuing erosive demineralisation, appears relatively resistant against mechanical impacts, so the notion that eroded dentine is particularly prone to toothbrush abrasion might be questioned. The present study sought to investigate if this holds true for clinically relevant brushing forces up to 4 N. Tissue loss was investigated by optical profilometry (PM) and longitudinal microradiography (LMR). Specimens (20/group) were subjected to cyclic de- and remineralisation for 9 days. All specimens were demineralised with HCl (6 × 2 min/day; pH 1.6) and stored between exposures in a remineralisation solution. In group 1 specimens were eroded only (control), and in groups 2–4 additionally brushed with a powered toothbrush (2 × 15 s/day) directly after the first and the last erosion with a brushing force of 2, 3 or 4 N. Specimens were subjected to LMR and PM before and after removal of the superficial demineralised organic matrix with collagenase (15 kU collagenase in 150 ml remineralisation solution for 36 h). Tissue losses (μm, mean ± SD) for groups 1–4 were: 98.4 ± 33.2, 86.6 ± 33.3, 78.6 ± 27.2, 91.0 ± 35.1 (LMR) and 11.7 ± 5.1, 13.4 ± 10.9, 30.7 ± 18.9, 25.5 ± 20.3 (PM) respectively prior to collagenase treatment and 110.0 ± 44.7, 98.5 ± 32.2, 107.2 ± 28.9, 106.3 ± 36.7 (LMR) and 111.7 ± 11.6, 121.9 ± 11.8, 121.9 ± 15.7, 123.0 ± 12.0 (PM) respectively after collagenase treatment. No significant differences were found except with PM before removal of the organic matrix where a brushing force of 3 N (p ≤ 0.01) and 4 N (p ≤ 0.05) re-
A Light Cola Drink Is Less Erosive to Enamel than a Regular Cola: An in situ/ex vivo Study

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This in situ/ex vivo study compared the erosive potential of a light cola drink with that of a regular one. 8 volunteers took part in this crossover and double-blind study performed in 2 phases (each 14 days). In each phase, they wore acrylic palatal appliances containing 2 human enamel blocks. Half of the surface of each block was coated with nail varnish to provide a profilometry reference surface. The treatments were erosive challenges with either a light cola drink (Light Coca Cola; Companhia Fluminense de Refrigerantes, Porto Real, Brazil) or a regular cola drink (Coca Cola; Companhia Fluminense de Refrigerantes, Porto Real, Brazil). For 14 days, erosive challenges were offered extra orally 3 x/day. In each challenge, the device was immersed in a cup containing 150 ml of one of the cola drinks for 5 min and then re-inserted into the mouth. The drinks had pH of 3.0 (light cola) and 2.6 (regular cola), the calcium concentrations (atomic absorption spectrometry) were 13.7 and 32.1 mg/l and the phosphate concentrations (colorimetry) 15.5 and 18.1 mg/l respectively. Enamel alterations were measured using percent change in hardness (%SHC) and wear profile tests. The data were tested using paired t test (H9251 = 0.05). The highest enamel losses were significantly less with light cola drink than that of a regular one. Enamel alterations were measured using percent change in hardness (%SHC) and wear profile tests. The data were tested using paired t test (H9251 = 0.05). There was no difference in %SHC between the groups (light – 63.9%; regular – 78.5%). The data suggest that the light cola drink is less erosive than the regular one.  
Supported by FAPESP (Proc. 2006/03874-8).

The Importance of Several Drink Parameters in Explaining Erosive Potential against Enamel

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The objective of this study was to determine which parameters are most important in explaining erosive potential of acidic drinks. 19 drinks were included: bottled water (control), 3 alcopops, 2 beers, 2 sport drinks, 2 fruit juices, 2 water based fruit drinks, and 7 soft-drinks. The pH, Ca and P\textsubscript{i} concentration, titratable acidity to pH 5.5 and 7.0, fluoride concentration and the viscosity were measured. The degree of saturation with respect to hydroxyapatite (DS\textsubscript{HAp}) was calculated. 180 bovine buccal enamel specimens were embedded in resin and polished flat. For each drink 10 specimens were used: 5 for chemical analysis and 5 for profilometric analysis. For calculation of DS\textsubscript{HAp} chemical analysis of the drinks before and after exposure of the enamel specimens was performed. Erosive potential was determined as the loss of enamel height after immersion in drinks for 63 min, measured with an optical profilometer. Correlation of drink parameters with erosive potential was evaluated using linear correlation. The highest loss of enamel was found for Sprite (9.78 mg/l) followed by apple juice (9.13 mg/l). No enamel loss was found for one of the beers or for bottled water. A good correlation was found between the erosive potential and three of the studied parameters, namely pH (r = 0.87), titratable acidity (r = 0.80) and calcium concentration (r = 0.81). Although it was concluded that pH, titratable acidity and calcium concentration are more closely related to enamel loss than other drink parameters, it seems necessary to develop a model in order to predict the erosive potential of the drinks.

Impact of Mineral Supplements to an Erosive Soft Drink on Inhibition of Enamel Erosion in vitro

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The objective of this in vitro study was to evaluate the effect of low levels of calcium, fluoride, iron and phosphate supplements, alone or in combination on erosion of enamel with an acidic drink. 90 enamel specimens were randomly allocated to 9 groups of 10, and eroded with Sprite Light containing the following supplements: 1, none (control, pH 2.96); 2, 1 mM Ca (pH 3.03); 3, 0.047 mM F (pH 3.04); 4, 1 mM Fe (pH 2.82); 5, 1 mM P (pH 2.91); 6, 1 mM Ca + 0.047 mM F (pH 3.02); 7, 1 mM Ca + 1 mM P (pH 2.96); 8, 1 mM Fe + 0.047 mM F (pH 2.90); 9, 1 mM Ca + 1 mM P + 0.047 mM F + 1.0 mM Fe (pH 3.00). For 24 h, the specimens were subjected to 6 cycles in which they were immersed in pure or modified Sprite Light for 1 min and in artificial saliva for 59 min. For the remaining 18 h, the specimens were stored in artificial saliva. Enamel alterations were assessed by profilometry and percent change in surface hardness (%SHC). Data were tested using ANOVA and Tukey’s tests (H9251 = 0.05). The highest enamel losses were observed in the controls (Group 1) and in Groups 4 and 8, containing Fe. Groups 2 and 6, containing Ca, showed significantly less wear than the control. All groups revealed similar values of %SHC, except for Group 5 (pH). The modification of an erosive soft drink with low concentrations of Ca, with or without F, may exert a protective effect against enamel erosion.  
Supported by FAPESP (Proc nos. 05/54203-3 and 05/04017-9).
This in vitro study aimed to evaluate the effect of Ca- and Fe-rich foods on dental enamel erosion. 75 enamel blocks from bovine teeth were randomly divided into 5 treatment groups as follows: 1, cola drink with prior milk immersion (Ca-rich); 2, cola drink with prior immersion in cheese extract immersion (Ca-rich); 3, cola drink with prior immersion in liver extract (Fe-rich); 4, cola drink with prior immersion in broccoli extract (Fe-rich); Treatment 5 cola drink (control). Half the surface of each specimen was coated with nail varnish to provide a profilometry reference. For 24 h, the samples were submitted to 3 pH cycles: immersion in the test food for 5 min, then immersion in cola for 5 min and finally storage in artificial saliva for 110 min. After the pH cycles the samples were stored in artificial saliva for 18 h. Erosive enamel loss (profilometry) and percent surface hardness change (%SHC) were measured. Data were tested using ANOVA and Tukey’s tests (*p < 0.05). The enamel loss in treatment groups 1 (mean enamel loss 0.46 μm), 2 (0.55 μm), 3 (0.64 μm) and 4 (0.54 μm) were significantly different from that in group 5 (1.18 μm) but not from each other. The data suggest that all the Ca- and Fe-rich foods tested could reduce the enamel loss caused by an erosive challenge on dental enamel. Supported by FAPEMIG.

The prevalence and severity of dental erosion have increased in recent years, with the increased consumption of acidic soft drinks a likely aetiological factor. The aim of this study was to investigate the effect of adding 0.1% and 0.2% w/v casein phosphopeptide-amorphous calcium phosphate nanocomplexes (CPP-ACP) to 4 commercially available soft drinks, 2 of which were carbonated. 39 enamel specimens were sectioned from sound, extracted human teeth and polished to a mirror surface, on which exposed enamel windows were created. 4 soft drinks and deionised water (DDW) were tested. pH values for three blinded subgroups, containing 0.0%, 0.1% and 0.2% CPP-ACP nanocomplexes w/v respectively were: Drink 1 – 2.27, 2.59, 2.83; Drink 2 – 2.42, 2.77, 2.91; Drink 3 – 2.37, 2.51, 2.90; Drink 4 – 2.24, 2.43, 2.90. The specimens (n = 3) were placed into 50 ml of solution at 37°C for 30 min, rinsed and varnish removed. The samples were profiled with a white light profilometer and erosive depths recorded. Erosive depths decreased with increasing concentration of the CPP-ACP nanocomplexes. The erosive depths for all solutions with 0.2% and 0.1% CPP-ACP nanocomplexes were not significantly different from those of DDW. The addition of 0.1% and 0.2% w/v CPP-ACP nanocomplexes significantly reduced erosive depth in all test solutions when compared to the original solutions (p < 0.05). The pH of all solutions increased with increasing concentration of the CPP-ACP nanocomplexes. Erosive depth correlated with solution pH (Pearson r = 0.891) and titratable acidity was less well correlated (r = 0.181). We conclude that the addition of CPP-ACP nanocomplexes in low concentrations (0.1–0.2% w/v) significantly decreased the test soft-drinks erosivity. The erosivity of the soft-drinks with 0.1% and 0.2% CPP-ACP nanocomplexes did not differ significantly from DDW.

This in situ study evaluated the protective effect of green tea on dentin erosion (ERO) subjected or not to abrasion (ABR) immediately or 30 min after an erosive challenge. 10 adult volunteers wore intraoral palatal devices with 6 bovine dentin specimens divided into 3 rows of 2 specimens, each exposed to one of 3 treatments. During 2 experimental 5-day crossover phases, the volunteers rinsed with green tea or water (control) between the erosive and abrasive challenges. The erosive challenges were performed ex vivo by immersion of the device in cola drink (pH 2.6) for 5 min, 4×/day. The device was then re-inserted into the mouth and the volunteers rinsed with 10 ml of tea or water for 1 min. Abrasion was performed ex vivo either immediately (ERO + I-ABR) or 30 min after erosion (ERO + 30-min-ABR) using an electric toothbrush with non-fluoridated dentifrice for 30 s. One row was not abraded (ERO). Dentin wear was measured by profilometry. Abrasion treatment led to significantly higher wear than erosion (ERO) alone (p = 0.008), but no differences were detected between ERO + I-ABR and ERO + 30-min-ABR. The green tea reduced the dentin wear (μm) significantly for all conditions (p = 0.001): T (ERO: 0.59 ± 0.18; ERO + I-ABR: 0.90 ± 0.36; ERO + 30-min-ABR: 0.74 ± 0.23); C (ERO: 0.98 ± 0.13; ERO + I-ABR: 1.23 ± 0.35; ERO + 30-min-ABR: 1.22 ± 0.23). Green tea seems to be a promising treatment to reduce dentin wear under erosive/abrasive conditions.

Supported by FAPESP (Proc. 07/04209-0).
The aim of this study was to investigate the surface restorative properties of two occluding desensitizing toothpastes on dentine in vitro. Dentine is affected by erosion, leads to hypersensitivity and the use of toothpastes with a desensitizing claim. Therefore, two toothpastes that occlude dentinal tubules were compared in this study: Test toothpaste (Theramed Sensitive), containing a hydroxyapatite/protein composite (HPC), 5% KNO₃ and 1450 ppm fluoride (as NaF) and Control toothpaste, containing strontium chloride. Bovine dentine specimens were polished flat, eroded (30 s, 5% citric acid) and randomly divided into 2 groups. The amount of eroded enamel (wear) was measured using a Proscan 3D profilometer (Scantron Ltd, UK). The eroded surfaces were brushed (1 min, 150 g load, room temperature) with 1:1 slurries (30 s, 5% citric acid) and randomly divided into 2 groups. The wear of eroded enamel was measured using a Proscan 3D profilometer (Scantron Ltd, UK). The eroded surfaces were brushed (1 min, 150 g load, room temperature) with 1:1 slurries of the pastes followed by re-mineralisation in artificial saliva [ten Cate et al.: Eur J Oral Sci 1995;103:362–367] (37°C, 8 cycles). During erosion and treatment, reference areas were covered by adhesive tape. Treated surfaces were rescanned by profilometry. The restoration was determined by subtraction of wear values from post- and pre-treatment scans at three positions per specimen using customised software. The model employed is based on a mild erosive challenge and material restoration rather than material loss under harsh conditions. It proved suitable to measure restorative effects on eroded dentine. The average wear of dentine after etching was 1.33 ± 0.25 μm. Test toothpaste yielded a net re-deposition of 2.19 ± 0.34 μm, which was stable against brushing. Control toothpaste led to a slight additional loss of material (0.60 ± 0.54 μm). Test toothpaste showed through its HPC content a significant and mechanically stable re-deposition of mineralized material and therefore reversed the erosive loss. Control toothpaste also led to the precipitation of material which was visible on the specimens. However, under the conditions employed it did not produce a mechanically stable deposition of re-crystallized material on the dentine. In conclusion, profilometry showed that a toothpaste containing a hydroxyapatite/protein composite can reverse dentine erosion.

12 Restoration of Eroded Dentine by Desensitizing Toothpastes

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13 Short Term Effect of NaF, SnF₂ and Dilute HF Treatments on Enamel Erosion-Like Lesions in vivo

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Dental erosion is a clinical problem in many countries today partly because of increased exposure to dietary acids. Citric acid for example is commonly found in fruit juices, mineral water, flavoured teas and bottled still water. Recent studies have shown that several acidic fluoride preparations hold promise in the prevention and treatment of dental erosion. This study aimed at comparing the effect of sodium fluoride (NaF, pH 7.3), stannous fluoride (SnF₂, pH 2.9) and dilute hydrofluoric acid (HF, pH 2.0) solutions, on enamel dissolution in an experimental in vivo model [Young et al.: Eur J Oral Sci 2006;114:180–183]. Four healthy anterior teeth in each of 29 subjects were isolated using plastic strips and a light bodied impression material for complete palatal coverage. The labial surfaces of the test teeth were exposed to 5 ml citric acid (0.18%) using a peristaltic pump (6 ml/min), before (etch 1) and 5 min after (etch 2) application of test fluoride preparations. The acid was collected in a test tube for later analysis. NaF, SnF₂ or HF solutions (all 0.1 M F) were applied to the labial surfaces of the teeth for 1 min using a peristaltic pump (6 ml/min). Enamel dissolution was examined by measuring calcium content in the citric acid using atom absorption spectrometry. Mean values for (etch 1) – (etch 2) were statistically analysed with the Bonferroni t-test for between-group comparisons. HF and SnF₂ gave mean calcium reductions of 0.37 ppm (76%) and 0.67 ppm (68%) respectively (NS). NaF gave no calcium reduction (–0.34 ppm (–52%)). In conclusion, while neutral NaF had no protective effect 5 min after treatment, SnF₂ and dilute HF markedly reduced calcium loss.

14 Screening of the Effect of Different Fluoride Compounds and Preparations Thereof on Erosive Tissue Loss in Enamel in vitro

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This in vitro study aimed to investigate the effect of different fluoride compounds and preparations thereof on erosion progression in enamel. Human enamel specimens were subjected to a cyclic de- and remineralisation procedure for 10 days, with 6 min demineralisation periods per day. Erosion was performed with 0.05 M citric acid (pH 2.3), directly followed by treatment with test preparations 6 × 2 min per day. Test preparations were: ZnF₂ solution (0.42% w/v ZnF₂, 2,650 mg/l Zn), AmF/NaF/CuSO₄ solution (0.03% w/v CuSO₄, 100 mg/l Cu), TiF₄ solution (0.26% w/v TiF₄, 1,000 mg/l Ti), TiF₄/AmF/ZnF₂ solution (0.17% w/v TiF₄, 670 mg/l Ti, 0.07% w/v ZnF₂, 450 mg/l Zn), TiF₄/AmF/ZnLa solution (0.17% w/v TiF₄, 670 mg/l Ti, 485 mg/l Zn), and AmF/SnF₂ solution (positive control [meridol], pH 4.2, 0.05% w/v SnF₂, 409 mg/l Sn, 250 ppm F). Except for the meridol solution, all preparations had a fluoride concentration of 1,500 ppm and were adjusted to pH 4.5. In the negative control group, specimens were not fluoridated. Tissue loss was determined by profilometry after the last experimental day. Highest tissue loss (μm, mean ± SD) was found in the negative control group (36.1 ± 4.6). Tissue loss was reduced about 15% by ZnF₂ (30.7 ± 4.4; p ≤ 0.001) and AmF/NaF/CuSO₄ (30.5 ± 3.3; p ≤ 0.001). Except the TiF₄/AmF/ZnLa solution, which reduced tissue loss by about 60% (14.0 ± 3.8; p ≤ 0.001 compared to control group), the titanium-containing prep-
The Effect of Fluoride on Tooth Surface Loss of Enamel under Erosive Cycling Challenge


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The aim was to compare the effect of 5 different concentrations of sodium fluoride in matched toothpastes on tooth surface loss of human enamel in vitro using an erosive cycling technique. In a randomised, blinded experiment 5 groups of 8 enamel slabs were cut and mounted into resin blocks, ground and checked for surface flatness using a scanning profilometer (Scanscan Proscan 2000). The surface of each slab was covered with nail varnish except for a small window. The slabs were immersed under static conditions for 2 min, 5×/day in fresh 200 ml aliquots of 0.3% citric acid (pH 3.6). In addition, each group was immersed in one of 5 fluoridated toothpastes (0, 250, 500, 1,150, or 1,450 ppm NaF) 2×/day (morning and evening), for 2 min each time. The total cycling period lasted 21 days during which the slabs were incubated overnight and between erosive challenges in artificial saliva at 37°C. A 60-min gap was left between daytime immersions. The amount of surface loss was measured with the scanning profilometer at 7, 14, and 21 days. Surface loss ± SD of enamel at day 21 caused by cycling with 0, 250, 500, 1,150, or 1,450 ppm NaF toothpastes was 25.8 ± 2.8, 25.2 ± 2.7, 23.1 ± 2.7, 17.1 ± 2.4 and 15.5 ± 0.9 μm respectively. In conclusion, enamel surface loss was reduced significantly (p ≤ 0.05) with fluoride concentrations in toothpaste of>500 ppm.

Supported by GlaxoSmithKline.

Reduction of Enamel Erosion by Low-Concentration TiF₄ Solutions in vitro

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TiF₄ solutions of 1–4% have been shown to reduce dental erosion, but through their acidity they also cause erosion. It was our aim to evaluate the surface loss by application and the reduction of subsequent erosion of TiF₄ solutions of lower concentration. 60 enamel specimens prepared from bovine incisors were embedded in methacrylate and ground flat (800 grit). Specimens were randomly divided into 2 groups: single versus daily treatment with TiF₄ solution (ST vs. DT), which were subdivided into 5 groups of: 0% (control), 0.1, 0.5, 0.75 and 1% TiF₄ concentration. Specimens were treated by immersing them in 10 ml of the TiF₄ solutions for 5 min and rinsing them with tap water for 30 s. ST specimens were treated once only, DT specimens were treated before each erosive cycle. All specimens were subjected to 4 erosion cycles: 6 immersions of 2 min in Sprite (Coca Cola Enterprises Nederland BV, Dongen, NL); rinsing in tap water for 1 min and storage in artificial saliva (Saliva Orthana, Pharmachemie, Haarlem, The Netherlands) for 1.5 h. Erosive enamel loss was measured using light profilometry (Proscan 2000, Scanscan, UK), after each treatment and each erosive cycle. At first application, TiF₄ (ST and DT) caused between 1.24 μm gain and 1.62 μm loss of surface height. At the 4th application (DT) this had fallen to 0.08 μm gain and 0.35 μm loss. After 4 erosion cycles ST and DT showed similar treatment rankings with 0.5% showing least (ST: 3.45 μm, DT: 1.08 μm) and 1% showing most enamel loss (ST: 4.87 μm, DT: 6.56 μm). Treatment with 0.5% TiF₄ showed significantly less enamel loss than all other concentrations and control for DT.

The Effect of 4% Titanium Tetrafluoride Varnish and Solution on Erosion of Enamel in vitro

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This in vitro study assessed the effect of 4% titanium tetrafluoride (TiF₄) varnish and solution on enamel erosion. 72 bovine enamel samples were randomly allocated to one of 6 treatments: Duraphat (NaF, 2.26% F, pH 4.5; Colgate-Brazil), Duofluorid (NaF, 2.71% F, pH 8.0; FGM-Brazil), TiF₄ varnish (2.45% F, pH 1.0; FGM-Brazil), placebo varnish (pH 5.0; FGM-Brazil), TiF₄ solution (2.45% F, pH 1.0) or control (not treated). The varnishes were applied in a thin layer and removed after 6 h. The fluoride solution was applied to the enamel surface for 1 min. Then the samples were transferred to an artificial mouth for 5 days at 37°C for alternating de- and remineralization (6 ×/day). Demineralization was performed with the beverage Sprite (1 min, 3 ml/min) and remineralization with artificial saliva (during day: 59 min at 0.5 ml/min; during night: 18 h at 0.1 ml/min). Each day, enamel loss was measured with a profilometer. Data were tested using analysis of covariance and Tukey’s test (α = 0.05). The mean daily cumulative wear (μm) was significantly lower for the TiF₄ varnish (0.80 ± 0.61) than for all other treatments. Wear did not differ significantly between Duraphat (1.76 ± 0.86) and Duofluorid (2.01 ± 0.94) or between placebo varnish (2.46 ± 1.18), TiF₄ solution (2.41 ± 1.41) and control (2.49 ± 1.18), but these groups...
of treatments were significantly different. In conclusion, TiF₄ varnish seems to be a promising treatment to reduce enamel loss under erosive conditions.

Supported by FAPESP (Proc. 2005/54203-3 and 2006/04628-0).

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**Abstracts**

**18**

**The Effect of Nd:YAG Irradiation and Fluoride Application on Enamel Resistance to Erosion**


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This in vitro study assessed the effect of Nd:YAG laser irradiation and fluoride application on enamel resistance to erosion. 100 bovine enamel blocks were randomly divided into 10 treatment groups: Group 1, untreated (control); Group 2, AF (1.23% F) 4 min; Group 3, F varnish (2.26% NaF) 6 h; Group 4, 0.5 W Nd:YAG laser (250 μm pulsewidth, 10 Hz, 35 J/cm²); Group 5, 0.75 W Nd:YAG laser (52.5 J/cm²); Group 6, 1.0 W Nd:YAG laser (70 J/cm²); Group 7, AF + 0.75 W Nd:YAG laser; Group 8, 0.75 W Nd:YAG laser + AF; Group 9, F varnish + 0.75 W Nd:YAG laser; Group 10, 0.75 W Nd:YAG laser + F varnish. After the treatments, half of the enamel surface was protected with nail varnish for reference. For 10 days blocks were subjected to an erosive cycle: in each day, 4 cycles of immersion in Sprite light (30 ml/block) for 1 min, followed by immersion in artificial saliva (30 ml/block) for 39 min; for the remaining 20 h, immersion in artificial saliva (25°C). Erosive wear was evaluated by profilometry. After 5 and 10 days, mean wear (μm) was, respectively: Group 1, 1.83/2.67; Group 2, 1.04/2.60; Group 3, 1.03/2.48; Group 4, 1.13/2.47; Group 5, 1.07/2.44; Group 6, 1.02/2.35; Group 7, 0.75/2.27; Group 8, 0.80/2.12; Group 9, 0.76/2.47 and Group 10, 1.09/2.46. ANOVA and Tukey’s tests showed a significant difference between Group 1 and the other groups, as well as between days 5 and 10 (p < 0.05). The results suggest that laser irradiation, fluoride application, and the combination of the two can increase enamel resistance to short-period erosive challenge.

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**19**

**Erosive Effect of Citric Acid on Glass Ionomer Restorations and Adjacent Enamel**

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The aim of this pilot study was to assess the erosive effects of citric acid on glass-ionomer restorations in vitro. 18 pairs of buccal or lingual surfaces of molar tooth crowns were prepared with one of the pairs restored with either conventional GIC (Fuji IX) or resin modified GIC (RMGIC; Fuji II LC). The specimens were then painted with nail varnish, leaving a 2-mm space surrounding the restoration and also a window of about 2 mm diameter on the same tooth surface. The specimens were exposed to 5.0% citric acid solution for 16 h at 37°C. Following acid challenge, each sample was sectioned through the middle of the restoration and the unrestored window and photographed. While the effect of erosion was assessed using the photographs, the depths of erosion on GIC and enamel were measured using Leica Stereomicroscope (Germany). It was observed that the conventional GICs were significantly more eroded than the adjacent enamel (p < 0.05). Erosion depth on the surrounding enamel was significantly less (p < 0.05) when restored with conventional GICs (215 ± 45 μm) than when there was no restorations (249 ± 39 μm). In contrast, significantly more (p < 0.05) surrounding enamel was eroded when restored with RMGIC (452 ± 85 μm) than when there was no restoration (238 ± 113 μm). Furthermore, the margins of the conventional GIC restorations showed significantly more dissolution than the body of the restoration. It was concluded that although the conventional GIC materials are vulnerable to severe damage when tooth is exposed to erosive challenge, using it for tooth restoration might reduce the effect of erosion on surrounding tooth enamel.

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**Section 2**

**Clinical Studies/Risk Assessment**

**20**

**Evaluation of Caries Risk in Sardinian Schoolchildren Using the Cariogram**

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Various caries risk prediction models have been developed in recent years. Cariogram, a software program that evaluates an individual’s caries risk profile and illustrates it graphically, appears to classify patients more correctly than other risk prediction methods. The objective of this study was to determine the caries risk of a child population by means of the Cariogram and examine the relationships between the different variables used by the Cariogram and the risk of caries determined by this program. 958 Italian 6–9-year-olds (49.4% males and 50.6% females) were examined. Data on general health condition, diet frequency and use of fluoride were obtained, dmfs/DMFS and CPI scores were calculated and salivary levels of Lactobacillus and mutans streptococci evaluated. Data were scored and en-
tered into the Cariogram model and the chance to avoid caries (CTAC) calculated for each child. Caries prevalence was 57.3%, df mean and standard error was 2.1 (0.1) while DMFS was 1.2 (0.1), and 35.3% had detectable levels of oral mutans streptococci. The distribution of the calculated risk profile according to the Cariogram was: 34.7% of the subjects at very low risk (81–100% CTAC), 16.2% at low risk (61–80% CTAC), 20.4% at medium risk (41–60% CTAC), 8.6% and 20.1% at high risk and very high risk respectively (21–40% and 0–20% CTAC). Caries medium risk (41–60% CTAC), 8.6% and 20.1% at high risk and (81–100% CTAC), 16.2% at low risk (61–80% CTAC), 20.4% at very-high-risk level. The presence of at least one carious surface was the most pertinent factor in children’s caries risk profile. One third of the subjects exhibited a low chance of avoiding caries in the near future.

One third of the subjects exhibited a low chance of avoiding caries. Saliva was the most pertinent factor in children’s caries risk profile. With OR of 1.5 (95% CI 1.1,2.0) being found among males at the very-high-risk level. The presence of at least one carious surface was the most pertinent factor in children’s caries risk profile. One third of the subjects exhibited a low chance of avoiding caries in the near future.

**21 Caries Risk Assessment by Pac-Specific IgA and Streptococcus mutans Detection System**

A novel semi-quantitative detection system has been developed to monitor levels of salivary immunoglobulin A (IgA) to mutans streptococci. This system generates a result of either high or low level of IgA in 15 min. The aim of this study was to explore the feasibility of assessing caries risk using this system. Saliva samples were collected from 69 3-year-old children, 0.1 ml saliva was diluted with buffer and 0.3 ml of diluted saliva was applied into the test device. After 15 min, if a red line appeared, it was classified as high IgA (H-IgA), otherwise as low IgA (L-IgA). In addition, a semi-quantitative enumeration system was applied to determine Streptococcus mutans concentration in saliva by treating 0.25 ml of the same saliva with 0.05 ml Tris-NaOH buffer, and then mixing with 0.1 ml Tris-citrate buffer and applying the treated saliva into the test device. After 15 min, if a red line appeared, it was classified as high S. mutans (H-SM), otherwise as low S. mutans (L-SM). From these 2 test results, each subject was treated into the Cariogram and the chance to avoid caries. 95 subjects aged >60 years were enrolled into the study. Timed stimulated whole saliva samples were obtained from each subject. Plaque and gingival indices, caries scores and denture-wearing status were recorded. The numbers of bifidobacteria, mutans streptococci (MS), lactobacilli and yeasts per millilitre of saliva were determined using conventional methods. For the bifidobacteria MPTY medium [Rada and Petr: J Microbiol Methods 2000:43:127–132], modified by addition of raffinose and 10 μg/ml mupirocin, was used. The mean saliva concentration of L-SM was determined by the use of xSE) of organisms (frequency of isolation) were 4.68 ± 0.17 (94%), 4.75 ± 0.14 (97%), 4.13 ± 0.17 (94%) and 2.05 ± 0.17 (65%) for bifidobacteria, MS, lactobacilli and yeasts, respectively. The bifidobacteria counts were significantly correlated with MS (r = 0.49), lactobacilli (r = 0.59), yeasts (0.35) and salivary flow (r = 0.27). The total caries score was significantly correlated with the salivary bifidobacteria concentration (r = 0.43; p = 0.001) and in a multiple regression model only salivary concentrations of bifidobacteria and yeasts were significant predictors of the total caries score (p < 0.001). In these subjects bifidobacteria were widely distributed, present at salivary concentrations equivalent to those of MS, and their concentration was significantly associated with caries. Salivary counts of bifidobacteria may be a new marker of caries risk, but fuller data are required.

Supported in part by the Dunhill Medical Trust and the Biomedical Research Centre of Guy’s and St Thomas’ Foundation Trust Hospital.

**22 Isolation of Bifidobacteriaceae from the Saliva of Elderly Subjects**

Bifidobacteriaceae are aciduric and acidogenic bacteria sporadically isolated from the oral cavity, occasionally from infected dentine. We have investigated the use of a novel selective culture medium to determine the prevalence of this genus in the mouth and the relationships between it and the organisms conventionally associated with caries. 95 subjects aged >60 years were enrolled into the study. Timed stimulated whole saliva samples were obtained from each subject. Plaque and gingival indices, caries scores and denture-wearing status were recorded. The numbers of bifidobacteria, mutans streptococci (MS), lactobacilli and yeasts per millilitre of saliva were determined using conventional methods. For the bifidobacteria MPTY medium [Rada and Petr: J Microbiol Methods 2000:43:127–132], modified by addition of raffinose and 10 μg/ml mupirocin, was used. The mean saliva concentration [log10(cfu/ml) ± SE] of organisms (frequency of isolation) were 4.68 ± 0.14 (97%), 4.75 ± 0.18 (93%), 4.13 ± 0.17 (94%) and 2.05 ± 0.17 (65%) for bifidobacteria, MS, lactobacilli and yeasts, respectively. The bifidobacteria counts were significantly correlated with MS (r = 0.49), lactobacilli (r = 0.59), yeasts (0.35) and salivary flow (r = 0.27). The total caries score was significantly correlated with the salivary bifidobacteria concentration (r = 0.43; p = 0.001) and in a multiple regression model only salivary concentrations of bifidobacteria and yeasts were significant predictors of the total caries score (p < 0.001). In these subjects bifidobacteria were widely distributed, present at salivary concentrations equivalent to those of MS, and their concentration was significantly associated with caries. Salivary counts of bifidobacteria may be a new marker of caries risk, but fuller data are required.

Supported in part by the Dunhill Medical Trust and the Biomedical Research Centre of Guy’s and St Thomas’ Foundation Trust Hospital.

**23 Fracture Resistance of Occlusally Restored Teeth after Different Stages of Simulated Caries Excavation**

The aim of this in vitro study was to evaluate differences in fracture resistance of teeth with occlusally restored caries lesions after different stages of simulated caries excavation. Extracted...
sound third molars were prepared and restored according to 6 protocols (n = 15). In groups 1–3, preparations with a 2.3-mm-wide opening and undermined dentine lesions were ground. In groups 4–6, identical shaped but larger preparations (access opening of 2.9 mm) were ground. Tempbond-cement (Kerr) with a Vicker’s Hardness close to soft carious dentine was used to create a caries-simulating layer (CSL). In groups 1 and 4, a CSL was placed in the whole preparation except in the access opening, simulating that just an opening in enamel was made and infected tissue was left behind. In groups 2 and 5, a CSL was placed only at the bottom of the preparation, simulating removal of infected dentine and leaving affected dentine behind. In groups 3 and 6, no CSL was placed, indicating total caries removal. All teeth were restored with an etch and rinse adhesive system and a composite dentine and leaving affected dentine behind. In groups 3 and 6, no CSL was placed, indicating total caries removal. All teeth were restored with an etch and rinse adhesive system and a composite resin. Group 7 was the control group, consisting of sound teeth. All teeth were occlusally loaded on the restoration until fracture occurred. Failure loads were statistically compared using multiple regression analysis. Analysis revealed that fracture resistance decreased significantly (p < 0.001) with increased layer thickness of simulated dentin caries. Groups simulating complete caries removal showed the highest fracture resistance. In groups with CSL, most fractures occurred in the tooth, while in groups 3 and 6 fractures either occurred in the tooth or in both the restoration and the tooth. From this study it can be concluded that fracture resistance of occlusally restored teeth after incomplete simulated caries removal may be lower than that of teeth after complete excavation.

24
X-Ray Microtomography Study of the Efficacy of Chemomechanical Removal of Carious Dentine
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The aim was to use a high-definition X-ray microtomography (XMT) scanner to study the mineral concentrations (Cmin) of infected and affected carious dentine in deciduous molars, and the efficacy of chemomechanical caries removal (Carloslov) in removing the infected dentine. Carious deciduous molars with open cavities were bisected. The two halves were mounted on a kinematic stage and were scanned using a MuCat XMT scanner (developed at Barts and The London Dental School). This scanner uses a novel aluminium step wedge to calibrate the multi-energy X-ray beam to one with an effective energy of 40 keV and a novel scanning methodology to improve contrast ratio. After scanning, in one half of the tooth, carious dentine was removed using a new spoon excavator until the dentine was ‘felt’ clinically hard. On the other half, carious dentine was removed chemomechanically. Then, the specimen was re-scanned. Analysis was carried out comparing the linear absorption coefficient (LAC) of the XMT slices from the two scans. The mineral concentrations of the dentine removed were measured, determined from LACs assuming the mineral being pure hydroxyapatite and the organic phase being collagen. 21 pairs of deciduous molars were scanned. The Cmin for the body of the carious dentine was ~0.37 g · cm⁻³ and for the demineralised zone, 0.57–1.07 g · cm⁻³. Dentine with higher Cmin, up to 1.37 g · cm⁻³, was removed using the excavator whereas dentine with Cmin up to 0.87 g · cm⁻³ was removed chemomechanically. In conclusion, the chemomechanical technique removed less sound dentine, leaving a layer of partially demineralised dentine which might correspond to the affected dentine. This method may be a useful tool in minimally invasive dentistry.

25
White Spot Lesions in Orthodontic Patients following Bracket Removal
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The aim was to describe the baseline distribution of white spot lesions (WSL) in a clinical trial conducted in a post-orthodontic population. Adolescent subjects were recruited from private orthodontic practices in metropolitan and rural regions. Inclusion criteria specified that subjects exhibited a minimum of two WSL on the buccal surfaces of teeth 14–24 and 34–44, which were at least 2 mm² in area. Baseline examinations were conducted within 7 days of bracket removal, and included a prophylaxis followed by clinical assessment, initially of wet surfaces and then after 5 s drying with air. Each WSL identified for study inclusion was scored for lesion severity and activity using the ICDAS II criteria. Subjects were randomised into either an intervention or a placebo-control group. The 45 subjects (mean age 15.5 years) recruited contributed a total of 408 WSL (range 3–19 per subject). On average, approximately half the 16 buccal surfaces had at least one WSL (range 18.8–87.5%). Three subjects had WSL on 14 of the 16 surfaces. Lesion severity was scored as Code 1 in 7.8%, Code 2 in 89.7% and Code 3 in 2.5% of WSL. The majority of WSL (86.3%) were classified as active. The prevalence of WSL was slightly higher on the upper jaw (56.1%). WSL were more common on the lateral incisors and canines in the upper jaw, and on the canines and first premolars in the lower jaw. In conclusion, the majority of lesions in the study were classified as active and had a greater likelihood of transition (progression or regression). The number of WSL per subject and the distribution of WSL were similar to those reported in other studies of post-orthodontic subjects.

Supported by CRC for Oral Health Science and GC Corporation, Japan.
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 3.07; p < 0.001). In conclusion, significantly more post-orthodontic WSL regressed with Tooth Mousse compared to a placebo control over a 12-week period.

Supported by CRC for Oral Health Science and GC Corporation, Japan.

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) in patients with fixed orthodontic appliances. 24 patients were divided into two groups of 12 according to baseline bacterial counts, creating high (≥10^5 CFU/ml saliva) and low (≤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, χ² 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; χ²). 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; χ²). In conclusion, conventional etch and bond remains the better approach for sealant application at this time.
Abstracts

29 Efficacy of Sealing Approximal Lesions on Primary Teeth: 1-Year Results
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It is well established that cavitated caries lesions among 3-year-olds may develop on molars, mainly on the distal surface of the first primary molar [Mejàre. Consensus Conference on Caries in the Primary Dentition and its Clinical Management. Stockholm, Förlagshuset Gothia, 2002, pp. 57–68] and that dentinal lesions progress twice as fast in primary teeth as in permanent teeth [Schwartz et al.: Arch Oral Biol 1984; 29: 529–536]. The aim of this study was to assess the efficacy of sealing approximal initial lesions on primary molars relative to flossing instructions (control). 50 4–6-year-olds from Colombia (n = 27) and Denmark (n = 23) participated in this split-mouth design. Patients had to have a minimum of two approximal lesions scored 1–3 according to the following radiographic classification system: (1) lesion restricted to enamel outer half; (2) lesion from the inner half of the enamel to the enamel-dentine junction; (3) lesion restricted to outer third of dentine. Standardized baseline and follow-up bite-wing radiographs were obtained. Lesions were sealed with an adhesive [Martignon et al.: Caries Res 2006; 40:382–388]. Patients’ parents/caregivers received a brochure with flossing instructions for their children’s teeth. Progression of the lesions was assessed by pair-wise reading of conventional bitewing radiographs. McNemar’s test was used to assess differences between test and control (α = 0.05). At one-year follow-up, 6 test and 9 control lesions were restored. 15 (30%) test lesions and 30 (60%) control lesions had progressed (p < 0.01). The sealing technique was superior to flossing instructions after one year of follow-up. This could be a preventive measure for use on distal surfaces of first primary molars.

30 Visualisation of Infiltrant Penetration into Natural Enamel Caries with Two Dual Fluorescence Techniques in vitro
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Fluorescence confocal microscopy is a useful tool to analyze the infiltration of enamel caries lesions with low- viscosity resins (infiltrants) in vitro. However, the conventionally used staining technique, which comprises dye labelling of the resin, has been shown to be limited by chromatographic separation of the resin-dye-mixture during penetration. The aim of this study was to develop an improved dual staining technique and to compare validity and reproducibility of both methods. Twelve human molars with proximal white spots were cut across the lesions. After vanishing the cut surfaces, paired lesion halves were infiltrated with a pre-product infiltrant (DMG, Hamburg, Germany) using one of two staining techniques. For the conventional direct technique (DT) the infiltrant was labelled with 0.1% rhodamine isothiocyanate (RITC) prior to application. Using the new indirect technique (IT) lesions were stained with 0.1% RITC solution (12 h) and subsequently infiltrated with pure infiltrant. After light curing, unbound dye was bleached by immersion in 30% H2O2 (12 h). Remaining lesion pores were stained with 0.1 mM sodium fluorescein. Penetration depths (PD) and lesion depths (LD) were evaluated by 5 examiners using confocal microscopy and compared with PD by scanning electron microscopy (SEM) and LD by microcradiography (TMR). The indirect technique showed better correlation (intraclass coefficient: ICC) with SEM (0.990) and TMR (0.982) than the direct technique (SEM 0.513, TMR 0.702). Inter-rater reliability was higher for IT (ICC: PD 0.904, LD 0.911) than DT (PD 0.841, LD 0.560). It can be concluded that the new indirect technique is a more valid and reproducible method to visualize infiltrant penetration into natural enamel caries lesions compared with the conventional method.

Supported by the Deutsche Forschungsgemeinschaft (PA 1508/1-1).

31 Influence of Application Time on Infiltrant Penetration into Natural Caries Lesions in vitro
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Compared to adhesives, resins with high penetration coefficients (infiltrants) showed superior penetration into natural lesions after etching with HCl gel for 2 min when applied for 5 min. Since shorter application times seem to be more feasible, the aim of this in vitro study was to compare the penetration depths of a pre-product infiltrant (DMG, Hamburg, Germany) into natural caries lesions after various application times. Extracted permanent human molars and premolars showing proximal white spots were etched for 2 min (15% HCl gel; DMG). After rinsing and drying, the lesions were immersed in 0.1% rhodamine isothiocyanate for 12 h. Subsequently, lesions were dried again and the infiltrant was applied for 0.5, 1, 3, or 5 min (18/group). After removing surplus, infiltrants were light cured and ground sections were prepared. Subsequently, specimens were immersed in 30% hydrogen peroxide for 12 h to remove all red fluorescence not adherent to the infiltrant. Specimens were then immersed in 0.1 m M sodium fluorescein and lesion depth and penetration depth were measured by confocal microscopy (up to 9 measurements per lesion). Penetration depths (median and interquartile range) after 0.5 min [113 (27–206) μm] and 1 min [144 (62–228) μm] differed significantly from those after 3 min [275 (150–378) μm] and 5 min [290 (127–410) μm] (p < 0.001; Mann-Whitney), but not among themselves in each case (p > 0.05). Deep lesion parts (>500 μm)
could only be penetrated after 3 min [100% (50–100)] as well as 5 min [100% (58–100)] almost completely. It can be concluded that 3 min application of a pre-product infiltrant seems to be sufficient to allow penetration into natural caries lesions in vitro.

Supported by the Deutsche Forschungsgemeinschaft (PA 1508/1-1).

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Therapeutic Sealing of Proximal Tooth Surfaces: Three-Year Clinical and Radiographic Follow-Up

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The purpose of this investigation was to test the effect of a new treatment for proximal caries. In 50 patients with two proximal caries (D1–3 without cavitation, bitewing X-ray) orthodontic rubber rings were applied to gain access to the interproximal space. One of the lesions was sealed with a thin polyurethane-dimethacrylate foil using a bonding agent (Heliobond, Vivadent, Schaan/Liechtenstein); the other lesion received oral home-care with dental floss and fluoridated toothpaste and was left as control. In clinical and X-ray follow-up 3 years after application, clinical retention of proximal tape and the underlying sealant, marginal adaptation, discoloration, tooth vitality, plaque and gingivitis could be checked in 25 patients; in addition, caries was assessed clinically and radiographically. The sealants showed good retention, marginal adaptation and colour. After 3 years, vitality of all teeth was positive and no differences in plaque accumulation or gingival status were found between sealed and control teeth. One sealed surface and one control surface were filled by another dentist. 8 sealed lesions showed regression, while two progressed. In contrast 6 control lesions regressed and also two showed progression. The loss of foil had no significant influence on the lesion progression, indicating the effect of the underlying bond. All other sealants and control lesions were stable, indicating an arrest of the lesion. In conclusion, sealing initial proximal lesions showed no clinical problems and mostly stabilization of the lesions after 3 years, as did the oral home care with flossing and fluorides.

Supported by Ivoclar Vivadent AG.

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Arrestment of Non-Cavitated Carious Lesions in Permanent Teeth Using Toothbrush and Fluoride or Non-Fluoride Dentifrice

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This study aimed to evaluate the clinical aspect of white spot lesions (WSL) after 6 weeks of daily mechanical removal of dental plaque using toothbrush and dentifrice with or without fluoride. To be included, children should present WSL on the buccal surface of at least one permanent upper incisor or permanent upper first molar. Parents signed an informed consent. The study was designed as a double-blind randomized clinical trial with 30 children (88 white spot lesions) randomly divided into two groups: fluoride (F) with 15 children and 41 WSL and nonfluoride (NF) with 15 children and 47 WSL. Children and parents were trained to brush the children’s teeth daily, with emphasis on the WSL. Dental surfaces were clinically examined once a week, during 6 weeks, by only one examiner to evaluate caries lesion activity [Nyvad et al.: Caries Res 1999;33:307–313] (kappa 0.95), presence of plaque and gingival status [Ekstrand et al.: Caries Res 1998;32:41–45] (kappa 0.86). Statistical analysis used chi-square, Mann-Whitney and Wilcoxon tests, and logistic regression. At baseline, groups F and NF were similar in age, sex, localization of lesions, and for the caries, plaque and gingival indices (p > 0.05). During the study, a significant improvement of the plaque and gingival indices was observed in both groups (p < 0.001). After 6 weeks, 73 (83%) lesions were considered inactive, with better results for the incisors (p < 0.001). The arrestment of the lesions was statistically associated with better plaque and gingival indices (p < 0.001) independently of the groups F and NF (p > 0.05). It was concluded that daily mechanical disturbance of plaque was able to arrest a large number of caries lesions.

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Remineralisation of Advanced Dentine Lesions in situ

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In previous in situ experiments standard dentine lesions (7 days, 8 wt% methylcellulose, 0.1 mol/l lactate, pH 5.0) were fully remineralised after three weeks of applying fluoride toothpaste 3×/day. In future research we want to study the effects of various oral hygiene protocols on the remineralisation of dentine lesions. Therefore lesions are needed that do not fully remineralise under standard oral hygiene procedure. The aim of this study was to monitor the remineralisation of advanced dentine lesions in time. Cylindrically-shaped dentine specimens, diameter 6 mm, were prepared from bovine central incisors. Advanced lesions were formed by incubating the specimens in 8 wt% methylcellulose containing 0.1 mol/l lactate at pH 5.0 during 17 days. 14 participants received two specimens placed contralaterally in the buccal flanges of their partial prosthesis. The participants brushed their teeth 2 ×/day with an amine fluoride toothpaste containing 1,400 ppm fluoride. After 2 and 3 weeks, specimens were removed and integrated mineral loss (IML) and lesion depth (LD) measured by transversal microradiography. The starting lesions showed a mean IML of 1,720 vol%·μm (range 1,441–2,118) and a mean LD of 121 μm (range 103–133). During the in situ period, on average, the lesions significantly remineralised (Mann-Whitney test; p < 0.005): after 2 weeks mean IML was 959 vol%·μm
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.

**35 Dentine Regeneration in the Carious Cavity**

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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 circumpulpal 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.

**36 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.

**37 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 μm 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.
The aim of the study was to evaluate the risk factors of early childhood caries in toddlers in Riga, Latvia. 110 randomised selected toddlers, mean age 30 months (62 males, 48 females), were involved in a socio-epidemiological study. The toddlers were examined for caries (d1, mft), initial caries lesions, plaque on upper front teeth and gingivitis. Their mothers answered a structured questionnaire concerning the children’s oral health behaviour and social aspects as well as diet protocol. Salivary mutans streptococci of toddlers were determined by Caries Risk Test Bacteria (Ivoclar Vivadent, Schaan, Liechtenstein). Pearson’s correlation coefficients between caries experience and continuous variables (α = 0.05) were calculated. 77.3% of the children were caries free. The mean caries experience was 1.01 d1, mft (dt = 0.88; ft = 0.13; mt = 0.05). The majority of the manifest caries lesions (d1, m) were located on the upper front teeth. 47% of the toddlers showed visible plaque on anterior teeth. 5.6% of the toddlers had gingivitis and 72% harboured mutans streptococci. Correlations were found between cariogenic meals and mutans streptococci. Furthermore, a positive correlation was found between visible plaque and the caries decay of upper incisors. The correlation coefficient between visible plaque and the caries decay of upper incisors (r = 0.47, p = 0.005) was 0.47.

To have teeth with untreated caries in comparison to the weakest parental LoC quintile were 0.35 (95% CI 0.15, 0.81, p < 0.05) times less likely to have teeth with untreated caries in comparison to the weakest parental LoC quintile. The findings support the hypothesis that higher internal parental LoC is associated with better control of caries in their pre-school children and highlight that a more internal LoC within the family is advantageous in the prevention of dental caries.

Supported by the IGA, Czech Ministry of Health (Project no. NR/8331-3).

To get a representative national survey of the caries prevalence, 6-year-old Austrian children were investigated in 2006 with respect to the educational level and migrant status of the parents. Migrant status was defined if at least one parent was not born in Austria. At the beginning, the investigators attended a 2-day calibration workshop. 563 children were investigated with ICDAS II-criteria according to WHO standards. 45.3% of all children were caries free. For all children the mean dmft was 2.7 and for caries-positive children 5.0. Also the number of decayed surfaces was 3.7 and the number of filled surfaces was 1.4. Of the decayed surfaces, 13.2% belonged to ICDAS 3; 22.4% to ICDAS 5 and 64.3% to ICDAS 5. The SiC Index of caries-positive children was 5.7. Education and migration status of the parents had an influence on the caries experience of the children. A treatment need for caries-positive children was given in 58.0% of children with migrant status and in 31.9% of Austrian children. Among children whose parents had at least attended a gymnasium, 30.3% needed dental treatment, but among children of parents with lower education, 45.8% needed treatment. 62.5% of children with low education and migrant status, but only 21.6% of Austrian children with more highly edu-
cated parents needed dental treatment. Migration probably has a great influence on caries prevalence and the costs of dental treatment in the future. Children of parents with migrant status and low education especially have a need for preventive measures.

From 1977 to 1998 preschool children in Hamburg have been subject to repeated epidemiological field studies for caries, showing a steady caries decline. The aim of the present study was to determine the caries prevalence in 3- to 6-year-old kindergarten and day-care children in Hamburg in 2006 and to compare it with data from 1998. The visual caries registration followed the WHO criteria with additional recording of initial caries. 1,643 children were examined. The dmft value was 1.2 ± 2.6 for all children and ranged from 0.5 in 3-year-olds to 2.2 in 6-year-olds. Including initial caries lesions the dmft value for the entire study cohort was twice as high (2.4 ± 3.5). 72.7% of all children were free of dentin caries experience. The remaining children with caries experience exhibited a mean dmft value of 4.2 ± 3.4, and 15.8% of the 3-year-olds who showed caries had on average 3.4 ± 2.5 dmft teeth. The caries experience was strongly related to the socio-economic status of the children’s families and ranged from 0.3 in upper-class children to 1.8 in lower-class children (p < 0.001, Kruskal-Wallis test). While in 9.6% of all children symptoms of nursing bottle tooth decay could be detected, 13.6% of the children with low socio-economic background exhibited this finding. Compared with the investigation from 1998, the caries decline in Hamburg preschool children has come to a halt, initial lesions even showing a reversal. This development has been accompanied by a distinct caries polarization. It can be concluded that there is need for intensive preventive care in risk groups, and that the dental care for children should start well before the age of three.

The study investigated caries trends in 1–5-year-old children attending public nursery schools in the Federal District of Brazil – the municipality of Brasilia and its satellite cities. The populations studied were 1,465 children (1996 cohort) and 2,511 children (2006 cohort). Dental caries was diagnosed according to severity and activity by a thorough visual examination, after professional brushing and drying. Intra-examiner reliability of diagnosis (kappa) was 0.93, 0.71, 0.90 in the 1996 cohort and 0.90, 0.93 in the 2006 cohort. The kappa value for inter-examiner reliability was 0.86 in the 2006 cohort. For all teeth and tooth surfaces, the clinical examination diagnosed whether they were sound or presented active lesions (non-cavitated and cavitated), inactive lesions (non-cavitated and cavitated) or fillings, or were indicated for extraction or extracted. Significant increases in the proportion of caries-free children between the 1996 and 2006 cohorts, and significant decreases in mean deft/s was observed for all age groups but only when both non-cavitated active and cavitated lesions were included in the decayed component. E.g. at age 5, proportion of deft/s = 0 was 28% (1996) versus 47% (2006) (p < 0.05; chi-square); mean deft/s = 8.87 ± 0.59 SE (1996) versus 4.27 ± 0.29 (2006) (p < 0.05; t-test). During the study period the rate of caries progression was reduced significantly in all age groups, but reduction in caries experience at cavitated level was only observed at the age of 5: i.e. def/s = 5.52 ± 0.46 (1996) versus 3.70 ± 0.26 (2006) (p < 0.001).

Supported by Gaba International.

The aim of the present study was to investigate factors associated with caries in a sample of 2,511 Brazilian 1–5-year-old children attending public nursery schools in the municipality of Brasilia and its satellite cities. The children were lifetime residents of municipalities with fluoridated water and had limited access to oral health care. 2,102 parents were interviewed about demographics, their children’s oral health and dietary habits. As the children attended nursery full time, a questionnaire was also answered by each institution (n = 31). The children’s teeth were professionally brushed and dried before clinical examination, which for all teeth and tooth surfaces diagnosed whether they were sound or presented active lesions non-cavitated or cavitated, inactive lesions or fillings, or were indicated for extraction or extracted. Intra-examiner kappa for caries diagnosis (10% of sample) was 0.90 (examiner 1) and 0.93 (examiner 2) and inter-examiner kappa was 0.86. The decayed component represented both non-cavitated active lesions and cavitated lesions. The proportion of caries-free children (dmfs1 = 0) ranged from 95% at 1 year of age to 47% at 5 years. 97% of the children used fluoride toothpaste and 72% brushed twice daily. Regular dental appointments were not frequent (26%) and a feeding bottle was used by 30% of children. In a multivariate logistic regression (dmfs1 = 0/dmfs1 ≥1) the following factors were significantly associated with caries experience: educational level of the mother; family income; toothbrushing without adult assistance; feeding bottle during the night at ≥2 years of age and dental appointments for treatment (p < 0.01). Children’s dental health might be achieved by enhancing parents’ co-operation in this regard.

Supported by Gaba International.
Evidence suggests that communities with higher level of social capital have better health. However, no study has looked into the relationship between the oral health and social capital. The aim of this study was to clarify the relationship between social capital and children's caries experience in Japanese children. The participation rates in hobbies and amusements, sport, volunteer and social activities, and social life in each prefecture in Japan were obtained from surveys on time use and leisure activities reported by Ministry of Internal Affairs and Communications, Japan in 2001. Data on caries experiences of children aged 1.5, 3, 5 and 12 years were obtained from reports by the Ministry of Health, Labor in Japan. The relationship between caries experience and 4 indices was analyzed by Spearman’s rank correlation coefficient. The proportion of caries-free children and the rate of hobbies and amusement were positively correlated, with coefficients 0.53 (p < 0.001), 0.65 (p < 0.001), 0.52 (p < 0.001) for 1.5-, 3- and 5-year-olds, respectively. DMFT and the rate of hobbies and amusement were negatively correlated (rₛ = –0.331; p < 0.001). Fluorosis and caries were also negatively correlated (rₛ = –0.16; p < 0.01). The differences between males and females in fluorosis were small (p = 0.3), but were statistically significant for socio-economic status/school type (‘Gymnasium’ mean Dean Index 0.25, ‘other schools’ 0.17; p = 0.01). In conclusion, fluoride use was associated with lower caries prevalence and minimal degrees of clinically irrelevant fluorosis in Greifswald, East Germany.

The aim of this study was to determine the prevalence of dental caries and fluorosis in a Brazilian town that recently started a water fluoridation program, beginning in 2006. The sample comprised 146 public school children (11–13 years old). The clinical examinations were carried out by a single, previously calibrated examiner (kappa = 0.98 and 0.61 for dental caries and fluorosis, respectively). Dental caries (DMF-T) and treatment needs were assessed at cavity level [WHO, 1997]; dental fluorosis was assessed using the TF index [Thylstrup and Fejerskov: Community Dent Oral Epidemiol 1978; 6:315–328]. 22% of the children were classified as caries-free and 34.3% of the children with caries presented DMFT ≤ 3. The mean ± SD DMFT was of 2.6 ± 2.6 (CI 95% 1.9, 3.2), 3.7 ± 2.9 (CI 95% 2.9, 4.5), 4.8 ± 3.4 (CI 95% 3.3, 6.3) for 11-, 12- and 13-year-old children, respectively. Regarding treatment needs the most frequent procedure was restoration of one tooth surface only. There was no statistically significant difference in caries with respect to gender, parents’ educational level or family income (p > 0.05; χ²). Dental fluorosis was observed in 39% of the children and the most frequent score was TFI 1–3. All children reported using toothpaste since childhood and 46% reported having swallowed fluoridated dentifrice during toothbrushing. About 75% of the children used the water from the public water supply. It can be concluded that the caries experience increases with age and can be regarded as a health problem in this community. The prevalence of dental fluorosis was moderate for a city without water fluoridation in previous years.
The aim of this pilot study was to investigate the influence of long-term exposure to antiasthmatic medications on caries experience in children. 96 asthmatic 3–15-year-old children (mean age 7.2), who were under treatment for asthma and who had used antiasthmatic medications for at least one year, were matched with their healthy siblings (1–2 years younger or older), who served as controls. Caries experience was determined as the number of decayed (non-cavitated = D1, cavitated = D2), missing, and filled surfaces in permanent (DMFS) and deciduous (dfs) teeth by two calibrated dentists using the ICDAS II criteria. Mann-Whitney U test was used to compare the D12MFStotal (combined primary/permanent teeth; D1 = non-cavitated caries, D2 = cavitated) of the groups (α = 0.05; n = 72/group). D12MFStotal was significantly higher (p < 0.001) in Groups A (28.4 ± 16.9) and B (23.9 ± 13.9) than in Group C (12.16 ± 10.34). The difference in D12MFStotal between Groups A and B was not statistically significant. This pilot study highlights the possibility that the difference in caries prevalence between children with and without asthma may be caused by anti-asthma medications with caries risk factors such as high sugar content or reduced salivary function.

Supported by Slovenian Ministry of Science and Education (No. J3-8713-0381-99).

The relationship between oral condition and Body Mass Index (BMI) gives rise to controversy. The aim of this study was to assess weight, height, caries experience and periodontal health in a sample of 12-year-old children. Subsequently the association between BMI and DMFT as well as between BMI and Community Periodontal Index (CPI) was analyzed. In 2006, 20% of the 12-year-old schoolchildren from Montpellier (France) were randomly selected by taking into account the proportion of public and private schools. The 803 children were examined in the schools by the same calibrated dentist, and the D1, DMFT and CPI scores were recorded following WHO recommendations. Overweight and obesity were defined according to the international cut-offs of BMI (kg/m2): BMI(1) < 18 = insufficient; BMI(2), between 18 and 25 = normal; BMI(3), between 25 and 30 = overweight; BMI(4), ≥ 30 = obese. The mean BMI was 18.9 (C.I.: 18.7, 19.1), with a minimum of 12.9 and a maximum of 37.8. The distribution of BMI from level 1 to 4 was 50.5%, 44.3%, 4.6%, 0.6%, the corresponding mean DMFT values were 1.38, 1.53, 1.31 2.00, and the mean CPI scores were 0.69, 0.68, 0.72, 0.73, respectively. The mean BMI was 18.7 in boys and 19.1 in girls (p = 0.01; Mann-Whitney test). The mean BMI was significantly different between children attending public schools and private schools (p < 0.001). There was a significant Spearman correlation between BMI and DMFT (rS = 0.10; p = 0.02), but not between BMI and CPI (rS = 0.03; p = 0.40). In conclusion, BMI was significantly correlated with caries experience. However, the prevalence of overweight and obesity was lower in this sample than is generally found in the French population.

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Prevalence of Unrestored Dentin Caries and Deep Dentin Restorations in Swedish Adolescents

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The objectives of this longitudinal study were to assess: (a) the prevalence of unrestored dentin caries among 15-year-olds, (b) the proportion of these lesions that had progressed to deep dentin lesions (inner half of dentin) since the immediately preceding examination at age 14, and (c) the frequency of deep restorations (extending into the inner half of the dentin). The sample consisted of all 15-year-olds (n = 2,487) born in 1990 and included in the Public Dental Service in Malmö, Sweden. Bite-wing radiographs taken during 2005–2007 and the immediately preceding radiographs were analysed and scored by 2 examiners. The main radiographic scores were: sound; radiolucency in outer or inner half of dentin; and restored surface. 22% of the individuals had one or more dentin lesions left unrestored from the time of the examination at age 14 until the next recall examination at age 15. During the observation period (median time 1.2 years), 9% of the unrestored outer dentin lesions progressed to deep dentin lesions. The majority of these (93%) were molars. One or more deep restorations were found in 22% of the 15-year-olds; the majority involved occlusal surfaces of first molars. In conclusion, unrestored dentin lesions were common in 15-year-olds. Progression to deep dentin lesions occurred in 9% of these lesions and was most common in first molars. Occlusal surfaces of first molars had the highest frequency of deep restorations.

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Disparities in the Distribution of Dental Caries among Adolescent in the Metropolitan Region of Chile

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The aim was to investigate gaps of inequity in the distribution of dental caries between groups of the population of different socio-economic levels and different genre in a cross-sectional oral health survey. The random sample consisted of 748 12-year-old individuals from 75 public, semi-public and private schools in the Metropolitan Region during 2006–2007. Dental exams were performed by 3 calibrated exam teams in the same setting, using artificial light, CPI probes and mirrors and following WHO recommendations for oral health surveys. The socio-economic status was established by means of the classification of the schools attended by the individuals and by the commune in which they live. The prevalence of dental caries, mean number of decayed, missing and filled permanent teeth (DMFT), the Significant Caries Index (SiC) and their 95% CI were determined and compared according to socio-economic status and gender. The prevalence of dental caries in the permanent dentition was 64.4% (95% CI 60.8, 67.87). The mean DMFT was 1.88 (1.78, 1.98). The SiC Index was 4.3 (4.00, 4.57). Statistical differences were found among the high socio-economic status group and medium and low socio-economic status groups in caries prevalence, DMFT Index and SiC Index. The data suggest the existence of disparities in the distribution of dental caries between adolescents of different socio-economic status and no differences by means of gender.

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Trends in Dental Caries in Australian Army Recruits from 1996 to 2008

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Dental caries has declined substantially in Australian children over the past 30 years, with much of the effect attributed to water fluoridation. There are few available data to support a similar effect in young adults. The objective of this paper was to evaluate the trends of dental caries prevalence in young adult Australian Army recruits aged 18–35 years, by three cross-sectional studies conducted in 1996, 2002/3 and 2008. In all cohorts, dental caries (DMFT/S) was measured using clinical and radiographic examination. There was a decline in mean DMFT scores across all age groups of between 22.3 and 32.3% between 1996 and 2002/3, with a larger decline in the youngest age group. The mean DMFT in 1996 was 4.53, and in 2002/3 was 3.32, with an increase in the proportion of caries-free recruits from 15.2% in 1996 to 27.3% in 2002/3. However, there was little change in untreated caries, with 1.50 teeth in 1996 and 1.20 teeth in 2002/3 with untreated caries. Multivariate Poisson regression was used to determine the impact of education, socioeconomic status and lifetime exposure to water fluoridation on caries experience. Subjects with a lifetime exposure to fluoridated drinking water had a 23% reduction in caries experience in 1996 and 26% reduction in 2002/3, compared with subject who had no lifetime exposure. In conclusion, this study showed that there has been a significant improvement in dental health among Australian Army recruits between 1996 and 2008, with significant differences between those with and without a lifetime exposure to fluoridated drinking water.

Supported by the Australian Dental Research Foundation.
(65–74 years old, n = 1,040). Scoring was performed by three calibrated dentists and differentiated between open accessible but sound surfaces, cavitated root lesions as well as filled root surfaces. 21.5% of the adults exhibited root caries experience (males 25.9%, females 17.0%; p = 0.001; chi-square). In senior citizens, 45.0% showed root caries (males 46.8%, females 43.5%; p = 0.294). Related to the number of free root surfaces, the Root Caries Index (RCI) was 8.8% in adults (males 10.2%, females 7.2%; p = 0.186) and 17.0% in senior citizens (males 16.5%, females 17.4%; p = 0.735). Compared with a corresponding survey from 1997, the root caries values were slightly changed with respect to RCI (1997: adults 9.9%, senior citizens 12.6%). While the root caries prevalence was unaltered in adults (1997: 22.1%), there was a significant increase in root caries experience in senior citizens (1997: 29.9%). Thus, the study shows that the higher number of remaining own teeth is accompanied by a distinct increase in root caries prevalence in senior citizens. This finding shows that strategies for effective root caries prevention and root caries treatment should be established.

Abstracts

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Caries Prevalence in an Older Population from Santiago, Chile

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The aim of this study was to assess the prevalence of dental caries in old individuals of a low socio economic status in Santiago, Chile. A sample of 109 subjects (74 female, 35 male), aged 64 to 74 years (mean age 69.4 ± 3.2), was selected using the probability-proportionate-to-size sampling technique. The data were collected by means of an interviewer-administered questionnaire and an oral examination performed by a calibrated dentist. DMFT scores were recorded following WHO recommendations using diagnostic criteria at the caries into dentin threshold. The results were evaluated and compared by chi-square and Student’s t-test (α = 0.05). The results showed a mean DMFT of 21.61 ± 5.1. 45.9% of the analyzed subjects had dental caries, of whom 52% presented one, 18% two, 14% three, 12% four and 4% five carious lesions, respectively. Of the analyzed population, 20.1% in the maxillary and 38.53% in the mandible required partial prosthetic rehabilitation. In addition, 20.2% of the individuals were edentulous in both maxilla and mandible. In conclusion, the population studied presented high prevalences of partial edentulousness, total edentulousness and caries.

Session 4

De- and Re-Mineralization

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Is the Rate of Demineralisation in a Caries Lesion Diffusion or Surface Reaction Controlled?

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The aim was to correlate deductions from published studies of enamel and in vitro caries systems that used a variety of experimental approaches to determine whether a consistent answer could be provided to the title question, which is fundamental to physical chemistry of caries. Historically, diffusion processes in enamel were regarded as rate determining. However, X-ray scanning microradiography has shown that rate of in vitro lesion progression is approximately linear, which is inconsistent with diffusion control and indicates surface reaction control at the advancing front [Gao et al: J Dent Res 1993;72:923–930]. This is consistent
with often observed [Yanagisawa et al.: J Electron Microsc 2003; 52:605–613] angular and keyhole structures in TEM of crystals from carious enamel. This assertion derives from comments [Berner: Amer J Sci 1978;278:1235–1252] concerning early diagenesis in sedimentary rocks subsequent to water deposition. Berner wrote that when dissolution is controlled by surface reactions, there is selective dissolution in different crystallographic directions and of specific crystal regions (e.g. dislocations), thus forming angular features with crystallographically determined orientations. On the other hand, under transport control (e.g. diffusion) smooth surfaces without angular features are formed. Nevertheless, rate determination solely by surface control seems contrary to our X-ray microtomography observation of very anisotropic dissolution from 150–300-μm adventitious windows [Dowker: Caries Res 2003;37:237–245], but part of this might originate from microscopic solubility inhomogeneities as observed by SEM [Shells: Arch Oral Biol 1996;41:473–484]. We have also recently used computer modelling to demonstrate a continuum from diffusion to surface reaction control in model systems. We conclude that more detailed analysis is required to understand the potential interplay between reaction and diffusion in carious dissolution of enamel.

Supported in part by Medical Research Council.

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Longitudinal Assessment of Dynamic Process of Caries Lesion with Microfocus Computed Tomography
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The objective of this preliminary study was to evaluate the suitability of μ-CT for longitudinal assessment of the caries process using a demineralization (demin)/remineralization (remin) model. A total of 12 ground and polished human enamel specimens were prepared. Specimens were chemically demineralized for 48 or 96 h. Specimens were subsequently remineralized with 1,100 ppm F as NaF for 6 or 12 days. μ-CT images of sound, demineralized and remineralized enamel were acquired (SkyScan1172, Aartselaer, Belgium). 67 μ-CT cross-sections from the middle portion of each specimen were used for the μ-CT analyses. The volumetric mineral contents (MC, μm 3 × 10 10) were determined [100–96 (Vol 100−96 , 96–91 (Vol 96−91 ), 91–87 (Vol 91−87 ), 87–82 (Vol 87−82 ), 82–73 (Vol 82−73 ), 73–0 (Vol 73−0 ) wt%]. The change in mineral content from sound (MCC, μm 3 × 10 10) was calculated as (MCC − MCC sound ), where n signifies a measurement after demin or after remin. Vol 100−96 and Vol 96−91 MC decreased through 96 h demineralization and then increased through remineralization. For Vol 87−82 , Vol 82−73 , and Vol 73−0 MC increased through 96 h de- mineralization then decreased through remineralization. Generally there were significant differences between the treatment periods for most of MC groups (p < 0.05), except between 48 h demin and 12 d remin. The MCC data also showed a similar trend. For comparison, at the end of each treatment period, three specimens were removed for TMR analysis. There was a correlation between integrated mineral loss and MCC at Vol 87−82 , Vol 82−73 , and Vol 73−0 (r = 0.68, 0.78, and 0.83, respectively). It can be concluded that the μ-CT shows promise for non-destructive longitudinal assessment of mineral content.

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The Development of Secondary Lesions in a pH-Cycling Model
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In the past years pH-cycling has proved to be a valuable method to assess the potential of new preventive products to affect demineralization. It has been observed frequently that during pH-cycling of preformed lesions a secondary lesion is formed beyond the original lesion when a fluoride product is used. Our aim was to study the development of secondary lesions in time under various conditions. 8 groups of 5 enamel specimens with preformed lesions were each pH-cycled up to 3 weeks. Specimens were treated once daily for 5 min with either a 250 ppm F solution or water. Fluoride treatments were continued for 1, 2 or 3 weeks, after which pH-cycling was either continued with water treatment or stopped. One additional group was treated daily for 5 min with 250 ppm F solution for 1 week prior to and during the 3-week pH-cycling period. During this first week specimens in this group were stored moist for the remaining time. Calcium uptake and loss were monitored by measuring the calcium concentration in solution. At the end of the experimental period specimens were prepared for transversal microradiography (TMR). Treating specimens with fluoride resulted in lower overall calcium loss values. When the treatments were stopped, daily calcium loss increased but did not reach the level of the control group. TMR data showed that secondary lesions occurred only in the fluoride groups and that their size increased with time. Formation of a secondary lesion was not inhibited by the fluoride pre-treatment of the lesions.

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Relationship between Gap Size and Secondary Caries: A Preliminary Study on Microcosm Biofilm
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The association between presence of marginal gaps and caries adjacent to restorative materials is still controversial, mainly with respect to fluoride-releasing capability of the materials. The aim of this study was to assess the relationship between various gap sizes and secondary caries for two restorative materials using a microcosm biofilm model in a constant depth film fermentor.
Dentine discs restored with resin composite (Z250) or glass ionomer (Vitremer) with gap sizes of 0, 50, 100, 180 or 250 μm were mounted on CDFF pans. Microcosm biofilm was formed in the CDFF by alternate pulsing with 10% sucrose solution (8×/day) and an artificial saliva medium for 14 days. Mineral loss and lesion depth were determined after the demineralisation period by transverse microradiography. ANOVA showed higher mineral loss and lesion depth adjacent to composite compared to glass ionomer (p < 0.001), while gap size affected neither mineral loss (p = 0.449) nor lesion depth (p = 0.328) in the model. However, Spearman correlation showed that mineral loss and lesion depth increased in relation to gap size for composite restorations (p < 0.05). Our results suggest that fluoride released from glass ionomer restorations inhibited demineralisation along the dentine surface in contact with the restoration, regardless of the marginal condition.

Supported by CAPES (BEX 0574/06-6; BEX 1482/06-8).

### Abstracts

#### 60 Microleakage and Fluoride Effect on Enamel-Dentine Demineralisation around Dental Restorations

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Besides being controversial, the association between microleakage and secondary caries could be obscured in the presence of fluoride (F) at the tooth-restoration interface. Thus, a double-blind, crossover study was carried out to evaluate in situ the effect of leakage on caries around enamel-dentine restorations in the presence of F from dental materials or dentifrice, either alone or in combination. In 4 phases of 14 days each, 14 volunteers wore palatal devices containing dental slabs restored with composite resin (CR; Z250), or resin-modified glass ionomer cement (GIC; Vitremer). Restorations were made without leakage (L–), following the recommended adhesive procedures, or with leakage (L+), in the absence of adhesive procedures. Biofilm was allowed to accumulate on the restored slabs, which were exposed extra-orally to 20% sucrose solution 10×/day. The volunteers used a non-F (NFD) or a F (FD) dentifrice 3×/day, depending on the experimental phase. After each phase, the biochemical and microbial composition of the biofilm formed was analysed and mineral loss in enamel and dentine adjacent to restorations was also assessed. Higher demineralisation around CR restorations was observed under NFD use (p < 0.05). No differences were found between L+ and L– restorations (p > 0.05). F concentration in biofilms exposed to FD or formed on GIC restoration was greater than in biofilms formed exposed to NFD or formed on CR restored slabs (p < 0.05). The results suggest that, while microleakage does not affect caries development, GIC or FD may maintain increased F levels in the biofilm and reduce caries progression.

Supported by FAPESP (Proc. 04/00412-8) and CAPES (BEX 0574/06-6).

#### 61 A Statherin-Like Peptide Reduces the Rate of Enamel Demineralisation under Artificial Caries and Erosion Conditions

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Kosoríc et al. [Int J Pept Res Ther 2007;13:497–503] demonstrated that exposing permeable hydroxyapatite slabs to solutions containing a synthetic peptide (STN21) comprising the first 21 N-terminal residues (DSpSpEKEKFLRIRGERGFGYGYGPY) of the salivary protein statherin reduced the rate of demineralisation under artificial caries conditions by about 40%. The aim of this study was to investigate the influence of STN21 on reducing the rate of demineralisation of human enamel under artificial caries and erosion conditions. STN21 was prepared using solid-state FMOC synthesis and dissolved in phosphate buffer at pH 7.4 at a concentration of 0.2 mM. Enamel pieces (2.0 × 2.0 mm, thickness 2.5 mm) were cut from caries-free human molars. The pieces were first treated with a 0.1 mM acetic acid, pH 4.5 or artificial erosion conditions (0.02 M citric acid, pH 3.8). The first treatment took place after 80 h demineralisation, and repeated thereafter every 12 h for a further 72 h. The rate of demineralisation was measured through the use of SMR. The average reduction in the rate of demineralisation of enamel following repeated STN21 treatments was 45% under artificial caries conditions, and 25% under artificial erosion conditions. Mann-Whitney tests showed a significant difference (p < 0.001) between treated and control specimens. Statherin plays an important role as an inhibitor of enamel mineral destruction. STN21 shows promise as a potential therapeutic treatment for reducing the rate of demineralisation in human enamel during an acid challenge. Supported by the Heptagon Fund (QMUL/TC03).

#### 62 A Shortened Statherin Analogue Reduces Enamel Demineralisation Rate Similarly to the Full-Length Protein

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Previous work [Kosoríc et al.: Int J Pept Res Ther 2007;13:497–503] demonstrated that exposing permeable hydroxyapatite slabs to a solution containing a synthetic peptide (STN21) comprising the first 21 N-terminal residues of the salivary protein statherin reduced the rate of mineral loss under artificial caries conditions by about 40%. The aim of this study was to test that this shortened


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analogue behaved similarly to the full-length 43 residue statherin protein (STN43) in reducing the rate of demineralisation in human enamel under artificial caries conditions. STN21 and STN43 were prepared using solid-state Fmoc synthesis and dissolved in phosphate buffer at pH 7.4 at a concentration of 0.2 mM. Enamel pieces (2.0 x 2.0 mm, thickness 2.5 mm) were cut from human caries-free third molars. The enamel pieces were varnished except for the natural surface and located in scanning microradiography (SMR) cells. Treatment consisted of rinsing the exposed surfaces with buffer, then to either STN21 or STN43 for 3 mins. For demineralisation, the pieces were exposed to artificial caries conditions (0.1 M acetic acid, pH 4.5). The first treatment took place after 80 h demineralisation, and was repeated thereafter every 12 h for a further 72 h. The rate of demineralisation was measured throughout using SMR. A Mann-Whitney test showed no significant difference between the rate of demineralisation in human enamel exposed to STN21 and STN43. STN21 inhibits the demineralisation of enamel resulting in a similar reduction in rate as the full-length STN43, suggesting that the N-terminal of statherin is responsible for the inhibition of demineralisation process by this protein.

Supported by the Heptagon Fund (QMUL/TC03).

63 Effect of Fermented Milk on Bovine Enamel Demineralization and Dental Biofilm Formed in situ
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This in situ study assessed the effect of fermented milk on enamel demineralization. Bovine enamel blocks were randomly allocated to the experimental phase, that consisted of 3 stages of 14 days each, separated by intervals of 7 days. In each phase, volunteers dripped 20% sucrose solution 8x/day on the blocks. In the controls there was no further treatment, but in the treatment groups fermented milk was dripped on the blocks after 5 min: either Brand A milk (treatment A) or Brand B milk (treatment B). After the study period, the dental biofilm was analyzed for F, Ca, and P, and alcali-soluble carbohydrate. Ion concentrations were higher for treatment B (0.093, 23.7, 8.82 μg/mg F, Ca, P, respectively) than for treatment A and controls. After treatment B alkali-soluble carbohydrate concentration (20.1 μg/mg) was lower than for treatment A and controls (38.8 and 47.2 μg/mg, respectively). The final superficial hardness (SH) and the percent change in SH (%SHC) of the enamel blocks was measured. %SHC was lower in the experimental treatment groups (A –38.6% and B –20.1%) than in the controls (~54.6%) but not significantly so. It is concluded that all treatments produced demineralisation, but treatment with one brand of fermented milk produced a higher biofilm ion concentration that could increase protection against enamel demineralization.

64 Synchrotron XMT Analysis of the Anti-Caries Potential of Sealants with Amorphous Calcium Phosphate

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The aim of this study was to analyse the anti-caries potential of sealants with amorphous calcium phosphate (ACP) using synchrotron XMT. 50 enamel blocks were selected by baseline surface hardness (SH3) (5 impressions spaced 100 μm apart, 300 and 600 μm from the edge). 10 specimens were made out of each of 5 materials: sealant + ACP; sealant + ACP + F (experimental); sealant + F; sealant with no F; resin-modified glass-ionomer. Next, the specimens attached to the enamel blocks were individually submitted to a pH-cycling model to promote demineralization [Vieira et al.: Caries Res 2005;38:514–520] and SH measured again (SH3) at 300 and 600 μm from the enamel/sealant interface. Synchrotron XMT was performed to analyze the mineral concentration profile of each lesion, the mineral concentration (gHAp cm–3) being measured every 2.8 μm from the surface to a depth of 221.2 μm, and ΔZ (mineral loss; gHAp cm–3 μm). SH3 and ΔZ were positively correlated in each group (300 μm, r = 0.97 and p = 0.160; 600 μm, r = 0.99 and p < 0.001). Groups of specimens exposed to products containing ACP, F or both had a higher mineral content in the outermost layer of enamel than those in the F-free control group. Specimens including sealant with no F and sealant + ACP had the lowest mineral concentration at the bottom of the caries lesion. It is concluded that sealants with ACP have a small effect on enamel mineral loss, but that the effect is enhanced when F is combined with ACP.

Supported by the US Department of Energy, Contract No. DE-AC02-06CH11357.

65 In vitro Hardness of Carious Enamel after Placing Two Different GIC Fissure Sealant Materials
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The aim of this in vitro study was to evaluate the hardness of naturally carious enamel fissure surfaces after placing a glass ionomer (GIC) surface protector cement with increased fluoride content, a conventional GIC and a resin fissure sealant. 56 extracted human molars with natural enamel caries were divided into two groups according to their DIAGNODent values (Group 1, 9–11; Group 2, 15–22) and inserted in acrylic blocks. Each main group was then divided into 4 subgroups according to fissure sealant placement: In Subgroups A and B, fissures were conditioned with...
20% polyacrylic acid and sealed with GC Fuji VII (Subgroup 2) or with GC Fuji IX (Subgroup B); in Subgroup C, fissures were etched with 37% orthophosphoric acid and sealed with resin fissure sealant (Fissurit, Voco); Subgroup D was untreated (control). Each group of teeth was left in artificial saliva for one month. The teeth were then sectioned and Vickers hardness (VHN) was measured on the basal and lateral walls of the fissures. The results were compared with data for non-carious enamel [Mentes and Haznedaroğlu: Caries Res 2007;41:271]. In group 1, Fuji VII and IX subgroups had significantly higher VHN than subgroups C and D (p < 0.01) and in Fuji VII subgroup, VHN of lateral walls was more affected than that of basal walls. In group 2, only Fuji VII subgroup had significantly higher VHN than control (p < 0.05). It was concluded that fluoride in GIC materials seemed to be effective in increasing the hardness of naturally demineralized enamel but to a lesser degree than that of sound fissures.

Materials provided by GC Corporation.

66 Fluoride Varnishes with Different Amount of Resin: In vitro Evaluation of Enamel Demineralization


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The effectiveness of fluoride varnishes has been demonstrated in many studies. The aim of this study was to evaluate whether the resin content of fluoride varnishes affected their anticaries action. 120 bovine enamel blocks were selected through surface hardness (SH) and randomized into 5 groups of 24: varnish without fluoride (control), 5% NaF varnish (Duraphat), 6% NaF + 6% CaF₂ varnish (Duofluorid XII), Duofluorid with increased resin content and Duofluorid with reduced resin content. 12 blocks from each group were used to measure CaF₂ formed after 6 h treatment. The remaining blocks were treated with the varnishes for 6 h and individually pH-cycled for 7 days. Surface microhardness was then re-measured and the percent change of surface hardness (%SHC) calculated. CaF₂ retained on enamel after the pH-cycling was also measured. The data were analysed using the Kruskal-Wallis test. The varnish with increased resin content showed the lowest %SHC (–25.6 ± 8.0) and the highest CaF₂ formed (16.1 ± 4.1 μg/cm²) and CaF₂ retained (3.53 ± 1.5 μg/cm²) (p < 0.05). There were no differences in %SHC between the Duraphat (–35.2 ± 6.4), Duofluorid (–36.3 ± 3.0) and Duofluorid with reduced resin (–33.4 ± 12.7) groups. Regarding CaF₂ formed and retained, there were no differences between the Duofluorid (12.0 ± 4.6 and 2.3 ± 0.7 μg/cm², respectively) and Duofluorid with reduced resin (10.4 ± 4.6 and 1.89 ± 0.3 μg/cm²) groups (p < 0.05). It was possible to improve the anticariogenic action of fluoride varnishes by increasing the resin content.

Supported by FAPESP (Grant #04/00538-1), FGM.

67 Efficacy of Fluoride Dentifrices in a De-/Remineralization Caries Cycling Model Investigated Using Multiple Interrogation Techniques

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The primary aim was to determine the effect of sodium fluoride dentifrices on promoting enamel fluoride uptake (EFU) and lesion remineralization in a caries de-/remineralization model in comparison to a marketed dentifrice. The secondary aim was to compare surface (SH) and profile hardness (ΔKHN) and enamel fluorescence (ΔF) in their assessment of remineralization. Test products were experimental NaF/silica dentifrices containing 0 ppm F – NaF(1), 650 ppm F – NaF(2), 1,426 F2O – NaF(3) or 2,700 ppm F – NaF(4). The marketed dentifrice contained 1,000 ppm as MFP and 450 ppm as NaF (NaFMPF). Lesions were formed in bovine enamel specimens by cycling for 20 days, the daily regime comprising 1 1-min dentifrice treatments (human saliva), 1 4-hour acid treatment in the lesion forming solution, and remineralization in artificial saliva. Measurements and time points: EFU – microdrill to four depths (20 days), SH – Vickers (10 and 20 days), ΔKHN – profile Knoop hardness data (20 days), ΔF – QLF (10 and 20 days). A fluoride dose-response was established for EFU at all depths. NaFMPF was less efficacious in EFU than NaF(3) (similar [F]). A fluoride dose-response was also observed for 10- and 20-day SH. NaFMPF was less efficacious in rehardening enamel lesions than NaF(3). The SH order was NaF(4) > NaF(3) > NaF(2) > NaFMPF > NaF(1). For ΔKHN, only directional differences were observed between NaF(2–4) with a poor fluoride dose-response. NaFMPF was less efficacious in promoting lesion remineralization than NaF(3), with a ΔKHN order of NaF(4) = NaF(3) = NaF(2) < NaFMPF < NaF(1). ΔF was not obtained for NaF(1), and the fluoride dose-response was poor for 10 and 20 days. NaFMPF was less efficacious in increasing lesion fluorescence than NaF(3) with a ΔF order NaF(4) = NaF(2) = NaF(3). Under the conditions of the study, 1,426 ppm F as NaF was more efficacious in remineralizing caries lesions than 1,000 ppm F(MFP) + 450 ppm F (NaF). SH was more sensitive in determining remineralization than ΔKHN and ΔF.

68 Effect of Fluoridated Toothpaste and CPP-ACP Paste on Enamel Surface Hardness after pH-Cycling

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An agent applied onto the tooth surface in the oral environment is continuously washed away by oral fluid. The aim of this study was to test the effect on enamel surface hardness of a fluoridated (F) toothpaste and a casein phosphopeptide amorphous
In vitro Remineralization after Topical Application of Calcium Phosphate or Fluoride by Synchrotron MicroCT and Microhardness


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The aim of this study was to evaluate enamel remineralization after topical application of amorphous calcium phosphate (ACP) or fluoride gel using a pH-cycling model, by synchrotron microCT (XMT) and surface hardness (SH). Early carious lesions were produced in bovine enamel blocks selected by surface hardness. The blocks were treated once with a placebo gel 1 min, ACP for 3 min, fluoride gel (neutral NaF 2%) for 1 min, fluoride gel for 1 min + ACP for 3 min, ACP for 3 h and ACP for 8 h. The blocks were pH-cycled for 6 days at 37°C to evaluate treatment effect on enamel remineralization [Vieira et al.: Caries Res 2005;39:514–520]. SH was measured and synchrotron XMT was used to analyze the profile of mineral concentration in the lesion, the mineral concentration (g HAp cm⁻³) being calculated every 2.8 µm, from the surface to a depth of 221.2 µm, and mineral recovery (−ΔZ; g HAp cm⁻³). ANOVA was used to test differences between treatments (α = 0.05). Fluoride and ACP produced a significantly higher mineral concentration in the outermost 2.8 µm layer of enamel than the other treatments. ACP for 3 or 8 h, or fluoride + ACP showed different responses. The XMT analyses of lesions treated with ACP for 3 and 8 h showed two layers of reduced mineral concentration. Lesions treated with placebo, with ACP for 3 and 8 h showed similar results for SH and −ΔZ (p > 0.05); better values were achieved after fluoride gel application (p < 0.05). SH and ΔZ were significantly correlated (Pearson’s r = –0.86; p = 0.027). It is concluded that synchrotron XMT can show differences in remineralization profile for different treatment groups, although −ΔZ did not do so as well as hardness analysis.

Supported by U. S. Department of Energy, Office of Basic Energy Sciences, Contract No. DE-AC02-06CH11357.
Correlation between Remineralization and Recrystallization of the Early Caries Lesion by Phosphoryl Oligosaccharide Calcium

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Phosphoryl oligosaccharides of calcium (POs-Ca) prepared from potato starch have been demonstrated to efficiently enhance remineralization of early dental caries. POs-Ca is highly soluble in water, even at neutral pH in the presence of inorganic phosphate, and carries both calcium and phosphate ions to the lesion. The aim of this study was to assess the correlation between the change in the quantity of minerals, using transversal microradiography (TMR), and that of hydroxyapatite (HAp), using electron microbeam X-ray diffraction. Sub-surface lesions created in bovine enamel using an acid gel system were treated at 37°C by mineral solution, pH 6.5, containing 3.6 mM phosphate and 6 mM calcium as either POs-Ca or CaCl\textsubscript{2}. After 24 h, thin enamel sections were prepared and changes in the mineral volume measured by TMR. The rates of remineralization were 45.3% with POs-Ca and 8.9% by CaCl\textsubscript{2} (mineral loss) and 29.7% by POs-Ca and 8.3% by CaCl\textsubscript{2} (lesion depth). In the same specimens the crystal volume as HAp was analyzed by electron microbeam X-ray diffraction. The solution containing POs-Ca significantly increased the quantities of mineral and crystal as HAp, whereas the solution containing calcium chloride did not. The profiles of mineral and crystal volumes were quite similar. The same enamel sections were examined by transmission electron microscopy. With POs-Ca images of crystal growth with the same orientation as the original crystals were observed. We concluded that POs-Ca enhanced not only remineralization but also recrystallization of early dental caries lesions.

Remineralization Potential of a Novel Chewing Gum

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The aim was to evaluate the in vitro pH and calcium ion release from a novel chewing gum that contains calcium sodium phosphosilicate (NovaMin). Multi-layer compressed chewing gums were made using patented technology to contain 4 wt% NovaMin. Gums were placed in a chewing machine with 20 ml of phosphate-buffered artificial saliva (pH 7.00, 37°C) and chewed for a total of 20 min. The buffer solution was recovered and replaced after 2, 5, 10, and 20 min. Recovered solutions were filtered through 0.2-μm membranes and analyzed for changes in pH and calcium ion concentration (inductively coupled plasma mass spectrometry). 16 NovaMin-containing and 16 placebo gums were analyzed. Data were statistically analyzed using t-test; pH of solutions after chewing for 2, 5, 10, 20 min (mean ± SEM; n = 16) were, for NovaMin gums: 8.50 ± 0.08, 9.04 ± 0.18, 9.15 ± 0.12, 9.35 ± 0.03 and for placebo gums: 7.00 ± 0.00, 7.00 ± 0.00, 7.00 ± 0.00, 7.00 ± 0.00. Cumulative calcium ion release from NovaMin gums at 2, 5, 10, 20 min (ppm in solution): 20.2 ± 0.78, 40.0 ± 1.75, 46.0 ± 1.35, 53.2 ± 5.50 and from placebo gums: 1.44 ± 0.02, 3.79 ± 0.32, 5.62 ± 0.05, 6.35 ± 0.32. At all time points, NovaMin gums produced significantly higher pH and calcium concentration than placebo gums (p < 0.01). In conclusion, preliminary in vitro data indicate that when chewed, NovaMin-containing compressed gums trigger a modest pH rise and release calcium ions into surrounding buffered aqueous solution. In the mouth, a pH rise and calcium ion supplementation may increase the remineralization potential of human saliva. Clinical research is needed to fully elucidate the remineralization benefits.

The Remineralization Effect of Dentifrice Containing Bamboo Salt

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The aim of this study was to evaluate the remineralization effect of bamboo salt, which has been used as a traditional remedy in Korea, in dentifrice. Incipient artificial caries lesions of bovine enamel were formed in pH 5.0 lactate carbopol buffer system for 48 h. Then specimens were allocated to 3 groups of 7 without statistical difference in Vickers hardness number (VHN). Experimental groups were: negative control (fluoride free); positive control (NaF 1,100 ppm, P & G Crest Cavity Protection) and test dentifrice (3.0% bamboo salt with NaF 1,000 ppm). The specimens were pH cycled for 20 days: specimens were immersed in dentifrice slurry for 2 min 3 ×/day, demineralized for 4 h and placed in mixed saliva for the remaining hours. After pH cycling, VHN change and integrated mineral loss (transversal microradiography) were assessed. VHN showed a significantly greater increase in test dentifrice (160 ± 17 ΔVHN) and positive control (138 ± 28) than in the negative control (26 ± 7) (p < 0.01). The integrated mineral loss showed a significantly greater decrease in test dentifrice (1,187 ppm) and positive control (1,443 ppm) than in the negative control (2,001 ppm) (p < 0.05). Also, the lesion depth by TMR showed a significantly greater decrease in test dentifrice (45 ± 12 μm) than in the negative control (70 ± 13 μm) and positive control (59 ± 12 μm) (p < 0.01). The dentifrice containing bamboo salt showed a remineralization effect; so it could be used as an effective remineralization agent for prevention of dental caries.
Infrared laser irradiation is extensively investigated for reducing enamel demineralization, but the mechanisms of laser interaction with dental enamel are still unknown. This study aimed to identify the changes in crystalline structure of enamel after irradiation with high-intensity lasers causing physical modifications in enamel, intended to prevent caries. 15 enamel slabs from sound deciduous anterior teeth of human and bovine teeth were randomly divided into 3 groups: 1, untreated; 2, irradiated with Er:Cr:YSGG laser (λ = 2.79 μm, 140 μs pulse width, repetition rate 20 Hz) at 2.8 J/cm²; 3, irradiated with Nd:YAG laser (λ = 1.064 μm, 100 μs pulse width, repetition rate 10 Hz) at 85 J/cm². The crystalline structure of enamel was evaluated by X-ray diffraction using a synchrotron monochromatic X-ray beam. Wavelength 0.0954 nm. Peaks were indexed using the International Centre for Diffraction Database. The X-ray diffraction pattern of irradiated enamel showed peaks similar to those observed in non-irradiated enamel. However, 6 new peaks of crystallographic phases for the Er:Cr:YSGG- and 4 for the Nd:YAG-irradiated enamel were identified, indicating that irradiated enamel contains tetracalcium phosphate and also α- and β-tricalcium phosphates as well as the original hydroxyapatite. The decreased solubility of the irradiated enamel may be associated with the changed crystal size as well as the carbonate loss. It is concluded that high intensity infrared laser irradiation of enamel promotes the formation of new crystallographic phases mixed with the hydroxyapatite, which may contribute to an improved overall resistance of irradiated enamel to demineralization.

Supported by FAPESP (Proc. 04/02229-6), CNPq (Proc. 155445/2006-5) and PROCAD/CAPES (Proc. 0349054).

Session 5

Diagnostics/Epidemiology

Impact of Scoring Single or Multiple Occlusal Lesions by ICDAS-II on Estimates of Diagnostic Accuracy

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Carious lesions can occur at different discrete sites on the occlusal surfaces of posterior teeth and can differ in appearance and severity. In assessing diagnostic accuracy, the presence of multiple lesions on an occlusal surface may cause problems for evaluating individual lesions and the group of lesions are not statistically independent. This study aimed to evaluate how estimates of reproducibility and accuracy of ICDAS-II were affected by whether all lesions on occlusal surfaces, or only a representative lesion, are scored. 100 unrestored permanent posterior teeth were selected and all sites (1–4) within the pit and fissure system of each tooth were investigated by four examiners (total sites 181) using the ICDAS-II criteria. The teeth were re-examined after 3 weeks. Then the teeth were serially sectioned and assessed for lesion depth (Downer classification). Weighted kappa, specificity and sensitivity were calculated for all investigation sites. Additionally, one investigation site per tooth was randomly selected to represent each surface. To test whether specificity and sensitivity differed systematically between the two sets of data, tests of equivalence were performed for each examiner. For all lesions inter- and intra-examiner k were 0.61–0.83, specificity and sensitivity (D₃ threshold) were 0.73–0.84 and 0.54–0.69 respectively and at D₄ threshold 0.81–0.91 and 0.51–0.79. For the single representative lesions inter- and intra-examiner k were 0.62–0.83, specificity and sensitivity (D₄ threshold) were 0.74–0.91 and 0.59–0.73 respectively and at D₃ threshold 0.82–0.94 and 0.48–0.83. Assuming a range of equivalence of [–0.20; 0.20] in each of the 16 tests (for sensitivity and specificity) equivalence could be shown (p < 0.05). This may support the view that dentists can be site specific in applying ICDAS-II criteria as a result of their education and clinical experience.

Methodology and Potential of the Caries Progression Index (CP Index) for Quantitative Caries Extension Measurement

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For assessment of caries extension in histological slices the semi-quantitative indices of Marthaler [Helv Odontol Acta 1966;10:1–18] and Ekstrand et al. [Caries Res 1998;32:247–254] are commonly used. To improve the precision of histological caries quantification the Caries Progression Index (CP Index) was developed. In calculating the CP Index, the first step differentiates between caries-free slices/sections (score 0), demineralisations in enamel (base value = 0.5, y) or dentine (base value = 1.5, y), and lesions reaching the pulp (score 2). For enamel/dentine lesions, the base value has to be combined with the ratio of the caries extension into enamel/dentine (x) to the enamel/dentine overall thickness (y) as a second step. The aim of this pilot study was to assess the potential of the CP Index. 65 third molars with no apparent occlusal cavitations were selected and visually scored according to the Universal Visual Scoring System [UniVisS; Kühnisch et al.: Caries Res 2007;41:289]. After sectioning the teeth, histological validation was performed using a stereomicroscope. There was excellent agreement of the histo-
logical findings between the CP Index and the Marthaler and Ekstrand criteria. While the mean values of the CP Index correlated with the UniViSS scores 1F, 1E, 2F, 2E and 3F in enamel lesions, dentine involvement was clearly registered for scores 4E, 2M, 4M, 1D, 2D and 3D. Brown discolorations (3E) showed a mean value of 1.2 (0.3 SD) close to the enamel-dentin junction. This pilot study underlines the potential of the new CP Index to measure caries extension quantitatively and to calculate for each possible diagnostic score mean, standard deviation, minimum and maximum etc. Furthermore, this index gives a good image of the caries progression.

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Do Caries Lesions on Upper Incisors Justify the Prescription of Bitewing Radiographs in 15-Year-Olds?

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Up to 90% of all approximal caries lesions [Poorterman et al.: Caries Res 2000;34:159–163] and up to 50% of all occlusal dentin lesions [Weerheijm et al.: ASDC J Dent Child 1992;59:408–412] were detected during clinical examination. Therefore, bitewing radiographs (BR) have been recommended as an additional diagnostic method to detect enamel and dentin lesions on interproximal surfaces and dentin lesions on occlusal surfaces. Bearing in mind the potential biological risk of ionizing radiation the prescription of BR should be objectified. Our study aimed at correlating easily detectable clinical predictors with the corresponding caries lesions in premolars and molars scored on BR. 143 15-year-olds were involved in the study. The clinical examination revealed a mean (± SD) DMFS of 6.3 ± 8.6; the clinical-radiographically detectable DMFS value was 8.0 ± 9.3. The increased value was attributable to an increased D-component. The presence of ≥1 non-cavitated caries lesion (D1–2 level) and the presence of ≥1 cavitated caries lesion (D3–4 level) on upper incisors were used as clinical predictors for approximal and occlusal enamel and dentin lesion in premolars and molars. Following odds ratios between the chosen clinical predictor and the event of possible caries lesions on BR were found: D1–2/incisors-BR/premolars >3.4; D1–2/incisors-BR/molars >2.1; D3–4/incisors-BR/premolars >7.1; and D3–4/incisors-BR/molars >4.5. The registered odds ratios suggest that the indication of bitewing radiographs in 15-year-olds suffering from caries on upper incisors is justified.

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Comparison of Digital Camera Method and Quantitative Microradiography for Evaluating in vitro Artificial Lesions

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t Using standard color scale and digital image analysis technique, in vivo white spot (WS) enamel lesions have been evaluated [Caries Res 2007;41:321]. The aim of this study was to compare mineral change values by quantitative microradiography with gray values by digital camera method. 21 human enamel specimens were demineralized (0.1 M lactic acid, 3.0 mM Ca, 1.8 mM P, pH 4.5) for 3 days. With a digital camera (Nikon D100, Japan), images of demineralized lesions were captured after thorough drying at room temperature. WS lesions were well demarcated. Specimens were sectioned, microradiographed and evaluated by integrated mineral loss (ΔZ) and lesion depth (LD). 8-bit RGB data were used to characterize the tooth color. The image analysis software was set to calculate the mean gray level and histogram data. Gray levels are expressed on a scale of 0–255 (0 = black, 255 = white). Integrated mineral loss was linearly related to gray values. Pearson’s correlation coefficients were similar but were slightly higher for LD (r = 0.474, p < 0.05) than for ΔZ (r = 0.442, p < 0.05). In conclusion, it seems that gray value by digital camera method may be appropriate for monitoring and assessing mineral changes in WS lesions without sample destruction. Reproducibility and reliability of assessment of changes in mineral of WS lesions by the digital camera method must be considered in future studies.

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Diagnostic Accuracy of an Optimised AC Impedance Device to Aid Caries Detection and Monitoring

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Accurate caries detection and an ability to monitor initial lesions over time are both important in modern clinical caries management. Traditional detection methods have recognised limitations in sensitivity, but enjoy high specificity, while newer methods typically trade-off decreased specificity for improved sensitivity. The aim of this work was to balance both components of diagnostic accuracy in an AC impedance device in order to be able to inform the dental team and patients. Building on earlier work [Los et al.: Caries Res 2007;41:296], a device certified to international standards was developed with reference to repeatability data from an in vivo study of 304 measures and with sensors adapted to occlusal and free smooth surfaces in the first instance.
AC impedance measurements were undertaken by 4 dentists on 137 extracted teeth (80% humidity, ~ body temperature). Reference evaluations were a consensus of at least 3 dentists using micro-CT and magnified visual techniques. Results were presented in 3 groups: Green (G) – Sound (n = 76), Yellow (Y) (n = 355) – caries where preventive care is advised and Red (R) (n = 115) – caries where operative care is advised. Using a probability map method (which takes into account the prevalence in each ‘zone’, for occlusal surfaces: sensitivity (R vs. G + Y) = 92.5%; specificity (G vs. Y + R) = 92.5%; accuracy for Y lesions alone = 79.4%. For smooth surfaces: sensitivity (R vs. G + Y) = 92.5%; specificity (G vs. Y + R) = 92.5%; accuracy for Y lesions alone = 80.5%. It is concluded that an AC impedance device can be optimized to aid caries detection and monitoring by displaying results with symmetrically high values for both sensitivity and specificity.

Supported by IDMoS Dental Systems.

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**Evaluation of Demineralization and Remineralization of Root Surface Incipient Lesion in vitro**

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This study aimed to quantify remineralization of root surface lesions in vitro. 64 specimens cut from extracted human tooth roots were demineralized in a microbial caries model [Fontana et al.: Caries Res 1994; 28: 315–321] for a month, with additional treatments as follows: Group 1, no fluoride (F); Group 2, treated twice daily with 1,100 ppm F; Group 3, treated twice daily with 1,100 ppm F and also with Bifluoride 12 F varnish weekly. Specimens were evaluated by QLF, ultrasound, laser fluorescence (LF) and CLSM at baseline and after demineralization and remineralization; changes were tested by Wilcoxon and Student t test (α = 0.05). With CLSM, there were no significant reductions in lesion depth (initial lesion depth 71.8 ± 27.2 μm; Group 1, 69.3 ± 30.4 μm; Group 2, 68.6 ± 12.4 μm; Group 3, 67.0 ± 28.5 μm). QLF differentiated between sound (ΔF 26.8 ± 12.5 ΔQ 9.9 ± 4.1) and demineralized enamel (38.4 ± 10.8 ΔQ 15.5 ± 3.9) (p < 0.01), and between demineralized enamel and Groups 2 (ΔF 31.7 ± 7.7 ΔQ 11.6 ± 4.3) and 3 (ΔF 28.3 ± 9.5 ΔQ 10.8 ± 3.5). There was a significant difference in LF between sound (8 ± 4) and demineralized enamel (30 ± 3) (p < 0.01), but there were no significant reductions after any of the remineralization treatments (Group 1, 29 ± 3; Group 2, 28 ± 2; Group 3, 27 ± 3). Ultrasound detected no significant differences between sound, demineralized and remineralized enamel. It is concluded that remineralization of root surface lesions could be detected and quantified by QLF in vitro. Bifluoride 12 varnish seems to enhance remineralization with 1,100 ppm fluoride.

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**Hyperspectral Near-Infrared Imaging for Caries Detection**

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Near-infrared (NIR) has a number of advantages for caries detection compared to visible light imaging. One of the most important confounding factors in caries diagnosis is stain. NIR wavelengths exhibit low absorption by stain and penetrate deeper in teeth. In addition, analysis of the reflectance spectrum allows differentiation between water-filled porosity and sound enamel and dentine. The aim of this study was to investigate the utility of NIR spectral imaging for the detection and measurement of dental caries. Diffuse reflectance measurements have been taken from a total of 12 extracted teeth (premolars and molars) with lesions of different extent by means of a hyperspectral camera. The wavelength range studied was from 1,000 to 2,500 nm and characteristic curves for sound, white spots (enamel lesions) and dentine lesions have been investigated. We will describe an image processing algorithm based on the selection of appropriate wavelengths that identifies the degree of the lesion. Teeth were ground for histological examination after the measurements. The data obtained with NIR imaging method correlated significantly (Pearson’s r = 0.89; p < 0.01) with the corresponding histological section score. Results yielded a sensitivity of 75% and a specificity of 87.5% for enamel lesions and a sensitivity of 87.5% and a specificity of 100% for dentine lesions. The nature of the technique offers a number of advantages including the ability to map the lesion distribution rather than obtaining single-point measurements, it is also non-invasive, non-contact and stain-insensitive. These results suggest that NIR spectral imaging is a potential clinical technique for quantitative caries diagnosis and can determine the presence of occlusal enamel and dentine lesions.

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**Comparison of Visual and Photographic Methods for the Surveillance of Dental Fluorosis**


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This study was part of a larger investigation that aimed at developing objective methods for fluorosis diagnosis. It assessed whether digital photography may be a valid, reliable and feasible technique for fluorosis diagnosis. 123 previously-consented children were examined for dental fluorosis by a calibrated investigator using the Tooth Surface Index of Fluorosis (TSIF). They were also photographed (facial view) using an extraoral camera system (Fuji Finepix SIPro/Micro Nikkor 105-mm lens/
Nikon SB 21 ring-flash), under field conditions, following a standardized protocol. Children were assigned as TSIF 1–5 cases according to the highest score received for anterior teeth. Images were later scored by a second calibrated examiner and were also assigned as TSIF cases. Visual examination scores were compared to the scores given to photographs percentage agreement and kappa values were calculated. 47.0, 33.3, 7.3, 8.3 and 4.1% of the children were diagnosed in vivo as TSIF 0, 1, 2, 3, or 4/5 cases, respectively, while 41.5%, 39.4%, 8.1%, 7.1% and 4.1% were similarly diagnosed using the photographs. When results of examinations were compared, weighted kappa was 0.73, showing a substantial agreement between original fluorosis scores and those from photographic scoring. The observed agreement demonstrated that, for surveillance purposes, it is feasible to obtain photographs on the field, which can be later scored by calibrated examiners.

Supported by NIDCR grant R21DE016034-02.

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Comparison of Visual and Photographic Methods for the Assessment of Dental Fluorosis
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This study is part of a larger investigation that aims to develop objective methods for diagnosis of dental fluorosis. It aimed to compare the use of intraoral digital images with visual examination for fluorosis and to assess if they may be valid, reliable and feasible for the surveillance of fluorosis. 61 consented children received an oral exam and fluorosis was assessed using the Tooth Surface Index of Fluorosis (TSIF) by a calibrated investigator. Tooth surfaces were photographed using a Suni USB digital intraoral wand type video camera (SUNI Medical Imaging, San Jose, CA); 1,280 × 960 pixel images were obtained. Children were classified as cases (TSIF 0 to 3) according to the highest score received clinically. Digital images were blindly scored by a second calibrated investigator and results obtained were compared with visual scores using kappa values. Visually, 33% of the children were diagnosed as sound; 38% were TSIF 1 and 29% were TSIF 2–3. Using digital images these proportions were 47%, 36% and 16% respectively. When results of the 2 methods were compared, weighted kappa was 0.62 for visual diagnosis versus intraoral digital images. For mild fluorosis, the use of digital images tended to over-score when compared to visual exams, while for the moderate cases it tended to under-score. Intraoral digital images appear to be moderately useful for the detection of dental fluorosis.

Supported by NIDCR grant R21DE016034-02.

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Differentiation of Demarcated Enamel Opacities from Fluorosis Using Quantitative Light-Induced Fluorescence
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The clinical appearance of demarcated enamel opacities can be confused with fluorosis. This phenomenon is further confirmed by diagnostic criteria of fluorosis indices, for example ‘questionable’ fluorosis using Dean’s Index. This could have an impact on the prevalence of fluorosis at relatively low levels of fluoride exposure when a sensitive index requiring a diagnosis of fluorosis (e.g. the Thylstrup-Fejerskov Index or Tooth Surface Index of Fluorosis) is employed. It has been observed that the appearance of demarcated enamel opacities differs from that of fluorosis when viewed under Quantitative Light-Induced Fluorescence (QLF). A convenience sample of 150 images was selected from a fluorosis prevalence study. The clinical images were separated into normal, fluorosis, and demarcated opacities. 38 images were deemed to have a normal appearance, 64 images demonstrated fluorosis, 42 images demonstrated demarcated opacities, and 6 images were deemed to show fluorosis and demarcated opacities. 24 (57%) of the images with demarcated opacities showed an increase in the fluorescence signal relative to ‘sound’ enamel in QLF images, 12 (29%) demonstrated a decrease in fluorescence signal, and 6 (14%) contained areas that demonstrated both increases and decreases in fluorescence signal. Of the 6 images showing both fluorosis and demarcated opacities, 5 revealed an increase in the fluorescence signal in the region of the demarcated opacity. Where decreases in fluorescence were observed, lesions were well circumscribed in contrast with the appearance of fluorosis. The results suggest that QLF may be useful as a tool to differentiate fluorosis from non-fluorotic opacities. Histological investigation of opacities may provide information for the different signal changes observed and thus the aetiology of the lesion.
PRS measurements were acquired from a total of 13 approximal surfaces: 16 measurements from unstained regions of sound enamel, 29 from stained regions and 9 from white spot lesions. From these measurements, mean Raman depolarization ratios were calculated from the areas under the main hydroxyapatite peak (~959 cm⁻¹) with parallel- and cross-polarization. The mean depolarization ratios and standard deviation values were 0.089 ± 0.059 for stained sound enamel, 0.046 ± 0.022 for unstained sound enamel and 0.155 ± 0.020 for white spot lesions. As observed from our previous studies on unstained specimens, the depolarization ratio was consistently higher for carious enamel compared to sound enamel with mean differences that were statistically significant. Post hoc ANOVA, a Tukey HSD test to compare the mean depolarization ratios among the three groups, showed that the values were significantly different (p ≤ 0.01). Very dark regions of stain could not be measured with PRS since background fluorescence was too intense. Since depolarization ratios of sound enamel even in the presence of moderate stain are lower than depolarization ratios for white spot lesions, this preliminary study demonstrates that Raman spectroscopic measurements of stained enamel are statistically different from those of white spot lesions and are not misinterpreted as false-positives for caries.

Supported by the Canadian Institutes of Health Research Institute for Musculoskeletal Health and Arthritis and the National Institutes of Health National Institute of Dental and Craniofacial Research.

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Investigating Regions of Enamel Hypocalcification Using Optical Coherence Tomography and Polarized Raman Spectroscopy
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Optical coherence tomography (OCT) and polarized Raman spectroscopy (PRS) are being developed as potential clinical tools for incipient caries detection. The aim of this preliminary study was to investigate how regions of hypocalcification might affect early caries detection using these optical methods. The approximal enamel surfaces of four extracted human premolar teeth were clinically examined to identify regions of hypocalcification, demineralization and sound enamel. From these various regions, triplicate OCT images at 850 nm were obtained. In addition, 31 independent PRS measurements were collected with the following distribution: 13 spectra from hypocalcified regions, 9 spectra from demineralized enamel and 9 spectra from sound enamel. The OCT images showed that regions of hypocalcification have slightly higher light back-scattering intensity at the surface compared to sound enamel. However, the intensity is confined to the surface region and rapidly decays with depth. In contrast, OCT images of demineralized enamel displayed characteristic back-scattering intensity with increased depth into the enamel, as observed in our previous studies. The mean (± SD) Raman depolarization ratios calculated from the hydroxyapatite ~959 cm⁻¹ phosphate peak were 0.12 ± 0.03 for hypocalcified enamel, 0.15 ± 0.02 for demineralized enamel and 0.07 ± 0.02 for sound enamel. Using one-way ANOVA, followed by Tukey HSD post-hoc comparison, the mean depolarization ratios among the three groups were found to be significantly different with p < 0.001 in all cases except p < 0.004 between demineralized and hypocalcified enamel. Therefore, this initial study indicates that regions of hypocalcification, which visually may be mistaken as white spot lesions, are statistically different from sound and carious enamel regions when examined with the optical methods of OCT and PRS.

Supported by the Canadian Institutes of Health Research and the US National Institutes of Health.

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A Luminescent Calcium-Ion Binder in the Assessment of Dental Erosion Risk/Susceptibility – An in vitro Feasibility Study
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Assessment of susceptibility of individuals to dental erosion is important in clinical treatment-decision making. Currently, there are few sources of objective data to help clinicians in determining the risk of individual patients experiencing dental erosion. During the erosive process calcium and other ions are released. The aim of this initial study was to assess, in vitro, the feasibility of using a luminescent calcium-ion-binding agent as a marker for surface changes due to release of calcium ions after erosive attacks on enamel (patent pending). 116 extracted human molar and premolar teeth were exposed to a variety of erosive challenges. After stopping the challenge by rinsing with deionised water the surfaces were photographed in white light and then immediately after a luminescent calcium-binding agent was placed on the enamel. The intensity of the luminescence signal varied with the nature (pH, inclusion of saliva) of the erosive challenge. Unexposed enamel produced significantly less luminescence signal than exposed enamel. For example, treatment with gel or solutions below pH 5 led to significantly (t test p < 0.01) more light output than when using materials of above pH 5: gel pH 4.7, mean 64 ± 13 SD (n = 10); gel pH 6.4, 22 ± 5 (n = 10); solution pH 3.4, 45 ± 10 (n = 6); pH 7.2, 25 ± 9 (n = 5). The results indicate that the luminescent calcium-ion-binding agent can identify surface changes due to release of calcium ions from enamel after an erosive attack. Thus it may be possible to develop an assay technique, based on this agent, to quantify an individual’s susceptibility to enamel erosion. In conclusion, the possible use of a luminescent calcium-ion-binding agent for assessing erosive risk/susceptibility is worthy of further investigation.
Caries prevalence in Western countries has declined since the 1970s, so the distribution of caries indices (DMFT/S) became positively skewed. These changes have had the effect of increasing the proportion of zeros in the distribution of dental caries indices. In the literature several models are applied to describe the nature of the index distribution and applied in multiple regression techniques to estimate the covariates of caries disease. The aim of this paper was to determine the best model to estimate the proportion of caries-free and the dependence of dmfs index to the influence of childhood socio-demographic factors (SDF). The data set from the first National Pathfinder survey of 4-year-old Children’s Oral Health in Italy was used. Negative binomial distribution (NBD) and Poisson distribution (PD), as the most common models for dental caries, were compared to the recent related zero-inflated models (ZIP and ZINB). NBD proved to be more appropriate for analyzing the distribution of caries data in children than PD (p < 0.001). Similarly, ZINB goodness-of-fit was better (p < 0.001) than that of ZIP, that of ZIP better than that of PD and, finally, ZINB was better than NBD. The significant background variables in bivariate analysis (parent’s nationality and educational level, pre-term birth, breastfeeding and age of tooth eruption) were considered as predictors for dmfs in the ZINB regression model. Children from parents with high educational level (mother or father) or from a father of Italian nationality had higher probability of being caries free (0.82 vs. 0.73, 0.83 vs. 0.74, 0.81 vs. 0.61, respectively). Investigation of caries distribution via new models might be useful to provide new insight into caries patterns.

Molar-Incisor Hypomineralisation among Children Living in Slovenia

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The aim of this study was to determine the prevalence and possible aetiological factors for molar incisor hypomineralisation (MIH) among Slovenian children. 478 children, aged 6–11.5 years (mean age 9.1), with all first permanent molars (FPM) present, attending 3 schools from different parts of Slovenia, were examined in a dental chair by one calibrated dentist. Developmental defects of enamel were assessed using the modified Developmental Defects of Enamel Index on buccal, occlusal, and lingual surfaces of FPM and buccal surfaces of incisors. Demarcated opacities, hypoplasia, post-eruptive enamel breakdown, and atypical fillings were recorded. Questionnaires completed by the parents provided information on mother and child risk factors which can result in MIH (e.g. demographics, health and smoking by the mother during pregnancy, gestational age, birth weight, duration of breast-feeding, child’s health during its first 5 years, medication usage, and fluoride exposure). 9.4% of FPM were affected, 7.6% had demarcated opacities and 0.8% hypoplasia and/or post-eruptive enamel breakdown and/or atypical fillings (other defects). 2.7% had more than one surface affected, and 1.1% had defects on more than one third of the surface. 6.4% of incisors were affected, 4.8% had demarcated opacities, and 1.8% had other defects. 0.7% had defects on more than one third of the surface. 21.6% of children had at least one FPM affected, 18.2% had demarcated opacities, and 2.7% had other defects. The mean number of affected FPM per affected child was 1.75 ± 0.94. Among 308 children with all permanent incisors erupted, 10.4% had at least one FPM and incisor affected. No significant differences were found between groups of children with and without MIH for possible aetiological factors.

Supported by Slovenian Ministry of Science and Education (No. J3-8713-0381-99).

Distribution of Tooth Erosion in Adolescents

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The objective of this study was to investigate the distribution of tooth erosion in young adolescents. The study sample was selected from a regular attenders at a pediatric dental clinic and consisted of 622 adolescents (51% boys, 49% girls, mean age 13.4 ± 0.9 SD). Tooth erosion was recorded on all accessible tooth surfaces of the permanent dentition, according to the index of van Rijkom et al. [Caries Res 2002;36:147–154]. All clinical examinations were done by one examiner, who received training and was calibrated by an experienced examiner. Duplicate measurements were conducted in 11% of the participants. The kappa for intra-examiner agreement was 0.76. The distribution of erosion showed a predominance on occlusal surfaces of first molars, especially the lower ones, and on palatal surfaces of upper anterior teeth. The prevalence of erosion on the upper first molars was 13.9%, on lower first molars 27.4% and on upper anterior teeth 3.2%. To examine palatal surfaces of upper anterior teeth and occlusal surfaces of first molars more closely, these surfaces were subdivided into areas. For upper anterior teeth with erosion, the percentage of affected cervical one-third of the palatal surface was 88.5% and 96.7% for the remaining part of this surface. In first molars, cusps tops were more frequently affected than cusp slopes. In upper molars with erosion, the proportion of affected cusp slopes was 16.3 versus 46.0% for cusp tops. For lower molars these proportions were 9.2 versus 37.2%. Of the cusp tops the mesio-buccal cusp top was most frequently affected.

Protocol of study approved by the research ethics committee of Radboud University (CEOM: No 2003/207).
The Reproducibility of a New Tooth Wear Index Tested on a Cohort of University Students Aged 18–30

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In this study the reproducibility of a modified tooth wear index (MTWI) was tested on a cohort of 1,010 university students. Previous indices combine the grading of enamel and dentine within the same index. However, dentine exposure occurs following enamel loss and its extent and spread is not incorporated into other indices. MTWI graded enamel and dentine wear separately. Enamel loss was graded from 0 over 4 levels: <10%; <1/3; 1/3 to 2/3 and >2/3. Exposed dentine was separately graded on 5 levels with an additional level to that in enamel and representing pulpal exposure. The 1,010 college students were aged 18–30 years with a mean age of 21.9 (1 SD) with 707 females and 303 males. Three previously calibrated and trained examiners measured tooth wear using the Smith and Knight Index. Grading was performed under optimal lighting in a dental chair. Wear was graded on the cervical, buccal, occlusal/incisal and palatal/lingual surfaces from first molar to first molar in the upper and lower arches. Restorations covering >25% of the tooth surface and missing teeth were recorded separately. 50 subjects were randomly selected for intra-examiner reproducibility and a kappa 0.84 was observed in enamel and 0.95 for dentine. Inter-examiner kappa scores were performed on a separate group of 50 subjects and the results varied between 0.95 to 0.85 for enamel and 0.9 to 0.95 for dentine. The new index appears to discriminate enamel and dentine wear more accurately than previous indices.

Supported by Sensodyne, GSK.

The Prevalence of Tooth Wear in a Cohort of University Students Aged 18–30

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In this study the prevalence of tooth wear in a cohort of university students was investigated with a modified tooth wear index (MTWI). Following ethical approval, 1,010 (707 female and 303 male) students aged 18–30 and attending a campus-based college were recruited. Three previously calibrated and trained examiners measured tooth wear based on the Smith and Knight Index. Grading was performed under optimal lighting in a dental chair. Wear was graded on the cervical, buccal, occlusal/incisal and palatal/lingual surfaces from first molar to first molar in the upper and lower arches. Wear was graded separately on enamel and dentine using MTWI. Enamel was graded from 0–4, ranging from no wear to enamel loss involving greater than 2/3 of the surface. Dentine was graded from 0 to 5 with an additional level (compared to the Smith and Knight scale) involving pulpal exposure. Restorations covering >25% of the tooth surface and missing teeth were recorded separately. The mean age of the subjects was 21.9 (SD 1) and a total of 946 teeth were missing (mean 1 tooth/subject). The total number of restored surfaces was 2,033 (mean 2/subject). Enamel and dentine wear were observed most commonly on the lower central incisor and the most common surface was the palatal/lingual (36.1% score >2). Enamel wear was observed on 96.8% of surfaces, whereas dentine was exposed on 2.71% of tooth surfaces. In conclusion, enamel wear was common in this age group whilst dentine exposure was relatively uncommon. The new index provides an opportunity to measure enamel wear in a large population.

Supported by Sensodyne, GSK.

Contamination of Toothpaste Tubes by Streptococcus mutans

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Few studies have been carried out to test the presence of Streptococcus mutans on toothpaste tubes. The aim of the study was to examine the transmission potential of S. mutans through toothpaste tubes used by individuals with high salivary concentrations of these microorganisms. 40 toothpaste tubes (Colgate Protection Caries, Colgate-Palmolive) used during 3 weeks by subjects who were previously diagnosed, using Dentocult SM Strip mutans test (Orion Diagnostica, Espoo/Finland) as high S. mutans carriers (≥105–6 CFU/ml), were assessed for contamination 6–8 h after the last use of the dentifrice. Afterwards, every toothpaste tube orifice area was sectioned under sterile conditions from the tube and incubated in a thioglycollate culture medium for 24 h at 37°C. Colonies were then plated onto selective media for S. mutans (TYCSB agar) for 48 h at 37°C. The results showed low tube contamination by S. mutans; the bacterium was present in only 3 toothpaste tubes (7.5%). This finding shows that contamination of this brand of toothpaste tube does not represent a favorable transmission route for S. mutans. Factors such as toothpaste properties that can inhibit the growth of certain microorganisms could be involved. In spite of the fact that these results cannot be assumed to apply to all commercial dentifrices, it seems that future analyses could be needed, more from a general hygienic point of view than from the risk of transmitting this particular cariogenic pathogen. In conclusion, under the presented study conditions, the contamination of toothpaste tubes by S. mutans was low.
Denaturing Gradient Gel Electrophoresis to Study Microbial Populations in Relation to Primary and Secondary Caries

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The role of individual species as opposed to complex communities in the caries process requires re-evaluation. Previously, we investigated plaque on restored and unrestored dentin and enamel samples in an in situ study in 8 subjects after 1 and 20 weeks. Culture of plaque samples from 4 sites/subject after 1 and 20 weeks (n = 32) revealed a shift towards cariogenic species. The objective of this study was to use denaturing gradient gel electrophoresis (DGGE) to (1) study in the same 64 plaque samples the shift in microbial composition from 1 to 20 weeks; (2) compare the microbiological populations on primary and secondary caries lesions; (3) identify predominant species in subjects with a high caries progression rate. After DNA extraction, part of the 16S rRNA gene was amplified by PCR and separated by DGGE, resulting in banding patterns roughly representing single species. The average number of bands per lane increased from 9 (range 3–13) at 1 week to 12 (range 7–18) at 20 weeks, reflecting an increase in bacterial diversity. Similar banding patterns were found for plaque from restored and unrestored sites. Lesion depth measurements at the sampled sites displayed 22 caries-active (CA) and 10 non-caries-active (non CA) sites. We found an increase in S. mutans, but in both CA and non CA sites. In CA sites from 1- to 20-week-old plaque a decrease of bands identified as Streptococci (e.g. S. mitis and S. salivarius or S. vestibularis) and an increase in lactobacilli (e.g. L. paracasei) were found. Intense bands representing Scardovia-like species were present only in 13 samples from CA sites at 20 weeks, indicating that this species may play a role in the caries process.

Differentiation of Streptococcus mutans from Saliva and Carious Dentine by MALDI-TOF-MS

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The aim of the study was to trace the origin of oral Streptococcus mutans strains isolated from stimulated saliva (SMS) and from carious dentin (SMC) which were phenotyped by matrix-assisted laser-desorption/ionization-time-of-flight-mass-spectrometry (MALDI-TOF-MS) and classified by a statistical learning algorithm (support-vector-machine, SVM). A primary set of 27 SMS of 10 infants before dentition (class 0: SMS) and a further 38 SMC of 5 children (class 1: SMC) were used to train the SVM algorithm: an error estimate of the class prediction was carried out by calculation of a 10-fold cross-validation error of the training group. The cross-validation accuracy was the percentage of data which were correctly classified. A specificity of 91%, a sensitivity of 97% and a correctness rate of 94% was achieved for the classification of the training groups. The SVM algorithm was applied to another set of samples originating from different children: class 0 (423 SMS, 16 children with mixed dentition including 4 caries-free children) and class 1 (222 SMC, 10 children with carious primary molars). The sensitivity for the correct classification of the SMC was 99%, the specificity for the SMS was 75% with an interval of 46–99%. For caries-free children, the specificity for the correct classification of the SMS was 80%, whereas 72% of the SMS from caries-active children were correctly classified. A phenotypic tracing of SMS and SMC proved to be possible with the chosen MALDI-TOF-MS/SVM method. The greater heterogeneity of the SMS can be attributed to sampling method since S. mutans in plaque and carious lesions are mobilized by saliva stimulation during paraffin chewing. Further investigation will be necessary to clarify which factors trigger phenotypic heterogeneity during caries initiation and progression.

Relationship between Glucosyltransferase Expression and Extracellular Polysaccharide Production by Different Strains of Streptococcus mutans

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The aims of this work were to determine the ability of different strains of Streptococcus mutans to produce extracellular polysaccharides (EPS) under various conditions and to investigate the relationship between glucosyltransferase (gtf) gene expression level and EPS production in specific strains. 20 S. mutans strains from clinical isolates and laboratory strain UA159 were selected, and their ability to produce EPS in TPY medium (initial pH 7) containing 1% glucose, 1% sucrose and 2% sucrose, respectively, was tested in triplicate. Under the same solution conditions, the relative quantities of mRNA for gfts A, B, C, D of the two strains which produced the highest and the lowest amounts of EPS were examined by real-time PCR methods. In all strains, the quantity of EPS was markedly enhanced by sucrose. Clinical strain 502 produced the highest amount of EPS and UA159 produced the lowest amount. The expression levels of gfts A, B, C and D were increased in 1% sucrose for both these strains. In 1% sucrose, the expression levels of gtfB and C of clinical strain 502 were 30% and 60% higher than those of UA159, respectively. In 2% sucrose, expression of gtfB and C decreased in UA159, whereas their expression did not change much in the clinical strain. In 1% sucrose, the expression level of gtfA was 60% higher in the clinical strain than in UA159, whilst in 2% sucrose, expression of gtfD was 3X higher in the clinical strain. Thus, expression of gtfB and C appeared...
closely related to EPS production by S. mutans, whilst the expression of gtfA and D may enhance EPS production in different conditions. We conclude that S. mutans produces gtfIs in proportions that are optimum for adhesion to the tooth surface.

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Diversity of Lactobacilli in Deep Carious Lesions of Deciduous Molars

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Before the wide acceptance of mutans streptococci as the major etiological agent, acidogenic and aciduric lactobacilli were generally thought to be associated with dental caries. Lactobacilli are today considered as secondary invaders after lesion formation. Up to now most clinical studies simply report lactobacillus counts on selective media. The aim of the study was to characterize lactobacilli from carious dentine of 70 deciduous molars with deep carious lesions. From soft (35 molars) and hard carious dentine (H1 and H2 after 12 months of clinical observation) 90 isolates, grown on brain-heart-blood agar and presumed to be lactobacilli, were identified by current methods to prove their possible specificity in the caries process. The isolates were identified by physiological and biochemical characteristics, ratio of lactic-acid isomers, electrophoretic mobilities of lactic-acid dehydrogenases, and shotgun mass mapping based on determining mass spectra profiles by MALDI-TOF mass spectrometry of tryptsinised isolates and database comparison with mass spectra from 20 reference strains. Lac
tobacilli dominated soft carious dentine with 71% of cfu (total cfu 3.6 × 10^9), L. paracasei paracasei (39.4%, 12 molars), L. p. tolerans (34.9%, 4 molars), L. rhamnosus (67.1%, 2 molars), and L. gasseri (34.3%, 2 molars) were identified. On the hard cavity floor, the total cfu was decreased to 3.7 × 10^7 (H1) and 0.1 × 10^7 (H2), respectively. L. p. paracasei (46.2%, 15 molars), L. p. tolerans (53.8%, 6 molars), L. rhamnosus (31.6%, 4 molars), L. gasseri (4%, 1 molar), and L. delbrueckii (8.2%, 1 molar) were determined at H1 and L. p. paracasei (53.7%, 2 molars) and L. casei (2.8%, 1 molar) at H2. It was shown that L. paracasei paracasei and L. p. tolerans are the predominant lactobacilli in carious progression.

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Occurrence of the Family Bifidobacteriaceae in Dental Caries

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The frequency of occurrence and the distribution in human dental caries of the family Bifidobacteriaceae were studied. Sam
ples were taken from 15 active root caries lesions, 15 leathery lesions, from 15 sound exposed root surfaces and from 18 occlusal caries lesions in the deciduous dentition. Bifidobacteria were iso
lated on mupirocin-containing trypticase phytone selective media and presumptive bifidobacteria were Gram-stained and sub
cultured. Total anaerobic counts were determined using FAA me
dium. Identification to species level was based on morphological and growth characteristics and detection of fructose-6-phosphate phosphoketolase. The isolates were identified using genus-specific PCR primers and subsequent partial 16S rRNA sequencing and BLAST searching. 979 bifidobacteria were identified and these included Bifidobacterium dentium, B. subtilis, Parascardovia denticolen, Scardovia inopicta, S. genomosp. Cl, B. breve and B. longum. B. dentium was present in the majority of the samples and was the predominant species. Bifidobacteria formed 7.1% of the cultivable flora from the active root caries lesions, 1.7% from the leathery lesions and 0.06% from the sound root surfaces (p<0.01) while from the occlusal lesions they formed 9.5% of the flora, which was not significantly different from the proportion in the active root caries lesions. Bifidobacteria were isolated from all active and leathery lesions, from 40% of the sound exposed root surfaces and from 89% of the deciduous occlusal lesions. The occurrence of the family Bifidobacteriaceae in dental caries was more complex than previously reported and there was a clear tendency of increased prevalence and proportion of bifidobacteria with root lesion severity.

Supported in part by the Biomedical Research Centre of Guy’s and St Thomas’ Foundation Trust Hospital.

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Reliability and Discriminatory Power of Methods for Dental Plaque Quantification

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The aim of this in situ study was to evaluate the discriminatory power and reliability of methods for dental plaque quantification, and if there is a relationship between two methods to detect mature plaque. 6 volunteers used palatal appliances with 6 bovine enamel blocks for 4 days, dripping 20% sucrose solution 8×/day. On 3 blocks, a plastic mesh was fixed to the appliance. After this period, two blocks per appliance were cleaned. Two examiners independently evaluated the presence of plaque (Silness and Löe Visible plaque index, Quigley and Hein modified by Tuveski and a modified Ekstrand index), with or without disclosing dye, which stains in different colors, showing mature plaque in purple. Images obtained with a novel fluorescence camera (FC) and with digital photographic camera were also evaluated. Images of the area covered by plaque were analyzed independently by the two examiners using image analysis software (Leica Qwin, Leica Microsystems, Heidelberg, Germany). It was expected that the cleaned blocks would have less plaque than the others,
and that the methods should express this difference. Different treatments were compared by Kruskal-Wallis test, and the inter-examiner reproducibility was evaluated by kappa test. Almost all methods presented good reliability, but the Tureski index and the assessment of area covered by disclosed plaque in the images obtained with FC presented better discriminatory power since they showed differences among all groups. The two methods for detecting mature plaque – red fluorescence with FC and plaque stained with two-tone dye – were correlated. In conclusion, the Tureski index and images with FC with disclosing agent showed good reliability and discriminatory power for plaque quantification.

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**Indwelling Wireless Telemetry for Long-Term Measurements of Intra-Oral pH Profiles**

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The aim of this study was to develop and evaluate the effectiveness of wireless pH telemetry, which can last longer than 24 h in the mouth to overcome the limits of the conventional wire telemetry used for salivary and plaque pH measurement. We developed a wireless pH telemeter which can measure and store the pH profile data during more than 24 h. It consisted of an intraoral part (antimony electrode pH sensor, battery and microprocessor for data storage) and an extraoral part (control/data receiver and data-analyzing software designed for this device and installed in a computer). The position of the electrode could be changed according to the target site (saliva or interdental plaque). After calibrating the electrode in standard buffer solutions, it was attached to the removable intraoral appliances and given to the four volunteers who were told to wear it full-time except during toothbrushing. The devices were retrieved after 24 h and the pH profile data were extracted and analyzed. This process was repeated 2–5 times for each volunteer. When compared with conventional wire telemetry, where the intraoral electrode is connected to an extraoral pH meter, this device showed almost the same results, induced less discomfort to the volunteers and enabled long-term measurement. The pH curves showed changes according to the various intakes of foods and beverages of the examinees. With this wireless pH telemetry, the accurate measurement of intraoral pH changes for longer duration will become possible. And we expect it to widen the scope of studies on the oral environment in relation to the individual’s dietary lifestyle and effects of various foods, definitely reducing the discomfort inflicted to the examinees’ daily life.

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**Development of a Preclinical Complex Dental Plaque Biofilm Model**

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Different single and multispecies in vitro biofilm models including selected bacterial populations have been used to study various aspects of either biofilm formation and caries development or prevention of dental caries, e.g. by fluoride or antimicrobial agents. However, dental plaque is a very complex microbial community that comprises about 700 species. The aim of this study was to develop a complex dental plaque biofilm model simulating oral conditions and to visualize the structures and viability patterns of biofilms in situ during the early stages of biofilm formation using confocal laser scanning microscopy (CLSM). A flow chamber system was established for biofilm formation using whole human saliva as inoculum and human enamel slices (mean surface roughness 0.2 μm) as adhesion substrata under dynamic flow conditions. After 24, 48 and 72 h the specimens were carefully removed from the flow chamber without destroying the three-dimensional architecture of the adhering biofilm. Subsequently they were stained using a fluorescent-based two colour assay (Syto 9/P1) without drying or fixation and investigated in situ by CLSM in fluorescence mode. The biofilm thickness and area of colonization were estimated, as well as the proportion of vital micro-organisms in every 1 μm layer of the whole biofilm. In addition, pH and % vitality were monitored in the salivary suspension. Each experiment was repeated 10 times. After 72 h film thickness was 24.6 μm (15.9, 33.3), area was 25.2% (10.8, 39.6) and mean% vitality was 69.3% (63.7, 74.8) (mean, 95% CI). In conclusion, this preclinical biofilm model allowed for the reproducible formation of complex dental plaque biofilms simulating the conditions in the oral cavity and might be used as a screening model for new antiplaque or caries-preventive therapies.

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**Increase of Red Autofluorescence of Dental Biofilm Grown under Cariogenic Stress**

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It is believed that mature biofilms associated with caries can produce red fluorescence (RF). The aim of this study was to evaluate RF from biofilms grown under cariogenic challenge as a function of time. Microcosm biofilms were grown in grooves and acrylic discs in a constant depth film fermenter (CDFF) for 4, 7, 14, 21 and 28 days by alternate pulsing with su-
crose solution (8 ×/day) and saliva analogue medium. Biofilm fluorescence (RF area and ΔR) were assessed using QLF. Harvested biofilms were assessed for CFU/mg biofilm (blood-agar; BHI-agar at pH 7.2 or 4.8) and RF (blood-agar). In dentine after 4 days, only the grooves showed RF (3.5 ± 2.5 mm²) increasing to nearly the entire surface (19.2 ± 1.2 mm²) after 7 days (p < 0.01). Acrylic specimens were covered with RF from 4 days (17.7 ± 2.9 mm²) onwards, which was higher than for 4-day dentine (p < 0.01). ΔR for dentine increased up to 14 days of biofilm growth (23.0 ± 1.2% at 4 days; 70.1 ± 9.5% at 14 days) and stayed constant thereafter, whereas ΔR for polyacrylate increased with time (t² = 0.5; p < 0.01) from 44.2 ± 5.9% at 4 days to 84.2 ± 13.5% at 28 days. Dentine exhibited lower ΔR after 4 and 28 days’ growth than acrylic (p < 0.05). Total CFU on blood-agar and BHI-agar (pH 7.2) were similar and higher (~1 × 10⁸ CFU/mg) for 4-day dentine biofilm compared to other times or polyacrylate (~5 × 10⁶ CFU/mg, p < 0.05). Total aciduric flora (BHI-agar pH 4.8) was ~4 × 10⁷ CFU/mg after 4 days and increased (p < 0.05) to ~5 × 10⁷ CFU/mg after just 7 days for polyacrylate and 14 days for dentine (p < 0.05) after which the values stabilized. Species harvested from acrylic at all times showed RF on blood agar plates, whereas the species harvested from dentine did not show visible red fluorescence on blood agar except for 28 days CDFFF-biofilm. We conclude that RF develops as a function of time (biofilm maturation) and is substratum-dependent.

### 103 Effects of Probiotics on Dental Plaque Ecology

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Probiotics are live bacteria which affect the host by improving its intestinal microbial balance. Most probiotic strains are lactic acid-producing bacteria that may contribute to increased plaque acidogenicity. On the other hand, some probiotic strains inhibit mutants streptococci and hence might reduce plaque acidogenicity. The aims of this study were to assess the effects of probiotics on acidogenicity and composition of oral microbial community. Sixteen healthy individuals rinsed their mouth for 14 days 3×/day with a mixture of 6 probiotic strains (Ecologic 950; Winclow Bio Industries, Amsterdam): 2 *Lactobacillus salivarius* strains W24 and W57, 1 *L. acidophilus* W74C, 1 *L. plantarum* W21, *Streptococcus thermophilus* W69 and *Lactococcus lactis* W19. Plaque was sampled 0, 7, 14 and 28 days after the rinsing period. At each visit, resting plaque (pre-sucrose) and plaque 10 min after 2 min 10% sucrose rinse (post-sucrose plaque) were sampled and organic acids (lactate, acetate, succinate, butyrate, propionate and formate) were determined by capillary electrophoresis. The results were expressed as μmol acid/mg protein. Duplicate plaque samples were collected for fingerprinting by microbial community profiling and characterization (MCPC) technique (TRFLP-based technique; van Haeringen Laboratorium B.V., Wageningen). No statistically significant changes (General Linear Model Repeated Measures test, α = 0.05), either in pre- or post-sucrose plaque acidogenicity, were observed throughout the study. Discriminant analysis of MCPC profiles indicated a shift in microbial community 4 weeks after the rinsing period. In conclusion, 2 weeks’ exposure to a mixture of 6 probiotic bacteria resulted in a microbial shift in plaque composition but did not affect plaque acidogenicity.

### 104 Effect of Starch and Sucrose on Dental Biofilm Composition and on Root Dentine Demineralization

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It has been suggested that the combination of starch with sucrose is more cariogenic to enamel than sucrose alone, but the effects on dentine demineralization and on dental biofilm have not been totally explored. Also, there is consensus that starch is not cariogenic to enamel, but there is no clear evidence that it would be safe to dentine. A crossover blind study was conducted in 4 steps of 14 days each, during which 11 volunteers wore palatal appliance containing 10 slabs of root dentine to which the following treatments were applied extraorally: 1, 2% starch solution; 2, 10% sucrose solution; 3, a solution of 2% starch + 10% sucrose, and 4, 2% starch solution followed by 10% sucrose solution. (The 2% starch solutions were gel-like.) On the 14th day of each phase biofilms were collected from the specimen surfaces for biochemical and microbiological analysis, and dentine demineralization was assessed as percent surface hardness change (%SHC). ANOVA was used for statistical analysis with Tukey’s post-hoc comparisons. Mean results ± SD for treatments 1–4 were, respectively: %SHC at 20.3 ± 16.7; 47.0 ± 8.4; 52.6 ± 16.9 and 52.8 ± 12.0; Soluble extracellular polysaccharide (EPS) 1.2 ± 1.0, 7.6 ± 4.8, 5.5 ± 4.7 and 4.8 ± 6.8 μg/mg wet weight; Insoluble EPS 0.9 ± 0.9, 15.8 ± 12.0, 16.2 ± 15.5 and 12 ± 12.5 μg/mg wet weight. The starch treatment group (1) showed significantly lower values of all response variables than the other groups (p < 0.001) but these did not differ significantly (p > 0.05). The same trend was observed for mutants streptococci and lactobacilli counts in dental biofilm, but neither treatment differed statistically with regard to actinomyces (p = 0.287). The findings suggest that starch is cariogenic to dentine but its combination with sucrose may not be more cariogenic than the isolated effect of these carbohydrates.

Supported by FAPESP (Proc. 04/00688-3).
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Inhibitory Activity of Pomegranate Extract and Fractions on Streptococcus mutans

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Ethnopharmacological studies identified pomegranate (Punica granatum L.) fruit as one of the most frequent plants used to prepare natural oral medicines in Northeastern rural communities of Brazil. The aim of this study was to evaluate the antibacterial activity of the crude ethanolic extract of pomegranate fruits and its fractions against Streptococcus mutans. Pomegranate fruit peels were dried, powdered and extracted with ethanol (80% v/v). The ethanol was subsequently removed under vacuum using a rotary evaporator. Four different polarity fractions isolated from the crude extract (CE) were obtained: hexane (HX), chloroform (CH), ethyl acetate (EA), n-butanol (NB). For testing, the CE and the fractions were diluted in DMSO (dimethyl sulfoxide). Minimal inhibitory concentrations (MIC) were determined (in duplicate) by broth macrodilution and microdilution methods using Mueller-Hinton broth, followed by subculture. The inoculum was derived from a logarithmically growing culture of S. mutans ATCC 25175 adjusted to 0.5 McFarland turbidity standard. MIC was considered the lowest concentration of the extract and its fractions that yield negative subcultures. Chlorhexidine (CHX), DMSO (with and without inoculum) and deionized water were used as controls. The MIC values for CE, HX, CH, EA and NB were 50.0, 30.0, 2.6 and 0.36 µg/ml respectively. These results indicate that the antibacterial activity of pomegranate against S. mutans is related to compounds in the ethyl acetate (EA) and n-butanol (NB) fractions.

Supported by CNPq grant no. 306234/2004-1.

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Effect of Psidium cattleianum Leaf Extract on Dental Biofilm: An in situ Study


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Natural products have been studied with relation to chemical control of dental biofilm. The aim of this study was to evaluate the effect of Psidium cattleianum leaf extract on the acidogenicity and microbiological composition of dental biofilm formed in situ. Harvesting of leaves was authorized by the Brazilian Environment and Natural Resources Institute (permit #0129208). The extract was obtained by decoction in deionized water. Bovine enamel blocks were selected on the basis of their surface hardness (SH). The study was divided into 3 experimental phases according to the treatment solution: water, extract and Listerine. There was a washout period of 1 week between each phase. Ten volunteers wore palatal appliances containing the enamel blocks for 14 days and dripped 20% sucrose 8 × /day and the treatment solution 2 × /day outside the mouth. On days 12 and 13 of the experiment, dental plaque pH was measured with a microelectrode. On day 14, biofilms were harvested, diluted in phosphate-buffered saline and plated on brain-heart infusion agar, mitis-salivarius agar and mitis-salivarius/sucrose/bacitracin agar to determine counts of total anaerobic microorganism (TM), total streptococci (TS) and mutants streptococci (MS), respectively. Surface hardness (SH) was also analyzed after the experimental phase to calculate the percent change in SH (%SHC). The results showed a smaller pH drop immediately after using the extract or Listerine than after water. %SHC showed a decrease in enamel demineralization by the extract or Listerine compared to water. Using the extract also gave a reduction in TM, TS and MS. It can be concluded that using P. cattleianum leaf extract may reduce plaque acidogenicity and micro-organism counts.

Supported by ACTA, CAPES – PDEE (4446/05), CNPq (471634/2007-7), FAPESP (06/00726-8).

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Inhibitory Activity of Stevia rebaudiana against Lactobacillus acidophilus and Streptococcus mutans

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The aim was to determine the inhibitory activity of extracts of Stevia rebaudiana (leaves with natural sweetness) in vitro against Lactobacillus acidophilus and Streptococcus mutans. Vancomycin and 0.2% chlorhexidine was used as a positive control. 200 g of S. rebaudiana leaves were extracted with water, ethanol, methanol, ethyl acetate or hexanol, then dried by evaporation. L. acidophilus and S. mutans were cultivated for a day in trypticase-soy agar. 20 ml trypticase agar were mixed in a test tube with 10 µl of S. mutans or L. acidophilus (0.5% on McFarland’s scale) and poured into Petri dishes. Afterwards, 20 µl of different concentrations of each extract, and also of vancomycin, were placed on the agar and the plates incubated anaerobically at 37°C. Growth was observed at 24, 48 and 72 h. The experiment was run in duplicate. After 48 h the mean inhibition halo obtained with vancomycin was 16.5 mm (95% CI 15.5, 17.5). Similar values were obtained with hexanol extracts of S. rebaudiana (mean 16.1 mm; 95% CI 15.1, 17.1) and chlorhexidine (15.0 mm; 13.1, 17.0). The halo obtained with the other extracts was significant lower compared to the positive control (p < 0.05). After 72 h, there was a late inhibitory activity against L. acidophilus where inhibition by the extract in water was greater (mean halo diameter 32.2 mm; 31.8, 32.8) followed by methanol (31.3 mm; 30.5, 32.2) and ethanol (29.8 mm; 28.1, 31.6). The corresponding values with vancomycin were 30.3 mm (95% CI 29.8, 30.7). In conclusion, the study shows the potential of Stevia rebaudiana for inhibiting growth of the cariogenic microorganisms Lactobacillus acidophilus and Streptococcus mutans.
Oral bacteria exist as a biofilm (dental plaque) on the surface of the tooth. Streptococcus mutans has been implicated as the major causative agent of caries, although the contribution of other acidogenic supragingival plaque species is believed to be significant. The antimicrobial peptide Kappacin consists of the nonglycosylated, phosphorylated forms of bovine caseinomacropeptide [κ-casein(106–169)] and has been shown to have antibacterial activity in vitro against oral bacteria. Kappacin complexed with the known bacteriostatic agent zinc (KappaZinc) has increased in vitro activity against oral bacteria. The Kappacin complex for 10 min 4× over 98%. In conclusion, the KappaZinc antimicrobial complex produced a sustained effect against a S. mutans biofilm that was more efficacious than chlorhexidine. The KappaZinc complex also had a sustained effect on a polymicrobial biofilm that is representative of supragingival dental plaque.

Effect of the KappaZinc Antimicrobial Complex on Oral Bacterial Biofilms
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Remineralization is generally defined as crystal re-growth of hydroxyapatite in incipient caries lesions by salivary calcium and phosphate. A previous in vitro study suggested that mineral recovery of bovine enamel lesions significantly depends on salivary Ca concentrations [Inaba et al.: Caries Res 2007; 41: 317]. The objective of this study was to observe distributions of salivary Ca and phosphate among Japanese adults. Paraffin-stimulated whole saliva samples were collected from 5,304 persons aged 43.5 years. The saliva samples were then analyzed for calcium and phosphate by OCPC and phosphomolybdate methods, respectively using a biochemical analyzer (Hitachi 7700 DDP). The salivary Ca and phosphate concentrations ranged widely: Ca from 0.10 to 4.92 mM (mean ± SD = 1.17 ± 0.33 mM) and phosphate from 0.19 to 28.9 mM (5.15 ± 2.23 mM). Neither Ca nor phosphate concentrations showed statistical significances between male and female in any age group, but were significantly correlated with age (p < 0.001; one-way ANOVA). In conclusion, it was found that concentrations of salivary Ca and phosphate vary extensively between individuals and are significantly related to age suggesting differences in remineralization potential among populations.

Distribution of Salivary Calcium and Phosphate Concentrations among 5,304 Japanese Adults
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This study aimed to evaluate the effect of the fluoride sustained slow-releasing device (FSSRD) on fluoride (F), calcium (Ca), and phosphate (Pi) concentrations in undisturbed plaque biofilms recovered after 7 days and its relationship to patient’s age. 77 participants took part in this randomised controlled double-blind crossover study, of whom 65 participants (age range 6–35, mean age 19.2) remained. Each participant had a FSSRD or placebo device (PD) and a plaque generation device (PGD) attached to any available combination of molar/premolar, permanent or deciduous teeth, on the same upper dental quadrant. The FSSRD or PD was always attached distal to the PGD. Test and control groups were compared using paired sample t-test, while correlations between patient’s age and plaque weight with F, Ca and Pi concentrations were assessed using Spearman’s coefficient. No statistically significant differences were found between test and control groups in dental plaque recovered after 7 days with respect to concentrations of F (1.29 ± 2.50, 2.89 ± 6.22 ppm), P (523 ± 609, 627 ± 829 ppm), and Ca (180 ± 203, 194 ± 238 ppm). There was no relationship between patient’s age or F, Ca and Pi concentrations, but plaque wet weight was inversely correlated with these concentrations. The data suggest that the FSSRD was not effective in raising F, Ca or Pi concentrations in plaque biofilms after 7 days. Plaque age and inverse relationship with plaque weight may relate to concentration of these ions in plaque layers which form a smaller proportion of thicker plaques.

Effect of Fluoride Slow-Releasing Devices and Patient’s Age on Ion Concentrations in Plaque Biofilms
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This study aimed to evaluate the effect of the fluoride sustained slow-releasing device (FSSRD) on fluoride (F), calcium (Ca), and phosphate (Pi) concentrations in undisturbed plaque biofilms recovered after 7 days and its relationship to patient’s age. 77 participants took part in this randomised controlled double-blind crossover study, of whom 65 participants (age range 6–35, mean age 19.2) remained. Each participant had a FSSRD or placebo device (PD) and a plaque generation device (PGD) attached to any available combination of molar/premolar, permanent or deciduous teeth, on the same upper dental quadrant. The FSSRD or PD was always attached distal to the PGD. Test and control groups were compared using paired sample t-test, while correlations between patient’s age and plaque weight with F, Ca and Pi concentrations were assessed using Spearman’s coefficient. No statistically significant differences were found between test and control groups in dental plaque recovered after 7 days with respect to concentrations of F (1.29 ± 2.50, 2.89 ± 6.22 ppm), P (523 ± 609, 627 ± 829 ppm), and Ca (180 ± 203, 194 ± 238 ppm). There was no relationship between patient’s age or F, Ca and Pi concentrations, but plaque wet weight was inversely correlated with these concentrations. The data suggest that the FSSRD was not effective in raising F, Ca or Pi concentrations in plaque biofilms after 7 days. Plaque age and inverse relationship with plaque weight may relate to concentration of these ions in plaque layers which form a smaller proportion of thicker plaques.

**Session 7 Diagnostics**

**111 A Simple Blue Light to Visualize Caries by Means of Fluorescence**

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Caries that remains undiscovered by visual examination (‘hidden’ caries) poses a problem in clinical dentistry. The use of fluorescence has been suggested to aid the detection of such lesions. In this paper we describe the use of a simple blue light to visualize caries lesions including dentinal lesions underneath seemingly intact enamel. 38 teeth, extracted for various reasons, were collected from a local dentist. 10 were rejected because of severe calculus or damage from extraction. Teeth were cleaned and autoclaved (134 °C, 20 min). Teeth were first examined visually (dry, corrected white light) for caries (enamel, outer-third dentine, deep dentine) and radiographs were subsequently obtained. Teeth were illuminated by a 405-nm LED, and observed for green or red fluorescence by looking through combined yellow and red filters possessing transmission peaks around 500 and 630 nm. Caries was confirmed after hemisectioning, which showed that 27 teeth were carious (4 enamel, 7 outer-third dentine, 16 deep dentine), while only 1 tooth was considered sound. By visual inspection 13 teeth were found to have lesions (1 outer-third dentine, 12 deep dentine) and 15 teeth were considered sound. Radiographs confirmed all caries except the 4 cases where this was limited to enamel. Red fluorescence was seen in all but two enamel lesions. Two cases of occlusal fissures with caries limited to enamel displayed red fluorescence, while enamel caries on the buccal surfaces showed up as dark spots on green fluorescing teeth. We conclude that fluorescence had very good agreement with actual caries presence. Given the amount of visually undetected dentinal caries in this sample, fluorescence seems a promising aid for the detection of caries including so-called hidden caries.

![Image](https://via.placeholder.com/150)

**112 Porphyrins Are the Cause of Red Fluorescence of Carious Dentine: Verified by Gradient Reversed-Phase HPLC**

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Porphyrin derivatives have been suspected to cause red fluorescence relevant for detection of carious enamel and dentine, but this has not been proven so far. The aim of this study was to verify the presence of selected porphyrins in carious dentine. Carious dentine samples were collected by bur or hand excavation from 40 patients during caries excavation under rubber dam. The samples were transferred to 220 µl extraction medium (pH < 3.5) containing methanol, dimethylsulfoxide and phosphoric acid, ultrasonicated, separated from solid material, and injected into the solid-phase column (Cromsystems, Munich, Germany) of a reversed-phase high-performance liquid chromatography (HPLC) system ( Dionex, Idstein, Germany). Two mobile phases were used with a decreasing ratio of the polar to non-polar phases over a period of 18 min (gradient reversed-phase HPLC). The eluate was excited at 405 nm. Fluorescence emission at 620 nm was detected by a photomultiplier detector and mV recorded. In order to allocate the chromatographic peaks to porphyrins, a calibration solution was used comprising uroporphyrin, hepta-, hexa- and pentacarboxyporphyrin, coproporphyrin I and II, protoporphyrin IX and Zn-protoporphyrin IX. All samples contained red fluorescing compounds. 95% of the samples contained protoporphyrin IX, 97.5% either one or both coproporphyrins, and 10% contained Zn-protoporphyrin. One sample only contained uroporphyrin. None of the other investigated porphyrin-compounds could be identified. However, a variety of peaks throughout the chromatograms leads to the assumption that other compounds, most likely porphyrin derivatives, contribute to the red fluorescence of carious dentine. It is concluded that the cause of the red fluorescence of carious dentine when excited with violet light is mainly due to protoporphyrin IX, coproporphyrin I and II and other as yet unidentified porphyrin derivatives.

**113 In vivo Performance of a Laser Fluorescence Pen for Interproximal Caries Lesion Measurement**

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The aim of the present study was to assess the supplementary value of an interproximal laser fluorescence device (DIAGNODent Pen, KaVo, Biberach, Germany) in vivo. 20 adult patients presenting 28 carious lesions were scheduled for interproximal restorative treatment in a private dental clinic. In this setting, decision making for operative treatment was achieved with bitewing
radiography (DIGORA Optime, Soredex, Tuusula, Finland). Prior to excavation, fluorescence measurement were made from both buccal and oral aspects. The higher of both readings was repeated once. In order to judge the extension of the lesion, photographs were taken during excavation. LFpen showed a sensitivity of 16.7% at D3 level and a specificity of 90%. The intra-examiner reliability was low. The intra-class correlation (ICC) was 0.27, while Cohen’s Kappa was 0.25. LFpen performed poorly in this clinical setting. Our results indicate that LFpen has restricted supplementary value for clinical decision making with respect to interproximal caries. The main reason appears to be the large probe size, which restricts the area of measurement cervical, buccal or lingual to the site of the carious lesion. Consequently, caries expansion into dentine was underestimated in 22 cases. In 6 cases, when proper location of the probe was feasible, LF measurement was accurate. The ICC indicates that two measurements performed at the same site yield better results than one measurement alone. However, anatomic factors significantly interfere with optimal measurement conditions being achieved as under laboratory conditions. LFpen in its current design seems to be of little supplementary value for detection of interproximal D3 caries under in vivo conditions. The thickness of the probe should be reduced significantly.

The aim of this in-vitro study was to determine the validity and reproducibility of the DIAGNOdent Pen (KaVo, Germany). 112 posterior primary teeth were cleaned, mounted in impression putty in anatomical alignment and stored in water. Three examiners screened the occlusal and approximal surfaces of each tooth with the instrument according to the manufacturer’s instructions and the worst reading for each surface recorded. Prior to examination the teeth were dried with a 3-in-1 syringe. The readings were taken on two separate occasions at least 24 h apart. Subsequently the teeth were serially sectioned and histological validation was undertaken by two examiners using compatible histological criteria [Ekstrand et al.: Caries Res 1998;32:247–254]. For approximal surfaces at the D3 (enamel and dentine) diagnostic threshold the mean specificity was 90.0%, and the sensitivity was 74.2%. For approximal surfaces specificity and sensitivity were 93.1% and 28.1%, respectively. For approximal surfaces at ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) mean specificity and sensitivity were 88.5% and 51.6%, respectively. For approximal surfaces the equivalent values were 98.3% and 24.0%. For approximal surfaces the mean intra-examiner reproducibility was a Kappa value of 0.32 at the D3 diagnostic threshold and at the ICDAS II codes 2/3 threshold 0.62. The mean inter-examiner reproducibility was 0.55 at the D3 threshold and 0.60 at the ICDAS II codes 2/3 threshold. For approximal surfaces the mean intra-examiner reproducibility was 0.55 and 0.65, at the D3 and ICDAS II codes 2/3 thresholds, respectively. The mean inter-examiner reproducibility was 0.57 and 0.65 at the D3 and ICDAS II thresholds, respectively. In conclusion, the laser fluorescence pen gave high levels of specificity but lower than expected levels for sensitivity and reproducibility.

The Validity and Reproducibility of a Laser Fluorescence Pen for Caries Detection in Primary Teeth

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The aim of this in-vivo study was to compare the performance of 3 different methods of approximal caries detection in primary molars. 50 5–12-year-old children were selected and 2 different examiners performed the detection methods in all approximal surfaces of primary molars. The methods used were: visual inspection (ICDAS II); bitewing radiographs (BW) and laser fluorescence pen device (LFpen; DIAGNOdent pen). As standard reference method, teeth were separated using orthodontic rubber rings for 7 days and the approximal surfaces were evaluated by two examiners to check the presence of white spots (WS) or cavitations. In the case of discrepancy, a consensus was achieved between the examiners. Area under ROC curve (AUC), sensitivity, specificity and efficiency were calculated and compared using McNemar test at WS and cavitation thresholds. Inter-examiner reproducibility was calculated using ICC (absolute values) and kappa test (at both thresholds). ICC for inter-examiner reproducibility of standard reference procedure was 0.94. At WS threshold, the tested methods did not present good performance (sensitivity: ICDAS 0.20, BW 0.19, LFpen 0.16; specificity: ICDAS 0.95, BW 0.99, LFpen 0.95). BW showed higher efficiency (0.66) than LFpen (0.62). At cavitation threshold, both LFpen and BW demonstrated higher sensitivity (0.68 and 0.60, respectively) and AUC (BW 0.89 and LFpen 0.92) than ICDAS (sensitivity 0.30 and AUC 0.73). All methods presented high values of specificity (ICDAS >0.99; BW 0.99; LFpen >0.99). For all methods, ICC were similar (ICDAS 0.72; BW 0.77; LFpen 0.75), but kappa for LFpen at WS threshold was lower (0.44) than for other methods. In conclusion, both LFpen and BW can detect the presence of cavitations in approximal surfaces of primary molars. Thus, LFpen may be used as an alternative to BW radiographs.

Supported by CNPq (476372/2006-2).
In this in vitro study aimed to assess the reliability of a novel fluorescence camera and two other laser fluorescence methods in detecting occlusal caries lesions in primary teeth. 86 sites on 61 primary molars were investigated by two trained examiners, with the fluorescence camera (Vista Proof-VP) and two laser fluorescence devices: DIAGNOdent (LF) and DIAGNOdent pen (LFpen). Teeth with enamel defects and extensive caries lesions were excluded. Before the readings, teeth were defrosted, and the time of drying was standardized at 3 s. One examiner repeated the investigation after a week. The maximum reading for each site achieved with each device was recorded. Intra-class correlation coefficient (ICC) was used to assess the intra/examiner reproducibility. Correlations among the three methods were assessed using Pearson’s coefficient. Intra-examiner reproducibility presented ICC for LFpen of 0.86 and for VP of 0.85. LF showed the best inter-examiner reproducibility (ICC 0.86). VP showed similar inter-examiner value (ICC = 0.85). Significant positive correlations were observed among all methods (p < 0.001), but correlation coefficient was higher between the two diode laser fluorescence methods (LF and LFpen) (0.83) than between VP and laser fluorescence (VP/LF 0.65; VP/LFpen 0.70). It was concluded that all methods show good reliability, and the methods yield correlated measurements.

The aim of this in vitro study was to evaluate the influence of pit and fissure sealants on fluorescence readings. 166 permanent molars were selected and randomly divided into four groups for sealing (Delton Clear, Delton Opaque, Helioseal Opaque, Experimental Nanofilled Clear). The teeth were independently measured twice by 2 experienced dentists, before and after sealing, using DIAGNOdent 2095 (LF), DIAGNOdent 2190 (LFpen) and VistaProof (FC). Then they were thermocycled (1,000 cycles 5 ± 2 and 55 ± 2°C, dwell time 30 s) and measured again using the same devices. The fluorescence values were compared by Wilcoxon test (α = 0.05). Reproducibility was assessed by calculating the intra-class correlation (ICC). LF and LFpen fluorescence values increased after sealing with clear materials and decreased after sealing with opaque materials. FC fluorescence values decreased after sealing for all groups. In the Delton Clear group, the change in LF and LFpen values after sealing was not significant but the increase in LFpen values after thermocycling was significant. For FC values, changes after sealing and after thermocycling were statistically significant for all groups except for the Experimental Clear sealant. The ICC was 0.54–0.96 for inter-examiner and 0.28–0.94 for intra-examiner reproducibility. In conclusion, pit and fissure sealants influence fluorescence readings, increasing or decreasing the values according to the sealant material used. It can be suggested that the LF device could be useful as an adjunct to monitor surfaces under Delton Clear pit and fissure sealant.

The purpose of this 6-month clinical study was to investigate the activity of early caries lesions by QLF in the nursery and elementary school children. The subjects were 52 children (4 or 5 years of age) in nursery school and 90 students (age 9.6 ± 1.2 years) in elementary school. Informed consent was given by their parents before study. Visual examination was performed by WHO method, the buffer capacity was measured by Dentocult-stripe and the salivary concentration of of mutans streptococci by Dentocult-SM. QLF images were acquired for occlusal surfaces of 2nd deciduous molars from nursery school children and 1st permanent molars from elementary school students and ΔQ was measured. Based on initial ΔQ, activities of early caries at 6 months were evaluated as progressing, arrested or recovering lesions. Visual examination showed that dft index was 2.59 in nursery school and DMFT+dft index was 3.5 in elementary school children. Among nursery school children QLF showed that 23 students had progressing lesions and 28 students had recovering lesions. Among the elementary school children, 23 students had progressing lesions, 56 students had recovering lesions, and arrested lesions were found in 11 students. In cross analysis between initial visual examination and QLF examination, students with progressing early caries lesions tended to have higher DMFT and dft than other students in both types of school. There was no relation between ΔQ and saliva tests. In conclusion, early caries activity assessed by QLF examination may be related to caries experience in nursery and elementary school children.
The aim of this in vivo study was to compare visual, combined FOTI/visual and radiographic examination for detection of dentinal caries adjacent to composite resin restorations. 115 Class III composite restorations in upper anterior teeth in 41 patients with at least 1 approximal restoration were examined independently by one examiner by visual and FOTI/visual examination. The diagnostic criteria assessed the depth of caries lesions and microleakage. 5 periapical radiographs per subject were taken. The restorations were evaluated by modified Ryge criteria. The visual, FOTI/visual and radiographic methods found, respectively: 23, 10 and 63 sound restorations, 3, 6 and 2 enamel lesions and 55, 69 and 15 dentinal lesions. The visual, FOTI/visual and radiograph examination were repeated in 53 surfaces and the intra-examiner reproducibilities (Cohen’s Kappa) were 0.88, 0.84 and 0.55. The 104 surfaces assessed as dentinal caries by at least one method or scored Charlie or Delta by Ryge criteria were validated through removal of the restoration. The sensitivities for visual, FOTI/visual and combined FOTI/visual or microleakage by visual and combined FOTI/visual methods did not show dentinal caries after validation. The specificities were 0.72, 0.53, 0.91 and the areas under the ROC curves were 0.77, 0.70, 0.53, respectively. The radiographic examination showed unacceptable lowest values for sensitivity and area under ROC curve. By ROC analysis, both visual and combined FOTI/visual examination showed unacceptable lowest values for sensitivity and area under the ROC curve. The radiographic examination (15%). 82–100% of surfaces judged as no caries by Ryge criteria were validated through removal of the restoration. The diagnostic criteria assessed the depth of caries lesions and microleakage. 5 periapical radiographs per subject were taken. The restorations were evaluated by modified Ryge criteria. The visual, FOTI/visual and radiographic methods found, respectively: 23, 10 and 63 sound restorations, 3, 6 and 2 enamel lesions and 55, 69 and 15 dentinal lesions. The visual, FOTI/visual and radiograph examination were repeated in 53 surfaces and the reproducibilities (Cohen’s Kappa) were 0.88, 0.84 and 0.55. The 104 surfaces assessed as dentinal caries by at least one method or scored Charlie or Delta by Ryge criteria were validated through removal of the restoration and tactile validation of dentinal caries presence. The FOTI/visual examination presented the highest proportion of surfaces judged as dentinal secondary caries (60%) followed by visual (48%) and radiograph examination (15%). 82–100% of surfaces judged as no caries or microleakage by visual and combined FOTI/visual methods did not show dentinal caries after validation. The specificities were 0.72, 0.53, 0.91 and the areas under the ROC curves were 0.77, 0.70, 0.53, respectively. The radiographic examination showed unacceptable lowest values for sensitivity and area under ROC curve. By ROC analysis, both visual and combined FOTI/visual examination showed an acceptable performance for dentinal caries detection adjacent to anterior composite resin restorations.

Using ICDAS-II and QLF-I for Assessing Caries Incidence within a Short Interval

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The International Caries Detection and Assessment System (ICDAS-II) and the Quantitative Light Induced Fluorescence with modified ICDAS criteria (QLF-I) are being used as caries detection methods in a 4-year longitudinal study in schools in the Commonwealth of Puerto Rico. The objective of this study was to assess whether these indices can identify lesion transitions after 8 months. 484 children were examined both at baseline and 8 months with ICDAS-II and QLF-I by one examiner on occlusal and buccal surfaces of all erupted permanent molars, along with lingual surfaces of upper molars. Lingual grooves and buccal pits were scored separately. Surfaces with fillings or sealants were excluded from QLF examination. The visual scoring was done according to the ICDAS-II system, while the QLF examination included acquiring images of the surfaces and performing a ranked scoring of the lesions on the computer screen using a ranked scale similar to the ICDAS II. A total of 43,878 calls were made. At 8 months, caries increment from 0.3 to 2.7% were observed for all baseline ICDAS II scores except for scores 2 and 6 which had a decrement (–1% and –1.9% respectively). This may be a reflection of the increase in number of sealants (1%) and fillings (2.9%) observed at 8 months. Most of the transitions occurred at scores 0–2 and were mostly on buccal and occlusal surfaces. QLF-I scored slightly more lesions at scores 1 (0.88%) and 2 (0.56%) than ICDAS II. In conclusion, both indices could detect early changes in lesions scores. Longitudinal assessment will determine at which threshold these increments are valid.

Supported by NIH/NIDCR RO1DE017890-01.

Aiming at early lesions detection and progression, 484 children aged up to 5 years in Detroit, MI, USA. 788 children (77% follow-up rate) were examined at baseline and after 2 years. The examinations were conducted by trained and calibrated dentists who achieved good to excellent reliability. Data on risk factors were collected by trained interviewers at baseline. Statistical tests were performed using the general linear model to model the predictors of progression. Of the 78,800 surfaces examined only 274 (0.3%) were classified as 'first visible sign' (ICDAS code 01) or 'distinct visual change' (02). Data on progression of caries were collected from a random sample of low-income African-American children aged up to 5 years in Detroit, MI, USA. 788 children (77% follow-up rate) were examined at baseline and after 2 years. The examinations were conducted by trained and calibrated dentists who achieved good to excellent reliability. Data on risk factors were collected by trained interviewers at baseline. Statistical tests were performed using the general linear model to model the predictors of progression. Of the 78,800 surfaces examined only 274 (0.3%) were classified as 'first visible sign' (ICDAS code 01) or 'distinct visual change' (02). Data on progression of caries were collected from a random sample of low-income African-American children aged up to 5 years in Detroit, MI, USA. 788 children (77% follow-up rate) were examined at baseline and after 2 years. The examinations were conducted by trained and calibrated dentists who achieved good to excellent reliability. Data on risk factors were collected by trained interviewers at baseline. Statistical tests were performed using the general linear model to model the predictors of progression. Of the 78,800 surfaces examined only 274 (0.3%) were classified as 'first visible sign' (ICDAS code 01) or 'distinct visual change' (02). Data on progression of caries were collected from a random sample of low-income African-American children aged up to 5 years in Detroit, MI, USA. 788 children (77% follow-up rate) were examined at baseline and after 2 years. The examinations were conducted by trained and calibrated dentists who achieved good to excellent reliability. Data on risk factors were collected by trained interviewers at baseline. Statistical tests were performed using the general linear model to model the predictors of progression.
The aim of this in vitro study was to assess the influence of examiners’ clinical experience on the reproducibility and validity of radiographic examination in detecting occlusal caries lesions. Double standardized bitewing radiographs were obtained from 160 permanent molars. Occlusal surfaces were photographed and one occlusal site per tooth was visually chosen. Radiographic examination was performed by last-year dental students from 2 universities (A, n = 5; B, n = 5) and by dentists with 5–7 years’ experience working in different countries (C, n = 5; D, n = 5). All examinations were repeated (one-week interval). The teeth were histologically prepared and assessed for caries extension. Unweighted Cohen's kappa values were respectively 0.90 and 0.85 for LF, 0.93 and 0.87 for LFpen and 0.85 and 0.76 for FC. The ICDAS II kappa values were 0.51 (inter-examiner) and 0.61 (intra-examiner). The post-test probability for dentine caries detection was high for BW and LF. It can be concluded that the performance of each method changes according to the sensitivity and specificity. The ICDAS II combined with BW showed the best performance, and this is the best combination to detect caries on occlusal surfaces.

This study aimed to determine the relationship between ICDAS-II, laser fluorescence (LF; DIAGNo dent), and radiographic readings of approximal surfaces and histological depth of lesions. The sample consisted of 160 carious/sound surfaces on 140 extracted permanent teeth stored in thymol water at Universidade El Bosque. The surfaces were initially scored visually using the ICDAS criteria (scores 0–6). After a few days LF readings were performed. Radiographs were then taken, scanned and examined on the computer screen using a program allowing change of contrast. Re-examinations using the different detection systems were conducted. The teeth were finally sectioned and the involved surfaces classified using a stereomicroscope as: 0, sound; 1, caries restricted to outer half of enamel; 2, caries between inner half of enamel and outer third of dentine; 3, caries in the middle third of dentine; 4, caries in the inner third of dentine. The original LF readings were used, while the radiographs were scored in 5 stages like the histological classification. Spearman correlations were: ICDAS-II versus histology, 0.81 (n = 151); LF versus histology, 0.54 (n = 151); radiographs versus histology, 0.86 (n = 131). Intra-examiner reproducibilities for ICDAS-II and radiographs were both 0.72 (kappa) and that for LF was 0.83 (Spearman correlation). To conclude, ICDAS-II and radiographs are satisfactorily correlated with lesion depth on approximal surfaces. LF is moderately correlated with approximal lesion depth, which might be explained by the fact that the teeth were stored in thymol water and that there were many brown spot lesions in the sample.
Validity and Reproducibility of a Meticulous Visual Caries Detection System (ICDAS II) in Primary Teeth

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The aim of this in vitro study was to assess the validity and reproducibility of the ICDAS II criteria in primary teeth [Pitts: Community Dental Health 2004;21:193–198]. 3 trained examiners independently examined 112 extracted primary molars under dental surgery conditions, using ICDAS II criteria. The teeth were cleaned and set-up in groups of 4, to mimic their anatomical positions. The condition of the teeth used ranged from clinically sound to cavitated; extensively broken down teeth were excluded. Following the ICDAS II criteria, a 3-in-1 syringe was used during the examinations. The examiners examined the occlusal and approximal surfaces and recorded the site of the most advanced caries. Subsequently the teeth were serially sectioned and histological validation was undertaken by two examiners using compatible histological criteria [Ekstrand et al.: Caries Res 1998;32:247–254].

For occlusal surfaces at the D1 (enamel and dentine) diagnostic threshold the mean specificity and sensitivity were 87.0 and 78.1%, respectively. For proximal surfaces the equivalent values were 90.6 and 73.3%. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively. The mean intra-examiner reproducibility (kappa) ranged from 0.74 to 0.83 at the D1 diagnostic threshold and at the ICDAS II codes 2/3 threshold (the first visible sign of cavitation or dentine caries) the mean specificity and sensitivity were 87.0 and 78.1%, respectively.

In conclusion, the validity and reproducibility of the ICDAS II criteria were acceptable when applied to primary molar teeth.

Supported by CNPq (476372/2006-2).

Assessment of Activity Scoring by Lesion Progression over 8 Months

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The aim was to examine transitions of lesions over 8 months in relation to activity scoring at baseline. In a longitudinal study in Puerto Rico, 484 children, age 6–13, were examined at baseline and again at 8 months. All teeth and all surfaces were scored visually, following thorough plaque removal, using ICDAS-II with an additional activity component. Lesions were scored as active or inactive, based on factors of plaque stagnation area, location relative to the gingiva, surface roughness and texture, surface appearance, enamel translucency, and color. A total of 2820 lesions were scored at baseline, with 17% called inactive. Distribution by ICDAS codes was 1, 55.2%; 2, 32.2%, 3, 4.4%; 4, 2.3%, 5, 2.6%, and 6, 3.3%. The odds ratio for progression of active lesions was 1.19 (p = 0.143), while the odds ratio for progression of active lesions was a little higher (1.33; p = 0.101). For individual surfaces the odds ratio for progression was only significant for occlusal surfaces (1.73; p = 0.045).
0.037). A problem in estimation of transitions based on activity was reversals to sound, but 28% of the inactive and 24% of the active lesions were scored as sound at 8 month exam: mainly occlusal surfaces. In conclusion, although a trend was noted, indicative greater progression of active lesions, the 8-month follow-up time was not able to reveal significant differences except on occlusal surfaces. Supported by NIH/NIDCR RO1DE017890-01.

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A Luminescent Calcium-Ion Binder as a Possible Marker for Caries Activity – A Feasibility Study

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The ability to differentiate clinically between active and inactive caries lesions would enhance current detection methods, facilitating appropriate preventive and operative care. Currently assessment of activity is based on subjective clinical judgement and/or longitudinal bitewing radiography. During the caries process, calcium and other ions are released from tooth structure. This in vitro study was aimed at assessing the feasibility of using a luminescent calcium-ion-binding agent as a possible marker for caries activity (patent pending). 45 deciduous and permanent teeth, freshly extracted from children were studied. For each of 54 surfaces (with or without caries lesions), white-light images and luminescence images, taken after applying the calcium-ion marker, were captured. One active area and one sound area on each tooth surface was identified by the clinician and circled on the visible image. The image generated from the luminescence assay was then overlaid and the brightness from circled regions determined with proprietary software. All teeth had at least one caries lesion. Luminescence signals were produced by all samples, emanating primarily from sites of visible caries, although also occasionally derived from adjacent, apparently visually sound, enamel, as well as from retained soft tissue remnants. There was 67% agreement in the circles predicted to be active by the clinician with the circles generating more light using the luminescence assay. Since the teeth were extracted (from young children) because of caries, it is likely the lesions identified visually were active. In conclusion, the luminescent calcium-ion binding agent is worthy of further investigation as a marker for characterising the activity of caries lesions.

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Session 8

Fluoride

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Fluoride Concentrations of Soft Drinks Available in the Iranian Market

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Parallel with the increasing amount of industrial production of food and drink, the risk of fluoride exposure from this source in nonfluoridated areas has increased. The aim was to compare the fluoride concentrations of different types of ready-made...
drinks available in the Iranian market, with the fluoride concentration in tap drinking water in the city of Isfahan, central Iran. As part of an investigation into fluoride intake and excretion in Iranian children, the most popular drinks were identified and categorized into 5 groups. Several different brands of each group were purchased randomly from scattered areas of the city. Home tap water samples were collected randomly from different areas of Isfahan. In total, fluoride concentration was measured in triplicate for 200 drink and 120 home tap water samples. The fluoride concentration of non-milk-based drink samples was measured directly using fluoride-ion-selective electrode. The hexamethyldisiloxane diffusion method was used to measure the fluoride concentration of milk-based samples. The drink samples were compared on the basis of fluoride concentration and the area of manufacture. The mean fluoride concentration (± SD) of drinks was 0.15 ± 0.12 mg/l with a range from <0.02 to 0.75 mg/l. The values for tap water were 0.10 ± 0.006 mg/l. The mean fluoride concentration of noncarbonated juices (0.24 ± 0.15 mg/l) was the highest amongst the groups of drinks tested. More than 85% of the brands purchased had been produced and transferred from distant areas to the city. In conclusion, although both mean concentrations of fluoride in drink and tap water samples were less than 0.2 mg/l, the wide range of fluoride concentrations and variety of water sources of all drinks should be considered in community fluoride studies.

Supported by grants from The Borrow Foundation and Isfahan University of Medical Sciences.

131 The Impact of Fluoridated Milks on the Availability of Trace Elements in Milk

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Milk is a nutritious food, and a vehicle for fluoride administration. However, the impact of added fluoride on the nutritional profile of milk is unknown. Therefore, the effects of added fluoride in cow’s milk with varying fat contents, on trace element (Fe, Zn, Cu, Cr, Mo and Se) availability in milk were investigated. In vitro model of simulated gastrointestinal digestion with enzymic steps was designed to measure and compare the availability of trace elements in pasteurised milk samples with 2 levels of fat content – whole (4% fat) and skimmed (0.3% fat) milk – and 4 concentrations of fluoride dose – none, 0.5, 0.75 and 1.0 mg F/200 ml milk – as well as non-fluoridated and fluoridated (0.5 mg F/200 ml) UHT 4% fat milks. Trace element concentrations were measured by inductively coupled plasma mass spectrometry. Availability of each trace element in samples was calculated from concentration in the supernatant in the digestion tube following centrifugation, after each stage of digestion. The results showed a negative effect of F on Cu availability. The mean (SD) concentration of Cu in the supernatant from pasteurised 4% fat milk with 5 ppm F was 5.73 (1.87) and 6.11 (0.32) µg/100 g before digestion, and after stomach, duodenal and jejunal digestion, respectively, while for the equivalent non-fluoridated milk the mean concentrations were 14.3 (3.17) and 11.1 (0.46) µg/100 g respectively. Fat removal increased the availability of Cu, Zn, Cr and Se, and decreased the availability of Cr from UHT milk compared with pasteurised samples. These initial data suggest that adding F to milk does not have a marked effect on its trace element profile with the exception of reduced Cu availability.

Supported by The Borrow Foundation.

132 Dietary Fluoride Intake in British Children Aged 4–18 Years and Receiving Optimally Fluoridated Water

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Knowledge of total dietary fluoride intake is important at a population level for monitoring community fluoridation and at an individual level for fluoride prescribing. The aim was to investigate the fluoride intake from non-alcoholic drinks including water and starchy staple foods and baked goods in British children aged 4 to 18 residing in fluoridated areas. Detailed quantitative information on food/drink consumption came from 1,659 individuals aged 4 to 18, who participated in the National Diet and Nutrition Survey (NDNS) [Gregory et al., NDNS: young people aged 4 to 18 years, 2000]; a nationally representative, dietary survey conducted in 1997 in the UK. Fluoride concentration values for each food/drink item consumed were derived from the fluoride database developed by the School of Dental Sciences, Newcastle University. Fluoride intake for each child was calculated from fluoride concentration of each food/drink and the amount consumed. The mean (SD) estimated dietary fluoride intake from drinks and staple foods was 0.032 (0.018), 0.026 (0.014), 0.017 (0.011) and 0.017 (0.011) mg/kg bw/day for 4–6-, 7–10-, 11–14-, and 15–18-year-olds, respectively. Fluoride intake from these dietary sources was less than the suggested optimum level of total fluoride intake of 0.05–0.07 mg F/kg bw/day in 94% of the children, while the intake of 2% of the children exceeded this level. The results suggest that foods as well as drinks are a considerable source of fluoride intake for some children and therefore, studies monitoring total fluoride exposure should consider dietary fluoride intake from all foods and drinks.

Development of the fluoride database was supported by The Borrow Foundation.


## Caries Incidence and Increment Five Years after Cessation of School Fluoride Administration Methods

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The aim was to analyse the long-term influence of school fluoride administration in caries incidence and increment five years after cessation. This was a longitudinal study with two different observations: one of 9–10-year-old children and another with 14–15-year-old adolescents, from the county of Vizela. The sample was divided into 2 groups: Group A, a cohort of 61 adolescents who had experienced one of two different fluoride delivery methods in school but had stopped it in the last 5 years; Group B (control), with 72 children observed first and then 42 adolescents that had not experienced any school fluoride delivery method. DMFT and DMFS index were determined using WHO criteria. Group A results were compared and Group B results were compared between both control groups. The caries increment of group A and B were compared. In the statistical analysis \( \alpha \) was 0.05. Results showed statistically significant dental caries increment in adolescents who experienced fluoride delivery methods and also in the control group. The DMFT increment was non-significantly higher in group A (1.36 ± 1.74) than in group B (0.85 ± 2.34). It is concluded that cessation of fluoride administration results in an increase of dental caries similar to the control group. The topical protection provided by different fluoride delivery methods is not enough to deal with the high dental caries experience in this population.

## Caries Incidence before and after Cessation of Water Fluoridation

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The aim was to determine the changes in the incidence of dental caries after cessation of water fluoridation. Different cohorts of schoolchildren in grades two and three (6–8 years of age) were examined in 1993–94 and re-examined in 1996–97, another cohort was examined in 1996–97 and re-examined in 2002–03, and a final cohort was examined in 2002–03 and re-examined in 2005/06 to determine changes in the incidence of dental caries following cessation of fluoridation of the public water supplies in 2001 in Kamloops, British Columbia, Canada. The D1/D2 MFS Index was used to assess dental caries. Residence and dental histories were confirmed for all children to determine the extent of exposure to all types of fluorides. Comparisons between the two series of surveys were used to establish the influence of fluoridated water, other fluoride sources and other risk factors on the incidence of dental caries. Data showed caries increments from two cohorts of children over the time period from 1993–94 to 2002–03 of 0.20 and 0.19 DMFS per year, and a caries increment of 0.30 DMFS for the final cohort of children from 2002–03 to 2005–06. Logistic regression showed that the only significant factor explaining the difference in caries increments was exposure to fluoridated water. All other demographic and preventive exposures failed to influence significantly the caries increments. Although the increments were small, because of the age of these children, this study looked primarily at permanent first molars. In conclusion, in this community, following cessation of water fluoridation, caries increments increased by about 50%. Exposure to fluoridated water was the only significant variable influencing the change in caries incidence.

## Dilution-Dependent Fluoride Release from NaF Dentifrices Containing Unique Forms of Calcium

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The purpose of this study was to evaluate fluoride release at four dilutions from NaF dentifrices with and without calcium. 8 dentifrices were examined in triplicate: 1, Crest Cavity Protection (100 ppm F); 2, Enamel Care with Liquid Calcium (1,000 ppm F); 3, MI Paste Plus (900 ppm F); 4, Sensodyne Pronamel (1,450 ppm F); 5, Theramed SOS (1,450 ppm F); 6, Prevident Booster (5,000 ppm F); 7, ControlRx (5,000 ppm F); 8, modified ControlRx (5,000 ppm F). Groups 2, 3, 5, and 8 comprised CPP-ACP, ACP, nano-sized HAP, and functionalized TCP, respectively. Following the American Dental Association’s guidelines, the dentifrices in all groups were diluted with distilled water (1:1, 1:3, 1:10, and 1:100), mixed at 400 rpm for 10 min and centrifuged for 10 min at 9000 rpm. Aliquots of each supernatant were mixed with TISAB II (1:1), and fluoride concentration measured by electrode. Relative to the labeled amounts of fluoride in the dentifrices, the mean (± SD) percent levels of ionic fluoride for Groups 1–8 at 1:1, 1:3, 1:10, and 1:100 dilutions were: Group 1, 60.5 ± 3.8; Group 2, 89.2 ± 5.0; Group 3, 77.3 ± 3.3; Group 4, 33.4 ± 1.0; Group 5, 53.2 ± 1.9; Group 6, 62.1 ± 3.9; Group 7, 80.6 ± 0.3; Group 8, 96.8 ± 0.4. These laboratory results indicate that fluoride released from dentifrices is inherently dilution-dependent but can be especially sensitive to calcium-containing dentifrices formulated to minimize calcium-fluoride interactions (e.g. those with CPP-ACP, ACP, or nano-sized HAP).
Availibility of Fluoride from Toothpastes in Peru and Belgium Measured with Different Methods

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Many well-controlled studies have proved that toothpastes containing fluoride reduce the risk of caries. In many developing countries, toothpastes are the only available method to prevent caries, so it is important to evaluate the amount of fluoride from toothpastes available in those countries. To achieve maximal effect, it is necessary that fluoride is present in its free ionizable form. In our study, 23 toothpastes were examined: 4 were bought in Belgium, 19 came from local shops in Peru. To establish the concentration of free ionizable fluoride, toothpastes were diluted 1:10 and the supernatant was analysed using a fluoride electrode after adding TISAB III. Furthermore, ionized fluoride was determined after overnight digestion of the supernatant with acid phosphatase (4 units per 1.25 g of toothpaste). These methods were compared to gas chromatography. In toothpastes containing sodium monofluorophosphate only 5 to 15% of the total fluoride was ionized. This amount increased to values between 9 and 25% after digestion with acid phosphatase. Measurement with gas chromatography showed that the full declared amount of fluoride was present. In sodium fluoride toothpastes, between 73 and 90% of the declared fluoride content could be retrieved using the ion-selective electrode. From our results, we conclude that the fluoride electrode method is the best method to measure the amount of free ionisable fluoride, but is not suitable to check the declared fluoride content. Fluoride toothpastes from Peru did contain the declared amount of fluoride.

Supported by Deutsche Zahnmedizinische Entwicklungshilfe e.V.

In vitro Remineralization of White-Spot Enamel Lesions from NaF Dentifrices with and without Calcium

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Support by Indiana Nanotech, Therametric Technologies Inc/Indiana University Research & Technology Corporation, Indianapolis, Indiana, and OMNI Preventive Care, West Palm Beach, USA

Multi-mineral formulations and treatments comprising fluoride and calcium can be effective in enhancing anticaries benefits relative to fluoride alone. In this work, we report on the remineralization of white-spot enamel lesions from the following five groups: (1) Deionised water; (2) MI Paste Plus (900 ppm F + CPP-ACP); (3) Theramed SOS (1,450 ppm F + Nanit active); (4) 1,000 ppm F dentifrice; (5) 1,000 ppm F modified dentifrice with functionalized TCP. Bovine enamel specimens were prepared and initially softened in a carbopol-lactic acid solution [White: Caries Res 1987;21:228] for 36 h (37°C). Following initial softening, specimens were stratified (mean Vickers hardness, VHN = 35) into groups of 10 and cycled for 10 days in a lesion reversal model consisting of 2 x 1.5 min treatments (diluted 1:3 with deionized water) and one 4-h acid challenge (carbopol-lactic acid, pH 5.0) per day. Between these events, specimens were immersed in artificial saliva [ten Cate et al: Caries Res 1988;22:20]. After cycling, surface hardness and enamel fluoride uptake were evaluated. Mean VHN recoveries (± SEM) were: (1) –2.7 ± 1.5; (2) 9.0 ± 0.9; (3) 95.8 ± 5.5; (4) 40.6 ± 5.2; (5) 84.3 ± 10.4 with 1 < 2 < 4 ≤ 5 < 3 (ANOVA and multiple pair-wise t-tests, p < 0.05). Fluoride uptakes (µg F/cm³) (± SEM) were: (1) 238 ± 16; (2) 406 ± 32; (3) 5,268 ± 334; (4) 3,275 ± 140; (5) 5,187 ± 237 with 1 < 2 < 4 < 5 < 3 (p < 0.05). Model validity was confirmed by statistical differences between deionized water and the fluoride-containing group. Group 5 with functionalized TCP was statistically different to both MI Paste Plus and unmodified 1,000 ppm F dentifrice. Despite the significant difference in fluoride content between groups 3 and 5, no statistical differences were found between these groups. Therefore, incorporation of functionalized TCP into a 1,000 ppm F dentifrice may boost anticaries performance.
μg/mm² for both groups) (p < 0.05). All products led to an appreciable uptake of fluoride by enamel; amine fluoride toothpaste plus mouthwash led to the highest concentrations and the concentration remained high 48 h after cessation of use.

Electron Probe Microanalysis for Imaging the Fluoride Distribution within Incipient Caries Lesions

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The aim of this study was to evaluate electron probe microanalysis (EPMA) for imaging fluoride distribution within incipient caries lesions in vitro after treatment with different NaF-containing toothpastes. Two NaF/silica based toothpastes (A and B) containing 1,100 ppm F were commercially sourced. Enamel fluoride uptake (EFU) studies were based upon method #440 described by the US Food and Drug Administration testing procedure. The fluoride depth profile of each enamel specimen was determined by removing successive layers of enamel at 30, 60, 90 and 120 μm from the surface using a microdrill technique (n = 15). EPMA was performed on a polished longitudinal cross-section of the treated incipient caries lesions (n = 6). Images (128 × 64 μm) were recorded using an 80-nA beam current and a 900-ms dwell time per step (1 μm step size). The EPMA images showed noticeable differences between the treatments in terms of fluoride uptake, with toothpaste A resulting in a higher EFU within the lesion compared to that of toothpaste B. These images were supported by the quantitative depth profiling data (microdrill); the EFU at 30, 60, 90 and 120 μm for toothpastes A and B were 1,207, 694, 382, 205 μg/cm² and 826, 521, 310, 178 μg/cm², respectively. The results demonstrate that EPMA can not only image fluoride gradients within caries lesions, but can also discriminate fluoride uptake differences between treatments.

Promotion of Fluoride Uptake into Demineralised Enamel by Casein Phosphopeptide-Amorphous Calcium Phosphate Nanocomplexes


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Casein phosphopeptides-amorphous calcium phosphate nano-complexes – CPP-ACP (CASRN691364-49-5) – have been shown to have an additive anticariogenic effect with fluoride in the rat. The aim of this in situ study was to examine the remineralisation of enamel subsurface lesions and their uptake of fluoride by three dentifrice formulations (placebo, 1,100 ppm fluoride and 2% CPP-ACP plus 1,100 ppm fluoride). A randomised, double-blind, three-way cross-over study was conducted utilising a maxillary appliance containing human enamel subsurface lesions. A 15-ml water slurry of the dentifrice (1 g/4 ml) was rinsed for 60 s four times a day for 14 days. At the end of each treatment period a portion of each treated slab was acid challenged in vitro by the Carbopol demineralisation buffer used to form the lesions. Mineral changes were determined using transverse microradiography and fluoride localised using electron microprobe analysis with wavelength dispersive spectrometers. The CPP-ACP plus fluoride dentifrice slurry produced significantly more remineralisation (21.0 ± 5.9%) compared to the fluoride alone (8.2 ± 2.0%) or placebo slurries (3.1 ± 1.6%). The net mineral remaining following the acid challenge was greatest for the CPP-ACP plus fluoride treatment (17.4 ± 1.2%) compared to the fluoride-only treatment (7.1 ± 1.3%). Fluoride uptake by demineralised enamel following CPP-ACP plus fluoride treatment (0.30 ± 0.13 wt% fluoride) was significantly higher than that of the fluoride-only (0.23 ± 0.09) and placebo treatments (0.05 ± 0.05). In conclusion, CPP-ACP enhanced the uptake of fluoride into demineralised enamel compared to fluoride alone and produced the greatest level of remineralisation in situ.

Supported by the National Health and Medical Research Council of Australia, and the Cooperative Research Centre for Oral Health Science.

Effect of Low-Concentration Fluoride Agents on Demineralization: An in situ Study

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The effect of combination of fluoride methods in low concentration on the inhibition of enamel demineralization under a high cariogenic challenge is not clearly established. This in situ cross-over study aimed to assess whether the additional daily use of fluoride mouthrinse (FR) to fluoride dentifrice (FD) is equivalent to increased FD application frequency on enamel surface changes, demineralization and fluoride content. During 3 phases of 14 days each, 12 volunteers wore mandibular appliances, containing bovine enamel blocks which were submitted to 3 treatment groups: 1, twice/day FD; 2, twice/day FD + once/day FR; 3, 3 times/day FD. Each treatment was followed by a 1-week washout period. At the end of the experimental phase, the enamel blocks were removed from the appliances and assessed with respect to surface clinical changes, microhardness and fluoride concentration. The 3 times/day FD usage had the same efficacy in reducing the enamel surface changes and demineralization than the combined use of twice/day FD + once/day FR, although it was more efficient than twice/day FD usage (p < 0.05). All treatments showed a significantly increased enamel fluoride concentration compared to control blocks (p < 0.05), but the differences among them were not significant (p > 0.05). These findings indicate that increased frequency of application of fluoride dentifrice can replace the use of FR in addition to conventional toothbrushing with fluoride dentifrice.
Camellia ext. MJ (MJ) is a green tea extract with a very high fluoride concentration. However, besides fluoride, MJ has many components: minerals and polyphenols such as catechins, caffeine, etc. The aim of this study was to evaluate the effect of the fluoride component of MJ on enamel acid resistance. De-fluoridated MJ (MJ-F) was fabricated by using microdiffusion analysis method and artificial MJ (artMJ) from chemicals. 63 bovine enamel specimens were demineralized in demineralizing solution (DEM: 0.1 M lactic acid, 3.0 mM Ca, 1.8 mM P, pH 5) at 37°C for 3 days, and were then remineralized in several types of remineralizing solution (REM: 3.0 mM Ca, 1.8 mM P, pH 7) at 37°C for 14 days. For remineralization, we prepared 5 REM solutions; REM_NC (REM only), REM_PC (1 ppm F as NaF), REM_MJ (1 ppm F as MJ), REM_MJ-F (MJ-F), REM_movie (artMJ without F). After remineralization, specimens were demineralized again in DEM solution (acid resistance test: ART). The change of mineral loss (ΔΔZART) and lesion depth (ΔLDART) after ART were measured by microradiography. Compared with REM_NC, the mineral loss after ART decreased when REM_MJ was used. Both ΔΔZART and ΔLDART were statistically significant (p < 0.05). These effects were similar to REM_PC. The same recovery effects as REM_MJ were also observed and statistically significant (p < 0.05) when 1 ppm F was added to REM_MJ-F and REM_movie. These data suggest that fluoride content of MJ, not the polyphenols, plays the major role in increasing enamel acid resistance.

Effect of Varying Concentrations of Fluoridated Milk on Enamel Remineralisation in vitro

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A decline in caries levels in children has been observed with the consumption of fluoridated milk [Bian et al.: Comm Dent Oral Epidemiol 2003;31:241], but there is still a need to find the optimum milk fluoride concentration for caries prevention. A modified pH cycling model [Malinowski et al.: Caries Res 2007; 41:278] was used to investigate changes in mineral concentration of sub-surface caries-like lesions exposed to different concentrations of fluoride in milk. An in vitro single blind experiment with 6 groups of 11 caries-like lesions exposed to varying fluoride concentrations (0, 0.25, 0.5, 1.0, 5.0 and 10.0 ppm F) in milk was carried out. On each of the 14 days of the cycling period the lesions were exposed to 5 2-min periods of cariogenic challenge (1.5 mM CaCl2; 0.9 mM KH2PO4; 50 mM acetic acid, pH 4.8) and 2 5-min periods in milk plus 10 min in a 1:3 milk/saliva slurry. Throughout the cycling period the slabs were stored at 37°C in artificial saliva and demineralisation/remineralisation assessed by TMR using dedicated image software (Inspecpter, Amsterdam). Remineralisation (ΔΔΔZ; %vol · μm) was observed in all fluoride groups (0.25 ppm F, 121 ± 273; 0.5 ppm F, 139 ± 370; 1.0 ppm F, 268 ± 391; 5.0 ppm F, 483 ± 224; 10 ppm F, 517 ± 486) in contrast to demineralisation in the non-fluoride control (–201 ± 75). Remineralisation was statistically significant for all concentrations >1.0 ppm. The results showed that fluoride concentration in milk exhibits a clear dose dependency in this model and that the presence of fluoride, even at low concentrations, promotes remineralisation in a pH cycling model.

Supported by The Borrow Foundation.
Amine fluorides are known for their antimicrobial properties and are widely used in oral health care. The aim of this study was to determine the viability of adhering and growing oral bacteria on amine fluoride treated pellicles with respect to a buffer control. Pellicles were adsorbed during 16 h from saliva to glass slides and subsequently treated with amine fluoride for 2 min. Hydrophobicities and zeta potentials of the pellicles were measured. Atomic force microscopy in tapping mode was used to determine the surface roughnesses, while adhesion and growth on the treated pellicles was studied in the parallel plate flow chamber for several oral bacterial strains. Pellicles became significantly (Student’s t test; $\alpha = 0.05$) more hydrophilic (water contact angle decreased from $56^\circ$ to $45^\circ$) and had rougher surfaces, increased significantly ($p < 0.05$) from $5.1 \pm 1.6$ nm to $28.8 \pm 9.9$ nm and zeta potentials became less negative, $-29 \pm 3$ mV to $-19 \pm 7$ mV after amine fluoride treatment. All strains showed a reduction of initial adhesion, ranging from 24% up to 70% after amine fluoride treatment. Contact killing was seen for 4/5 oral bacterial strains. A further decrease in viability of the adhering bacteria was observed after perfusing the flow chamber with an amine fluoride solution, which resulted in the inability of the adhering bacteria to grow. It is concluded that amine fluoride treatment of salivary pellicles reduces bacterial adhesion and viability with respect to a buffer control.

Supported by GABA International, Münchenstein, Switzerland.
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