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Abstracts

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Session 1 Microbiology and Pulp

1

Photodynamic Antimicrobial Chemotherapy Mediated by Curcumin on *Streptococcus mutans* Biofilm

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To evaluate (i) the effect of Photodynamic Antimicrobial Chemotherapy (PACT) using curcumin (C) and a blue LED (BL) on *S. mutans* (SM) mature biofilm in comparison to 0.12% chlorhexidine (CHX) and (ii) PACT optimization using a novel white light (WL) and C on planktonic SM. (i) PACT was performed using BL (48 J.cm², 3'33'') combined with C (2.5 mM). SM biofilms were formed on saliva-coated hydroxyapatite discs in batch culture, replacing the medium daily for five days. Treatments were C+BL+ (PACT); C only; BL only; CHX (60'') or no treatment. Assays: variable pressure scanning electron microscopy, confocal laser scanning microscopy and microbial viability (colony-forming units – CFU/mg of biofilm dry weight). (ii) SM was adjusted at 1 × 10⁶ cells/ml in PBS and the treatments were performed using WL (42 J.cm², 12'') with C (0.75 μM) using the same design of (i). The CFU/mg was calculated by inoculating the disrupted biofilm in blood agar per milligram of biofilm dry weight and transformed into log₁₀. Duplicate of three different experiments were performed (n = 6). One-way ANOVA followed by t-student test was applied to verify the differences among all the studied groups. The level of significance was 5%. (i) PACT resulted in a substantial higher number of dead cells (5.98 CFU/mg; reduction of 2.13 log₁₀) when compared to the control (8.11 CFU/mg) with no statistical difference to CHX (7.73 CFU/mg) (≤0.05). The morphology of biofilms did not differ among the groups. (ii) PACT group WL achieved a lethal photokilling rate (reduction of 5.37 log₁₀). PACT mediated

by curcumin was able to control the mature SM and the WL was an efficient light source to be used in photodynamic approach.

The study was funded by Brazilian Government Agency – CAPES Foundation grant BEX #8485/11-9 (scholarship to Marco Aurélio Paschoal).

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Microbial Recognition by the Immune System in Individuals with and without Caries

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More than half of oral microorganisms have not been cultured to date, hampering research to determine the specificity of bacterial and fungal species to different antibodies. We have combined the use of flow cytometry and pyrosequencing to describe the microbial composition of saliva samples from adult individuals (20–45 years old) and its interaction with the immune system by using fluorescent markers which are specific for different immunoglobulins. Proper fluorescent marking was assessed by fluorescence and confocal laser scanning microscopy. Anti-mouse Igs labelled with the fluorophore FITC were used to control for non-specific binding. By the use of Fluorescence-Activated Cell Sorting (FACS), bacterial cells were separated depending on whether they were coated with any of the three most frequent antibodies in saliva (IgA, IgG and IgM). The DNA of each bacterial population was extracted, PCR-amplified and pyrosequenced, characterizing the

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microorganisms which are ignored and recognized by these immunoglobulins. The proportion of bacteria coated with IgA IgG or IgM at 0, 6, 12 and 24 h after toothbrushing was 90.1, 48.8, 49.6, and 53% (average 60.4%) in caries-bearing subjects (>2 active cavities at the moment of sampling, average DMF index = 0.33) and 78.4, 87, 83.5 and 85.7% (average 83.6%) for caries-free (DMF index = 0) individuals (n = 9 in both groups). The frequency of many bacterial genera in the Ig-opsonized and non-opsonized fractions was different, suggesting a particular affinity of the antibody for some microorganisms. For instance, data for saliva samples from individual CA021 show that the most common genera in the IgA-opsonized fraction were *Propionibacterium*, *Streptococcus*, *Arthrobacter*, *Veillonella* and *Atopostipes*, whereas the latter three were absent in the non-opsonized fraction. The application of the technique to healthy and caries-bearing individuals unravels the contribution of the immune response to the disease.

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Comparison of Antibacterial Efficacies of Several Dentin Bonding Agents

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To Aim of the Work: In this study, antibacterial effect of several dentin bonding agents were compared which have different pH values and different active monomers. **Experimental Approach:** Infected dentin samples were taken from a depth of approximately 4–6 mm into isolated and air-dried dental caries. *Streptococcus mitis* (*S. mitis*) isolated and incubated into sheep blood agar plates (BAP) at 37 C for 18 h. Used liquids (60 µl) were impregnated antimicrobial susceptibility test disks (Oxoid Ltd, Hants, UK). All samples were grouped: group 1 is negative control group that applied nothing; group 2 is positive control group that applied %2 chlorohexidine digluconate (pH = 6); group 3 applied 3M ESPE Adper Single Bond Universal (included copolymer of itaconic acid and pH = 4.5); group 4 applied Kuraray Clearfil SE Bond 2 Primer (included 10-methacryloyloxydodecyl dihydrogen phosphate and pH = 2.5); group 5 applied Kuraray Clearfil S3 Bond Plus (included 10-methacryloyloxydodecyl dihydrogen phosphate and pH = 2); group 6 applied Kuraray Clearfil Protect Bond Primer (included 12-methacryloyloxydodecylpyridinium bromide (MDPB) and pH = 2). Disks were inserted into BAPs. Inhibition zone diameters were measured on the plates by well-educated specialist. **The Main Results:** 1.2×10^6 colony-forming unit (CFU) *S. mitis* proliferated in negative control group with no inhibition zones. In group 3; 7×10^4 CFU *S. mitis* were survived. No surviving *S. mitis* were in other groups. Inhibition zone diameters of group 2, 3, 4, 5, 6 were 21 mm, 14 mm, 30 mm, 26 mm and 34 mm respectively. **The conclusions:** According to Clinical Laboratory Stan-

dards Institute (CLSI), inhibition zone diameter of penicillin is 28 mm which needs the largest inhibition zone. This results showed that MDPB monomers have significant antibacterial effect.

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Immunomorphological Localization of Cartilage Matrix Proteins in Reparative Dentin of Deciduous Teeth

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Tertiary dentin is secreted as a response to pathologic stimulus, such as dental caries. From a morphological point of view in human dentine-pulp complex, tertiary dentin has been described as reactionary or reparative (osteodentin). At a histological level this reparative dentin matrix is composed of cellular inclusions embedded in an amorphous extracellular matrix which stains positively with several cationic histochemical stains, resembling cartilage. Despite this the composition of this reparative matrix is not completely understood. This study was designed to test the hypothesis that cartilage proteins are present in reparative dentin in deciduous teeth. One hundred deciduous molars with rapidly progressive caries, extracted due to dental emergency were used. The specimens were fixed in 10% buffered formaldehyde, demineralized, and embedded in histological paraffin. 6 µm longitudinal sections were stained with Safranin O, Fast green FCF or Giemsa. Type-II collagen, aggrecan, osteopontin and chondroitin sulfate were detected using four monoclonal antibodies. The antigen-antibody reaction was assessed by means of indirect immunofluorescence. As positive control, human tracheal cartilage was used. Results. Histochemically, the pericellular matrix was strongly stained in all specimens. A positive immunoreaction was detected in the pericellular matrix with all antibodies. The intercellular matrix remained negative to the immunoreactions. The distribution of immunolabeling, was coincident with the histochemical stain. In reactionary tertiary dentin, only chondroitin sulfate was detected within dentin tubules. In conclusion the localization of cartilage epitopes in reparative dentin, but not in reactionary dentin, suggest that the former had a chondrogenic phenotype and that both are morphological and immunochemically different.

We would like to acknowledge to Finis Terrae University for founding this research trough the Academic Vicerectory Competition Foundation.

Evaluation of Salivary *Streptococcus mutans* Test and Caries Incidence in Three Years with School Students

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A semi-quantitative enumeration system was developed to monitor levels of *Streptococcus mutans* in saliva. The system promptly detected salivary *S. mutans* in 15 min using species-specific monoclonal antibodies and classified the results into two levels. The purpose of this study was to evaluate the detection system with saliva samples collected from school students and compare the results of caries incidence after three years. Saliva samples were collected from 106 junior high school students. When a test result of the system was positive, it was classified as high *S. mutans* level. When a test result was negative, it was classified as low *S. mutans* level. The remaining saliva samples were subjected to the real-time PCR methods and salivary *S. mutans* numbers were enumerated. At the same time, caries prevalence was examined. After three years, the caries examination of those students were repeated. Real-time PCR methods revealed that the subjects with positive result of the system had significantly higher salivary *S. mutans* (median 1.7×10^5 cells/ml) than the subject with negative result (median 2.3×10^3 cells/ml) (Wilcoxon: $p < 0.01$). The caries incidence in the low *S. mutans* group was 0.0 and in the high *S. mutans* group, it was 1.0 (Wilcoxon: $p < 0.01$). The high *S. mutans* group has significantly higher caries increment (median 1.0) than the low *S. mutans* group (median 0.0) (Wilcoxon: $p < 0.05$) after three years. The detection system was a simple and reliable tool to detect salivary *S. mutans*. The current study showed the caries increment in high *S. mutans* group could be increased higher than that of low *S. mutans* group in the future. In conclusion, the detection system could be useful for caries risk screening at group examination.

Sugar Consumption of *Streptococcus mutans* Biofilms in the Presence of Sucrose and Xylitol

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Streptococcus mutans is known to be involved in caries development, e.g. by acid-associated tooth decay, in the presence of dietary sugars like sucrose and glucose. Among the most commonly

applied measures the use of nutritive sweeteners plays an important role of caries prevention. The application of xylitol may result in bacterial growth inhibition because streptococci are not able to metabolize this sweetener.

The aim of the study was to investigate the development of *Strep. mutans* biofilms grown under three nutritional conditions: 1. in control medium (C, 0.58% glucose), 2. in C with 5% sucrose (S) and 3. in C with 1% xylitol (X). The biofilms were grown on human enamel slides for 24 h and 37°C under anaerobic conditions. They were characterized by the microbiological approach: total bacterial cell counts and microbial growth as colony forming units. The vital proportion of biofilm bacteria was determined using live dead fluorescent staining analyzed by confocal laser scanning microscopy. In addition, glucose and sucrose consumption in the environmental growth media of mutans streptococci was quantified by means of an enzyme-based assay kit. Ten experiments were performed including microbial and fluorescent data, three representative glucose/sucrose analyses have been carried out. The statistical evaluation was conducted using ANOVA and Tukey Kramer tests.

Under glucose (C) and xylitol (X) exposition *Strep. mutans* biofilms metabolized glucose almost completely (>97%), after exposure to sucrose (S), 50.3% of sucrose was utilized. In the presence of glucose, xylitol did not cause growth inhibition. Sucrose supplement increased the biofilm vitality and depth slightly.

The availability of dietary carbohydrates in pure form or in combination affects the metabolism and cariogenic potential of *Strep. mutans* biofilms.

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Serotype *k* *Streptococcus mutans* Strains with Cbm Protein Contribute to Pathogenicity of Infective Endocarditis

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Streptococcus mutans, a pathogen of dental caries, is occasionally isolated from the blood of patients with infective endocarditis (IE). *S. mutans* strains are classified into 4 serotypes, *c*, *e*, *f*, and *k*, of which *c* and *e* are the major types in oral isolates with distribution frequencies of approximately 75% and 20%, respectively, while those of serotypes *f* and *k* are lower than 5%. Recently, we found that most examined serotype *k* strains were positive for the 120-kDa cell-surface collagen-binding protein termed Cbm. In addition, the serotype *k*-specific bacterial DNA was frequently detected in *S. mutans*-positive heart valve specimens extirpated from IE patients. In the present study, we evaluated the virulence of serotype *k* *S. mutans* strain for IE. We used the Cbm-positive *S. mutans* SA31 strain, its Cbm-defective mutant strain SA31CBD, and SA31 complemented mutant strain SA31comp. The adhesion and invasion properties of SA31 with human umbilical vein endothelial cells (HUVEC) were significantly higher than those of SA31CBD, while SA31comp showed recovery of those properties.

In addition, 93.3% and 60% of *Galleria mellonella* wax worm larvae infected with SA31 and SA31comp, respectively, died within 24 hours after infection, whereas only 20% of those infected with SA31CBD died during that time period. The virulence of these *S. mutans* strains was also evaluated using a rat endocarditis model and all rats infected with SA31 died within 6 days after infection. Pathological observations from Gram-stained sections showed that SA31 administration induced considerable bacterial vegetation formation in the injured aortic valves of all rats. On the other hand, no bacteria were observed in the aortic valves of those with SA31CBD infection, whereas rats infected with SA31comp displayed such formations. These results indicate that serotype k *S. mutans* strains with Cbm may be highly virulent for IE.

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Contribution of Cell Surface Proteins of *Streptococcus mutans* in Interaction with Fibrinogen

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Streptococcus mutans, associated with dental caries, is considered to be a cause of infective endocarditis (IE). One of the most important steps in IE development is bacterial attachment to impaired endothelium. Recently, 120-kDa cell-surface collagen-binding proteins (CBPs) of Cnm and Cbm were identified in *S. mutans*. In addition, *S. mutans* strains with CBPs have been shown to frequently lack expression of the cell surface 190-kDa protein antigen (PA). It is also widely known that fibrinogen is one of the extracellular matrix proteins of host tissue that form bridges between platelets by binding to GpIIb/IIIa surface membrane proteins. In the present study, we investigated the interaction between fibrinogen and *S. mutans* cells by focusing on cell surface proteins such as CBPs and PA. A total of 85 *S. mutans* clinical strains were classified into the Cbm+/PA- (n = 10), Cbm+/PA+ (n = 5), Cnm+/PA- (n = 10), Cnm+/PA+ (n = 20) and CBP-/PA+ groups (n = 40), and their fibrinogen-aggregation and -binding properties were analyzed. Fibrinogen-aggregation properties were identified mainly in the Cbm+/PA- group, while the most prominent fibrinogen-binding properties were also observed in the Cbm+/PA- group (133.6%), which were significantly higher as compared to the Cnm+/PA- group (57.2%) ($P < 0.05$) when those of the reference strain SA31 were defined as 100%. In contrast, the fibrinogen-binding rate for each of the other groups was 0%. Next, we analyzed platelet aggregation caused by *S. mutans* in the presence of fibrinogen. Addition of Cbm+/PA- *S. mutans* strains did not cause platelet aggregation without fibrinogen, whereas their presence resulted in elevated aggregation.

These results suggest that Cbm+/PA- *S. mutans* strains may be highly virulent for IE due to their ability to interact with fibrinogen, thus accelerating bacterial aggregation.

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Anti-Caries Activity of Egg Ovalbumin in an Experimental Caries Biofilm Model on Enamel and Dentin

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Limited evidence suggests a putative inhibitory effect of dietary proteins on hard dental tissue demineralization. The aim was to explore a potential anti-caries activity of the egg protein ovalbumin on a relevant *in vitro* approach. Frozen stocks of *Streptococcus mutans* UA159 were reactivated to form biofilms on saliva-coated enamel and dentin bovine slabs. Surface Knoop microhardness (SH) of the slabs was initially determined. Biofilms grown for 5 and 4 days for enamel and dentin, respectively, were subjected to cariogenic challenges with 10% sucrose three times per day for five minutes. Immediately after sucrose, biofilms were exposed to either a 200 µg/mL solution of ovalbumin or its serial dilutions of 1:10, 1:100 and 1:1000 (v/v), for the entire length of the experiment. Biofilms exposed to 10% sucrose followed only by 0.9% NaCl served as caries-positive control. Culture medium was replenished twice per day. Once completed the experimental phase, biofilms were separated from the slabs and biomass, viable microorganisms and polysaccharide formation was assessed. Final SH was obtained to calculate %SH loss (demineralization). Two independent experiments were conducted, each in triplicate (n = 6). Data were analyzed by ANOVA and a post-hoc test at the 95% confidence level. A reduction ($p < 0.05$) in biomass, extracellular polysaccharide formation, but not in the number of viable cells, was observed for both dental substrates. All ovalbumin concentrations tested showed lower demineralization than the positive control ($p < 0.05$), in a dose-dependent manner. The highest concentration showed a reduction in the %SH loss of about 30% for both enamel and dentin. In conclusion, egg ovalbumin presented to sucrose-challenged biofilms of *Streptococcus mutans* seemed to reduce cariogenicity of a biofilm-caries model. Clinical evidence is needed to confirm these findings.

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Reliability of Perpendicular Reflection Intensity Before and After Caries Induction with a Multi Species Biofilm

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The aim was to test the reliability of a chromatic-confocal sensor to assess reflection intensity measured from perpendicular direction.

Twenty-five enamel specimens were obtained by grinding 200 µm off the buccal surfaces. The specimens were polished until the surface had a roughness of 1 µm. Five measurements of perpendicular reflection intensity (PRI) were made on each specimen (Neuhaus et al.: Caries Res 2011;45:408–14). The measurement area of the PRI device was 20 µm. The specimens were then forwarded into a multi-species biofilm model with *S. mutans* ATCC 25175, *S. sobrinus* ATCC 33478, *A. naeslundii* ATCC 11975 and *L. rhamnosus* ATCC 12104 for 10 d at 37°C in an anaerobic atmosphere. The broth (brain heart infusion BHI) was inoculated with 1 ppmF from NaF and with 1% saccharose; it was exchanged every 2nd day. The numbers of bacteria in the formed biofilm after 10 d were in mean $8.42 \times 10^6 \pm 1.38 \times 10^6$ cfu/mm² surface. The biofilm consisted of 90.55 ± 5.40% of *S. mutans*, 0.72 ± 0.43% of *S. sobrinus*, 1.03 ± 0.02% of *A. naeslundii*, and of 7.70 ± 4.49% of *L. acidophilus*. After cleaning the specimens with sodium hypochlorite 3% the reflection intensity measurements were repeated. Intraclass Correlation coefficients (ICC) were calculated in order to assess the reliability of the method. Mean PRI values were 17 ± 0.5 at baseline and 29 ± 3.78 after inducing caries. ICC at baseline was 0.926 at baseline and 0.794 after inducing mineral loss. In conclusion, under standardized conditions PRI can be regarded as a reliable method. The increase of reflection intensity could be a result of superficial remineralisation.

Antibacterial Effect of *Galla chinensis* and Grape Seed Extracts

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This study was conducted to determine the antibacterial effect of two natural remineralizing agents, the *Galla chinensis* and grape seed extracts, against standard strains of *Lactobacillus acidophilus* (ATCC 4365) and *Streptococcus mutans* (ATCC 25175). **Materials and Methods:** Aqueous solutions of 0.4% *Galla chinensis* (GCE),

6.5% grape seed extracts (GSE), and 0.12% chlorhexidine (CHX) as positive control were prepared. The antibacterial effect was determined using agar diffusion test. Distilled water (DW) was used as negative control. The mean zones of inhibition from each filter paper (n = 15) were measured and recorded to the nearest mm. Data was analyzed using ANOVA and Tukey's post hoc test at p ≤ 0.05. Minimal inhibitory concentration (MIC) and Minimal bactericidal concentrations (MBC) were determined using broth dilution technique. **Results:** CHX, GCE and GSE were significantly more effective than DW (p < 0.05) against *L. acidophilus*. However, they did not differ between groups. For *L. acidophilus* strain MIC results were: 156 µg/ml for CHX, 2 mg/ml for GCE and 4 mg/ml for GSE. MBC results were: 312.5 µg/ml for CHX, 4 mg/ml for GCE and 8 mg/ml for GSE. Significant results (p < 0.05) were found comparing all three active agents with DW against *S. mutans*. There was no statistical difference between GSE and GCE. CHX was significantly more effective than the other samples. For *S. mutans* strain MIC results were: 78.12 µg/ml for CHX, 0.5 mg/ml for GCE and 2 mg/ml for GSE. MBC results were: 156 µg/ml for CHX, 1 mg/ml for GCE and 4 mg/ml for GSE. **Conclusion:** The natural remineralizing agents GCE and GSE present similar antibacterial effects to CHX against *L. acidophilus*. Moreover, they produced zones of inhibition against *S. mutans*. This fact might expand the use of GCE and GSE in both minimal invasive and adhesive dentistry avenues.

Saliva Samples Are Not Representative of Dental Plaque Bacterial Composition

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Saliva is one of the preferred sample types in etiological and epidemiological studies of oral diseases, probably because it is an easy and non-invasive way to obtain biological material. However, there are many different saliva sampling procedures and it is currently unknown to what extent salivary samples are representative of the bacterial population at disease sites. We have studied microbial composition by extracting DNA from saliva samples, followed by PCR and pyrosequencing, and present four lines of evidence that support that saliva is not an appropriate microbiological sample for caries research: 1) bacterial composition in unstimulated saliva samples changes through time during a 24 h-period; 2) the bacterial composition of saliva obtained with six different sampling methods (paper points, oral swabs, non-stimulated saliva by drooling, oral wash with saline solution, spitting and stimulated saliva after paraffin gum chewing) differed. For instance, spitting samples were dominated by *Prevotella* whereas paper points on the mouth floor showed high levels of Streptococci; 3) Independently of which saliva sampling method was used, the observed bacterial composition was heterogeneous, showing a high inter-individual variability, whereas dental plaque samples were more homoge-

neous and reproducible between subjects; 4) None of the saliva samples relate to the composition found in dental plaque from the same individuals; in addition, the bacterial composition in saliva resembled that of the tongue (especially stimulated saliva by chewing) and oral mucosa but not the one at disease sites (teeth surface and gingival crevice). It is therefore advised to use dental plaque when studying dental caries, as saliva samples will not be reliable to characterize microbial shifts between health and disease conditions in etiological and epidemiological studies.

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Antimicrobial Effect of Selenium-Containing Demineralization Inhibition Primer and Surface Sealant

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Enamel and root surfaces at risk of developing caries can be protected against demineralization by prevention of plaque growth. Selenium-containing (SeLECT Defense™) primer and surface sealant tailored to inhibit tooth tissue demineralization were investigated for potential antimicrobial effect against cariogenic plaque by assessment of biofilm formation and viability. 100 tooth blocks were produced and randomly assigned to five experimental groups (20 blocks/group). Following sterilization with ethylene dioxide, the blocks in each group were coated with their respective products as follows: A) Control – no treatment, B) SeLECT Defense™ (SD) primer, C) SD surface sealant, D) Pro-seal sealant, and E) Chlorhexidine varnish. Groups B, C, and D were light-cured for 20 seconds. Each block was incubated at 37°C in 2 mL of glutathione-added (150 µm/l) brain heart infusion (BHI) broth inoculated with 20 µl consortium of *Streptococcus mutans* (ATCC 55677) and *Lactobacillus acidophilus* (ATCC 11975). BHI was renewed every 12 hours. 5 blocks were removed from the BHI after 24, 48, 72 and 96 hours for confocal laser scanning microscope (CLSM) analysis. Following staining with Baclight™ live/dead Bacterial Viability Kit, biofilm viability was determined by calculating the overall live/dead ratios using CLSM. Test groups were compared with control at each time period using Mann-Whitney U-test ($\alpha = 0.05$). SD primer and sealant groups demonstrated significantly ($p < 0.01$) lower live/dead ratio at all time points when compared with the control. After 96 h, SD primer, SD sealant, and chlorhexidine groups exhibited statistically significant ($p < 0.001$) lower live/dead ratio compared to control. At no time did Pro-Seal group show any significant difference when compared with control. In conclusion, selenium-containing primer and surface sealant showed antiplaque properties comparable to chlorhexidine against cariogenic plaque, while Pro-Seal did not demonstrate any antiplaque effect.

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Dental Caries, Probiotic Bacteria and Human Beta Defensin 3 in Chilean Preschool Children

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The aim of this study is to establish changes in human beta defensin 3 (HBD3) expression adjusted by caries experience among preschool children that consumed probiotic bacteria. 28 children, 2–3 years-old, attending nursery schools in Santiago, Chile participated in this study. The nursery schools were randomly assigned to two parallel groups. Intervention group: children were served 150 ml milk supplemented with *Lactobacillus rhamnosus* LHR08 (10^7 CFU/ml) and Control group: children were served 150 ml of standard milk. The observation time was set at 3 month of intervention. Clinical examinations were performed according to ICDAS II criteria for base line. Beta-defensin-3 levels in saliva samples were determined using enzyme-linked immunosorbent assays according to manufacturer's instructions. Briefly, samples (20 fold diluted) and standard protein were added and plates were incubated for 1 h. Then, they were washed 4 times and biotinylated anti-human BD3 (1:100) was added and incubated for 1 h. Plates were treated with streptavidin-HRP (1:100) during 30 minutes and washed 5 times. Finally, proteins were detected after 10 minutes of incubation with TMB Solution. The reaction was stopped using Stop Solution and plates were read at 450 nm. In the group of caries free preschool children the media of HBD3 was 160.7 pg/mL and in the group with dental caries was 567.5 pg/mL. After 90 days of Probiotic bacteria consumption the group of caries free preschool children the media of HBD3 was 200.6 pg/mL and in the group with dental caries was 208.9 pg/mL. The difference of HBD3 levels among individuals from the probiotic group and the control group are -239.2 pg/ml and 40.2 pg/ml respectively ($p < 0.05$). In conclusion, there are differences among preschool children in human beta defensin 3 (HBD3) expression adjusted by caries experience when they are exposed to probiotic bacteria.

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Immunomorphological Evaluation of Collagen in Rapidly Progressive Dentin Carious Lesions

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Early morphologic studies have reported that collagen in the external zone of carious dentin is demineralized and irreversibly denaturated. This fact has determined the principles of classic restorative dentistry. **Objective:** This study was designed to describe the immunomorphological properties of type-I collagen in the external zone of carious dentin. **Methods:** Fifty teeth with active dentin caries and ten healthy teeth were extracted for dental emergency or orthodontic reasons and fixed in formaldehyde or Carnoy, demineralized and embedded in paraffin. 10 µm sections were incubated with polyclonal or with monoclonal anti-type-I collagen antibodies and then treated with the biotin-streptavidin-peroxidase system. The tissue infection was visualized with the Gram's stain. A digital densitometric analysis, was performed to determine the intensity of the immunoreactions. Data were analyzed using Student's t-test ($p < 0.05$). Some immune-labeled sections were studied with transmission electron microscopy. For confocal microscopy, the secondary antibody was labeled with FITC. As negative control, the primary antibody was replaced by non-immune serum. **Results:** With both, immunoperoxidase and confocal microscopy, the intertubular matrix in the infected dentin was labeled with both antibodies. Statistically significant differences were found between experimental and negative control ($p = 0.0124$). At the ultrastructural level, collagen fibrils showed the characteristic band pattern. However, many fibrils were disorganized into the microfibrils. Despite this, fibrils and microfibrils were strongly stained with the antibody. In conclusion, the carious attack produces a modification of the supramolecular organization of collagen molecules in dentin, but not protein denaturation. This fact, is supported by the fact that the monoclonal anti-type-I collagen antibody only recognizes the native (helical) form of collagen and does not reacts with the denaturated molecule.

We would like to acknowledge to Finis Terrae University for the fund of this research through Academic Vicerectory's Concurable Foundation.

Effect of Calcium Hydroxide and Mineral Trioxide Aggregate on Tertiary Dentin Formation in Rats

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We investigated the pulpal response to direct pulp capping in rat molar teeth using mineral trioxide aggregate (MTA) and calcium hydroxide (CH).

A palatal cavity was prepared in rat maxillary molar teeth. Either MTA or CH was placed on the exposed pulp and all cavities were restored with composite. Rats were sacrificed for histological evaluation after 12 hours and at 2, 7, 14 and 21 days.

In both the MTA and CH groups, tertiary dentin formation was clearly observed on histology after 14 days. The MTA-capped pulps were found to be mostly free from inflammation, and hard tissue of a tubular consistent barrier was observed. In contrast, in CH-capped teeth, excessive formation of tertiary dentin toward residual pulp was evident. The pulpal cell response beneath the tertiary dentin layer was examined by immunofluorescence using antibodies against DSP (Dentin sialoprotein). After 2 days, a few DSP immunopositive cells, most of which showed a cuboidal shape, appeared beneath the predentin layer. At 7 days, DSP-immunopositive cells with columnar odontoblast-like cells were seen beneath the newly formed hard tissues. At 14 and 21 days, DSP was more abundant in the vicinity of the odontoblastic process along the dentinal tubules than in the mineralized tertiary dentin. The CH group showed strong expression patterns in terms of DSP immunoreactivity. Our results thus indicate that MTA may be a more effective pulp capping material as it induces the differentiation of odontoblast-like cells and the formation of tertiary dentin without the loss of residual pulp functions.

Session 2 Epidemiology

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Socioeconomic Inequality and Caries Prevalence: A Systematic Review and Meta-Analysis

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Socioeconomic inequality is frequently associated with an unfair and polarized distribution of health. We aimed at systematically reviewing and meta-analysing how socioeconomic and educational factors affect the prevalence of dental caries. **Methods:** We performed a systematic review and meta-analysis according to PRISMA guidelines. Electronic databases were screened for papers published between January 2000 and August 2013, and additional iterative hand searches performed. We included cohort, case-control and cross-sectional studies investigating the association between inequality of individual education, income or occupation and dental caries prevalence, experience or incidence. Here, we only report data on the association between these factors and caries prevalence. Effect estimates were extracted from the most extensive model, and inverse generic random-effects meta-analyses performed, with pooled Odds Ratios and 95% CI being calculated. Publication bias was assessed using Funnel plots and Egger test. Heterogeneity was assessed and underlying reasons explored using sub-group analyses and mixed-effect meta-regression. Effects of missing values were controlled by imputation. Risk of bias was assessed using a modified Newcastle-Ottawa-Scale (NOS). **Results:** Our search yielded 5,539 articles, and 157 studies with a total number of 458,935 observations were eventually included. Most studies showed low (NOS 3-4) and moderate quality (NOS 5-7). High educational status, income and occupational status significantly reduced the risk of having dental caries (OR [95%CI] = 0.60 [0.52–0.58], 0.67 [0.61–0.75], and 0.78 [0.63–0.96], respectively). Imputing missing values decreased the magnitude of the associations, eventually losing the association between occupational status and caries prevalence. Meta-regression did not indicate significant in-

fluence of study- or country-related co-factors. **Conclusions:** Prevalence of dental caries is significantly associated with socioeconomic status. Heterogeneity in study design and quality of most studies limit the strength of evidence.

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Validity of the Child Health Utility 9D Index in New Zealand Children

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Aim: To investigate the validity of the Child Health Utility 9D (CHU9D) Index in New Zealand children. **Method:** A survey was conducted of New Zealand children aged between 6 and 9 years attending for routine dental examinations in community clinics in 2012. Health-related quality of life data were collected, including the Child Perceptions Questionnaire (CPQ11-14) and the CHU9D. Sociodemographic characteristics (sex, age, ethnicity and household deprivation) were also recorded. Dental therapists undertook routine clinical examinations, with charting recorded for each child for decayed, missing and filled deciduous teeth (dmft) at the d3 level. **Results:** One hundred and forty 6-to-9-year-olds (50.7% female) took part in the study (93.3% participation rate). The mean d3mft was 2.4 (SD = 2.6; range 0 to 9). Both questionnaires detected differences in the impact of dental caries on quality of life, with scores in the expected direction: children who presented with caries had a higher CPQ11-14 score (indicating poorer oral health-related quality of life) than those who were caries-free. Children with no caries had a higher mean utility score than those with caries. The difference for the CPQ11-14 was statistically significant;

however, this was not the case for the CHU9D. There was a significant difference in the prevalence of CPQ11-14 and subscale impacts by mean CHU9D scores, with children experiencing no impacts having mean CHU9D scores closer to 1.0 (perfect health). **Conclusion:** The CHU9D shows promise as a utility measure in this population. The use of the CHU9D in child oral health studies will enable the calculation of quality-adjusted life years (QALYs) for use in economic evaluation.

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Untreated Severe Decay and Oral Health Related Quality of Life Among Preschool Children in Northwest Russia

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Aim: To assess the impact of untreated severe carious lesions on quality of life of pre-school children and their families in Northwest Russia. **Methods:** In 2012, a sample of 292 parents of 2–6-years-old children completed the Russian version of the Early Childhood Oral Health Impact Scale (R-ECOHIS) in Kotlas and Koryazhma cities. Their children were involved into dental examination and each coronal tooth surface was assessed using the Caries Assessment Spectrum and Treatment (CAST) index. All parents gave informed consent. Clinical consequences of untreated carious lesions were diagnosed as ‘code 6’ (pulpal involvement) and ‘code 7’ (abscess). Impact of dental caries to the quality of life was measured by comparing average ECOHIS scores for children with different caries status classified as follows: group 1 (n = 83) – caries free; group 2 (n = 97; d3mft ≥4); group 3 (n = 112; pufa >0). ECOHIS scores across the groups were assessed separately using Kruskal-Wallis tests with Mann-Whitney post-hoc tests. The study was approved by the Ethical Committee of the Northern State Medical University, Arkhangelsk, Russia. **Results:** The mean total ECOHIS-scores were 0.9±0.3, 3.1±0.5 and 5.4±0.5 for the three groups, respectively. The mean ECOHIS-scores for the child section were 0.8±0.2, 2.0±0.4 and 3.2±0.3 for the three groups, respectively. The corresponding means for the family section were 0.1±0.1, 1.1±0.2 and 2.1±0.2. Significant differences between mean ECOHIS scores were observed for both child and family sections (p < 0.001 for both omnibus tests). Post-hoc tests also revealed significant differences between each of the groups (p < 0.001 for all comparisons except the difference in child impact scores between the groups with caries free children and children with high caries activity, p = 0.002). In conclusion the highest ECOHIS scores were found among parents with children who had diagnosed untreated severe decay, particularly progressed into the pulp. Untreated severe carious lesions were associated with more negative impacts on the quality of life of preschool children and their parents/caregivers. Personal funds were used for this study.

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Outcomes 18 Years After a Caries Preventive Programme Was Implemented on Children in Moscow

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This study reports the long term effect (18 yrs) of the Nexö-method for preventing caries, initially implemented on groups of children in Moscow in 1994 [Ekstrand et al: Caries Res. 2000;34:8–19]. This study focuses on two of the three initially included groups of children: 6 years old children in 1994 (in this study termed Group A) and 11 years old children in 1994 (termed group B). These two groups were both divided in test- and control groups with 50 children in each of the 4 subgroups (A_{Test} , $A_{Control}$; B_{Test} , $B_{Control}$). In 2012, >80% the now 24-(group A) and 28-yr-olds (group B) were reexamined, by the original examiner, who was blinded to whether the participants initially belonged to the test- or the control-groups. After the reexamination, the participants were interviewed by a third person. Finally, caries data were collected from 100 24- and 100 28-yr-olds (external control groups A, B), who attended the dental school in Moscow (50%) and in a private clinic in Moscow (50%). Outcome variables: Plaque-and gingival status (each expressed by means of 3 severity scores) and DMFT/S. Chi-square analyses disclosed that the control groups in 2012 had significantly higher plaque scores than the participants in the test groups (p-values <0.05). No differences were seen with respect to gingivitis scores (p-values >0.41). Mean DMFT in 2012 were in group A: $A_{Test} = 6.98$ (1 SD = 4.29), $A_{Control} = 8.84$ (3.32) (t-test, p = 0.02). External control group A = 8.89 (4.86), (p- A_{Test} versus external control group A = 0.01; p- $A_{Control}$ versus external control A = 0.94). Group B; $B_{Test} = 6.74$ (1SD = 3.81), $B_{Control} = 8.70$ (3.84) (t-test, p = 0.02). External control group B = 9.03 (5.19) (B_{Test} versus external group B = 0.03; p- $B_{Control}$ versus external control B = 0.68). When data were expressed in DMFS, the tendency was the same as above mentioned. The interview indicated that the test-group participants had a higher level of emphasis on factors which are considered important for the individual to control for caries, than the participants in the control groups. To conclude, the data on this group of Moscow citizens indicate that there is a long lasting positive effect of the Nexö-method given during childhood.

Caries Experience in German Special-Olympic Athletes – Comparison of Regional Data

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The aim was to compare caries experience of athletes with intellectual disabilities participating in Special Olympics events in two German regions. **Methods:** Free voluntary dental examinations were offered to athletes participating in regional Special Olympics events in North Rhine-Westphalia (NRW) and Thuringia (TH) between 2011 and 2013. Dental examinations were performed according to WHO criteria by dentists. In addition, information about the athletes' oral hygiene habits and accommodation was collected by standardized questionnaire. All athletes or care takers gave written informed consent to participate at the Special Smiles Oral Health Program. **Results:** 516 athletes aged between 10 and 64 years (mean age: 27.4, SD = 11.0) participating in 5 regional events, 319 from TH and 197 from NRW, were examined. Caries prevalence of all athletes was 85.7% (95% CI = 0.56). The mean DMFT was 7.7 (SD = 6.8) and the SIC-Index 14.8 (SD = 4.3). Significant higher DMFT was found in athletes with gingivitis (8.3, SD = 6.5 vs. 7.0, SD = 7.1; $p = 0.02$), without fissure sealants (8.9, SD = 7.0 vs. 3.8, SD = 6.2; $p = 0.00$) and those performing oral hygiene without assistance (7.8, SD = 6.9 vs. 4.7, SD = 5.4; $p = 0.01$). Caries experience in TH and NRW did not differ significantly although in TH more filled teeth were recorded than in NRW (FT = 5.1, SD = 5.3 vs. FT = 4.4, SD = 4.9) and more decayed teeth (DT = 0.9, SD = 1.7 vs. DT = 0.7, SD = 1.4). Regarding accommodation, in TH higher DMFT was registered in athletes living at home (7.9, SD = 6.7 vs. 6.9, SD = 6.5) and in NRW higher DMFT was found in athletes living in institutions (9.3, SD = 7.3 vs. 7.0, SD = 6.9). **Conclusions:** In athletes with intellectual disabilities caries experience and treatment need are high in Germany with regional variances. Caries experience was rather influenced by assistance in oral hygiene, gingivitis and fissure sealants than by geographic region.

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Prevalence and Severity of Dental Caries in Lithuanian 4–6 Year-old Children Attending Kindergartens

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The purpose of the study was to describe the prevalence and severity of dental caries among Lithuanian 4–6 year-old children attending kindergartens. A cross sectional survey was conducted in five major cities and in the corresponding rural areas of Lithuania. The study population comprised 1,351 child selected by multistage cluster sampling, presenting a total of 25,081 deciduous teeth and of 1,529 permanent molars for the clinical examination. Dental caries was scored using Nyvad criteria for assessment of caries lesion severity and activity. Results: ninety percent of all children presented dental caries: mean deft (SD): 7.9 (4.9), where d component comprised 90% of the index [mean number (SD) of cavitated lesions was 4.4 (3.9), and of non-cavitated lesions 2.8 (3.0), respectively]. Prevalence of cavities and fillings increased significantly with age, while prevalence of non-cavitated lesions did not differ significantly among 4-, 5-, and 6-year olds. The mean number (SD) of active lesions was 4.4 (4.9), and of inactive lesions 2.7 (3.3), in the total study group, respectively. At the noncavitated level, prevalence of active lesions decreased, while prevalence of inactive lesions increased significantly with the age of children. Caries in the 1st permanent molars was detected in 41% of all children [mean DFT (SD): 1.1 (1.5)], 27% of the lesions were cavities, and 36% of all detected lesions were active. The survey revealed that dental caries was poorly controlled among Lithuanian pre-schoolers: lesion severity increased significantly with age. However, the increasing proportion of inactive lesions at the noncavitated level of caries detection indicates the potential for non-operative control of caries in this population.

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National Caries Strategy for Children in Greenland. Principles and Outcome Data for 3- and 9-Year-Olds After 4 Years

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Due to high caries prevalence in Greenland a national caries strategy (CSG) for 0–15-yr-olds based on Danish experience was

implemented in 2008. This report aimed to describe the principles of CSG, on the national level report the caries outcomes for 3- and 9-yr-olds in 2008 (baseline) and in 2012 and to assess the effect of CSG on the same age groups. GSC focused on predetermined visits/examinations related to dental ages, combined with individual risk related visits, an up to date program for oral health education and promotion, and a predetermined fluoride and sealing policy. Of the total cohorts, 75% of 3-yr-olds and 88% of the 9-yr-olds in 2012 had their caries status recorded. Corresponding figures in 2008 were 47% and 79%, respectively. Seventy-six % of the 3-yr-olds had a defs = 0 in 2012 compared to 64% in 2008 ($p < 0.0001$). Corresponding DMFS = 0 data for the 9-yr-olds were 65% versus 57% ($p = 0.003$). In both age groups, the caries experience in 2012 varied among the 17 Greenlandic districts and was significantly higher for children living in remote settlements than in towns ($p < 0.05$). The annual increase of percentage of 3- and 9-yr-olds with defs/DMFS = 0 after implementation of CSG in 2008 was twice the rate of increase in the years from 1996–2008. Fluoridated table salt was introduced on a national scale late in 2010. However, it never became a confounding factor because its sale was negligible. To conclude, by means of the CSG it has been possible to improve the dental health for children significantly from 2008 to 2012 exemplified by the caries experience for the 3- and 9-yr-olds.

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Caries Prevalence: def/DMF Index and ICDAS System 8–12 Years Old Children: Villavicencio Colombia

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In Colombia, dental problems are among the most common diseases. According to the Colombia National Oral Health survey from 1998, the eastern region of Colombia had the highest caries experience. The aim of this study was to determine the prevalence and severity of dental caries in children 8 to 12 years in Villavicencio Meta using the def/DMF index and the ICDAS scoring system. Samples of 459 children enrolled in educational public schools, with pre-signed parental consent were examined after professional prophylaxis. **Outcome Variables:** dmft, DMF-T and ICDAS at the tooth level. The reproducibility (Kappa) for the 6 examiners, previously trained in the ICDAS criteria from an expert examiner was higher than 0.79. **Results:** Of the 459 children examined 48.8% were girls and 51.2% were boys. The number of children in each age cohort was: 8-yr-olds = 83 (18.1%), 9-yr-olds = 82 (17.9%), 10-yr-olds = 90 (19.6%), 11-yr-olds = 94 (20.5%) and 12-yr-olds = 110 (24%). The mean deft at the age of 8 was 1.83 and at the age of 9 = 2.45. Expressed by the ICDAS score increased from 4.48 at age 8 to 5.53 at age 9. Mean DMFT among the participants were highly constant from the age of 8–12 around 1.6. Expressed by the ICDAS system the caries experience increased from 4.48 to 7.2. The f/F components made up about 30% of the deft/DMFT index and about 8% of the entire sample had teeth lost, due to caries. To

conclude, the samples in this study had a high caries prevalence, when expressed by the traditional def/DMF index, increasing 3 to 4 times when the caries experience was expressed by the ICDAS system. About 70% of the ICDAS-identified lesions were at the initial stages, indicating a very high need of non-operative interventions.

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Students' and Lecturers' Perception of the Degree of Difficulty of Caries Detection-Related Learning Topics

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We aimed to explore the degree of difficulty of caries-detection-associated-topics perceived by dental students and lecturers as a pedagogical step in the development of a teaching tool in this field. A convenience sample comprising different academic levels (undergraduate students vs. graduate students/pediatric dentistry lecturers) were invited for participation. Two spreadsheets (horizontal/vertical), were created considering some key topics in the caries detection process. A horizontal evaluation intended to explore each topic in an isolated way (Likert-scale 1-5) while a vertical one intended to classify comparatively, the participants' perceived difficulty per topic (1-11). Afterwards, these instruments were applied to the participants exploring the perceived difficulty in topics. For the analysis, the values in the spreadsheets were combined obtaining the subject's final perception. Associations between the subjects' degree of the perceived difficulty and academic level were estimated by Poisson and Linear Regression. Analysis of variance was used to determine differences regarding the perception among evaluated topics in groups. A total of 98 subjects participated. Caries histopathology and detection of proximal lesions (visual and tactile) were the topics perceived as the most difficult in the process of caries detection by both students and lecturers. Undergraduates considered more difficult the differentiation between an extrinsic pigmentation and a brown-spot (caries lesions) as well as differential diagnosis between caries and enamel developmental defects or non-cariou lesions as topics than graduates and lecturers reported (respectively, regression coefficient (Coef) = 14.54; Standard Error (SE) = 3.34; $p < 0.001$ and Coef = 8.40; SE = 3.31; $p = 0.01$). Some topics as histopathology and proximal detection of caries lesions are identified as most difficult despite the academic level. However, several topics are differently perceived by the each studied group. These results could be useful for developing teaching tools based on the students real learning needs.

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Oral Health of 6 and 12 Year-Old Children in Nepal: A Cross-Sectional Study

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The aim of the study was to assess the oral health of 6 and 12 year-old schoolchildren in Kathmandu Valley and to identify its social and behavioral determinants. The study was carried out between 11/06/2011 and 12/18/2011 by 2 calibrated dentists. dft and DMFT were recorded by mean of a clinical examination with a mirror, a probe in natural light. Six and 12 year-olds of public, private and community schools were included in the study. The study population included 1,322 children, 647 5–6 year olds (317 girls and 330 boys) and 675 12–13 year olds (350 boys and 325 girls) (participation rate = 99%). The mean (m±SD) dft in 5–6 year-olds was 3.9±4 (d = 3.8±3.9; f = 0.1±0.5) and 43.4% of the children had at least 4 decayed teeth. In the 12–13 year-olds, the mean DMFT was 1.0±1.5 (D = 0.9±1.5; M = 0.0±0.1; F = 0.1±0.3) and 9.0% had at least 4 decayed teeth. Among the 5–6 year-olds, 72.3% needed dental care (41.6% of the 12–13 year-olds). dft was significantly higher in private schools (4.3 [3.9–4.7]) than in public schools (3.2[2.7–3.7]) (Mann-Whitney test p = 0.0365). The 20% of the 5–6 year-olds declaring eating sweets everyday had more decayed teeth (dft = 5.7) than the others (3.7) (Mann-Whitney test, p = 0.0165). In the 12–13 year-olds, the 40% eating sweets every day, DMFT was 1.1 (0.9 for the others). Most children brushed their teeth only once a day (67% of the 5–6 and 74% of the 12–13) and mostly before breakfast (59% of the 5–6 and 41% of the 12–13).

Fluoridated toothpaste was used by 68% of the 5–6 years-old and 81.8% of the 12–13 year-olds. Among the 5–6 year-olds 63.3% had never been to a dentist (34.8% of the 12–13 year-olds). In conclusion, the impact of tooth decay is high in the 5–6 year-olds, especially in private schools, and in children eating sweet. The dental care needs are mostly unmet. Health and dietary habits should be improved.

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Prevalence of Developmental Tooth Defects in the Permanent Dentition of Schoolchildren

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Parallel with the distinct caries decline in children and adolescents in industrialized countries other dental hard tissue alterations have increasingly attracted scientific attention in recent years. The aim of this study was to determine the prevalence of developmental tooth structure defects in the permanent dentition of schoolchildren in Hamburg. In an epidemiologic survey, 1,580

schoolchildren aged 6 to 15 years were examined for developmental defects by two calibrated investigators. The findings differentiated between fluorosis, trauma of the preceding primary tooth, infection of the preceding primary tooth, molar-incisor-hypomineralisation, and alterations without any aetiological assignment. Statistical evaluation included prevalence rates and relations to gender and social class (education level of the parents). 36.0% of the school children exhibited developmental tooth defects (fluorosis 24.6%, molar-incisor-hypomineralisation 13.5%, infection of the preceding primary tooth 1.5%, trauma of the preceding primary tooth 0.5%, and alterations without aetiological assignment 1.1% – some children exhibiting more than one kind of defect). Less than 2% of the children exhibited severely fluorotic teeth. There was no gender related difference. However, the prevalence was significantly related to social class with a prevalence in upper class children of 33.3%, middle class 34.3%, and lower class 42.1% (p = 0.032, Chi²-test). In addition, there was a strong relation between the prevalence of developmental defects and caries: In caries free children (D1-level) the prevalence was 28.6%, while it was 44.4% in children with caries (p < 0.001). Compared with previous investigations the prevalence of developmental tooth defects seems to increase. Although the severity of most defects is low, the high prevalence requires attention. Health services should be ready to deal with increasing numbers of patients with such alterations.

Higher Serum 25-Hydroxy-Vitamin D Levels Are Associated with a Lower MIH Risk

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It is well-known that ameloblasts and odontoblasts are target cells for vitamin D and it plays, therefore, a key role in enamel and dentine formation. Therefore, we analyze the association between 25-hydroxy-vitamin D (25(OH)D) in serum and molar-incisor hypomineralisation (MIH) considering dental, medical, behavioural and socioeconomic data from 1,048 children of the 10-year follow-up from the Munich GINI-plus and LISA-plus birth cohorts. The study protocol was approved by the ethics committee at the Bavarian General Medical Council. The dental examination included the diagnosis of MIH according to the criteria of the European Academy of Paediatric Dentistry. Children who had at least one first permanent molar with a demarcated opacity, enamel disintegra-

tion or atypical restoration were categorized as MIH. Further, (non-)cavitated caries lesions were registered in permanent teeth according to ICDAS, UniViSS and WHO criteria. Serum concentration of 25(OH)D was measured by fully automated modular system by Roche and corrected for date of sampling to normalize for seasonal variability using generalized additive models with thin plate regression splines. Different types of logistic regression and Poisson hurdle models were calculated. 13.6% (143/1048) of the study population was identified as having MIH. 16.4% (172/1,048) of all 10-year-olds showed caries-related defects (D3-4MFS >0); mean D3-4MFS value amounted to 0.4 (SD = 1.1). The mean concentration of corrected 25(OH)D was 75.8 nmol/L (standard deviation 22.0 nmol/L). After adjusting for gender, age, BMI, parental education, equivalent income, time spent in front of TV/PC a 10 nmol/L increase in 25(OH)D serum concentrations was significantly associated with lower odds ratio of having MIH (OR = 0.89; $p = 0.006$). Furthermore, higher 25(OH)D values were associated with a lower number of caries-affected teeth. We conclude that higher 25(OH)D serum concentrations were with a lower number of caries-affected teeth.

The GINI study was funded for 3 years by grants from the Federal Ministry for Education, Science, Research and Technology (Grant No. 01 EE 9401-4), and the 6 and 10 year follow-ups of the GINI-10 plus study were partially funded by the Federal Ministry of Environment (IUF, FKZ 20462296). The dental investigation was funded by grants from the German Research Foundation (Deutsche Forschungsgemeinschaft, FKZ KU 2518/1-1, KU 2518/1-2, HE 3294/7-1 and HE 3294/7-2). The GABA GmbH, Lör-rach, Germany, supported the dental examination and provided oral health care packages for all of the participating children.

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Caries Experience of Immigrant Children Residing in the Metropolitan Region of Chile: A Pilot Study

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In Chile there has been a progressive increase in immigrant population, especially from neighboring countries attracted by economic expectations. The increase in the immigrant population affects country epidemiological indices. The aim of this pilot study was to describe the caries experience of immigrant children participating an oral health program in the Metropolitan Region of Chile and to compare the results with those of Chilean children.

Methods: A convenience sample of 391 children (318 Chilean children and 73 foreign children) aged 6–12 years-old, was select in basic schools by the ‘Santiago Children Oral Health Program’. The clinical exams were conducted by a calibrated examiner, according to World Health Organization criteria. **Results:** Foreign group included Peruvian (72.3%), Colombian (2.7%), Ecuadorian (20.5%) and Bolivian (4.1%) children. The mean DMFT was 1.07 (CI95%: 0.89–1.24). In Chilean children, the mean DMFT was 1.09 (CI95%: 0.89–1.29) and in foreign children, it was 0.95 (CI95%: 0.57–1.34). The resulting dmft for total sample was 1.62 (CI95%: 1.36–1.89).

In Chilean group the dmft was 1.55 (CI95%: 1.26–1.85) and in foreign group the mean was 1.93 (CI95%: 1.28–2.57). 25.4% of Chilean children and 34.2% of foreign children presented untreated caries lesions. t test showed no significant difference between the mean DMFT ($p = 0.54$) and dmft ($p = 0.28$) by groups. In conclusion we were not able to detect any significant differences between the caries experience in Chilean versus foreign children.

The present study was funded by the University of Chile.

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Gender-Dependent Prediction of High Caries Increment in Adults – A 5-Year Longitudinal Study from North-East Germany

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Objective: The aim of this study is to develop an easily applicable prediction model for high coronal caries increment in dentate adults (20–79 years) from a representative sample ($n = 2,565$) to identify a high risk-group for intensified caries-preventive programmes. **Methods:** The data from the Study of Health in Pomerania (SHIP-0: 1997–2001) and the 5-year follow-up (SHIP-1: 2002–2006) were used for the analyses. The oral health examination was conducted according to WHO criteria [1997] with half-mouth recording. 2,565 participants were included for statistical analyses, while recording 619 drop-outs who were significantly older, had a lower school education and who were more frequently current smokers, but still had a better self-perception of their teeth. **Results:** The majority of the study-population (76%) exhibited caries incidence in this 5-year period with a mean of 3.71 ± 4.70 Δ DMFS and a median of 2 Δ DMFS (half-mouth). Caries increment showed a polarized distribution, as the high caries increment group (≥ 9 Δ DMFS in half-mouth, 11.4% of the sample) comprised 40% of the total increment. The variables male gender, age ≥ 40 years, lower school education or lower income, current smoking, pain-associated dental visit, baseline caries experience and a non-satisfying self-perception of teeth showed a statistically significant influence on high caries increment. The optimized prediction model allowed a fair to good prediction on an epidemiological level for men (AUC = 0.75). Contrarily, a poorer prediction for women (AUC = 0.68) was attained, as the factors smoking, school education and pain-associated visit did not have a significant impact on the prediction of high caries increment in women. **Conclusion:** Due to very high caries prevalence and increment, a population-based prevention in adults should be optimized first, before risk-group specific preventive programmes might be implemented.

The Study of Health in Pomerania (SHIP-0 and SHIP-1) were financed by the ‘Bundesministerium für Bildung und Forschung’ (Federal Ministry of Education and Research) in the Grant period: 1st of January 1997–30th of June 2007 and by the ‘Kultusministerium’.

Caries Experience and Treatment Needs Among the Students of Nice-Sophia Antipolis University

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The Dental School in the University of Nice – Sophia Antipolis (UNS) proposed to involve their 6th year students in a preventive program to improve prevention and diagnosis of oral diseases among all other undergraduate students. The aim of this work was to present the 2012 results for scientific students (Medicine, Odontology, Mathematical Sciences, Biological Sciences, Technology, Sport Sciences). The Department of Preventive Medicine and Health Promotion invite students enrolled for the first time at the UNS for a systematic and compulsory medical examination. A dental screening was proposed during this first consultation for those who wished it. 6th year dental students performed standardised oral examinations (kappa value of 0.82). A questionnaire was used to collect both administrative and clinical data (DMFT index, Dean's fluorosis index, CPITN). Statistical analyses were performed thanks to SPSS 18.0. 410 students aged 18.8±1.76 years were included in this study. The mean DMFT score was 1.22±2.03 (D 0.38±0.83, M 0.04±0.28, F 0.80±1.71). Clinical signs of fluorosis were scarce (7.1%). A malocclusion was present in 15.1% of the cases. 67.1% had benefited from an orthodontic treatment. Overall, 74.1% needed an oral hygiene motivation, 48% a dental scaling and 23.4% a caries treatment. Conservative treatment needs were significantly related to malocclusion ($p = 0.006$) and presence of calculus ($p = 0.008$). The binary logistic regression revealed an OR of 2.28 [1.27–4.08] for malocclusion and 1.85 [1.16–2.96] for calculus.

Overall, the conservative treatment needs were low for this population of scientific students probably because they were motivated volunteers. Collective caries prevention programs carried out in France during the last decades may be responsible for this advance. Nevertheless, nearly three-quarters of the students still had a need for oral hygiene motivation.

Does the CAST Instrument Produces Comparable DMF/dmf Scores as the WHO Criteria?

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The Caries Assessment Spectrum and Treatment (CAST) is an epidemiological instrument that has been validated for face, content and construct, and has its clinical reproducibility tested reliably. That of WHO is the most used epidemiological criteria for caries detection worldwide. **Aim:** The aim of this study was to investigate whether both methods differ in determining caries outcomes using the DMF/dmf index in epidemiological surveys **Methods:** An epidemiological survey was carried out among 6–11-years-old children in Brazil. Three trained and calibrated examiners performed examinations at school premises using CAST and WHO criteria. Time of examinations was recorded. DMFT and dmft index were obtained according to WHO criteria and the CAST instrument using the most appropriate cut-off point for the decayed component (D = codes 5,6,7). **Results:** 419 children were examined using both methods. DMFT count for using CAST and WHO criteria was 0.20 and 0.19, respectively ($p = 0.72$: correlation coef = 0.78); whereas the dmft count was 1.88 (CAST) and 1.99 (WHO) ($p = 0.005$) with correlation coefficient of 0.94. DMFS count for using CAST and WHO criteria was 0.33 and 0.30, respectively ($p = 0.48$) with correlation coefficient of 0.72, whereas the dmfs count was 5.11 (CAST) and 5.34 (WHO) ($p = 0.09$) with a correlation coefficient of 0.93. Mean time spend on applying CAST and WHO was 66.3 and 64.7 sec, respectively ($p = 0.000$). **Conclusions:** DMFT/S and dmfs counts, calculated from using the CAST instrument in this age groups, are not statistically significantly different to those obtained from using WHO criteria and the correlation coefficients between them are high. There is a high equivalence between both methods when recording decayed, missing and filled teeth in epidemiological surveys covering this age group.

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Session 3

Demineralization- Remineralization

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Modification of Surface Pre-Treatment for Resin Infiltration to Natural White Spot Lesions

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The aims of this study were to investigate an alternative pre-treatment method for resin infiltration using 37% H₃PO₄ with a brush applicator, and to evaluate the penetration effect of the infiltrant for masking the natural white spot lesions (WSLs) in human teeth. Seventy extracted human molars and pre-molars with non-cavitated WSLs were collected. Thirty teeth that met criteria of ICDAS code 2 were sectioned, providing total of sixty paired halves. The experimental approach involved applying 15% HCl gel for 120 seconds as a control group, and 37% H₃PO₄ gel was gently rubbed with a brush applicator for 30 seconds. To evaluate the penetration effect of infiltrant by pre-treatment, the specimens were treated with infiltrant (Icon[®]). Thicknesses of removed surfaces and percentages of infiltrated areas (IA%) were evaluated by CLSM, and micro-morphological changes were observed by SEM. The main results were that there was a significant difference in the mean thicknesses of removed surface layers between the control (36±7.62 μm) and experimental group (13±2.76 μm) ($p < 0.001$), but that the means of IA% were similar in both groups ($p > 0.05$). The SEM images showed that the prism cores were preferentially dissolved in the control, while the prism peripheries were preferentially dissolved in the experimental group. It can be concluded that the use of 37% H₃PO₄ with a brush applicator for 30 seconds can increase permeability and minimize removal in the surface layer of natural WSLs. Moreover, the effect of resin infiltration was similar to the control that was pretreated with 15% HCl gel for 120 seconds *in vitro* study.

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Modification of Surface Pre-Treatment to Improve Safety and Efficacy for Resin Infiltration

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A low-viscosity resin (infiltrant) has been used to inhibit further progression of white spot lesions (WSLs) and to resolve its esthetical problem. The aim of the work was to investigate an alternative pre-treatment method to increase pore volume of the surface layer of WSLs, and to evaluate the penetration effects of the infiltrants according to the pre-treatments. Sixty-two artificial lesions were made on bovine teeth, and the experimental approach involved applying 15% HCl gel for 120 seconds as a positive control, and 37% H₃PO₄ gel was applied for 30 seconds using three different methods; H₃PO₄ only group, H₃PO₄ sponge group, and H₃PO₄ brush group. When a sponge or a brush was used, the acid gel was gently rubbed with the applicators during application time. To compare the effects of resin infiltration by pre-treatment, twenty paired halves of specimens were treated with infiltrants (Icon[®]). Thicknesses of removed surface layers and infiltrated areas were evaluated by CLSM. The main results were that there was no significant difference in removed thicknesses between the positive control and the 37% H₃PO₄ brush group ($p > 0.05$), but that the mean percentage of infiltrated area was significantly higher in the 37% H₃PO₄ brush group (84.13±7.58) than the positive control (63.51±7.62, $p < 0.001$). In the SEM observation, increased pore volume was observed more in the 37% H₃PO₄ brush group than in the positive control. The conclusions of the work were that the use of 37% H₃PO₄ with a brush applicator for 30 seconds could increase not only the pore volume of the surface layer of WSLs but also the percentage of infiltrated areas, compared with the use of 15% HCl for 120 seconds.

Effect of Exposure to Plaque-Fluid Representative pH-Cycling Conditions on Bovine Enamel Demineralisation

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The present study aimed to determine whether exposure to pH-cycling conditions representative of plaque fluid served to reduce mineral loss during a subsequent demineralisation. Polished bovine enamel blocks were exposed to a demineralisation solution representative of plaque fluid (pH 5.11, 2.25 mM calcium chloride dihydrate, 17.7 mM potassium dihydrogen orthophosphate, 32.9 mM lactic acid, 4.25 μ M fluoride (As NaF)) for 0, 1 or 3 h, before being subjected to a pH-cycling regime for 0, 2, 4, 6, 8, 10 or 12 days (30 min demineralisation at 9 am, 12:30 pm and 4 pm), stored in remineralisation solution (pH 6.58, 20 mM HEPES, 1 mM calcium chloride dihydrate, 12.7 mM potassium dihydrogen orthophosphate, 130 mM potassium chloride, 5.7 μ M fluoride (As NaF)). A 72 h demineralisation was conducted using a standard demineralisation solution and the results analysed using Quantitative light induced fluorescence (QLF-D), Multispectral imaging (MSI) and Transverse Microradiography (TMR). Whilst no differences between cycled and un-cycled enamel were observed in the 0 h group using QLF-D or TMR, MSI analysis highlighted decreased fluorescence loss in all cycled blocks (significant at 4 ($P = 0.04$), 6 ($P = 0.05$) and 8 days ($P = 0.022$)). For the 1 h condition, a significant decrease in fluorescence loss was observed at 12 days using both QLF-D ($P = 0.042$) and MSI ($P = 0.032$) with a non-significant decrease observed for all other time points and for mineral loss using TMR. A non-significant decrease in mineral loss was observed in the 3 h condition. QLF-D and MSI analysis both highlighted a decrease in fluorescence loss at all time points (QLF-D: significant at 8 ($P = 0.013$) and 12 days ($P = 0.036$)) except 2 days, in which there was a non-significant increase. These initial results indicate that exposure to plaque-fluid relevant pH-cycling conditions may serve to provide some protection against demineralisation.

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Tracking Dentin Demineralization Progress during Microcosm Biofilm Exposure Using Micro-CT

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Aim: Use micro-CT to determine the demineralization rate of bovine dentin subjected to a microcosm biofilm. **Experimental Approach:** Six dentin rings of 2 mm thick and 5 mm in diameter were obtained from bovine roots and divided into two groups of 3. For Group I, all surfaces were covered with 2 layers of nail polish except the top flat surface. For Group II, only the inner circular surface was uncovered. Specimens were exposed to an oral microcosm biofilm using a previously optimized CDC-bioreactor for 24, 48 and 72 hours. Sucrose was pulsed 5 times a day to simulate food intake. Micro-CT scans for each specimen were taken at each time point using the following parameters: 90 kV, 80 μ m, 720 projections and 4 frames/projection. Five through-thickness profiles of the attenuation coefficient factor were obtained per scan and converted into density profiles, from which the percentage of integrated mineral loss (IML) and demineralization rate was calculated. **Results:** Group I showed demineralization of 21.1% (± 2.2), 31.3.4% (± 3.2) and 40% (± 3.7) at 24, 48 and 72 hours, respectively, with an IML rate of 13.3% (± 1.2)/day. The R^2 value was 0.99 for the linear regression model. Group II displayed corresponding demineralization of 13.7% (± 4.1), 20.1% (± 3.6) and 28.3% (± 2.7) with an IML rate of 9.4% (± 0.9)/day. R^2 for this group was equal to 0.99. **Conclusions:** Micro-CT can be used to track demineralization generated by a microcosm biofilm. Using these dentin demineralization rates the CDC-bioreactor can be calibrated using clinical data measured in patients' mouths to better resemble the clinical situation.

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Remineralization Potential of Theobromine Containing Toothpaste on Initial Enamel Lesions: An In Situ Study

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Remineralization effect of theobromine, which is principle xanthine species in *Theobroma cocoa*, in repair of early caries lesions has received attention lately in literature. The aim of this in

situ study was to investigate the efficacy of theobromine containing toothpaste on remineralization of both permanent and primary teeth enamel in comparison with other toothpastes. 56 sound human primary and 56 permanent teeth specimens (4X4X3 mm) were sterilized and to be located in appliances. Half of specimens were demineralized for 72 hours which analyzed for surface microhardness (SMH) with digital Micro Vickers Hardness Tester and other half demineralized for 10 days to be analyzed for lesion depth and mineral density by X-Ray Microtomography. Seven children were given theobromine containing toothpaste (Theodent™ with Rennou™ Kids), 1450 ppm NaF containing toothpaste (Sensodyne Pronamel for Kids), 1450 ppm NaF containing toothpaste (Colgate Total) and F Free toothpaste (R.O.C.S. Baby) to brush their teeth not the specimens twice a day for 2 min, each for 3 weeks brushing period with 1 week wash-outs. SMH and micro-CT data was analyzed by repeated measures ANOVA. Difference between toothpastes for %SMHR values were not found statistically significant ($p > 0.05$), where mean±SD values of Colgate Total, Sensodyne Pronamel Kids, Theodent™ with Rennou™ Kids and R.O.C.S. Baby groups were 40.562±70.658, 30.517±42.376, 39.146±66.568 and 53.709±45.945 for permanent teeth and 16.721±33.036, 92.272±73.160, 106.08±85.107 and 104.59±430.53 respectively for primary teeth. According to results of micro-CT analysis, only at 0–50 µm depth for Colgate Total primary teeth group mineral density (mean±SD values after demineralization and after remineralization were 2.327±0.1320 and 2.248±0.08015 respectively) showed significant remineralization ($p < 0.05$). After remineralization % change for mean lesion depth (MLD) between toothpastes for primary teeth was not significant ($p > 0.05$) where mean±SD values of Colgate Total, Sensodyne Pronamel Kids, Theodent™ with Rennou™ Kids and R.O.C.S. Baby were 46.289±21.750, 32.315±21.449, 31.697±24.440 and 24.565±35.854 respectively but for permanent teeth difference between Sensodyne Pronamel Kids (45.552±22.030) and Theodent™ with Rennou™ Kids (44.138±12.276) vs R.O.C.S. Baby (14.323±15.251) groups were found significant ($p < 0.05$). Based on our results, it can be concluded that new theobromine containing toothpaste may have positive effects on enamel remineralisation. Information on the long-term performance of the new theobromine containing toothpaste necessitates further investigation.

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The Effects of Fluoride, Strontium, Theobromine and Their Combinations on Caries Lesion Rehardening

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Both strontium (anti-dentin hypersensitivity agent) and theobromine (alkaloid found in cocoa beans) have been reported to provide cariostatic benefits in their own right, and their mode of

action may suggest some synergistic effects. Therefore, the aim was to investigate the effects of fluoride, strontium, theobromine and their combinations on caries lesion rehardening and enamel fluoride uptake (EFU) under pH cycling conditions. Human enamel specimens were demineralized at 37°C for 24h using a pH 5.0 solution containing 50 mM lactic acid and 0.2% Carbopol 907 which was 50% saturated with respect to hydroxyapatite. Lesions were assigned to nine treatment groups ($n = 16$) based on Knoop surface microhardness indentation length. Treatment aqueous solutions were: placebo, 11.9 mM F as NaF (F), 23.8 mM F as NaF (2×F), 1.1 mM Sr as SrCl₂×6H₂O (Sr), 1.1 mM F theobromine (theobromine), Sr+theobromine, F+Sr, F+theobromine, F+Sr+theobromine. Lesions were pH cycled for 5 d (daily protocol: 3×1 min-treatment; 2×60 min-demineralization; 4×60 min & overnight-artificial saliva). Knoop indentation length was measured again and %surface microhardness recovery (%SMHR) calculated. EFU was determined using the acid-etch technique. Data were analysed using ANOVA. Model showed fluoride dose-response for both variables (2×F>F>placebo). For %SMHR, F+Sr±theobromine resulted in more rehardening than F, however less than 2×F. F+theobromine was similar to F. For EFU, F+Sr was inferior to F, F+theobromine and F+Sr+theobromine which were similar and inferior to 2×F. In absence of fluoride, Sr, theobromine or Sr+theobromine were virtually indistinguishable from placebo and inferior to F. It can be concluded that a) Sr aids rehardening but not EFU and only in presence of F; b) theobromine does not appear to offer any cariostatic benefits in this model; c) there are no synergistic effects between Sr and theobromine in the presence or absence of fluoride.

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In Vitro Remineralization Efficacy of CPP-ACP and Hydroxyapatite Containing Pastes

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Remineralization agents create a supersaturated environment around the early lesion; thus, preventing mineral loss and forcing calcium and phosphate ions in the vacant areas. Universally, these agents contain calcium phosphate with or without fluoride. The aim of this study was to evaluate the remineralizing potential of different remineralizing agents contain calcium phosphate (ReminPro and MI Paste Plus) submitted to a pH cycling model. 40 enamel pieces (3x2 mm) were prepared from the buccal surface of each extracted teeth. Only intact enamel areas on the buccal surface were used in this study. To produce demineralised lesions the samples were stored in acidic solution (pH 4.8) for 36 hours, in accordance with the method of Amaechi et al. (1998), after demineralization each group was subjected to pH cycle regimen with the products according to their group. Baseline microhardness of the

enamel surface was determined for each specimen initially before artificial demineralisation, after demineralization and after pH cycle regimen. A digital microhardness tester fitted with a Vickers diamond and a 200 N load was used to make indentations in the enamel surface. The loaded diamond was allowed to rest on the surface for 15 s. For each sample 3 indentations were made. The mean values of all three measurements at the three different times (baseline, after demineralization and after treatments were compared. The results were tabulated and statistically analysed. SPSS 18.0 software package was used for data analysis. SMH showed statistically significant differences between baseline, after demineralization and after experimental remineralization ($p < 0.05$). The Mean Rank baseline surface microhardness analyses of enamel blocks were 100.24; 131.67; 106.58; 88.13; 115.71 for F Toothpaste, ReminPro, MI Paste Plus, water as negative control, F Free Toothpaste respectively. The highest surface microhardness recovery (%SMHR) was found for ReminPro, but the differences of % SMHR between experimental groups was not statistically significant ($P > 0.05$). From the results it can be concluded that all treatments were equally effective.

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In Vitro Evaluation of Dentine Remineralisation by a Self-Assembling Peptide Using Scanning Electron Microscopy

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A biomimetic self-assembling peptide was recently introduced as a non-invasive treatment for early stage dental caries. The self-assembling peptides can diffuse into the subsurface micro-pores and form a 3D scaffold mimicking protein function during tooth development to support apatite crystallisation, promote remineralisation and reverse early tooth decay. The present study aimed to investigate the ability of a commercially available biomimetic self-assembling peptides in promoting mineralisation on a dentine surface. Dentine discs were polished and etched with 6% citric acid for 2 minutes. Each disc was halved, one for treatment with the self-assembling peptides and one as a control. Both halves were quartered as paired samples, and subjected to immersion in TRIS buffer (pH = 7.35) or artificial saliva (AS, pH = 6.5) for different time periods. The samples were further fractured for Scanning Electron Microscopy analysis on the etched and fracture surfaces. The controls exhibited open dentine tubules with a size between 2.3–4.2 μm in diameter and the fracture surfaces exhibited a characteristic funnel-shape of the open etched tubules with an etched smooth surface. No visible changes were observed for both the control and peptide treated dentine discs following immersion in TRIS. Similar results were observed for the controls immersed in AS. In contrast, plate-like structures present in, on and around

the dentine tubules were clearly visible in samples treated with the self-assembling peptides following 3 hours immersion in AS. The fracture surface demonstrated that the plate-like structures interacted with the smooth etched dentine surface. Occlusion of the dentine tubules with Calcium Phosphate was also evident. The biomimetic self-assembling peptides clearly promoted apatite formation compared with the controls and allowed the interaction between an apatite-like phase and the etched smooth dentine surface *in vitro*.

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Caries-Preventive Effect of Anti-Erosive and Nanohydroxyapatite-Containing Toothpastes

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The aim of the study was to investigate the caries protective effect of fluoride and nanohydroxyapatite-containing fluoride-free toothpastes developed for erosion prevention. Since the acid challenges during erosive episodes are much stronger than in caries process, it was hypothesized that these products might show even superior caries-inhibiting effect than regular fluoride toothpaste. Ninety bovine enamel samples were obtained and stored in thymol solution. After surface polishing they were immersed in Buskes's demineralizing solution for 21 days to create artificial caries lesions. Baseline mineral loss (ΔZ) and lesion depth (LD) were determined by transversal microradiography (TMR). The samples were then randomly divided into five groups ($n = 18$) and brushed 2x daily using: fluoride-free toothpaste, as negative control (NC); AmF (1400 ppm F) anti-caries (AC); AmF/NaF (475 ppm F) + SnCl₂ (800 ppm Sn), anti-erosion (AE1); NaF (1400 ppm F) + KNO₃, anti-erosion (AE2) and nanohydroxyapatite-containing (0 ppm F) (NH) toothpastes. Brushing was performed during pH-cycling for 14 days using slurries of the respective toothpastes with artificial saliva (1:3wt/wt, total contact time for each sample: 2 min). After cycling samples presenting lesion surface loss (mainly by NC and NH) were discarded and for the remaining 77 samples new TMR analyses were performed. The changes in mineral loss ($\Delta\Delta Z$; $\Delta Z_{\text{baseline}} - \Delta Z_{\text{treatment}}$) and in lesion depth (ΔLD ; $LD_{\text{baseline}} - LD_{\text{treatment}}$) were calculated and statistically analysed. All toothpastes caused significantly lower $\Delta\Delta Z$ than NC ($-4557 \pm 2178 \text{ vol}\% \times \mu\text{m}$, $p < 0.05$; Mann-Whitney test), except for NH ($-2119 \pm 1896 \text{ vol}\% \times \mu\text{m}$, $p = 0.683$). Both AE's and AC toothpaste did not differ significantly in $\Delta\Delta Z$ ($p > 0.05$). While both anti-erosive and the anti-caries toothpastes reduced mineral loss to a similar extent, the nanohydroxyapatite-containing toothpaste seemed not to be suitable for artificial caries inhibition *in vitro*.

Formation and Characterization of Hypermineralized Zone

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This in-vitro study found hypermineralized zone with higher mineral density than in sound dentin beneath dentin lesion body, hereinafter Hyp-zone, when the demineralized dentin lesion was treated by topical fluoride solution followed by remineralization treatment. Hyp-zone, which is novel to our knowledge, might be associated to lesion arrestment. **Purpose:** To characterize Hyp-zone by TMR, SEM and EDS. **Materials and Methods:** Acid demineralized bovine dentin specimens were treated by 9,000 ppm F APF or NaF solution followed by 2- and 4-week remineralization. The specimens were cut to slices for TMR analysis. SEM observation was performed to characterize the newly formed minerals. Moreover EDS analysis was carried out to examine relative contents of Ca, P and F. **Results:** TMR analysis indicated 8–9 out of 10 specimens in fluoride groups showing Hyp-zone, but not in fluoride-untreated group, and revealed a higher mineral density at Hyp-zone than in sound dentin (48 vol%) with ranging from 50 to 61 vol% and width with ranging from 197 to 344 μm for 4-week specimens. However SEM pictures taken at Hyp-zone showed no evident crystal-like deposits in dentinal tubules and no notable difference in intertubular dentin compared to normal dentin. EDS analysis demonstrated higher concentrations of Ca and P at Hyp-zone than in sound dentin, which corresponded to the TMR mineral profile. For exploring the mechanism, sound dentin specimens were treated by NaClO to remove some organic or organic substances, and were remineralized. The treated specimens showed hypermineralized layer just below the specimen surface. A hypothesis for the Hyp-zone formation was discussed that some substances which had mineral inhibitory property was removed during the pre-demineralization or NaClO treatment, and then crystal growth was activated for the Hyp-zone formation.

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Effect of Casein as a Model Pellicle Precursor Protein on In Vitro Dentin Remineralization

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Pellicle precursor proteins (PPPs) regulate tooth mineral processes but are usually absent in in-vitro experiments. Previously, we have found that casein inhibits hydroxyapatite growth and precipitation on sound tooth surfaces, indicating its potential to be a PPP analogue. This study aims to investigate the effect of casein, when incorporated in artificial saliva (AS) solutions, with and without fluoride (F), on dentin remineralization. Bovine root dentin blocks were demineralized at pH 5.0 and remineralized for 28 days in AS with casein (0, 10, 20, 50, 100 $\mu\text{g}/\text{ml}$) and F (0, 1 ppm). Mineral densities were obtained using transverse microradiography. Results analyzed using two-way ANOVA with Bonferroni post hoc revealed that surface mineral density of casein 0 $\mu\text{g}/\text{ml}$, F 1 ppm group (64 \pm 6 vol%) which exhibited surface mineral precipitation was significantly greater ($p < 0.001$) compared to the baseline (12 \pm 2 vol%) and all other groups (30–43 vol%) where surface precipitation was inhibited regardless of casein concentration. Mineral loss (ΔZ) was higher ($p < 0.001$) in the baseline group (2094 \pm 262 vol% $\times\mu\text{m}$) than the rest of the groups (251–616 vol% $\times\mu\text{m}$) which were not significantly different from each other. Lesion depth recovery (LDR) was lower ($p < 0.001$) for all F-containing groups (31–40%), which exhibited laminations, compared to non-F groups (67–75%). The laminated pattern of remineralization did not affect ΔZ but could have inhibited LDR. Based on present findings, it could be concluded that casein, regardless of concentration, inhibits the mineral precipitation on tooth surfaces but does not inhibit dentin lesion remineralization. This is consistent with literature claims regarding salivary proteins, thus indicating suitability of casein to be a model of PPPs for in vitro studies. It can also be suggested that homogenous patterns of remineralization could lead to better lesion depth recovery.

This work was supported by a grant from the Japanese Ministry of Education and Global Center of Excellence 'GCOE' Program; International Research Center for Molecular Science in Tooth and Bone Diseases.

In-Vitro Study of the Effects on the Dentin Caries by Phosphoryl Oligosaccharides of Calcium

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Phosphoryl oligosaccharides of calcium (POs-Ca[®]) is a new material containing calcium. POs-Ca has the following beneficial characteristics for the prevention of caries: i) in the presence of POs-Ca, calcium ions do not form calcium-phosphate precipitates in saliva, ii) POs-Ca cannot be assimilated by cariogenic microorganisms, mutans streptococci, and avoids the fall of plaque pH in vitro by mutans streptococci. POs-Ca has been demonstrated to efficiently enhance remineralization of the enamel early caries lesion. The aim of this study was to investigate the effectiveness of POs-Ca on early-stage dentin caries and occlusion of dentinal microtubules. Bovine dentin blocks with early-stage caries lesions were prepared by treating with the solution containing 50-mM acetic acid (pH 5.0), 1.5-mM CaCl₂, 0.9-mM KH₂PO₄ 0.5 ppm KF at 37°C for 24 hrs. Then, they were divided into two groups (n = 3), and treated with mineral solution containing 6-mM calcium from POs-Ca or CaCl₂, and 3.6-mM phosphate ions (pH 6.5) at 37°C. After 24-hour treatment, dentin blocks were cut perpendicularly into thin sections, and subjected to analyze the mineral volume by transversal microradiography (TMR). The analyzed samples were further studied by scanning electron microscopy (SEM) to evaluate the occlusion of the tubular openings. The results from TMR showed that the rates of remineralization were 64.3±6.8% by POs-Ca and 21.0±7.1% by CaCl₂. The solution containing POs-Ca perfectly occluded the exposed dentin tubules by forming smooth layer, whereas the solution containing calcium chloride did not. These results suggest that POs-Ca maybe useful for enhancing remineralization and occluding the exposed dentin tubules of early caries lesions.

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Peptide Scaffolds to Support Remineralization of Initial Carious Lesions – In Vitro

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With the knowledge about the limitation of fluoride compounds for carious prevention alternative methods to enhance remineralization in initial carious lesions have to be found. Peptides that penetrate into the enamel lesion assemble to scaffolds, serve as a nucleus for crystal growth and may support the uptake of minerals into the demineralized enamel. To test this hypothesis, bovine enamel samples were ground, polished, demineralized to form lesion volumes of 3000 Vol.-%-µm and randomly divided into 4 groups (n = 40). Group 1 and 2 were treated with a tryptophan containing short chained peptide that forms pH-driven scaffolds. Furthermore groups 2 and 3 were stored in toothpaste-saliva slurry (1450 ppm NaF; proportion 1:2 v/v) twice a day for 3 minutes. Group 4 served a negative control. To simulate moderate carious conditions all specimens were pH-cycled over a period of 14 days according to a well-established protocol. The change of the mineral content and the lesion depth were assessed by transverse microradiography. The results were statistically analyzed with ANOVA and Tukey test. In relation to the demineralized enamel the highest mineral uptake was found in group 3 (14.43% (95% CI: 6.08/22.8)). Significantly less remineralization was found in the group 2 (2.67% (95% CI: -5.67/11.02)) and group 1 (-5.52% (95% CI: -13.78/2.73)), indicating further demineralization. A significant demineralizing effect was measured in group 4 (-34.19% (95% CI: -42.45/-25.93)). The same de- and remineralizing tendencies could be found for the lesion depth, although being not statistically significantly different. The application of the peptides on enamel lesions decreased demineralization but hampered remineralization when fluoride is present. Peptide scaffolds, as a nucleus for crystal formation, do not enhance remineralization in a way fluoride compounds do. However a protective effect against demineralization can be observed.

Novel Self-Assembling Peptides, for the Treatment of Early Caries Lesions

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An emerging and innovative treatment, which can expedite the natural remineralisation process of teeth to cure early damage on enamel and dentin involves the use of rationally designed self

assembling β -sheet tape forming peptides. A pair of complementary peptides, P₁₁₋₁₃ (CH₃CO-E-Q-E-F-E-W-E-F-E-Q-E-NH₂) and P₁₁₋₁₄ (CH₃CO-Q-Q-O-F-O-W-O-F-O-Q-Q-NH₂) have been investigated to assess their potential viability in promoting mineralisation. FTIR and NMR studies indicate P₁₁₋₁₃ and P₁₁₋₁₄ exhibit a pH dependant phase behaviour. P₁₁₋₁₃ initially exists as monomeric low viscous non-Newtonian fluid (<3% β -sheet) at pH 6 to 12. Conversely P₁₁₋₁₄ is a monomeric low viscous non-Newtonian fluid at a pH 1 to 9 (<5% β -sheet). Upon equimolar mixing, the complementary peptides spontaneously self-assemble into anti-parallel β -sheets (>95% β -sheet) between pH 6–9 as indicated by FTIR and NMR. The biocompatibility of the peptide monomers and the peptide gel network has been demonstrated by contact cytotoxicity and extract cytotoxicity using L929 cells lines. TEM studies illustrate that the peptides form a fibrillar morphology with widths of 10–20 nm and lengths greater than μ m. Rheological techniques demonstrate that the complementary peptide gel has a elastic modulus (G') of ~1700 Pa and a viscous modulus (G'') of 170 Pa at 10 Hz in physiological conditions (pH 7.4, 37°C). The mineralisation potential of P_{11-13/14} gels was tested and demonstrated an increased mineral deposition when compared to positive and negative controls. In conclusion, complementary peptides have the potential to be used as a treatment for early damage on enamel and dentin. Peptides P₁₁₋₁₃ and P₁₁₋₁₄ have a phase behaviour that can be manipulated by pH, and form stable self-supporting gels when mixed. They are able to promote remineralisation and are biocompatible.

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P11-15 (NNRFEWFEENN): A Second Generation Biocompatible, Self-Assembling Peptide with Potential to Promote Enamel Remineralisation

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P11-family peptides show pH-dependent gelation, promote mineralisation and are being investigated as tooth-repair therapies. P11-15 (a novel analogue; 2–10 mg/ml) is a clear, low viscosity solution at pH 9.0 (<7% B sheet) but gels at pH <7.0 (>95% B sheet). TEM shows this gel comprises long, thin unbranched peptide fibres (20 nm width). Computational chemistry (AMBER11 software) suggests that in acidic conditions P11-15 monomers associate edge to edge to form bilayers of anti-parallel B-sheets with hydrophobic cores (composed of the PHE and TRP side chains), charged surfaces (composed of GLU and ARG residues) and hydrophilic edges. The predicted Ca²⁺ binding sites are formed by 4 x GLU residues at the 5 and 7 positions of two adjacent anti-parallel P11-15 monomers. Modelling further suggests that this binding site is stabilised by the ARG at positions 3. The spacing between

adjacent Ca²⁺ binding sites (9.4 Å) resembles that between columnar Ca²⁺ in hydroxyapatite.

Leachable extracts from P11-15 gels (5 mM) were non-cytotoxic (viability 96±1.1%; n = 8). Furthermore, L929 cells growing in contact with P11-15 gels stained +ve with MTT, confirming biocompatibility. Serum pre-treated P11-15 gels showed improved cell attachment and spreading in serum-free media indicating that P11-15 can bind serum-derived factors (n = 3). To assess in vitro mineralisation, gel plugs of P11-15 (30 mg/ml), agarose (–ve control), and agarose gel containing polyglutamate (10 μ g/ml; +ve control) were placed between reservoirs of calcium and phosphate solutions at 37°C for 6 days. Calcium assays showed P11-15 significantly increased mineral deposition (negative control 79.9±6.3 μ g; PGA 123.4±5.2 μ g and P11-15 277.7±2.1 μ g; n = 3; p < 0.001). Conclusion: Results indicate that P11-15 is a candidate second generation biocompatible, self-assembling peptide with potential to promote remineralisation.

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Caries Lesion Prevention in Approximal Surfaces Adjacent to GIC Restorations – A Systematic Review and Meta-Analysis

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This systematic review aimed to evaluate the ability of Glass ionomer cements (GIC) to prevent caries lesion in surfaces adjacent to occluso-proximal restorations. Experimental approach: Literature searching was carried out by two independent reviewers in PubMed through October 2013 to assess longitudinal trials, in situ and in vitro studies available associated with the outcome. The inclusion criteria were: (1) subject related to the outcome of this systematic review, (2) longitudinal, in situ or in vitro study, (3) not performed in specific groups, (4) have a comparison group. The meta-analysis was performed considering the outcome as caries lesion progression or arrestment. Results: The search strategy identified 742 potentially relevant studies and 10 of them were included in the review (six laboratorial studies and four longitudinal trials). The main reasons for the non-inclusion of the studies were related to criteria 1 (60%) or 2 (28%). For the laboratory studies, GIC was significantly associated with better ability to prevent artificial caries lesions (OR = 0.153, 95%CI: 0.060–0.391). When a meta-analysis was performed to verify if the type of GIC would influence the results, only high-viscosity GIC (OR = 0.260, 95%CI: 0.092–0.736) and resin-modified GIC (OR = 0.044, 95%CI: 0.004–0.504) were associated with artificial caries lesion prevention. For longitudinal clinical trials, there was no difference among the materials (OR = 0.680, 95%CI: 0.233–1.983). In conclusion, in laboratory studies, high-viscosity GIC and resin-modified GIC show better ability to prevent artificial caries le-

sion in adjacent surfaces, but it has not been confirmed in longitudinal clinical trials.

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The Role of pH on the Dissolution of Bioactive Glasses For Remineralising Additives for Toothpastes

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Bioactive glasses are increasingly being used as re-mineralising components for toothpastes and for occluding dentinal tubules in the treatment of dentine hypersensitivity. BGs dissolve in the mouth releasing calcium and phosphate and forming apatite. Recently it has been shown that glasses with higher phosphate content form apatite more rapidly and in larger quantities. The aim of this study is to investigate the pH dependence of the dissolution process and the ability of the BGs to form apatite. Five BGs with increasing phosphate content from (0 to 7 mole%) were synthesized by a melt quench route and ground to a powder <45 microns. Dissolution of the glasses in Tris buffer (0.1 M) at pH 7.3 and 9 and in acetate buffer (0.1 M) at pH 5 on was followed for up to 72 hours. The ions released were analysed by ICP-OES. The residual solid was collected after immersion and characterized by Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction and ³¹P solid-state nuclear magnetic resonance (ss-NMR). The results of the experiments show that the higher the content of orthophosphate in the initial glass the faster the formation of apatite. High pH is generally considered optimal for the apatite precipitation in solution. However, at pH 9 no FAP formed and this was due to the low rate of glass degradation. On the contrary, the highest rate of apatite formation was found at pH 5 due to rapid glass dissolution at this acidic pH. The fact that the dissolution of bioactive glasses occurs faster under acidic conditions suggests they will respond rapidly to a cariogenic challenge.

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Continuous ISE Studies of HAP Powder and Enamel Dissolution Inhibition by Statherin-Like Peptides

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X-ray microradiographic studies have demonstrated that statherin inhibits enamel dissolution under caries-like conditions. Ion selective electrodes (ISEs) measure concentrations of 'free' ions in solution not bound to proteins or other species. Computer-interfaced ISEs allows continuous measurements enabling monitoring of calcium concentration ($[Ca^{2+}]$) changes in solutions for kinetic and thermodynamic analyses. The aim was to develop continuous-ISE (Ca^{2+}) to investigate the cariostatic efficacy of STN21 (peptide comprising 21 N-terminal residues of statherin) resulting in reduction of Ca^{2+} release into caries-simulating solutions containing hydroxyapatite (HAP) powder or enamel surfaces. HAP powder (particle size 2–6 μm , specific surface area 6–8 m^2/g) was obtained (PlasmaBiotol, UK). Enamel surfaces were created by coating caries-free incisors with acid-resistant varnish except for windows 2 mm x 2 mm. Test solutions contained 0.2 mmol/L STN21 dissolved in phosphate buffered saline (PBS). Control solutions contained only PBS. Computer-linked Ca^{2+} -ISEs were immersed in stirred caries-simulating solutions (pH = 4.0) containing either 0.2 g HAP powder, or enamel surfaces, to measure $[Ca^{2+}]$ every 60 s for 9 h. The samples were then treated with STN21 solutions for 10 h, then demineralisation with ISE measurements continued for 9 h further. HAP powder: Reduction in Ca^{2+} release following StN21 treatment was 17.4 \pm 1.0%, 15.4 \pm 1.0%, 13.9 \pm 1.0%, 12.4 \pm 1.0%, 7.9 \pm 1.0%, 7.0 \pm 1.0%, 5.0 \pm 1.0%, 4.3 \pm 1.0%, 2.1 \pm 1.0%, at each hour time-point, demonstrating an initial rapid increase followed by decrease in cariostatic efficacy. Enamel surfaces: Reduction in Ca^{2+} release following StN21 treatment was 70.0 \pm 1.0%, 76.0 \pm 1.0%, 79.0 \pm 1.0%, 82.0 \pm 1.0%, 83.9 \pm 1.0%, 85.8 \pm 1.0%, 87.0 \pm 1.0%, 88.2 \pm 1.0%, 89.2 \pm 1.0% at each hour time-point, demonstrating an initial rapid increase in cariostatic efficacy of STN21 which subsequently increased slightly. Samples treated with control solutions containing only PBS demonstrated no reduction in Ca^{2+} release. In conclusion, continuous-ISE $[Ca^{2+}]$ studies demonstrate that StN21 reduces dissolution kinetics of HAP powder, and enamel surfaces, under simulated caries-like conditions.

High Fluoride Content Alone in Varnishes Does Not Guarantee High Surface Microhardness Remineralization

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Varnish is used to remineralize lesions due to high fluoride content. Although varnishes all contain 5% NaF, lesions treated with varnish exhibit varying surface microhardness gains. A high increase in microhardness signifies high remineralization of tooth structure. Aim: to compare surface-microhardness of bovine incipient lesions using two varnishes: 3MTMESPETMClinproTMWhite-Varnish (A); Colgate[®]Duraphat[®]Varnish (B); to artificial-saliva-control (C) in an in-vitro-remineralization study. Approach: Bovine incisor enamel was cut into 5x5 mm blocks, mounted in acrylic and polished. Lesions were prepared by immersing blocks in pH5.0-Carbopol-907/lactic-acid demineralization-solution (96 hrs, 37° C). Baseline surface Vickers microhardness (200 g load, 15 sec) was measured, and samples were divided into equivalent treatment groups (n = 8). Remineralization: coat samples with varnish, immerse in artificial-saliva (24 hr, 37°C, pH = 7), remove varnish-residue, immerse in artificial-saliva (24 hr), rinse with de-ionized (DI)-water, and measure microhardness. Acid-challenge: soak samples in demineralization-solution (24 hrs), rinse, and measure microhardness. Calculate and compare mean surface microhardness and percent-change from lesion for each treatment group using one-way-ANOVA and Tukey's-T-test (p < 0.05). Varnish effect on pH was measured by immersing varnish-coated slides into unbuffered pH4.9-DI-water (50 mg Varnish/10 mL water, n = 3) for 2-minutes. Results: Treated: mean surface-microhardness percent-change from lesion-state to treated (SD); A:42.0% (18.1); B:-11.0% (10.2); C:5.6% (7.8). Challenged: mean surface-microhardness percent-change from lesion to acid-challenge (SD); A:24.2% (20.8); B:-12.4% (8.5); C:-11.2% (18.0). Baseline microhardness values are not significantly different, demonstrating the treatment groups were balanced at study initiation. After treatment; A, B, C are not statistically the same, only A becomes harder. After acid-challenge, A retains net hardness gain from lesion state, and is statistically different than B and C. In water after 2-minutes, the pH of A-in-water does not change (pH4.9), B-in-water decreases (pH = 4.6), the blank-slide remains constant (pH = 4.9). In conclusion, Varnish A remineralized enamel lesions by hardening enamel while varnish B does not harden lesions. Therefore, additional factors beyond fluoride, including medium pH may also impact remineralization.

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In Vitro Detection of Remineralisation of Early Caries Using Curodont[®] Repair with The Canary SystemTM

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The aims of this study were to evaluate the efficacy of Curodont[®] Repair (CR) to regenerate enamel in natural early caries on smooth surfaces of extracted teeth, and to evaluate the ability of The Canary System[®] (CS) and The Canary Lab (CL) to detect and monitor CR-induced remineralisation of caries lesions. 129 sound and carious sites were scanned with CS at baseline. Three repeat measurements were made per site. Then 30 sound and 27 carious sites were randomly assigned to the 'Treatment Group' (TG), and 42 sound and 30 carious sites were randomly assigned to the 'Control Group' (CG). A total of 1357 sound and carious sites in the CG, and 1359 sound and carious sites in the TG were scanned with CL at baseline. CR was then applied to the TG. All teeth were then placed in remineralisation solution, and the same examination sites were re-scanned with CS and CL at 7, 14, 30, and 50 days. For carious sites in TG, mean Canary Numbers (CN) from CS decreased significantly (P < 0.05) from 44±SD at baseline to 24±SD at Day 50 (Related-Samples Wilcoxon Signed Rank Test; p < 0.05) while mean CN from CL decreased significantly (p < 0.05) from 49±SD at baseline to 36±SD at Day 50. In contrast, no significant changes were observed in CN of carious sites in the CG from both CS and CL. In both TG and CG, mean CN of sound sites from both CS and CL were less than 20±SD, which indicates healthy tooth structure, throughout the treatment period. This study demonstrated the efficacy of CR in inducing remineralisation of early caries and the ability of CS and CL to detect and monitor the remineralisation progress.

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In Vitro Assessment of Experimental Chocolate with Herbal Mixture Submitted to a pH Cycling Model

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An increased use of herbal products in various catalogues of fast moving consumer goods is evident. Recently, biological approaches focused on application of remineralization (RML) agents to early carious lesions, aimed at controlling demineralization (DML), and promoting RML. Chocolate with herbal mixture showed positive enhancement of remineralization of early enamel lesions in a recent study (Bilgin G., Thesis, 2012). The objective of this study was to evaluate the remineralization potential of ginger, chocolate and herbal mixture separately using human surface-softened enamel as substrate with a different treatment protocol, pH cycle regimen. 56 enamel specimens (3x2 mm) were prepared from the buccal surfaces of extracted teeth and randomly divided into 6 groups of 8 each. To produce demineralised lesions the samples were stored in acidic solution (pH 4.8) for 36 hours, in accordance with the method of Amaechi et al. (1998); after demineralization each group was subjected to the pH cycling regimen of Vieira AE., et al. (2005) with treatment products Ginger, F Toothpaste, Chocolate with Herbal mixture (Ginger and Rosemary were in powder form and mixed with honey (8 mg/ml). The mixtures then covered by chocolate which was bitter (15% sugar most). The chocolate

specially produced by Bind Chocolate (Tatlı Çikolata San. ve Tic. A.Ş., Tekirdağ, Turkey)), Honey, Bitter Chocolate, with Water as the negative control. Microhardness of the enamel surface was determined for each specimen initially before artificial demineralisation, after demineralization and after the pH cycling regimen. A digital microhardness tester fitted with a Vickers diamond and a 200 N load was used to make indentations in the enamel surface. The loaded diamond was allowed to rest on the surface for 15 s. For each sample 3 indentations were taken at each assessment. The mean values of all three measurements at the three different times (baseline, after demineralization and after treatments were then compared. The results were tabulated and statistically analyzed. SMH showed statistically significant differences between baseline, after demineralization and after experimental remineralization ($p < 0.05$). The Mean Rank baseline surface microhardness analyses of enamel blocks were 103.60; 100.24; 165.77; 72.04; 130.25; 88.13 for Ginger, F Toothpaste, Chocolate with Herbal mixture, Honey, Bitter Chocolate, Control respectively. The highest surface microhardness recovery (%SMHR) was found for Chocolate with Herbal mixture. The difference of % SMHR between the experimental groups was found to be statistically significant ($p < 0.001$). Within the limitations of our study, the Experimental Herbal Mixture Chocolate showed a remarkable protection of the enamel surface.

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Withdrawn

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Dental Health of 12-Year-Olds in Slovenia (1987–2013)

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The aim of the study was to evaluate the prevalence of dental caries in 12-year-olds in Slovenia over the period 1987 to 2013.

Methods: The first nationwide survey of oral health, conducted in 1987 in all nine geographical regions, covered about 2% of all 12-year-olds in Slovenia and contained an equal number of females and males. Caries experience was diagnosed at the cavitation level, using artificial light, plane mirror and sharp explorer. The presence of sealants was also recorded for each child. After the initial survey, five further nationwide surveys were conducted in the same way at intervals of five years. The statistical analysis of the results included the Mann-Whitney and Kruskal-Wallis tests. The level of significance was set to $p < 0,05$. The Statistical Package for Social Sciences (SPSS) was used. **Results:** The results showed that while the percentage of children with one or more sealed teeth increased from 6.0% (1987) to 92.0% (2013), the percentage of caries-free children increased from 6.4% (1987) to 36.0% (2013), and the mean DMFT value decreased from 5.10 (CI = 4.77, 5.43) in 1987 to 1.89 (CI = 1.66, 2.12) in 2013. **Conclusion:** The decline in dental caries among 12-year-olds in Slovenia was continuous from 1987 until 2008, but in the last five years it slightly increased. Meanwhile the percentage of 12-year-olds with sealed teeth kept increasing throughout all research periods and reached the high value in 2013. The continuous increase in the number of sealed teeth largely contributed to caries decline documented in the period 1987–2013.

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Level of Oral Health in a Sample of Croatian Suburb Pre-School Children

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The purpose of this study was to determine the level of oral health, the prevalence of oral habits and to identify the malocclusions associated with them in pre-schoolchildren. A convenience sample consisted of 100 children (55 girls and 45 boys) aged 2 to 6 years. The children were divided into three age groups: group I (n = 30) from 2 to 3, group II (n = 50) from 4 to 5, and group III (n = 20) at the age of 6 years. The children were screened in kindergarten for dmft index, oral habits (thumb sucking, mouth breathing, pacifier habit, lip and cheek biting) and malocclusions (open bite, crowding). In group I no habit was found in 23%, in group II in 36% and in group III in 45% of children. The most prevalent oral habit was thumb sucking but the prevalence decreased with age (from 37% in group I to 20% in group III). In group I 54% of children did not have malocclusion, in group II 36% and in group III 50%. The most prevalent malocclusion was open bite in all three groups. The prevalence of open bite increase from group I (35%) to group II (50%) and decreased in group III (33%). The dmft index for the whole sample was 7.7. In group I dft index was 4.96, in group II dmft was 9.24 and in group III dmft was 6.4. Results show low level of oral health and very frequent oral habits in Croatian suburb pre-schoolchildren. Consequently, we found a high incidence of malocclusions in the primary and early mixed dentition (47% in group I, 64% in group II and 50% in III).

Association of Sweet Taste Genotypes with Dental Caries Experience in School Children

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The peripheral taste response to sugar is mediated by the TAS1R2/TAS1R3 taste receptors. The aim of the study was to determine the relationship between TAS1R2 (rs35874116 or rs9701796) and/or TAS1R3 (rs307355) single nucleotide polymorphisms with dental caries experience in school children. Prior to study, ethical approval was received from Medical Ethical Committee of Marmara University (protocol 09.2012.0063-4). Hundred and eighty-four school children aged between 7–12 years (101 girls, 83 boys) were examined intraorally by pediatric dentists and dft and DMFT scores were recorded using WHO criteria. Consent forms and demographic charts including brushing and dietary habits were filled by parents. Genomic DNAs were extracted from saliva samples with high pure PCR template preparation kit (Roche) and the genotypes were identified by using appropriate FastStart DNA Master HybProbe kits with qPCR in nano lightcycler. Fisher Exact, chi-square and logistic regression tests were performed. Mean dft was 4.28±3.41 (0–16); DMFT 1.61±1.64 (0–7) and only 32 children were caries-free (17%). The genotype frequencies were 6.6% for homozygous wild type, 41.8% for heterozygous and 51.6% for homozygous polymorphic genotype carriers of rs35874116; 27.8% for heterozygous, 72.2% for homozygous polymorphic genotype carriers of rs9701796 and 83.1% for homozygous wild type, 16.9% for heterozygous genotype carriers of rs307355 polymorphism. A significant association was observed between total caries experience (dft+DMFT) and rs35674116 ($p = 0.008$), and rs307355 ($p = 0.04$) but not rs9701796. Logistic regression analysis revealed that rs307355 polymorphism is an independent risk factor for dental caries experience, increasing the risk of caries. In a moderate to high caries population such as our group, TAS1R2 and/or TAS1R3 genotypes seemed to influence the caries experience of children and could expand the accuracy of caries prediction.

Comparative Evaluation of Resin Infiltration, Bleaching and Bleaching-with-Resin Infiltration in Esthetic Improvement of Non-Pitted Fluorosis

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Dental Fluorosis is a clinical manifestation of chronic exposure to high intakes of fluoride. Its signs range from opaque-white-flecks to dark-brown-spots to pitting and is always a concern of esthetics. The aim of study was to compare esthetic improvement in non-pitted fluorosis treated with different interventional strategies. 80 patients, aged 8–14 years, with non-pitted fluorosis were selected from high fluoride belt and randomly divided in 4 equal groups. Bleaching with 35%-Hydrogen-Peroxide [Pola-Office, SDI-Australia] (BHP) was done in group-I, Resin-Infiltration [ICON, DMG-Germany] (RI) in group-II, Resin Infiltration two-applications (2RI) in Gr-III & for group-IV, patients were first treated with BHP followed by RI after 20 days (B+RI). Preoperative and immediate-postoperative photographs were taken and later evaluated using Visual Analogue Scale (VAS) in terms of 'Esthetic Changes' (EC) and 'Improvement in white/brown opacities/stains' (SC), by two observers [scores = 1–7, 1 = 'No-Change' 7 = 'exceptional-improvement'] and mean of two observers was used for comparisons. Best improvement for both parameters, EC & SC was seen by 2RI (mean EC = 5.53±1.20, mean SC = 5.18±1.30) followed by RI (mean EC = 5.50±1.00, mean SC = 4.98±0.980), B+RI (mean EC = 5.35±1.22, mean SC = 4.40±1.59) and least changes observed with bleaching group (mean EC = 1.90±0.95, mean SC = 1.53±1.07). Kruskal-Wallis test showed significant differences for intergroup comparisons ($pEC < 0.001$, $pSC < 0.001$). Mann-Whitney U test, showed significant results, when mean scores for RI, 2RI, B+RI were compared with bleaching ($pEC < 0.001$, $pSC < 0.001$) while non-significant differences were seen for RI Vs 2RI ($pEC = 0.838$, $pSC = 0.584$), RI Vs B+RI ($pEC = 0.837$, $pSC = 0.378$) and 2RI Vs B+RI ($pEC = 0.612$, $pSC = 0.160$). Postoperative photographs were also evaluated by independent examiner scoring 'requirement-for-further-treatment' [Score = 0-2 (0 = No need, 1 = May need, 2 = Need)]. Maximum patients reported to have scored 2 in BHP group while no patient scored 2 in 2RI-group. Chi-square test showed significant differences between 'requirement-for-further-treatment' among various groups ($p < 0.002$). Conclusively, Resin infiltration shows best results in terms of esthetic improvement and change in white/brown opacities with some additional benefit observed with double RI application.

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Practice-Based Randomized Clinical Trial on the Efficacy of Proximal Caries Infiltration After 18 Months Follow-Up

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Proximal caries infiltration has been shown to be efficacious to inhibit caries lesion progression when performed by dentists working in a university dental clinic. The aim of this randomized split-mouth placebo-controlled clinical trial was to assess the efficacy of resin infiltration of proximal caries lesions being performed in private practice in combination with self-applied non-invasive measures compared with non-invasive measures alone to inhibit lesion progression. In 87 children and young adults, 238 pairs of proximal caries lesions radiographically extending into inner half of enamel (E2) or outer third of dentin (D1) were randomly allocated to either one of two treatments. Test lesions were infiltrated (Icon; DMG). A placebo treatment was performed in control lesions by five German private practitioners. All patients received instructions for non-cariogenic diet, flossing and fluoridation. The primary outcome was radiographic lesion progression (pairwise comparison) evaluated by two independent evaluators being blinded to treatment allocation (SP and KB; kappa: 0.65). After approximately 18 months [mean (SD): 561 (81) days] 174 lesion pairs (74%) in 69 patients (79%) [DMFT at baseline: 9.0 (5.0)] of five dentists could be re-evaluated radiographically using individualized bitewing holders. No unwanted effects could be observed. Radiographic progression was recorded in 8/174 test lesions (5%) and 38/174 control lesions (22%) ($p < 0.001$; McNemar test; relative risk reduction = 79%); 2/174 test and 21/174 control lesions ($p < 0.001$; relative risk reduction = 90%) progressed from one stage to a deeper one. Thus, resin infiltration performed in a private practice-setting by various practitioners seems to be more efficacious in reducing lesion progression compared with self-applied non-invasive measures alone over a period of eighteen months.

The study was supported by DMG, Hamburg the producer of the infiltration kit (Icon) which is hereby acknowledged. SP and HML receive royalties and funding from DMG, the manufacturer of Icon. KB declares no conflict of interest.

Case Series on the Value of Re-Wetting Prior to Resin-Infiltration of Caries Lesions

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The aim of this case series was to assess the effect of resin infiltration to mask post-orthodontic white spot lesions and to evaluate colour differences during the re-wetting process in order to establish a time point to predict the final result. In three adolescents 20 non-cavitated caries lesions (ICDAS 2) were treated by resin infiltration. Prior to treatment digital pictures were taken. Following etching with 15% HCl (Icon etch, DMG) the dried teeth were re-wetted with alcohol (Icon dry) while serial pictures were taken every second for up to 10 seconds. During this re-wetting process the dentist decided whether the masking result was satisfying; otherwise the etching and re-wetting process was repeated (maximum three times). Finally, the infiltrant (Icon infiltrant) was applied and light-cured followed by polishing. Digital pictures of the final result were taken one week after treatment and colour differences (ΔE) of healthy and carious enamel were evaluated. Of the 20 teeth treated, three were etched and re-wetted once, seven twice and ten teeth three times. Overall we observed a significant ΔE (SD) reduction from 9.01 (3.30) to 2.77 (2.05) ($p < 0.000$; t-test). The etching process by itself did not result in a significant change of ΔE . However, during the re-wetting process a significant reduction of ΔE was observed within the first second with a further continuous reduction reaching a plateau between 4 to 10 sec. after re-wetting. The final result after resin infiltration even showed a further reduction of approximately 2 ΔE . Resin infiltration is able to mask post-orthodontic white spot lesions. Our data reveal that the period between 4 to 10 sec. after starting the re-wetting process is most suitable for judgement of the final result.

The study was supported by DMG, Hamburg the producer of the infiltration kit (Icon) which is hereby acknowledged. HML receives royalties from Icon sales.

Safety, Clinical Applicability and Effect of Curodont™ Repair in Children with Early Occlusal Caries

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Occlusal surfaces of erupting permanent molars are highly prone to caries. The self-assembling peptide (P11-4) has been proven to enhance biomimetic mineralization of early carious lesions. The aim of this study was to evaluate safety, clinical applica-

bility and effect of using P11-4 (Curodont™Repair) in non-invasive treatment of early occlusal lesions. 70 patients (28 females, mean age 10.03 yrs±2.7, dft 2.8±3.1, DMFT 1.3±2.5) with early occlusal lesions (ICDAS-II:1-3) on first or second permanent molars at eruption were allocated in this randomized, controlled, single blinded post-marketing study to test (Curodont™Repair+ Duraphat®) or control (Duraphat®) groups. Safety and applicability was evaluated using dentist's/patient's questionnaires about adverse events, difficulties of application and satisfaction with the procedure. Lesions were assessed at baseline and recalls after 3 and 6 months regarding clinical status (ICDAS-II) and with Diagnodent®. At every recall fluoride varnish was applied and patients received oral health instructions. Results: Preliminary data showed good patient acceptance for Curodont™Repair. Investigators considered the application as much easier as a composite filling or even a fissure sealant. In all cases, no adverse events or allergic reactions have been observed after application. At the 3-month recall (n = 49; 24 test lesions), one test and one control lesions had progressed and had to be sealed, while four test and one control lesions showed regression. All other test and control lesions were stable. 13 test lesions had not progressed in contrast to five control lesions (OR 4.7, p = 0.019). Regressions in Diagnodent readings were obvious in the test group in comparison to the controls (means = -13±16.1, -4.4±24.6 resp., p = 0.06). In conclusion, preliminary results suggest that Curodont™Repair may present a simple, safe and effective non-invasive treatment for early carious lesions.

Approved by the Ethic Committee/University of Greifswald, informed consent from patients and their parents was obtained. This study is supported by Credentis, Switzerland.

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Development of a Children's Story Book Series to Enhance Parental Self-Efficacy in Oral Health-Related Behaviours

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Child sugar control and twice daily toothbrushing need parental self-efficacy (PSE) to establish the behaviours within supportive environments. We have shown that a storybook with embedded Behaviour Change Techniques (BCTs) can improve PSE for toothbrushing. We have developed inter-linked storybooks with embedded BCTs for parents to read to children. The storybooks have anthropomorphised animal characters (Zip and Pop) set in their own world. **Aim:** To determine acceptability and feasibility of using storybooks to enhance PSE to prevent childhood caries. **Methods:** Children 5–7 years in three multi-cultural London schools randomly allocated to test and control. Test received Zip and Pop book and Controls, a children's book with no dentally-related

BCTs. Parents asked to read storybooks to children at bedtime over one month and complete questionnaires at start and end. **Results:** 144 families recruited; 105 completed. On 5-point scale (1 = strongly disagree; 5 = strongly agree); most families found the stories in both books were well understood (4.30 Test; 4.34 Control). UK-origin parents were more likely than non-UK to find Test story helped them talk about brushing their child's teeth: 3.55 Test compared 2.29 Control; but 3.41 Test and 3.25 Control for non-UK. Test story helped UK parents to talk to their child about healthy foods and drinks 3.70 Test compared to 2.07 Control; but 3.69 Test and 3.50 Control for non-UK. More UK parents felt that because they read the storybook together, it has helped their child co-operate better with getting their teeth brushed 3.10 Test v. 2.14 Control; but non-UK: 3.42 Test and 3.27 Control. **Conclusions:** Storybooks to enhance PSE are acceptable and feasible; UK-origin parents reported the book (in English) helped with some oral health-related behaviours but non-UK families did not and may need additional support.

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Effect of Systematic Toothbrushing on Changes of Toothbrushing Behaviour and Relation to Plaque

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Brushing teeth systematically is a common recommendation for proper oral hygiene. Whether this recommendation is meaningful, however, has not been verified in observational studies so far. Aim was therefore to investigate effects of a brushing systematic on observed brushing behaviour and on plaque levels. Video recordings and plaque index values (Turesky-modified QHI (T-QHI)) of an earlier controlled, randomized clinical trial on the adoption of a toothbrushing systematic [Schlueter et al.: Clin Oral Invest 2010;14:99–106] were used. Videos were in-depth re-analysed using the observation software INTERACT. Those participants who fully performed a predefined brushing systematic (SG; n = 22) were included in this reassessment and compared to an uninstructed control group (CG; n = 28). Parameters of interest were changes from baseline (T1 to T2) of the mean brushing duration on vestibular, occlusal and oral surfaces, and of mean and maximum T-QHI scores (total score and scores on oral and vestibular surfaces). Inter- and intra-group comparisons were calculated with independent-samples and paired-samples t-tests (Bonferroni-adjustment). Brushing duration in CG at T1 as well as T2 was vestibular>occlusal>oral with only a slight increase at T2 (13–20%; n.s.). Duration in SG was similar to CG at T1 (n.s.) but changed distinctly at T2 to oral>vestibular>occlusal (duration differences T1 to T2: +175%, p < 0.001, +53%; p < 0.001 and -24%, n.s. resp.) T-QHI (mean/maximum) decreased only in the SG group. The reduction was 20%/13% resp. for the total T-QHI and 32%/19% resp. for the oral T-QHI (all p < 0.001). These results show that systematic brushing leads to a distinct redistribution of

brushing duration. The smooth surfaces, particularly the previously neglected oral surfaces, were now brushed longer leading to a reduction of total and oral plaque values whereas the occlusal surfaces were brushed shorter.

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Association Between Caries Progression and GIC Sealant Retention in First Permanent Molars

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The aim of this study was to investigate the relationship between caries progression and the presence of a GIC sealant in first permanent molars. A 3-year randomized split-mouth trial was performed at schools, in southeast Brazil. The inclusion criteria involved 6 to 8 years old children with the 4 first permanent molars fully erupted without clinically detectable caries lesions in dentine evaluated by ICDAS-criteria (scores 0, 1 and 2), with cooperative behavior, good physical and medical health. One hundred and eighty seven patients were selected and had two molars sealed with GIC (Maxxion R, FGM or Fuji IX, GC Corp), while the other two molars remained without treatment, only controlled by tooth-brushing. All molars were evaluated with ICDAS and caries activity was assessed through clinical evaluation of roughness and brightness of the lesion at the baseline and after 3, 6, 12, 18, 24 and 36 months by a single examiner (κ intra-examiner = 0.98). Kaplan–Meier survival analysis, log-rank test and Cox Regression analyses were performed ($\alpha = 5\%$). The comparison between treated and untreated teeth revealed no statistical difference for caries progression outcome ($p = 0.103$). The relative risk for caries progression in case of sealant failure was 1.78 (95% CI 1.34, 2.38). The overall retention rate of sealants was 36.4% after 3 years assessment. In conclusion, the presence of GIC sealant is not related to incipient caries lesion progression in first permanent molars.

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MIH Increases the Need of Operatory Intervention in Permanent Teeth: A Case-Control Study

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MIH is relatively frequent worldwide and might represent a challenge for the clinical management of children. The aim of this case-control study was to investigate whether MIH increased the chance of having permanent teeth indicated for operatory intervention in a group of children assisted at the pediatric dental clinic of the State University of Rio de Janeiro, Brazil. Participants were screened from the list of patients assisted in 2011/2012. Those who were born in 2002/2003/2004 were considered eligible for the study. From 211 eligible patients, 193 attended the invitation, but 38 were excluded for several reasons. The final sample comprised 155 children, 57 CASES and 98 CONTROLS. Patients who had received or were indicated for operatory intervention in at least one permanent tooth (outcome) were the CASES. All patients were screened for MIH (exposure). A single calibrated examiner assessed MIH according to EAPD criteria and caries according to WHO. Odds ratio was calculated to evaluate the association between MIH and operatory intervention, chi-square was used to analyze the association between categorical variables, and t-test to compare means. The groups were similar regarding age, gender, and dft. The chance of receiving operatory intervention in permanent teeth was 5.89 (IC: 2.7–12.7) times higher in the CASE group. Among the CASES, the mean number of permanent first molars (PFM) and the mean number of tooth surfaces of PFM indicated for operatory intervention was higher for those with MIH ($p < 0.05$). Tooth surfaces of molars usually not affected by cavitated caries (buccal of upper and lingual of lower molars) were mainly restored when MIH was present ($p < 0.01$). MIH significantly increased the chance of having permanent teeth indicated for operatory intervention.

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Relationship Between DMFT in Young Adults at Initial Visit and Frequency of Invasive Treatments in the Following 10 Years

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Aim: This retrospective study was to investigate the relationship between DMFT in young adult patients at initial visit and frequency of invasive treatments in the following 10 years. **Method:** A total of 28 patients were selected from the patient database at

Sugiyama Dental Clinic, a private dental clinic in Japan, based on the following four criteria: between 20–39 years old at the initial visit, at least 28 remaining teeth, periodontia intact or mild periodontitis, and at least 10 years of maintenance period. Excluding two patients who requested for and received aesthetic treatments, the remaining 26 subjects were divided by the DMFT index into 3 groups, A (0–5), B (6–10), and C (>10). **Result and Discussion:** The number of total treatments were 178, of which 18.5% were treat primary caries lesions. The mean (SD) DMFT at the initial visit and the number of treatments, counting by events as to include secondary caries, were 2.4 (2.07) and 3.8 (3.90) in group A (n = 5), 8.4 (1.41) and 5.0 (5.01) in group B (n = 8), and 14.8 (3.61) and 9.0 (3.89) in group C (n = 13). The result showed statistical significance ($P < 0.05$, one-way analysis of variance). The greater DMFT, if interpreted as a higher caries risk, may tend to lead the dentist to more invasive treatments; this result may be replicating this tendency. Nonetheless, the result still makes a case for oral care at early age and is relevant in the clinical context. **Conclusion:** This result suggests that the higher DMFT index in young adulthood indicates more frequent interventions during the following 10 year maintenance period.

This study was supported by The Japan Health Care Dental Association.

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Non-Operative Control of Cavitated Approximal Caries Lesions in Primary Molars Over 6–24 Months: A Practice-Based Approach

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The effect of non-operative caries control at the cavity level is often questioned by dental practitioners. The aim of this practice-based observational study was to monitor the clinical characteristics and changes of active cavitated approximal caries lesions in primary molars over 6–24 months. 39 children from a community dental clinic, aged 5–10 years, DMF-S/dmf-s (cavity level) 0-1/1-13, were consecutively enrolled into the study based on the following inclusion criteria: i) presence of at least one active cavitated caries lesion in the distal surface of first primary molar or mesial surface of second primary molar extending up to 2/3 into the dentin, assessed radiographically, ii) absence of spontaneous pain. After informed consent by the parents one randomly selected lesion per child was opened (slicing method) and treated by non-operative procedures (site-specific oral hygiene instruction, topical fluoride treatment and dietary advice). The following lesion parameters were assessed at baseline and at follow-up: Pulpal pathology, presence/absence of plaque, lesion activity and sensitivity to air. Insertion of a filling or extraction was considered failure. 3 patients wanted operative treatment and 4 patients dropped out of the study. Of the remaining 32 lesions, 5 teeth failed because of pulpal pathology and 3 lesions received a filling. Twenty-four lesions (75%) survived without pain at 6–24 months follow-up, including

5 lesions on teeth which exfoliated without symptoms. Analysis of failures showed that 5, 2 and 1 lesions, respectively, failed because of insufficient slicing, insufficient compliance or sensitivity. Both children and parents were highly satisfied with the treatment approach. In spite of the relatively small sample size and short observation period we conclude that non-operative cavity treatment might be an alternative to conventional filling therapy in primary molars.

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Evaluation of Caries Management for Children between 7 and 12 Years Old

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This study aims to investigate by analyzing clinical data the effect of caries management for children between 7 and 12 years old at private dental clinics in Japan. Three clinics from Japan Health Care Dental Association, practicing prevention oriented dentistry for over 13 years participated in this study. These three clinics, Saito Dental Clinic (SDC) in Sapporo city, Tanaka Dental Clinic (TDC) in Kawaguchi city, and Takagi Dental Office (TDO) in Kobe city are located in suburbs of metropolises and the DMFT at 7 years old in those cities are 1.49 (2012), 0.81 (2011), and 0.91 (2011) respectively. 219 subjects (96 from SDC, 48 TDC, and 75 TDO) were selected from the clinical database at these clinics based on the following criteria: visited the clinic at the age of 7 and 12, 6 and 11, or 8 and 13. On the selected subjects, the rate and causes of DMFT increase were investigated. The increase of DMFT in the 5 year period was 0.27 (SDC), 0.31 (TDC), and 0.43 (TDO), and in total there were 75 newly filled surfaces, of which 66 were due to caries (88%) and 5 (6.7%) enamel hypoplasia. 51.5% of fillings due to caries were found on the first molar's mesial surface. In conclusion DMFT increase over 5 years was <0.5 teeth per patient. Still the result suggests a possibility of further improvement by site specific preventive care on the mesial surface of the first molar.

This study was supported by The Japan Health Care Dental Association.

Factors Influencing the Use of the Canary System™ to Detect Early Caries Lesions

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The Canary System™ (CS) is used to detect and monitor early tooth decay on all tooth surfaces. The purpose of this study was to examine some factors that may influence caries assessment by the CS. 40 extracted human teeth with early caries lesions were used. Twenty teeth had lesions on the occlusal pits and fissures, while the remaining 20 teeth had their lesions on the smooth surfaces. Each lesion was measured using the Canary System under the following conditions: (A) lesion dried for 5 seconds (the recommended condition for use [RC]), (B) lesion moistened, (C) lesion stained, (D) lesion covered by bacterial plaque, and (E) with the optical tip at varying distance (contact to 5 mm) from the lesion surface. Using ANOVA/Tukey's Multiple Comparison tests ($\alpha = 0.05$), data were analyzed separately for occlusal and smooth surfaces, and then with all surfaces combined. With combined data from all surfaces, the Canary Number (CN) was significantly ($p < 0.05$) increased by lesion staining (71.9 ± 21.9) and lowered by the presence of moisture (28.6 ± 16.0) when compared with RC (41.3 ± 17.2). Similar trend was observed on occlusal surfaces. On smooth surfaces, only lesion staining increased the CN (65.7 ± 16.89) significantly ($p < 0.05$) when compared to RC (43.2 ± 13.3). However, neither the presence of plaque nor the device's optical tip distance up to 5 mm away from lesion surface affected CN significantly. In conclusion, while caries assessment by the Canary System may be influenced by lesion staining on any surface and by both the presence of moisture and lesion staining on occlusal surface, it may not be affected by the presence of plaque on the lesion and with its optical tip up to 5 mm away from the lesion surface.

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Early Childhood Caries: Etiological Factors in a Child Population in Brazil

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The aim was to investigate the etiological factors associated with the presence of severe Early Childhood Caries (s-ECC) according to the American Academy of Pediatric Dentistry classification in Brazilian preschool children. **Methods:** from a total of 120 children (4 and 5 yrs-old) registered in a kindergarten located in a low socioeconomic area, 106 were orally examined using the Caries Assessment Spectrum and Treatment (CAST) instrument. Parents answered a validated questionnaire about family's life style, child's diet, oral hygiene habits and access to dental care.

Children were grouped into those having ECC (1 or more cavitated or non-cavitated, missing or filling tooth surfaces) and having s-ECC (1 or more cavitated, missing or filled smooth surfaces in the anterior teeth or a decayed, missing or filled score ≥ 5 or ≥ 6 for ages 4 and 5, respectively). A second analysis was carried out in which was considered the presence of dentine (CAST codes 4 to 7) versus non-dentine carious lesions (CAST codes 0 and 3). Data were analyzed using cross tables. **Results:** Caries prevalence was 97.2% including enamel and dentine carious lesions (CAST codes 3 to 7) and it was 56.6% when only dentine lesions were considered. There was no difference in etiological factors between the occurrence of s-ECC in comparison to the non-severe form of the disease (ECC). For the second analysis, among the etiological factors investigated, mothers' civil state ($p = 0.009$) and the use of fluoridated toothpaste ($p = 0.000$) were associated to the development of dentine carious lesions (CAST codes 4 to 7). **Conclusions:** being a single and/or divorced mother and not using fluoridated toothpaste were associated to the development of dentine carious lesions in preschool children.

This study was supported by Institutional funds.

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Effect of 10 Month Consumption of Milk Supplemented with Probiotic Lactobacilli on Dental Caries in Preschool Children

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Aim: The aim was to evaluate the effect of milk supplemented with probiotic bacteria on caries development in preschool children. **Methods:** Children 2–3 years of age ($n = 261$) attending 16 nursery schools in four municipalities of northern area of Metropolitan Region, Santiago, Chile, participated in the study. The nursery schools were randomly assigned to two parallel groups. Children in the intervention group were served 150 ml milk supplemented with *Lactobacillus rhamnosus* LHR08 (10^7 CFU/ml) while the control group received 150 ml of standard milk. The intervention lasted for 10 months considering only weekdays. Clinical examinations were performed according to ICDAS II criteria. The primary outcome was caries development and it was calculated regarding differences of prevalence and severity between baseline and 10-month-period exams. **Results:** The baseline prevalence of caries lesions ICDAS 2-6, was 39% in the intervention group and 42% in the control group and after 10 months of probiotic consumption, 54% and 65% respectively ($p > 0.05$). The baseline prevalence of caries lesions ICDAS 5-6 was 23% in the intervention group and 22% in the control group and after 10 months, 33% and 45% respectively ($p < 0.01$). The mean baseline caries lesions ICDAS 2-6 was 1.7 in the intervention group and 1.6 in the control group and after 10 months of probiotic consumption, 2.8 and 3.5 respectively ($p < 0.05$). The mean baseline caries lesions ICDAS 5-6 was 0.7 in the intervention group and 0.7 in the control group and after 10 months, 1.3 and 1.7 respectively ($p < 0.01$). **Conclusions:** Daily consumption of milk con-

taining probiotic bacteria reduces prevalence and severity of caries lesions in preschool children.

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Usefulness of MicroCT in Assessment of Calcium-Silicate Based Materials in Contact to Dentin

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In cariology research there is an increased demand for non-invasive and non-destructive techniques to evaluate mineral changes in hard tooth tissues. Also, the development of biomaterials has gained importance in the last years. MicroCT is a technology that allows recording of high spatial resolution of inner structures. The aim of the study was to evaluate the potential of microCT to study bioactive calcium-silicate based materials in contact to dentin. Human extracted molars (with signed agreement of every patient) were used to create cavities on the occlusal surface. Two biomaterials, Biodentine (Septodont, France) and MTA+ (Cerkamed, Poland), were each applied immediately after cavity preparation without prior conditioning of the dentin. For 3D visualization an X-ray Microtomography by v|tome|x m system (General Electric-Measurement&Control Solutions), first compact 300 kV microfocus CT system with < 1 µm detail detectability was used. The pictures showed a selective visualization of the biomaterials and the dentin. Firstly, bioactive materials were identified through morphological 3D teeth image analysis. Secondly, the location of both of them was mapped. Visual inspection of models demonstrated that there were noticeable differences between the biomaterials in adhesion to dentin in cavities. The border between dentin and the examined biomaterial showed no gap for Biodentine in contrast to MTA+. For MTA+, which has a less packed structure, a margin was visible on the interface. MicroCT seems to be a valuable method to assess structural changes during application of biomaterials, pinpointing precisely where material was lost. Further studies should be performed to provide additional information about the dynamics of the process of reactions with dental tissues. This method has a promising potential for future de/remineralization studies.

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Study Regarding the Composite Resistance to Microleakage Depending on the Use of Dental Spatula or Composite Brush

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The aim of this study was to establish if, while layering the composite, using the composite brush with composite primer improves or not the resistance to micro leakage for composite restorations by compare with using a dental spatula. Experimental approach: 108 extracted teeth were held in deionized water at 4°C for maximum 48 hours. Standardized class V cavities were completed on both buccal and oral sides of the teeth, resulting 216 cavities with 1 mm bevel. The cavities were filled using two different composites, Filtek Supreme (3M Espe) and Gaenial (GC), two equal groups. Each group was divided equally to use the composite brush or classic dental spatula. The teeth were isolated in nail polish and immersed in a 5% methylene blue. The specimens were sectioned and the dye penetration was assessed using dental loupes (EyeMag ProS 5X). Considering the presence or absence of penetration we formed: group A with no penetration or penetration <1/3 cavity depth and group B penetration >1/3 preparation depth. Results: We found a correlation between the instrument used for restoration adaptation and the presence of dye penetration (OR = 5.5, trust range = 1.2767–23.6928, Chi Square = 5.6) and no correlation between the composite used and the presence of dye penetration (OR = 1.75, trust range = 0.3977–7.7001, Chi Square = 0.5538). There was a higher prevalence of deeper dye penetration occurrence when the classic system with oral spatula was used ($p = 0.000729 < 0.05$). In conclusion there was a higher prevalence of dye penetration occurrence when the spatula was used then when composite brush with composite primer was used.

Randomised Control Trial of Three Caries Management Methods for Primary Molars: 1 Year Results

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Over the last decade, there has been a move away from the traditional treatment approach to managing caries towards more conservative techniques involving partial or even no caries removal. The aim of this study was to compare the clinical efficacy of three caries treatment approaches after 1 year: Non-restorative Caries Treatment (NRCT), the Hall Technique (HT), and conventional restorations (CR), for management of class II caries lesions (ICDAS 3-5) in primary molars. One-hundred and sixty-nine children (3–8-year-olds; mean = 5.56, SD = 1.45) were enrolled in this secondary care-based, three-arm parallel-group, randomized controlled trial. Participants were randomized to one of three arms: NRCT (opening-up the cavity and applying fluoride), HT (sealing in caries with stainless steel crowns without caries removal), CR (control arm, complete caries removal and compomer restoration), and treated by 12 paediatric dentists (specialists or postgraduate students). Only one tooth per child was included. There were 148 children (88%) with a minimum follow-up of 11 months. Nine patients (6%) experienced at least one 'Major' failure (irreversible pulpitis, abscess, unrestorable tooth): NRCT 4 (2%), CR 5 (3%), no major failures in the HT arm ($p = 0.002$, CI = 0.001 to 0.003). Twenty teeth were recorded as having at least one 'Minor' failure (reversible pulpitis, caries progression, and secondary caries): NRCT 8 (5%), CR 11 (7%), HT 1 (1%) ($p = 0.002$, CI = 0.001 to 0.003). By independently comparing two samples, no differences were observed between NRCT-CR ($p = 0.75$, CI = 0.73 to 0.76), nevertheless differences were still observed between NRCT-HT ($p = 0.002$, CI = 0.001 to 0.003) and CR-HT ($p = 0.001$, CI = 0.000 to 0.002). Overall, the HT exhibited more favourable outcomes than the NRCT and CR, while the outcomes of NRCT and CR were comparable (Trial registration no. NCT01797458).

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Clinical Evaluation of Two Prevention Programs in Adults. Six-Month Results

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This study investigated the application of two preventive and minimally invasive protocols in early caries lesions. A total of 21 adult patients of high and medium caries risk with 435 early caries took part in the present study. These patients were assessed for caries with ICDAS criteria and then were divided into 2 groups depending on their caries risk profile: A high risk (Group A) and a medium risk group (Group B). Each group was divided into subgroups (A1, A2, B1, B2). In groups A1 and B1 intensive preventive protocol was applied (A1: 5 high-risk patients with 167 incipient caries and B1: 5 medium-risk patients with 83 incipient caries) while in groups A2 and B2 just instructions for oral hygiene were given. (The control group; A2: 6 persons in high-risk with 116 incipient caries and B2: 5 medium-risk patients with 69 caries lesions). The invasive protocol included the topical application of fluoride, brushing with 5000 ppm fluoride toothpaste, use of ACP-CPP at lesions on smooth surfaces, applications of sealants at lesions of occlusal surfaces (ICDAS code 2). The control protocol program included patient motivation and twice daily brushing with 1450 ppm Fluoride toothpaste and inter-dental flossing. Patients were re-examined in six months. For the results the non-parametric Wilcoxon statistic test was applied in two different visits (baseline and after 6 months). There was no statistically significant difference in the number of lesions among high-risk and medium-risk patients group where protocols were applied (Group A1, $p = 0.498$; Group B1, $p = 0.066$) nor statistically significant difference in the control group (Group A2, $p = 0.498$; Group B2, $p = 0.414$). The actual change in levels of caries was: in Group A1 baseline DMFS was 22 ± 3.36 (mean \pm se) and after six months was 22 ± 3.56 (mean \pm se), in Group B1 DMFS was 16.6 ± 1.86 (mean \pm se) and after six months was 15 ± 1.87 (mean \pm se), in Group A2 DMFS was 19.33 ± 2.8 (mean \pm se) and after six months was 20.16 ± 1.3 (mean \pm se) while in Group B2 DMFS was 13.8 ± 0.97 (mean \pm se) and after six months was 12.4 ± 1.72 (mean \pm se). This study requires more time for its efficacy to be determined.

Caries-Preventive Effect of Supervised Toothbrushing, Composite Resin and ART Sealants After Three Years

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Objectives: To investigate the effect of three caries preventive measures on high-risk permanent first molars over a period of 3 years. **Methods:** This cluster (school)-randomized controlled clinical trial evaluated the presence of caries lesions in occlusal surfaces of first permanent molars of low socio-economic children (baseline age: 6–7 y) that received one of the following preventive measures: supervised toothbrushing at school every other day, STB (n = 71); composite resin sealants, CRS (Fluoroshield, Dentsply) (n = 169); high-viscosity glass-ionomer cement sealants, ART (Ketac Molar Easymix, 3M ESPE) (n = 69). Sealants were placed only on caries high-risk teeth which were assessed and identified at baseline based on fissure depth, and enamel caries lesions scores using ICDAS II. A dentine cavity (ICDAS score: 5,6) was considered a failure. Data were analyzed using Kaplan-Meier and Wald-test. ANOVA and Jackknife SE were used for effect testing on the survival rates. **Results:** A total 225 occlusal surfaces were evaluated at year 3. There were no effects of the backgrounds variables gender, jaw type, mean DMFT/dmft-score at baseline observed for the survival rates between the three groups. There were no statistically significant differences in the survival rates of dentine cavity free occlusal surfaces over 3 years between STB (95.6%±2.5%), CRS (91.4%±2.9%) and ART (90.2%±5.0%) (p = 0.59). **Conclusion:** Supervised toothbrushing at school premises was as effective as placing sealants in preventing dentine caries lesions in high-risk permanent first molars over a period of 3 years.

This study was supported by FAPDF, University of Brasília and Radboud University Medical Center Nijmegen.

Sealants Retention and Caries-Preventive Effect: Materials, Evaluation Techniques and Retention Rates Criteria

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Sealing pits and fissures is a good strategy to prevent caries. The aim of this study was to compare retention rates and caries preventive effect of two fissure sealants (Clinpro XT Varnish – CV and Fuji IX GP FAST – FIX) in newly erupted first molars of children with high caries risk using different evaluation techniques and retention rates categorization. **Methods:** 56 children, 6–7 years of age, were included in a split-mouth randomized clinical trial. 112 matched pairs of teeth received one of the tested sealants (CV or FIX). Clinical and standardized photographic evaluations were carried out after 6 and 12 months. All patients were evaluated by a calibrated and blinded examiner. Data were analyzed using chi-square test (p < 0.05). **Results:** Retention rates were analyzed using a traditional and a modified success/failure categorization [Chen et al.: Clin Oral Investig 2012;16(5):1443–50]. The modified categorization presented lower retention rates. Photographic evaluation resulted in higher retention rates than clinical evaluation. After 12 months, dentine caries free occlusal surfaces were encountered in 98.9% (CV) and 98.9% (FIX) of teeth under clinical evaluation and in 98.9% (CV) and 95.9% (FIX) of teeth under photographic evaluation. No statistical significant differences were found between materials. **Conclusion:** the tested sealants had similar retention rates and caries preventive effect after 12 months of clinical evaluation. The modified retention categorization was more strict in considering retention success.

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Saliva Buffering Capacity in Relation to Consumption of Beers

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Objective: to determine the effect produced by the buffering capacity of the saliva on the pH of beers. **Methods:** Seventeen beers on the market of Chile were examined. Stimulated saliva samples were obtained from five systemically healthy young patients (aged 18 to 28 years), who did not present with any pathology that affect the salivary glands and / or any defects in the quality and quantity of saliva. The saliva samples were grouped in a 'pool' of saliva in absence of oxygen, and its pH was measured three times. Each beer was opened and immediately the pH was measured. A solution was made of 2.5 ml of beers and 4 ml of pool of saliva. The measurements of pH were performed when the solution was mixed and, 10, 20 and 30 minutes after. This procedure was performed with each of the beers and repeated three times. **Results:** The pool of saliva on average had a pH value of 7.86, with a standard deviation of ± 0.04 . pH ranges of beers range from 2.97 ± 0.045 (Lemon Stones[®]) to 4.56 ± 0.03 (Paulaner[®]). The pH of the Lemon Stone[®] beer increased the most after adding the saliva, reaching a pH value of 5.14, while Kuntsmann Lager[®] reaching a pH value of 5.8 achieved the lesser pH increase. **Conclusion:** The buffering capacity of saliva is capable of considerably increasing the pH of the beers, exceeding a pH of 5.5 in 81.25% of samples analyzed. Further clinical studies are required.

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Self-Induced Vomiting and Dental Erosion

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In persons suffering from eating disorders characterized by vomiting (e.g. bulimia nervosa), the gastric juice regularly reaches the oral cavity, causing a high risk of dental erosions. This study aimed to assess the occurrence, distribution and severity of dental erosions in a group of Norwegian patients experiencing self-induced vomiting (SIV). The individuals included in the study were all undergoing treatment at clinics for eating disorders. They were referred to the university clinic for dental examination. One calibrated clinician registered erosions using the Visual Erosion Dental Examination (VEDE) system [Mulic et al.: Caries Res 2010;44:294–299]. Of 72 referred patients, 66 (63 females, mean age 27.7 years) were or had been experiencing SIV (mean duration 11 years; range: 9–37 years), and were therefore included in the study. Dental erosions were found in 46 individuals (70%), 19 had enamel lesions only, while 27 had both enamel and dentine lesions. Ten or more teeth were affected in 26% of those with erosions, and 9% had ≥ 10 teeth with dentine lesions. Of the erosions, 42% were found on palatal/lingual surfaces, 37% on occlusal surfaces and 21% on buccal surfaces. Dentine lesions were most often found on lower first molars, while upper central incisors showed enamel lesions most frequently. Though individuals experiencing SIV are commonly affected with dental erosions, 30% had no lesions, despite of SIV for up to 37 years. The majority of the erosive lesions (57%) were found in those with the longest illness period, and 75% of the lesions extending into dentine were also found in this group. SIV does not necessarily lead to dental erosion.

The study was funded by University of Oslo, Norway.

Risk Factors for Dental Erosion Development in Kindergarten Children Aged 2–5 Years

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Aim: To reveal risk factors for development of dental erosion in kindergarten children aged 2–5 years. A case-control study approved by the Volgograd Regional Ethics Committee was conducted to reveal potential risk factors for dental erosion in 2–5 year old kindergarten children in Krasnoarmeysky district, Volgograd, Russia. The study was arranged in randomly selected 18 of 54 kindergartens (33.3%) in Krasnoarmeysky district. 682 children aged 2–5 years attended these kindergartens. 159 of 682 children (23.3%) had dental erosion as were revealed in the previous study. The parents of 159 children with dental erosion were asked to fill in the questionnaire. The response rate was 53.3% (study group, $n = 85$). The control group was formed of randomly selected 159 parents of children without dental erosions (who attended the same kindergartens). The response rate was 20.1% (control group, $n = 32$). The control and study groups were comparable by age and gender of the children. The questionnaire consists of 42 questions about the parents (socio-demographic status) and their children (general health, allergic status, taking medicines, dietary habits, and oral hygiene practices). Mean (%), odds ratio (OR), 95% confidence interval (CI), chi-square (X^2) test and p-value were calculated to analyze the data. **Results:** The children who consumed citrus fruit several times a day (OR = 1.136, CI 0.615–2.114, $X^2 = 0.085$, $p = 0.7705$), citrus drinks (OR = 4.72, CI 2.437–10.047, $X^2 = 25.39$, $p < 0.0001$), acid candies (OR = 1.5, CI 0.824–2.787, $X^2 = 1.62$, $p = 0.2031$) and those who preferred vegetable marinades (OR = 2.88, CI 1.662–5.247, $X^2 = 1.620$, $p < 0.0001$) had a stronger tendency to develop dental erosion. **Conclusion:** Frequent consumption of citrus fruit, citrus drinks, acid candies and vegetable marinades can be identified as risk factors for dental erosion development in 2–5 year old kindergarten children.

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Toothpastes with Special Active Agents and Abrasive Systems: Effects on Enamel Erosion/Abrasion

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There is still little known about the influence of overall toothpaste formulation and of abrasives as well as of the interaction between abrasion effect and active agents regarding the anti-erosive/

anti-abrasive effect. Aim of this study was to compare different types of toothpastes: four conventional (1450 ppm F), three hydroxy-apatite (with (1450 ppm F) and without F), four F/Sn-containing formulations (1000–1450 ppm F; 3000–4200 ppm Sn) and four formulations containing special abrasives, e.g. with the indication for interdental cleaning, for use with an powered toothbrush or for sensitive teeth (1400–1450 ppm F). The study was conducted in two experiments. In E1, enamel samples (17 groups, $n = 15$ each) were demineralised (10 days, 6x2 min/day; 0.5% citric acid, pH 2.6), exposed to toothpaste slurries (2x2 min/day) and intermittently stored in mineral salt solution. In E2, samples were additionally brushed for 15 s during slurry immersion time. Negative control was immersion/brushing with placebo (experimental F-free formulation). Substance loss was quantified profilometrically. Comparison of formulations within one experiment was performed with ANOVA/Tamhane's post hoc. Interaction between active ingredients and abrasive effects were analysed with a factorial ANOVA. Approximately half of non-Sn formulations reduced tissue loss significantly compared to placebo (E1:12.0±3.0 µm; E2:17.5±3.8 µm) between 36% and 51% in E1 and between 30% and 57% in E2. Between the non-Sn toothpaste types no distinct differences were found. The Sn-containing formulations were effective in both experiments (significant reductions between 48% and 77% in E1 and between 49% and 76% in E2). The comparison of the results of E1 and E2 showed that the increase of loss by abrasion was highly variable; a significant interaction between the active agent and the abrasive effect was found ($p \leq 0.001$). In conclusion, additional to the active agents, the abrasive system of toothpastes seem to be an important aspect of anti-erosive toothpaste formulations.

This study was supported by Justus-Liebig-University Giessen.

F/Sn Increases the Durability of an Adhesive Used as Erosion Sealant under Erosion-Abrasion Conditions

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Adhesives can be used for sealing erosive lesions; however, clinical studies revealed limited efficacy. There is evidence that tin potentially increases bond strength of self-etching adhesives. Aim of the present study was to investigate whether the pre-treatment of dentine with different F/Sn solutions can increase the durability of adhesive sealings. Dentine specimens (eight groups, $n = 16$ per group) were freed of smear-layer (0.5% citric acid, 10 s), treated (15 s) either with none (control), amine fluoride (AmF, 500 ppm F⁻; pH 4.5), SnCl₂ (800/1600 ppm Sn²⁺; pH 1.5), SnCl₂/AmF (500 ppm F⁻, 800 ppm Sn²⁺; pH 1.5/3.0/4.5) or elmex ErosionProtection mouthrinse (EP, 500 ppm F⁻, 800 ppm Sn²⁺, pH 4.5, GABA Int.), rinsed with tap water (30 s), gently air dried and individually covered with Clearfil™SE (Kuraray). Specimens were demineralised (0.5% citric acid, 2 min) and brushed automatically (15 s, 200 g, NaF-toothpaste, RDA 80) for 1320 cycles. One cycle was demineralisation (alternately 5°C/55°C), rinsing (tap water, 60 s),

brushing and rinsing. Adhesive coverages were stable up to 1320 cycles. Therefore, cycles were modified by prolonging brushing duration (30 min/cycle); specimens under went a total of 60 modified cycles. Loss was measured profilometrically after 480/1320 cycles and after 20/60 modified cycles. Comparison of values between time points within one group was performed with paired t-tests ($p \leq 0.005$, Bonferroni-adjustment). SnCl₂/AmF pH 4.5 and EP distinctly increased sealant durability; sealant was worn only by 17% and 33% ($p \leq 0.005$ compared to initial adhesive thickness) by erosion/abrasion, but survived on the surface. In conclusion all other groups sealant was totally lost. F/Sn application at pH 4.5 prior to adhesive application might offer an improved symptomatic therapy option for dentine erosion by durably sealing the lesion.

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Effect of Experimental Mouthrinses Containing NaF and TiF₄ on Enamel Erosion In Vitro

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This study analysed the anti-erosive effect of experimental solutions containing TiF₄ and NaF on enamel in vitro. Bovine enamel samples were treated with one of the solutions containing around 500 ppm F: (1)– commercial solution with SnCl₂/NaF (Erosion Protection®); (2)– experimental solution with 0.0815% TiF₄ (pH 2.5); (3)– 0.105% NaF (pH 2.5); (4)– 0.042% NaF + 0.049% TiF₄ (pH 4.4); (5)– 0.063% NaF + 0.036% TiF₄ (pH 4.5) or (6)– control (untreated). The samples were submitted to pH cycles (4x90 s in soft drink intercepted by artificial saliva) and daily fluoride applications (2x1 min/day) for seven days. The enamel loss was measured using a contact profilometer and analysed by ANOVA and Tukey's test ($n = 15$, $p < 0.05$). The best anti-erosive effect was found for TiF₄ solution (99% reduction in enamel wear, $0.03 \pm 0.04 \mu\text{m}$), followed by SnCl₂/NaF (78%, $0.75 \pm 0.18 \mu\text{m}$) and 0.049% TiF₄ + 0.042% NaF solution (41%, $1.97 \pm 0.90 \mu\text{m}$) compared to the control ($3.34 \pm 0.94 \mu\text{m}$). The other fluoride solutions did not differ from the control. The experimental solution containing a specific combination of TiF₄ and NaF has the ability to partially reduce enamel erosion. However, the best preventive effect was still found for TiF₄ alone.

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Relative Influence of Brushing Force Transmission Factors on Substance Loss of Eroded Dentine

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This study was carried out to evaluate the influence of factors (such as tip form, brush tension and slurry viscosity) transmitting brushing force onto eroded dentine surface by measuring thickness of substance loss using an Electron Probe Micro-Analyzer. Dentine slabs each with an exposed window (4 mm²) made using nail-varnish were eroded through immersion into 0.83 M acetic acid for 30 minutes. Half of the window area were then covered with nail-varnish and served as the control area. The slabs were fixed and the tips (flat trim or tapered) of the bristles were placed on the eroded window, applying adequate pressures (2 g or 4 g per mm²) respectively. The slabs were then brushed for one minute using a sonic toothbrush (480 Hz) with slurry containing silica abrasive and aqueous solutions of carboxymethylcellulose (0.5% or 1%) in a ratio of 10 g to 50 mL. The slabs from which the nail-varnish was removed were then sputter-coated with platinum and embedded in methacrylate to be cross-sectioned in the middle of the windows. The cross-sectional surface was polished, coated with carbon and examined using back-scattered electron imaging to measure the thickness of dentine loss in the brushed and the control areas within the window. The loss values (μm , mean \pm SD) in the treated and the control areas were 24.6 ± 3.61 and 21.5 ± 3.36 respectively. ANOVA analysis indicated that abrasion thicknesses defined as the differences of loss values between brushed and control surfaces were affected only by tip form with no significant interactions among the other factors. Eroded dentine wear by a sonic toothbrush was deeper with tapered tip (3.82 ± 2.73) than with flat trim (2.40 ± 1.54). The results suggested that the influence of factors transmitting brushing force on eroded dentine wear was limited.

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Exposure to Lactic or Citric Acid Changes the Proteomic Profile of Acquired Pellicles Formed on Dentine

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This study characterized, for the first time, the proteomic profile of the acquired pellicle formed on dentine. The changes in this proteomic profile after exposure to lactic (caries) or citric (erosion) acids were also evaluated. The experiments were conducted on three consecutive days. Each day, volunteers ($n = 9$) used a man-

dibular device containing 6 blocks (3X3 mm) of human root dentine. After the volunteers remained with the device in the oral cavity for 10 or 120 minutes in order to allow the formation of the acquired pellicle *in situ*, the blocks were immersed in citric acid (1%, pH 2.5) or lactic acid (0.1 M, pH 4.8) or deionized water for 20 seconds. Following, the pellicle was collected with an electrode filter paper soaked in 3% citric acid. This procedure was repeated for two additional days and 'pools' with the filter papers obtained from the 9 volunteers for each type of substrate, sampling time and type of acid were made. After extraction, proteins were subjected to reverse phase liquid chromatography coupled to mass spectrometry (nLC-ESI-MS/MS). MS/MS data were processed and submitted to SEQUEST software. Searches were done using SWISS-PROT and TrEMBL databases. In total, 223 distinct proteins were identified in the dentine acquired pellicle. After immersion of the 10-minute pellicles in deionized water, lactic acid and citric acid, 66, 76 and 16 proteins, respectively, were identified. The corresponding numbers for the 120-minute pellicles were 52, 51 and 6 proteins, respectively. Acid-resistant proteins, especially isoforms of mucins and collagen, were identified after short and long-term pellicles were exposed to lactic or citric acid. These proteins, or the peptides originated from them that are responsible for the protective effect, are candidates to be used in the enrichment of dental products, aiming to prevent dental caries and erosion.

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Randomized In Situ Trial on the Effect of Milk and CPP-ACP on Dental Erosion

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This randomized in situ study aimed to analyse the effect of milk (with or without 5 ppm F) and CPP-ACP pastes (with or without 900 ppm F) on dental erosion. The study was a seven phases (5 days each) crossover design involving 15 participants wearing intraoral appliances with enamel and dentin specimens. Specimens were extraorally eroded (erosive softdrink, 6 x 90 s/day) and brushed (2 x 30 s/day, 2 N) using a non-fluoridated (Calendula, Weleda, negative control) or fluoridated toothpaste (elmex, GABA). The test products were milk, milk + 5 ppm F (twice daily, each 100 ml in 2 min), CPP-ACP paste (Tooth mousse, GC, 3 min/day), CPP-ACP paste/900 ppm F (MI Paste Plus, GC, 3 min/day) or a SnCl₂/AmF/NaF mouthrinse (elmex EROSION PROTECTION, GABA, positive control, 30 s/day), which were applied immediately after erosion with the appliances in the oral cavity. In these test groups, a non-fluoridated toothpaste was used for brushing. Tissue loss was determined profilometrically after 5 days and statistically analysed by linear mixed models methodologies ($p < 0.05$). Enamel and dentin loss was significantly reduced by the use of fluoridated toothpaste (enamel: $1.1 \pm 1.0 \mu\text{m}$; dentin: $2.4 \pm 1.7 \mu\text{m}$) or the SnCl₂/AmF/NaF mouthrinse (enamel:

$1.5 \pm 1.5 \mu\text{m}$; dentin: $1.8 \pm 1.9 \mu\text{m}$) compared to the negative control (non-fluoridated toothpaste; enamel: $2.2 \pm 1.3 \mu\text{m}$; dentin: $3.8 \pm 2.2 \mu\text{m}$). Milk and CPP-ACP were not effective in reducing enamel and dentin loss significantly, independently of the presence of fluoride. Under the conditions of the present study, milk and CPP-ACP were not effective to reduce dental erosion. Clinical trials registration: NCT01566357.

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Using an Optical Pen-Size Reflectometer to Monitor In Vitro Erosion of Sound and Eroded Enamel

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This study aimed at applying an optical pen-size reflectometer to monitor *in vitro* erosion of enamel with or without signs of erosive wear. Twenty-three central incisors were selected: $n = 14$ were clinically sound (Sound) and $n = 9$ presented clinical signs of erosive wear (Eroded). The buccal incisal third of the teeth was embedded leaving a window of native enamel ($6.69 \pm 2.30 \text{ mm}^2$). Two examiners measured enamel surface reflectivity (ESR) initially and after each erosive challenge (7.5 ml, 1% citric acid, 25 °C, 70 rpm, total of 4, 8, 12, 16, 20 and 24 min). Calcium dissolution was measured with an atomic absorption spectrometer. Initially, ESR values were lower in Sound teeth than Eroded teeth: Examiner 1, 2.83 and 5.86 ($p = 0.007$), Examiner 2, 3.17 and 6.05 ($p = 0.033$), respectively. With the progression of erosion, ESR decreased in both groups to different extents: Sound teeth presented a significantly lower percentage decrease in reflectivity (PDR) than the Eroded teeth: 75.1% and 89.4% ($p = 0.033$) from examiner 1; and 70.7% and 86.9% ($p = 0.013$) from examiner 2, for Sound and Eroded teeth, respectively. Calcium dissolution increased, but presented no significant differences between Sound ($0.031 \mu\text{mol}/\text{mm}^2$) and Eroded ($0.032 \mu\text{mol}/\text{mm}^2$) teeth ($p = 0.643$) after 24 min. Satisfactory correlation was observed between calcium dissolution and PDR from examiners 1 ($r_s = -0.66$) and 2 ($r_s = -0.61$). The intra-class correlation (ICC) for the PDR between both examiners after 4 and 8 min were $\text{ICC} = 0.368$ and $\text{ICC} = 0.489$, and good agreement was reached after 12, 16, 20 and 24 min erosion ($\text{ICC} = 0.826$, 0.776, 0.911, 0.857, respectively). In conclusion, the optical pen-size reflectometer can be used to monitor *in vitro* erosion, and it was able to discriminate the effect of erosion on enamel with or without signs of erosive wear.

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Dental Erosion among Adolescents in the 1980s

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Oslo, Norway High prevalence of dental erosions is assumed to be a 'new' phenomenon as a result of modern lifestyle with high and frequent consumption of acidic beverages/diets. This study aimed to record the prevalence, incidence and progression of dental erosion among 15 year olds born in 1970. Dental erosions were registered on study models of 300 15 year olds in 1985 by two calibrated examiners, using the Visual Erosion Dental Examination (VEDE) system [Mulic et al., 2010]. New study models of 88/300 individuals were made in 1988 and of 35/300 in 1991. Absolute frequencies and proportions were obtained for descriptive analysis, and bivariate analysis (Chi-square) was used for testing possible associations between the variables. The level of significance was set at 5%. Of the 15 year olds, 31% had dental erosions: 27% in enamel only and 4% into dentine. Males had higher prevalence (36%) than females (25.3%) ($p = 0.04$), and more dentine erosions: 4.7% vs 3.3% (not significant). Mandibular first molars were most frequently affected (38%) followed by upper central incisors (17%). Among the 88 individuals re-examined after 3 years, the prevalence increased from 20% ($n = 18$) to 68% ($n = 60$); mostly in enamel (17% to 65%). The erosion severity remained unchanged in 10 adolescents, increasing from no erosion to enamel erosion in 27 and to dentine in one. Of the 35 individuals re-examined after another 3 years, the prevalence increased from 69% to 72%. Enamel erosions progressed into dentine in six individuals (17%). The prevalence of dental erosions among 15 year olds in 1985 was lower than reported in recent studies, and males and mandibular molars were most frequently affected.

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The Erosive Potential of Dental Rinses

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The aim of this study was to analyze the erosive effect of dental rinses available on the market. Premolars were selected and their crowns were cut in half. A total of 75 enamel specimens were ground and polished and randomly divided into 7 groups by draw: Tap Water (TW, $n = 11$), Orange Juice (OJ, $n = 11$), and 5 dental rinses (1. elmex sensitive® ($n = 11$), 2. Listerine teeth and gum protection® ($n = 10$), 3. Listerine Smart Kidz® ($n = 10$), 4. Odol-med3® ($n = 11$), 5. Signal Integral® ($n = 11$)). The pH of the rinses was measured using a pH meter. The enamel specimens had their initial surface microhardness (SMH) measured and were then individually immersed into the solutions (10 ml, 25°C, 70 rpm dynamic conditions) for a total of 3 and 10 min. SMH was measured

after each immersion. The pH values of the solutions were: pH 7.0 and 3.5 for TW and OJ, and pH 5.0, 4.0, 3.0, 6.5 and 4.5 for Rinses 1, 2, 3, 4 and 5, respectively. There was no difference in initial SMH between the groups (363.2 ± 14.2 KHN, $p > 0.05$). There was no significant change in the SMH values when the specimens were treated with TW and Rinses 1, 2, 4 and 5 ($p = 0.913, 0.060, 0.062, 1.000$ and 0.407 , respectively). A significant decrease in SMH was observed in the groups treated with OJ ($p < 0.001$) and Rinse 3 ($p < 0.001$): after 3 and 10 min OJ presented a decrease of 4.3% and 13.9%, respectively, and Rinse 3 presented a decrease of 7.5% and 17.7%, respectively. Rinse 3 presented a greater SMH decrease than OJ after both 3 and 10 min immersion ($p = 0.010$ and $p = 0.029$, respectively). In conclusion, most rinses had no significant erosive potential, but Listerine Smart Kidz® was able to significantly soften the enamel surface to a greater degree than Orange Juice.

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Effect of Tooth Surface and Type during In Vitro Erosion

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The aim of this laboratory study was to investigate the differences between molar and premolar teeth and buccal or lingual surfaces of teeth to a standardised erosion model. Extracted caries free molar and premolar teeth were sectioned to produce a total of 40 highly polished enamel samples. Each section was embedded in cold cure acrylic resin, with a window of exposed enamel (~1x3 mm) and separated into 4 groups. Initial Knoop hardness (KH) was calculated and samples outside a range of 330 ± 30 KH were rejected. Sections were eroded in 0.3% citric acid (pH 3.2) for 10 minutes. Each group of 10 samples underwent 5 cycles of erosion (1 cycle = immersion in acid/ rinse with deionised water) at room temperature, in 80 mL of solution, with agitation (Orbital shaker, 60 rpm). Samples were analysed with non-contact laser profilometry and KH (981.2 mN, 10 seconds). Single/mean step height (SH) was calculated with a surface analysis program and mean change in KH compared to an un-eroded reference area. 1 way ANOVA and multiple pairwise comparisons were performed. The mean SH loss \pm standard deviations (μm) for; molar buccal, molar lingual, premolar buccal and premolar lingual was 6.52 ± 0.78 , 7.74 ± 1.81 , 7.35 ± 1.03 and 8.16 ± 1.63 , $P = 0.084$. The mean change in KH \pm standard deviations (KH) for; molar buccal, molar lingual, premolar buccal and premolar lingual was 131.64 ± 16.34 , 166.32 ± 18.19 , 179.60 ± 20.01 and 193.20 ± 20.12 , $P < 0.001$. In conclusion, the differences in SH were small ($< 2 \mu\text{m}$) and detection depended on sample preparation and limitation of the instrument sensitivity so SH loss was not mirrored by KH.

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Erosive Potential of Vitamin Effervescent Tablets Available in Switzerland

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The aim of the present study was to investigate the erosive potential of seven commercially available vitamin effervescent-tablets (ET). Ninety-six bovine enamel samples were prepared and randomly assigned to eight groups (G1-G8). Per cycle, samples ($n = 12/\text{group}$) were immersed (2 min) in solution (10 ml/sample) freshly prepared from water supplemented with: none (control-group, G1), Actilife Multivitamin ET (G2), Sunlife Vitamin C ET (G3), Optisana Vitamin C ET (G4), Optisana Multivitamin ET (G5), Well&Active Multivitamin ET (G6), Kneipp Vitamin C+Zink ET (G7) and Sunlife Multivitamin ET (G8) according manufactures instructions. Enamel loss was measured using profilometry after 10 and 20 erosive cycles and compared by Scheffe's post hoc test ($p \leq 0.05$). Additionally, saturation of the solutions with respect to hydroxyapatite (HA), octacalciumphosphate (OCP) and calcium-fluoride (CF) was determined [Shellis: Comput Appl Biosci 1988;4:373-379]. After 10 and 20 cycles, no tooth wear was observed for G1 (control). In all other groups, a significant erosive tooth wear was recorded. Significantly highest enamel wear (mean \pm SD) after 10 and 20 cycles of erosion was observed for G8 ($3.16 \pm 0.38 \mu\text{m}$ and $8.45 \pm 1.08 \mu\text{m}$, respectively), while the lowest erosive wear was observed for group G2 ($2.23 \pm 0.73 \mu\text{m}$ and $5.61 \pm 1.08 \mu\text{m}$, respectively). In all groups, except G1, the tooth wear after 20 cycles was significantly higher compared to that after 10 cycles ($p < 0.05$, respectively). All solutions, except G1 (control), presented an acidic pH between 4.14 and 4.49 and were undersaturated with respect to HA, OCP and CF. In conclusion all effervescent tablets tested showed an erosive potential and patients should be informed accordingly.

The Influence of Different Bleaching Agents on Surface Roughness of Composite Resins

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The aim of this study was to evaluate the erosive effects of one in-office and two home bleaching agents on the surface roughness of a hybrid, microhybrid and nanohybrid composite resins. Three composite resins (Valux Plus, 3M ESPE, Filtek Z 250, 3M ESPE and Herculite XRV Ultra, Kerr) and three bleaching agents (Opalescence PF, Ultradent Products, Perfect Bleach, Voco and

Perfect BleachOffice +, Voco) were chosen for this study. Twenty-eight samples, $10 \times 7 \times 1 \text{ mm}$, of each composite resin were prepared by placing the composite resin in contact with plastic matrix strips between two glass slabs and lightcuring for 40 seconds. The samples of each composite resin were equally split in 4 groups: 7 samples were stored in distilled water (control group), 7 samples were subjected to 17% carbamide peroxide gel (Perfect Bleach) action for 3 hours a day, 7 days, 7 samples were subjected to 15% carbamide peroxide gel (Opalescence PF) action for 3 hours a day, 7 days and 7 samples were subjected to 35% hydrogen peroxide gel (Perfect BleachOffice +) action two times for 15 minutes. After cleaning the samples were submitted to roughness evaluation using atomic force microscopy (AFM). AFM images were taken in air, at room temperature, on a SPM SOLVER PRO-M instrument (producer: NT-MDT Moscow, Russia). A NSG10/Au Silicon tip with a 6 nm radius of curvature, semi-contact mode was used. The results were expressed as root mean square roughness. The highest roughness was recorded when 35% hydrogen peroxide gel was used (mean values \pm SD: $36.58 (\pm 0.15) \text{ nm}$ Valux Plus, $24.35 \text{ nm} (\pm 0.04)$ Z250 and $21.86 (\pm 0.05) \text{ nm}$ Herculite XRV), followed in descending order by 17% carbamide peroxide gel (mean values \pm SD: $22.45 (\pm 0.02) \text{ nm}$ Valux Plus, $20.51 (\pm 0.04) \text{ nm}$ Z250 and $15.19 (\pm 0.02) \text{ nm}$ Herculite XRV), and 15% carbamide peroxide gel (mean values \pm SD: $20.14 \text{ nm} (\pm 0.03)$ for Valux Plus, $11.33 (\pm 0.03) \text{ nm}$ for Herculite XRV and $13.86 (\pm 0.02) \text{ nm}$ for Z250). The hybrid resin recorded the highest roughness after bleaching, followed in descending order by microhybrid and nanohybrid composite resins. In conclusion, bleaching agents that contains 35% hydrogen peroxide and 17% or 15% carbamide peroxide have erosive effect on composite resins surface.

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The Efficacy of Fluoride Containing Products on Tooth Surface Loss in an Erosion/Abrasion In Vitro Model

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The primary aim was to evaluate the effect of fluoride containing products alone and in combination on tooth surface loss (TSL) of permanent enamel under erosive/abrasive conditions in vitro. A secondary aim was to evaluate the effect of high fluoride dentifrices on TSL. 15 enamel slabs/group were obtained from extracted teeth. The test groups were: Pronamel toothpaste twice/day (PT), Pronamel toothpaste plus Pronamel Mouthrinse twice/day (PT/PM), Pronamel toothpaste twice/day plus Leeds Slow Release Fluoride Glass Device (PT/SRFD), Pro-Expert toothpaste twice/day plus Pro-Expert Mouthrinse twice/day (PET/PEM), and two high fluoride dentifrices, 2800 ppm twice/day and 5000 ppm twice/day. F Free toothpaste served as a control. A non-static pH cycling

model was used with a 1% citric acid at pH 3.6, with an abrasive challenge using 200 g load from a brushing machine for 2 minutes twice/day. Toothpastes were applied as 3:1 slurry. TSL was measured using a non-contact laser profilometry after 28 days. Data were analysed using one-way analysis of variance (ANOVA) with Tukey multiple comparisons post-test. Results showed that significantly lower TSL was observed in all the test groups compared with control. Although 5000 ppm dentifrice showed significantly lower TSL compared with control (Mean Diff \pm SE) ($-3.97 \pm 1.0 \mu\text{m}$) ($p < 0.003$), it had the least protective effect of all the test groups, being significantly different to PT ($4.25 \pm 1.0 \mu\text{m}$) ($p < 0.001$), PT/SRFD ($3.22 \pm 1.0 \mu\text{m}$) ($p < 0.03$), and 2800 ppm TP ($4.96 \pm 1.0 \mu\text{m}$) ($p < 0.000$). Interestingly, no dose response was evident from 2800 ppm to 5000 ppm, with 2800 ppm dentifrice being significantly more protective for TSL as compared with 5000 ppm ($p < 0.000$). It was concluded that all fluoride containing test products, alone and in combination provided protection against TSL in our model, but a dose response in the high fluoride dentifrices was not evident.

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Prevalence and Severity of Dental Erosion Among a Group of Vietnamese Adults

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Dental erosion is a multifactorial condition involving lifestyle and is the subject of extensive research in developed countries. Vietnamese lifestyle has been changing with the increase of soft drinks and acidic foods available in the market. However this condition still is of no interest to both clinical dental practice and dental public health. Is this really a concerned condition in Vietnam? This is the first study on erosive dental wear to assess the prevalence and the severity of a group of Vietnamese adults. First-year students of the Ho Chi Minh University of Medicine and Pharmacy were invited to participate. Students were interviewed about their dietary habits and knowledge of dental erosion. Three examiner recorded caries and periodontal status and one examiner recorded dental erosion using the Basic Erosive Wear Examination (BEWE). 107 male and 194 female participated in both the interview and dental examination. The student's mean age was 29 (23–43) years. Of the 301 individuals examined, 30.9% showed no signs of erosion and 69.1% presented dental erosion. Regarding severity, mild erosion (BEWE scores 1) was observed in more than a half of students (57.8%) while BEWE score 2 and BEWE score 3 (severe erosion) was 28.5% and 15.7% respectively. Most of erosion observed on anterior teeth was score 1 (83.5% upper and 65.7% lower anteriors). In posterior teeth the first molars were the most often affected. There was no significant difference in both prevalence and severity between gender. The results suggested that erosive dental

wear is a matter of concern for this group of selected university students.

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Erosive Tooth Wear and Its Impact on Quality of Life in Children with Cerebral Palsy

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The aim of this study was to evaluate the presence and associated factors of Erosive Tooth Wear (ETW) in children with Cerebral Palsy (CP), as well as its impact on the oral-health-related quality of life (OHRQoL). Parents of 60 CP children, between 6 and 14 years of age, answered the Brazilian version of the Parental-Caregivers Perception Questionnaire (P-CPQ). The ETW diagnosis was performed by a single trained and calibrated examiner according to the O'Brien's modified index. Data collected also included associated factors such as family income, behavioral factors and type of CP. OHRQoL was measured through P-CPQ domains and total score, and Poisson regression was used to correlate ETW to associated factors and to the scores. **Results:** Dental erosion was present in 48.3% of the CP children. The multivariate adjusted model showed that the presence of ETW was significantly associated with more than 2 days of soft drink intake per week ($p = 0.003$); daily intake of powdered juice ($p = 0.002$) and reported gastro-esophageal reflux ($p = 0.016$). Concerning P-CPQ total scores, the family income higher than one Brazilian minimum wage showed a positive impact on the CP children's OHRQoL ($RR = 0.53$; $p < 0.001$). In conclusion, ETW in CP children is associated to frequent consumption of soft drinks, powdered juices and reported gastro-esophageal reflux; however, ETW had no significant impact on the OHRQoL of these children according to parent's proxy-reports.

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Session 6

Diagnostics 1

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Use of Digitally Optimized Images of ICDAS Caries Codes by Undergraduate Dental Students

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Images of teeth with sound and carious surfaces are often used as a tool for training purposes in the cariology curriculum. This study aimed to evaluate the diagnostic performance of ICDAS applied by undergraduate students when digitally optimized images of extracted teeth are used. **Material and Method:** 60 extracted teeth with ICDAS codes 0–6 were photographed (Image conventional). Then, digitally optimized images were produced from the same investigation sites (Image optimized). Ten third-year dental students without previous experience in using ICDAS (group A) and ten fifth-year students experienced in the use of ICDAS (group B) participated in the study. All images were examined and a random selection of 60% was reassessed after one week. Statistical analysis was performed using MedCalc 12.4.0. Examiner reproducibility was calculated using weighted kappa-values. Diagnostic performance (area under the ROC-curve) was calculated at the D1 and D3 diagnostic threshold using ICDAS consensus scores of two experienced examiners (reference scores). **Results:** Kappa values for intra- and inter-examiner reproducibility were 0.43–0.90 for group A and 0.45–0.87 for group B. No significant difference between the mean kappa values of the two groups was observed (*t*-test, $p > 0.05$). The AUC for Image conventional were: group A: 0.921 (D1) and 0.853 (D3); group B: 0.935 (D1) and 0.859 (D3). The AUC for Image optimized were: group A: 0.915 (D1) and 0.856 (D3); group B: 0.947 (D1) and 0.856 (D3). In group B the AUC was significantly higher at the D1 diagnostic level when optimized digital images were examined (nonparametric test, $p = 0.004$). **Conclusion:** The use of optimized images by students with ICDAS experience led to a significantly better performance in the detection of enamel lesions.

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Differential Diagnosis Between Caries and Fluorosis: Reproducibility Among Examiners on Visual Diagnosis and Histologic Confirmation

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In spite of a high prevalence of caries and fluorosis in Colombia, there are no clear clinical diagnosis or treatment-decision guidelines. The aim of this study was to assess the reproducibility of 4 trained examiners in the differential diagnosis of early-stage caries (ICDAS) and mild/moderate fluorosis (TFI) confirming it with stereomicroscopy (SM) and polarized-light microscopy (PLM). 22, 78 and 40 extracted permanent teeth (out of approximately 300), were classified by an experienced examiner in TFI/ICDAS-0, TFI 1-4 and ICDAS 1-2, respectively, specifying the examined surface. Other 3 examiners then assessed them. One week later all assessments were repeated independently. Inter- and intra-reproducibility kappa values were calculated. SM-surface, 250 μm -SM and 100 μm -PLM longitudinal-section images were obtained of 5-randomly selected teeth from each category and standard examiner assessed them. **Results:** 142 teeth were classified as follows: TFI/ICDAS-0: $n = 22$, ICDAS-1: $n = 19$, ICDAS-2: $n = 21$, TFI-1: $n = 18$, TFI-2: $n = 20$, TFI-3: $n = 26$, TFI-4: $n = 14$. Inter- and intra-reproducibility kappa values for caries (ICDAS) were, respectively: examiner-1: 0.82, 0.88; examiner-2: 0.83, 0.88; examiner-3: 0.81, 0.81. Inter- and intra-reproducibility kappa values for fluorosis (TFI) were, respectively: examiner-1: 0.77, 0.82; examiner-2: 0.65, 0.78; examiner-3: 0.67, 0.70. Standard intra-reproducibility kappa values were 0.93 for caries (ICDAS) and 0.87 for fluorosis (TFI). SM-surfaces' images confirmed fluorosis (hypomineralized appearance as fine/wide horizontal white-opaque lines to all-over-the-surface with/without cusp-tip/incisal-edge/

marginal-ridge 'opaque-snow' areas or whole-surface marked chalky opacity) and caries (demineralization pattern with opaque/shiny white/brown appearance located only in natural plaque-stagnation areas) characteristics. Both SM- and PLM-section images confirmed fluorosis affected larger surface areas, showing a birefringence-band pattern and no lesion body (PLM) as opposite to caries, which also showed a more localized pattern. Both pathologies showed a higher porosity subsurface covered by highly mineralized enamel with a fluorosis-band and a caries prism-direction pattern. Increasing severity showed deeper structure affection in accordance with indices. **Conclusion:** differential diagnosis between caries and fluorosis was feasible with substantial inter- and intra-reproducibility for the examiners.

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Effectiveness of Fluorescence-Based Methods in Monitoring Non-Cavitated Caries Lesions on Smooth Surfaces

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The aim of this in vitro study was to evaluate the effectiveness of fluorescence-based methods (DIAGNOdent – LF, DIAGNOdent pen – LFpen and VistaProof fluorescence camera – FC) in monitoring non-cavitated caries-like lesions on smooth surfaces. Sixty bovine enamel blocks were used and caries-like lesions were developed using a bacterial model that consisted of *S. mutans* and *L. acidophilus*. Enamel blocks were evaluated at baseline (I), after the first cariogenic challenge (8 days) (II), and after the second cariogenic challenge (further 8 days) (III) by two independent examiners using LF, LFpen and FC. Blocks were submitted to surface microhardness (SMH) and cross-sectional microhardness (CSMH). The integrated loss of surface hardness (Δ KHN) was calculated. The intraclass correlation coefficient for intraexaminer and interexaminer reproducibility ranged from 0.51 (FC, phase II) to 0.94 (LF and LFpen, phase III) and from 0.49 (FC, phase I) to 0.93 (LFpen, phase III), respectively. SMH and fluorescence values presented significant differences among all three phases ($p < 0.05$). Fluorescence values for phases I, II and III were: 7.8 ± 2.5 , 12.3 ± 7.0 , 24.8 ± 10.7 for LF; 15.9 ± 4.8 , 30.0 ± 11.3 , 60.2 ± 20.2 for LFpen; 1.0 ± 0.0 , 1.2 ± 0.1 , 1.6 ± 0.3 for FC. Higher sensitivity, specificity and area under the ROC curve values were observed for FC in phase II and for LFpen in phase III. There was a significant correlation between fluorescence values and SMH in all phases and Δ KHN in phase III ($p < 0.05$). In conclusion, the fluorescence-based meth-

ods were effective in monitoring non-cavitated caries lesions on smooth surfaces developed in vitro, which moderate correlation with SMH hardness to differentiate sound and demineralized enamel.

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In Vivo Reproducibility of CarieScan Pro in Detecting Initial Occlusal Caries Lesions

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Objective: To evaluate the reproducibility of the CarieScan Pro device (AC Impedance Spectroscopy technique) used on occlusal surfaces and to correlate the measurements to visual findings.

Methods: The multicenter study was approved by the ethics committees and informed consent was given by the participants. Examinations were carried out by one investigator per university (A, B). Prior to the main study, ICDAS training was performed to determine examiner reproducibility. 308 permanent unrestored teeth of 30 patients were selected (189 premolars, 119 molars). Only teeth with ICDAS codes 0–2 were included in the study. Teeth were cleaned, isolated with cotton rolls and dried for 5 s. First, ICDAS score of each investigation site was obtained as reference standard. Then, CarieScan Pro (CS) reading of each site was recorded. After 6 months examination was repeated. Reproducibility of CS readings of the first and second examination was described by Intra-Class Correlation Coefficient (ICC). Correlation between ICDAS codes and CS measurements were calculated using Spearman's correlation coefficient (r_s , $\alpha = 0.05$). **Results:** Rater's reproducibility (weighed Kappa) for ICDAS ranged from 0.55–0.92. Reproducibility of CS (ICC) varied depending on location [A: 0.61 (95% CI: 0.47–0.72), B: 0.83 (95% CI: 0.76–0.87) and A+B: 0.71 (95% CI: 0.64–0.77)]. A significant positive correlation (r_s) was found between ICDAS-II and CS measurements for both examiners at baseline and after six months: A: 0.25 ($p = 0.0024$) and 0.43 ($p < 0.0001$), B: 0.89 ($p < 0.0001$) and 0.74 ($p < 0.0001$). **Conclusions:** The CS showed in vivo a high reproducibility for detection of initial occlusal caries lesions and significant positive correlation to ICDAS findings. Therefore, the CS could be used as a diagnostic tool to monitor early non-cavitated caries lesions.

The CarieScan Pro devices were provided by CarieScan Ltd., Dundee, UK and orangedental, Biberach, Germany.

Volumetric Assessment of Occlusal Caries Lesions Using Micro-CT: A Methodological Pilot Study

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Micro-CT is a non-destructive 3D imaging system which can be used to visualise and quantitatively assess dental hard tissues and carious lesions. Most studies have analysed individual tomographic cuts. This study aimed to devise a quantitative method for volumetric analysis of occlusal lesions from Micro-CT images and to determine the relationship between ICDAS scores and lesion volume. Twenty extracted teeth were selected with varying appearance from sound to non-cavitated lesions on the occlusal surface. Three trained examiners independently examined one investigation site per tooth representing the worst site if present according to the ICDAS criteria. Teeth were scanned using a desktop μ CT 40 Scanco system with an isotropic resolution of 20 μ m. One examiner measured the total volume of the occlusal lesion in enamel and dentine (when present) at each investigation site using a Photoshop CS6 software programme and a modified script to measure the sum of pixels after slicing the tooth in 0.1 mm thickness slices and setting the threshold to the lowest Linear attenuation coefficient (LAC) of sound enamel and dentine. The mean volume of demineralised enamel in the lesions detected according to ICDAS scores were 0.131 mm³ for code 1 (n = 8), 0.334 mm³ for code 2 (n = 4) and 1.88 mm³ for score 3 (n = 3). The mean total volume of demineralised enamel and dentine in the lesions detected according to ICDAS codes 1, 2 and 3 were 0.131 mm³, 0.525 mm³ and 9.63 mm³ respectively. One way ANOVA showed that there were significant difference in lesion volume between ICDAS scores (P value <0.05). In conclusion, a method of volumetric analysis of carious lesions using micro-CT has been devised which shows a difference in lesion volume between ICDAS scores.

Risk of Initial and Moderate Caries Lesions in Primary Teeth Progressing to Dentine Cavitation

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We investigated the risk of sound surfaces, non-cavitated caries lesions classified according to the International Caries Detection and Assessment System (ICDAS scores 1 and 2) and moderate lesions (ICDAS scores 3 and 4) to progress to dentine cavitation (ICDAS scores 5 and 6). A cohort study was designed using 639 children (12 to 59 months-old), who were examined by visual inspection using ICDAS. After 2 years, 469 children were re-examined regarding presence of dentine cavitations. The probability of progression was calculated for surfaces classified at baseline as scores 0 to 4. We calculated the relative risk (RR) of progression and 95% confidence intervals (95%CI) for each condition compared with sound surfaces using multilevel Poisson regression analysis. We also investigated explanatory variables associated with progression. We found that the higher the initial score attributed to the dental surface, the more likely was the progression. Probability of progression for sound surfaces was 1.9%, for sites scored as 1 and 2 were 4.8% and 10.7%, respectively, 45.0% for score 3 and 47.2% for score 4 lesions. We also observed that children with severe lesions at baseline had higher risk of having a sound surface cavitated after the follow-up compared with caries-free children (children with 1 to 4 cavitations: RR = 3.63; 95%CI = 1.56 to 8.44; more than 4 cavitations: RR = 7.03; 95%CI = 2.81 to 17.55). Considering non-cavitated lesions, children with 1 to 4 cavitations (RR = 4.26; 95%CI = 1.88 to 9.65) or with more than 4 cavitations (RR = 6.30; 95%CI = 2.76 to 14.37) also presented higher risk of progression. In conclusion, higher scored lesions have higher risk of progressing to cavitation than lower scored lesions. Moreover, presence of cavitated lesions is a significant predictor of progression of sound surfaces and non-cavitated caries lesions.

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In Vitro Study of the Canary System for Proximal Caries Detection

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This in vitro study investigated the diagnostic efficacy of the Canary System (CS) to detect proximal caries, and compare it to conventional methods, International Caries Detection and Assessment System (ICDAS) II and bitewing radiography (BW). Extracted human molars, premolars, canines and incisors with or without proximal caries were assessed by blinded examiners. Visible proximal surfaces were assessed by ICDAS-II before setting the teeth in five manikin mouth models. The contacting proximal surfaces in mouth models were assessed by BW and CS (50 teeth excluding third molars and incisors). Histological validation with polarizing-light microscopy (PLM) served as a gold standard. Pairwise comparisons were performed on area under the curve (AUC), sensitivity and specificity of the three detection methods, and were corrected for multiple comparisons using Bonferroni's method. Sensitivities and specificities were compared using a test of proportions and AUC values were compared using DeLong's method of nonparametric testing of AUC values. CS presented significantly higher sensitivity (0.933) than ICDAS II (0.733, $p = 0.01$) and BW (0.267, $p < 0.001$), and ICDAS II significantly higher sensitivity than BW ($p < 0.001$). There were no significant differences between their specificity values: 0.825 (CS), 0.65 (ICDAS II), and 0.875 (BW). In the sample with prevailing early lesions, the highest negative and positive predictive values were calculated for CS, 89.2% and 88.9%, respectively. AUC of CS (0.862) was significantly higher than of ICDAS II (0.681, $p < 0.001$) and BW (0.577, $p < 0.001$). This in vitro study show that the diagnostic efficacy of CS in detecting proximal caries lesions is greater than that of ICDAS II and BW. The Canary System can be a valuable method for proximal caries lesions diagnosis.

Radio-Opaque Tagging of Deep Caries Lesions after Incomplete Excavation

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One-step incomplete excavation seals caries-affected dentin under a restoration and seems advantageous when treating deep lesions. However, it is impossible to radiographically discriminate intentionally left arrested lesions from overseen or active lesions. The associated diagnostic uncertainty decreases the acceptance of minimal-invasive excavation and might lead to unnecessary re-treatment of incompletely excavated teeth. Radiopaque tagging of sealed lesions might allow to mask intentionally sealed and arrested lesions and to discriminate them from progressing lesions. Therefore, we screened aqueous and ethanolic solutions of SnCl₂, AgNO₃, CsF and CsCH₃COO for their micro-radiographic effect on artificial lesions ($n = 15/\text{group}$). Since aqueous stannous chloride (80% SnCl₂×Aq) most reliably masked artificial lesions and was most resistant to leaching, we further controlled its radiographic effects on progressing lesions ($n = 15/\text{group}$). Furthermore, 20 natural deep caries lesions were incompletely excavated and radiopaque tagging with SnCl₂×Aq was performed. After excavation and after tagging dental radiographs were taken, each with radio-opaque composite being placed in the cavity. Grey-value differences (ΔGV) between sound and carious dentin were determined and radiographs evaluated by 20 dentists for the presence of a carious lesion (radiolucency) beneath the restoration. Whilst radiolucency after tagging with SnCl₂×Aq was significantly decreased and resistant to leaching for non-progressing artificial lesions, it significantly increased during a second demineralization ($p < 0.001$, t -test). For natural lesions, tagging with SnCl₂×Aq significantly reduced ΔGV ($p < 0.001$, Wilcoxon). Tagged lesions were identified significantly less often (18%) by dentists than untagged lesions (66%) ($p < 0.001$). In conclusion SnCl₂×Aq was most suitable to mask caries-affected dentin beneath restorations.

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Detection of Secondary Caries Around Resin Composite Restorations in Primary Teeth

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Secondary caries has been reported as the main reason for restoration replacement. This *in vitro* study compared the performance of different conventional and light-induced fluorescence-based methods in detecting secondary caries in primary molars restored with resin composite. Two examiners evaluated independently forty-two sites adjacent to tooth-colored restorations using all methods: visual inspection (ICDAS-CARS), radiographic examination and quantitative light-induced fluorescence (QLF). As reference standard method, the lesion depth was determined after sectioning and evaluation in stereomicroscope (x40). The area under the ROC curve (Az), sensitivity, specificity, and accuracy of the methods were calculated at enamel (D1) and dentin caries (D3) lesions thresholds. The intra and interexaminer reproducibility were calculated using the intra-class correlation coefficient (ICC) and kappa statistics. There was no difference among the methods considering the area under ROC curve for enamel threshold. For dentin caries lesions, although the methods presented similar performance in detecting the dentin caries lesions, there was significant difference between the radiograph with QLF (scores) and QLF (ΔF %) with QLF (scores). At D1 threshold the visual inspection, radiograph and QLF (scores) methods presented similar sensitivities and significantly higher than the obtained with the QLF (ΔF %). The accuracies tended to be higher with the visual inspection with difference to QLF (ΔF %). At D3 thresholds, in general, there were no significant differences among the methods performances. In general, all methods presented high reproducibility. In conclusion, visual inspection alone seems to be enough to be used in clinical practice for detecting secondary caries lesions around tooth-colored restorations in primary teeth.

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Sealant Retention Is Best Assessed Through Colour Photographs

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Aim: To test the hypothesis that the colour photograph method has a higher level of validity for assessing sealant retention than the visual clinical examination and replica methods. **Method:** Sealed molars were assessed by two trained and calibrated evaluators. Scores obtained of the three assessment methods were compared against consensus scores derived through assessing retention from SEM-images (gold standard). Presence/absence (survival) of retained sealants on occlusal surfaces was determined according to the traditional and modified categorization of retention. Sensitivity, specificity and Youden-index scores were calculated. **Results:** Comparison of sealant retention assessment scores for visual clinical examinations and colour photographs with those of the gold standard was performed on 95, and for replicas on 33 surfaces. The highest mean Youden-index score for presence/absence of sealant material was observed for the colour photograph method, followed by that of the replica method whilst the visual clinical examination method scored lowest. The mean Youden-index score for the survival of retained sealants was highest for the colour photograph method for both the traditional (0.882) and modified (0.768) category of sealant retention, with the visual clinical examination method had the lowest Youden-index score (0.745–0.063). **Conclusion:** The colour photograph method had a higher validity than the replica and the visual examination methods for assessing sealant retention.

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Impact of Bitewing Radiographs on Restorative Decision of Caries Lesions in Primary Teeth

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Objective: To investigate the impact of bitewing radiographs in deciding treatment need for caries lesions in primary teeth by dental students. **Materials and Methods:** Students of the last three semesters of the Faculty of Dentistry, UFRGS, were invited to par-

ticipate in this study. The participation rate was 61.2%, comprising a sample of 79 students. A questionnaire of seven clinical cases was developed using photographs of primary teeth with caries lesions at different stages. Each case was presented on screen to students with the description of the lesion (ICDAS code and activity status), initially, only illustrated by the clinical photograph (visual examination). After completion of all questions, the cases were presented again, now showing clinical and bitewing photographs side by side. For each question the participants were asked about their treatment decision, whether invasive or non-invasive. The association between the treatment options considering the visual examination or visual and radiographic examination was assessed by the McNemar test. **Results:** Among four ICDAS – code 3 lesions, most students decided for invasive treatment (55.7%, 74.7%, 81% and 94.9%). In two of these lesions, the association of radiographic examination increased the percentage of students who decided for an invasive treatment, compared to visual examination ($p < 0.05$). Considering the two ICDAS – code 4 lesions, most students decided for the invasive treatment when bitewing radiographs were associated (82.3% and 68.4% – $p < 0.05$). In ICDAS – code 1 lesions, most students decided for a non-invasive treatment. The percentage of students who decided for an invasive treatment on visual examination was only 2.5%. This value increased to 17.7% when an additional radiographic exam was involved ($p < 0.05$). **Conclusion:** Most of participants decided for an invasive treatment, with a higher percentage when bitewing radiograph were associated. **Summary:** Students decided for invasive treatment in 57% of the questions through visual examination and in 85.7% associating radiographic examination, which influenced the treatment decision.

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Assessment of Compatibility of Toothpastes with the Calcivis Activity Imaging System

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The present study seeks to assess the compatibility of example toothpastes with the Calcivis Caries Activity Imaging Device with European Marketing Authorisation (CE Mark) in relation to potential interference by the presence of plaque, pellicle or calcium ions within tooth-cleaning agents. The device consists of a sensitive camera capable of taking images in visible light and very low level transient luminescence produced when a photoprotein (see: <http://www.google.com/patents/EP2682132A1?cl=en>) is applied to the tooth surface. The device was used to image freshly extracted teeth (4 teeth per tooth-cleaning agent), and assessed for a) cleanliness and b) 'hot spots' of elevated calcium ions (visualised as luminescence on the tooth surface), after cleaning the teeth for 60 seconds with tap water only, pumice or 4 toothpastes (Colgate, Sensodyne, Oral-B, Aquafresh), followed by rinsing with water. Water-only cleaned surfaces show contrast/brightness in the luminescence images whereas pumice-cleaned surfaces show brighter

images with greater contrast than the water-only images. Colgate and Sensodyne increased brightness and contrast, whereas Oral-B and Aquafresh show slightly increased brightness with no difference to contrast. In conclusion increased luminescence in the images post-cleaning (removal of plaque) indicates that the disclosing solution is detecting calcium ions within the enamel. Images with increased contrast and brightness are obtained when the tooth surface has been cleaned of surface integuments (pellicle/plaque). Some toothpastes may interfere with the Imaging system, whereas Pumice appears to be most compatible with the Calcivis System.

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Use of Scoring Systems Improves Accuracy of Visual Inspection in Detecting Caries Lesions

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Visual inspection is widely used for detecting caries lesions, but the effect of using scoring systems on accuracy is unclear. Therefore, we performed a systematic review with meta-regression to evaluate if the use of well described visual scoring systems would improve accuracy of visual inspection. Two independent reviewers searched PubMed, Embase and Scopus through 07/2013 to identify papers published in English. Non-published literature was also checked. Eligibility criteria were: (1) sufficient data about sample size and accuracy of visual method for detecting caries lesions on occlusal, approximal or smooth surfaces, in primary or permanent teeth; and (2) have a reference standard. We recorded data about method's accuracy and if it was performed using a well described scoring system or not. Meta-regression analyses were performed in order to compare the effect of the use or not of scoring systems, and relative diagnostic odds ratio (RDOR) and 95% confidence intervals (95%CI) were calculated. From 5,578 articles initially identified, 96 were included. Studies that used ICDAS presented better performance in detecting initial (4.22; 1.94 to 9.15) and advanced (4.74; 1.37 to 16.39) occlusal caries lesions of primary teeth and advanced caries lesions in approximal (18.60; 1.64 to 211.45) and occlusal (3.94; 1.67 to 9.30) surfaces of permanent teeth than studies that did not report any scoring system or used their own criteria. Other systems also presented superiority, namely ERK (2.17; 1.04 to 4.54) used in occlusal advanced caries lesions of permanent teeth, and ERK (2.60; 1.11 to 6.09) and Nyvad (7.82; 2.50 to 24.43) in occlusal initial caries lesions of primary teeth. In con-

clusion, studies employing widely recognised visual scoring systems present better performance compared with studies that used their own criteria.

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Correlation between Optical Coherence Tomography and Polarized Light Microscopy for Detection of Early Fissure Caries

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Aim: Ability of Swept Source Optical Coherence Tomography (SS-OCT) to detect and measure non cavitated Early Fissure Caries (EFC). **Methodology:** Thirty extracted permanent human premolars with ICDAS code 0, 1 and 2 were selected. Regions of interest (ROI) at fissures were marked using rotary instrument. The ROI were imaged using Thorlabs SS-OCT, (OCS-1300, Thorlab, Inc) and Polarized Light Microscopy (PLM) using NikonE90i microscope. The teeth were sectioned buccolingually to thickness of <200 µm and imaged under 4X magnification after imbibition in water. OCT B-Scan that corresponded with PLM section were selected from 104, 3-D OCT frames from X-Z plane. Small DR-2 was used for identification of EFC at base, walls and slopes of fissure or pit. The criteria used for OCT were presence of: i) demarcated, localized and subsurface areas of increased back scattered intensity (IBI) at base and walls of fissure ii) symmetrical bilateral IBI on fissure slopes. The lesions on PLM were identified as areas of birefringence loss (dark areas) and measured for width (WB) and height (HB) of base of lesion, width of lesion at the slopes (WS) and total area (A) for OCT and PLM images using NIS Element AR v4.2 software. **Results:** The Cohen Kappa test showed moderate agreement of identification of lesion between PLM and OCT with $k = 0.514$. WB and WS of OCT and PLM showed significant correlation ($P < 0.05$) while HB and A of OCT and PLM did not. The ICC with 95% CI for WB and WS were 0.655 (0.391, 0.819) and 0.301 (-0.061, 0.593) respectively. **Conclusion:** OCT was able to detect the ECF when compared with PLM. The OCT measurements of WB and WS correlated well with the corresponding PLM measurements.

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Which Visual or Tactile Features of Caries Lesions Can Better Predict Their Progression?

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Some clinical features of caries lesions have been used in the assessment of their activity status. However, few studies have concerned with the predictive power of these parameters in a long-term analysis. This study aimed to assess the predictive validity of evaluating some clinical features used in the caries activity assessment. For this, 208 children had their occlusal surfaces of primary molars examined in the baseline according to the Nyvad's system. A reference examiner also evaluated the sites by visuo-tactile inspection according to clinical features related to the caries lesions activity: potential for plaque stagnation, staining, opacity, cavitation, texture and estimated depth. One year after the first examination, 151 children (845 surfaces) were re-evaluated in order to assess caries lesions progression (cavitation into dentin, surface restoration or tooth extraction). This outcome was set to verify the predictive validity of the clinical parameters initially assessed. Multilevel Poisson regression analyses were performed to investigate the association between lesions progression and these clinical parameters. The relative risk (RR) for each condition tested was calculated considering a confidence interval of 95% (95% CI). From surfaces reassessed, 354 were sound, 373 had enamel caries and 118 had dentine caries in the baseline. Forty percent of this sample progressed after 1 year. Dentine caries lesions were approximately 10 times more likely to progress than sound surfaces (RR [95% CI] (9.65 [5.01–18.57])). Similarly, cavitated lesions showed highest risk to progress than non-cavitated lesions (5.80 [3.75–8.96]). Other parameters as roughness and opacity did not predict progression of cavitated lesions. When we considered only the non-cavitated surfaces, roughness proved to be a risk factor for progression (1.16[1.13–4.53]). In conclusion, dentine or cavitated lesions had a higher risk of progression compared to sound or non-cavitated surfaces. For non-cavitated lesions, roughness seems to be a parameter that may help to distinguish lesions that tend to have higher risk of progressing.

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In Vitro Validation of Optical Coherence Tomography for the Detection of Early Proximal Enamel Caries

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The aim of this in vitro study was to assess the potential of Optical Coherence Tomography (OCT) in the detection of artificially induced proximal caries lesion (PCL). The objectives were to determine the sensitivity, specificity and detection threshold of OCT.

Materials and Methods: Approximal surfaces of forty sound premolars were prepared and exposed to pH cycling daily for 0, 7, 14 and 21 days to produce artificial caries like lesions. High resolution Swept Source OCT B-scans in the bucco-lingual direction (parallel) and direct view (perpendicular) were done. The direct view was used to verify the increase in backscattered intensity of the artificial PCL. The parallel view images were assessed and given a dichotomous score by two trained and blinded examiners. The teeth were later sectioned and polished for cross sectional microhardness measurement. The Knoop hardness numbers measured were converted to volume % mineral and the mean value for each group at each measuring depth was calculated. **Result:** Cohen's kappa showed good intra- and inter-examiner agreement with kappa value of 0.93 and 0.60 respectively. The sensitivity and specificity of the OCT parallel view of 7, 14 and 21-day lesions are 0.6 and 1.0, 0.8 and 1.0, 0.8 and 1.0 respectively. Cross sectional microhardness measurement of the lesions showed that the mean volume % mineral of the 7, 14 and 21-day lesions at 100 µm subsurface is 77.3, 66.3 and 50.9 respectively. **Conclusion:** OCT is able to detect proximal caries lesion when imaged from a bucco-lingual direction in vitro. The detection threshold is a 14-day artificially induced lesion, which corresponded to a mean volume % mineral of 66.3 at 100 µm subsurface.

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Restorative Treatment Thresholds Among Chilean Dental Students: Differences throughout Undergraduate Education

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This study aimed to assess diagnostic criteria and restorative treatment strategies among Chilean students among different undergraduate courses during dental education. **Methods:** The Spanish version of a precoded questionnaire (Espelid, 2001) was distributed to 232 students at the Faculty of Dentistry in University of Chile, Santiago, Chile. Treatment thresholds for hypothetical approximal and occlusal caries, as well as most favored types of materials and restorative techniques were assessed. The questionnaire was applied to dental students from the 3rd, 4th, 5th and 6th year courses. **Results:** 33.3% of the students from 3rd, 24.7% from 4th, 20.0% from 5th and 43.8% from 6th year courses stated that they would automatically restore a primary approximal caries lesion confined to the enamel or that had reached the enamel dentin border. Moreover, 73.68% of the 3rd grade students, 62.19% from 4th, 75.0% from 5th and 80.8% from 6th would only consider immediate restorative treatment of an occlusal surface if obvious cavitation and/or radiographic signs of dentin caries could be observed. When a questionable occlusal surface was presented, 45.5% of the students diagnosed presence of enamel and/or dentin caries. The 76.2% of the students agreed that it was more important not to fill sound teeth unnecessarily, accepting the risk of not restoring some carious lesions. **Conclusions:** The results present disparities among the dental students' responses concerning diagnosis and treatment thresholds for both approximal and occlusal caries. Students tend to more invasive treatments as they become more habituated to clinical procedures at more advanced courses throughout dental education. Findings of this study might be a guideline for Chilean university teachers to find more consistent alternatives to teaching the subject of current caries management.

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Session 7

Diagnosics 2

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Intra- and Inter-Examiner Reliability of Radiographical Scorings of Caries within a Multi-National Network

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The radiographical definition of deep caries is often unclear in clinical trials. The aim of this study was to examine intra- and inter-examiner reliability scores of 3 stages of caries, within a clinical multi-national network. The network aims to investigate the beneficial and harmful effects of 1-step partial excavation of primary caries removal versus 2-step partial caries removal of deep caries (pulpal $\frac{1}{4}$ of the dentine) in permanent teeth. The network comprises currently centers located in India, Russia, UK, Sweden, Norway and Den-

mark. 3 stages of caries were used for the reliability test. Score 1: The lesion depth is $<\frac{1}{4}$ of the entire dentine thickness. Score 2: The lesion depth is $\geq \frac{1}{4}$ of the dentine with a radiodense zone separating the lesion from the pulp. Score 3: The lesion depth (translucent zone) is reaching the pulp. 50 x-rays of caries lesions *in-vivo* were selected, and scored twice. A 'gold standard' (GS) was defined using the most frequent scoring for each of the 50 caries lesions (based on the second scorings). The GS was then compared with the second scorings from all participating dentists (n = 50). Kappa-values were calculated using a standard statistic package. The intra-examiner kappa values (mean; sd.) from the six countries were in between 0.75 \pm 0.06 and 0.85 \pm 0.11. The overall mean was 0.78 \pm 0.12. The inter-examiner values from each of the countries were in between 0.63 \pm 0.14 and 0.84 \pm 0.02. In conclusion, the network has demonstrated a moderate to substantial agreement for the radiographical scoring of caries, involving lesions stages relevant for the enrolment of patients, in future clinical superiority trials of deep caries.

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Is an Additional Headlamp Beneficial for Visual Caries Detection Using ICDAS?

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Aim: To test the hypothesis that additional illumination by a headlamp enhances diagnostic outcome of ICDAS in vitro. **Methods:** Two trained examiners assessed occlusal surfaces of 139 extracted molars using ICDAS. The teeth were inspected using a Standard Operation Light (SOL) of a dental unit without or with an additional headlamp (HL) (Power-X Light, American Dental Systems, Vaterstetten, Germany) with 3 different white light intensity steps: weak (W), medium (M), strong (S). The teeth were re-examined 1 week after the first assessment. Sensitivities, specificities and areas under the ROC-curve were calculated. Weighted kappa statistics were used to obtain intra-examiner and inter-examiner reliabilities. Histology with rhodamine B staining served as

gold standard. **Results:** The distribution of caries prevalences was D0:30; D1:25; D2:34; D3:29; D4:21. On the D1 threshold, additional light intensities lead to a decrease of cumulative sensitivities from 0.84 (SOL) to 0.76 (SOL+HLS), while the cumulative AUCs remained largely unchanged. On the D3 threshold the same tendencies could be observed with sensitivities decreasing from 0.73 (SOL) to 0.59 (SOL+HLS). In separate examiner analysis, this observation was significant on the D1 threshold with sensitivities decreasing from 0.9 to 0.76 (HLM) and 0.75 (HLS), respectively. The inter-examiner wK values increased with increasing light intensities from 0.64 (SOL) to 0.73 (all headlamp intensities). Intra-examiner reliabilities were >0.8 when a headlamp was used, and with SOL they were 0.88 (examiner 1) and 0.71 (examiner 2). **Conclusions:** Too powerful additional light from a headlamp might impair visual caries detection. The authors suggest that too brightly illuminated teeth become too shiny to identify surface changes.

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Effect of X-Ray Beam Angulation on Subtraction Radiography Accuracy for Detecting Occlusal Demineralization In-Vitro

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This in-vitro study investigated examiners' accuracy in detecting occlusal demineralization using subtraction images produced from bitewing radiographs taken with variations in x-ray source to subject projection geometry. Forty extracted molar teeth with occlusal cavities were placed in anatomical arches. Baseline radiographs were taken with x-ray beam passing tangentially through contact points at 90° to the x-ray film (0° angulation). Baseline radiographs were also taken altering the horizontal (7° and 15°) and vertical (10° and 15°) beam angulation. Nineteen randomly selected teeth underwent occlusal cavity acid demineralization with 0° angulation radiographs taken at 12 and 24 hours of demineralization. These were digitally subtracted from the baseline radiographs at each angulation, producing 400 subtraction images. Twelve examiners independently viewed the subtraction images in random order, scoring demineralization on a five point certitude scale. One-way ANOVA post-hoc analyses with Bonferroni correction found no difference ($P = 1.0$) in the mean areas under the receiver operator characteristic (ROC) curves (MnAUC) when x-ray angulation was moved from 0° to 7° horizontal angulation at 12 hours demineralization (MnAUC = 0.86 and 0.83 respectively); or 24 hours demineralization (MnAUC = 0.95 and 0.90 respectively). However, when horizontal angulation was increased from 0° to 15°, the MnAUC decreased to 0.64 at 12 and 0.75 at 24 hours demineralization ($P < 0.001$). All changes in vertical angulation (10° and 15°) also resulted in statistically significant decreases in MnAUCs at both 12 and 24 hours of demineralization ($P < 0.001$). Examiners accurately scored occlusal cavity demineralization, using digital subtraction radiography, when the x-ray source to

subject projection geometry did not change more than 7° horizontally, compared to 0° angulation. Any change in vertical beam angulation, and horizontal changes over 7° led to inaccuracy.

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Evaluation of Reliability and Validity of QLF-D Planimetric Analysis

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The aim of this study was to evaluate the reliability and validity of the QLF-D plaque scoring system based on a planimetric analysis. The subject of this study included 50 persons aged between 20 and 60 years old. All subjects received information regarding the procedures and then agreed to refrain from oral hygiene behavior and food intake of minimum 4 hours before visiting. The buccal surfaces of 600 sound anterior teeth were examined. The subjects received two types of dental plaque examination using the Turesky modification of Quigley Hein plaque index (QH index) and the Löe & Silness plaque index (L&S index). The QLF-D photographs were taken on two separate occasions (before and after plaque examination) and then the Plaque Percent Index (PPI) was calculated based on the planimetric analysis. Correlation between two conventional plaque indices and the PPI of the QLF-D plaque scoring system was evaluated to find a ΔR value having the highest correlation with two existing indices. Randomly selected 60 teeth (10%) were used in intra-examiner and inter-examiner reliability test, and the area under the ROC curve analysis. As main results, the $PPI_{\Delta R 20}$ of this method had a moderate correlation with two existing indices (ρ of QH index = 0.479, L&S index = 0.506). This methodology showed an excellent reliability (intra-examiner ICC = 0.995, Inter-examiner ICC = 0.980) and it fell in the fair category based on the validity test (AUC of QH index = 0.779, L&S index = 0.747). In conclusion, the QLF-D plaque scoring system showed an excellent reliability and fair validity compared with two conventional plaque indices. Further study is needed to demonstrate characteristics of this methodology based on detecting red fluorescent plaque.

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Monitoring the Maturation Process of a Cariogenic Microcosm Biofilm Using the Quantitative Light-Induced Fluorescence-Digital (QLF-D)

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The aim of this study was to investigate whether QLF-D could monitor the degree of maturation of dental microcosm biofilms by observing the red fluorescence emitted from biofilms. Dental mi-

crocsm biofilms were grown on bovine enamel discs. They were initiated from human saliva for 4 h, and then grown in 0.5% sucrose growth media which were replaced daily for 10 days. On days 1, 2, 3, 7, and 10 after the inoculation, fluorescence images of the biofilms were captured using the QLF-D and the red fluorescence intensity was quantified by calculating the red/green ratio (R/G value). Total and aciduric bacteria within the biofilms were counted, and the percentage of surface hardness change (Δ VHN) and lesion depth in the enamel were evaluated. Data were analyzed by ANOVA, Bonferroni post hoc test, and Pearson's correlation. This experiment was repeated 3 times. The results showed that the R/G values of the biofilms increased significantly over time up to 7 days after inoculation ($p < 0.0001$). The mean amount of total and aciduric bacteria (\log_{10} CFU/ml) were increased from day 1 (7.31 ± 0.05 and 6.45 ± 0.15) to day 10 (8.55 ± 0.40 and 8.38 ± 0.51). The R/G values showed significant correlations with the total bacteria ($r = 0.74$, $p = 0.001$), aciduric bacteria ($r = 0.85$, $p = 0.001$), Δ VHN ($r = 0.65$, $p = 0.001$), and lesion depth ($r = 0.82$, $p = 0.001$). In conclusion, the red fluorescence detected by the QLF-D increased according to biofilm maturation and was significantly associated with the cariogenicity of the biofilm. Therefore, QLF-D could be used to monitor the biofilm maturation by observing the biofilm fluorescence in real time.

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Evaluation of Resin Infiltrant Using Optical Coherence Tomography and Quantitative Light-Induced Fluorescence-Digital

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The aims of this study was to investigate the correlation between the results of Optical Coherence Tomography (OCT, Spectral Domain setup OCT prototype, LG Electronics, Korea), Quantitative Light induced Fluorescence-Digital BiluminatorTM (QLF-D, Inspektor Research Systems BV, The Netherlands), and Confocal Laser Scanning Microscope (CLSM, LSM700, Carl Zeiss, Gottingen, Germany) for evaluation of Resin Infiltrant (RI, Icon[®], DMG, Germany) infiltrated into lesion. Total 30 demineralized specimens (mean lesion depth = 324.04 ± 30.26 μ m) were formed from polished bovine permanent sound anterior teeth with demineralizing solution containing 1% carbopol (pH 4.8). RI was applied by following the instructions of manufacturer. The depth of RI infiltrated into lesion was analyzed after standardization for grey scale of the images obtained by OCT. The mean fluorescence gain (Δ Δ F) for lesion infiltrated with RI was analyzed by comparing with sound tooth part using proprietary software (QA2 v1.15, Inspektor Research System BV, Netherlands). The depth of RI infiltrated into lesion was analyzed by CLSM as the gold standard. The correlations between the result values obtained from each analysis

method were analyzed by Pearson correlation. The high correlation was confirmed between the result values of CLSM and OCT ($r = 0.712$, $p < 0.001$). There were no significant correlations between the variables CLSM and QLF-D ($r = 0.176$, $p = 0.351$), and OCT and QLF-D ($r = 0.212$, $p = 0.261$), respectively. The OCT might be able to analyze the RI due to the principle of distinguishment between the materials result from the difference in compositions. QLF-D might not be able to assess the RI due to the damage on the tooth surface result from the process of etching. In conclusion, OCT can be used as alternative method of CLSM for RI treatment evaluation.

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Mesoscopic Assessment of Caries Infiltration of Non-Cavitated Carious Lesions by Optical Coherence Tomography

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Objective: Visualisation of etching process and of resin penetration at white spot lesions by spectral domain optical coherence tomography (SD-OCT). **Methods:** The non-cavitated proximal carious lesions of two visually preselected extracted human molars (ICDAS 2) were verified as E1 and E2 lesion by X-ray microtomography (100 kV, Sky-Scan 1172-100-50; Bruker MicroCT, Kontich, Belgium). One region of interest (ROI) per tooth was marked by two drill-holes in occlusal-cervical direction. The lesions were imaged by SD-OCT (2D, 3D image stacks; center wavelength 1,325 nm; Telesto SP5, Thorlabs GmbH, Dachau, Germany). Lesions were infiltrated with Icon (DMG) according to the manufacturer's instructions. During each treatment step and after light curing of the infiltrant, the ROIs were again imaged by SD-OCT (CamStudio 2.7.2, Apowersoft Free Screen Recorder 1.2.4). The teeth were sectioned through the ROIs and section layers were then imaged by scanning electron microscopy (Phenom G2 pro, Phenom World, Eindhoven, Netherlands) and again by X-ray microtomography, by OCT and light microscopy (1x) in order to validate the OCT signals and to assess the extent of lesion and resin. The image sequences for etching and infiltration were viewed in time lapse. **Results:** During the etching process, numerous mobile bubbles form on the lesion surface. Thus the etching proceeds inhomogeneously. The OCT signal can be assigned to the monomer, and the dynamic of the resin infiltration can be imaged by OCT. The E1 lesion was completely infiltrated by the resin whereas infiltration of the deeper E2 lesion was incomplete and inhomogeneous. **Conclusion:** Caries infiltration can be further increased, for example by optimizing the etching process.

This study was supported by Thorlabs GmbH (provision of OCT).

Enhancement of Detectability of Non-Cavitated Occlusal Caries Using Optical Coherence Tomography by Variation of Beam Angle and Glycerin Application

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Objective: Improvement of performance of spectral domain optical coherence tomography (SD-OCT) for detection and assessment of non-cavitated occlusal carious lesions. **Methods:** Nine extracted human molars with 18 occlusal lesions (ICDAS code 2) were visually selected. 18 regions of interest (ROI) were marked by two drill-holes each in mesio-distal direction and the lesions were imaged with SD-OCT (2D, 3D image stacks, center wavelength 1325 nm; Telesto SP5, Thorlabs GmbH, Dachau, Germany) under different beam angles (0° , $\pm 5^\circ$, $\pm 10^\circ$, $\pm 15^\circ$) and with/without surficial application of glycerin (at 0°). X-ray micro computed tomography images (μ CT; Skyscan1172-100-50, Bruker MicroCT, Kontich, Belgium) were used as a validation standard. The μ CT and OCT signals were categorized according to the lesion extent (score 1–4): 1-sound, 2-lesion limited to half of enamel, 3-lesion limited to enamel, 4-lesion into dentin. **Statistics:** Cohen's kappa coefficient (K), Friedman-/Wilcoxon-test ($\alpha = 0.05$). **Results:** For a beam angle of 0° agreement between methods was nonexistent (μ CT vs. OCT 0° ; $K = 0$, $p = 0.001$) or slight (μ CT vs. OCT 0° , glycerin; $K = 0.1$, $p = 0.004$). With variation of beam angle almost a perfect agreement was observed (μ CT vs. OCT various angles; $K = 0.94$, $p = 0.5$). OCT images without glycerin showed moderate agreement with results obtained with glycerin (OCT no-glycerin vs. OCT glycerin; $K = 0.57$, $p = 0.75$). The application of glycerin increased contrast of OCT images and provided additional morphological information about caries lesions. It was observed that glycerin reduced the penetration depth of OCT signal. **Conclusion:** Variation of OCT imaging beam angle improves image quality and detectability of non-cavitated occlusal carious lesions. Improvement of caries visualization by application of glycerin is little. Supported by Thorlabs GmbH (provision of OCT).

Approximal/Occlusal Caries Detection by General Dental Practitioners Using Radiograph, Diagnocam and QLF

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The aim was to explore how general dental practitioners perform caries detection with methods hitherto unknown to them in an in vitro set-up. Detection of caries is generally performed using

visual/tactile and radiographic techniques. Novel optical methods slowly integrate in daily practice. In the framework of a course on caries detection, practitioners scored caries in vitro. They used images of digital radiographs, Diagnocam and QLF. Sensitivity and specificity were calculated and compared. **Methods:** 16 extracted teeth with sound and carious surfaces were collected and assembled in phantom models. Images were taken using Diagnocam (DC, KaVo, Bieberach, Germany), QLF (Inspektor Research, Amsterdam, the Netherlands) and two solid-state digital radiographic sensors. The obtained images were integrated in an on-screen presentation and presented to the 6 participating practitioners. These scored the presence/absence of caries (sound, enamel caries, dentine caries). Reference standard was established with micro-CT (resolution 35 μ m), yielding 8 occlusal dentine lesions, 3 approximal enamel and 13 dentine lesions. Sensitivity and specificity were calculated. **Results:** Using a cutoff 'enamel caries', a sensitivity (SE) and specificity (SP) was established for DC: SE:0.39, SP: 0.70, QLF: SE: 0.48, SP: 0.61, X-ray A: SE 0.34, SP: 0.92, X-ray B: SE 0.45, SP: 0.91. Using a cutoff 'dentine caries', sensitivity (SE) and specificity (SP) was obtained as follows: DC: SE: 0.29, SP: 0.86; QLF: SE: 0.29, SP: 0.80; X-ray A: SE: 0.21, SP: 0.95; X-ray B: SE: 0.25, SP: 0.92. The sensitivity (cutoff enamel caries) was higher in occlusal surfaces using the optical methods compared to the radiographs (DC: 0.78, QLF: 0.75, X-ray A: 0.35, X-ray B: 0.46). In approximal surfaces, radiographs performed somewhat better (SE DC: 0.19, QLF: 0.28, X-rayA: 0.32, X-ray B: 0.41). **Conclusion:** New and established methods did not yield different global sensitivities, specificity of radiographs was higher in approximal surfaces whereas the optical methods performed better in occlusal surfaces. Due to bleaching of the red fluorescence, QLF may have performed better in approximal surfaces. This should be taken into account for further studies.

Diagnocam and QLF were kindly loaned by their manufacturers.

Assessment of Marginal Discolorations of Composite Restorations by Optical Coherence Tomography

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Assessment and characterization of marginally discolored composite restorations by spectral domain optical coherence tomography (SD-OCT). **Methods:** 15 extracted human incisors with composite restorations showing at least one region with a marginal discoloration were visually selected from a pool of extracted teeth stored in chloramine solution. Three regions of interest (ROI) were marked by two drill holes each in coronal-apical direction. The ROIs were imaged with SD-OCT (2D; Telesto SP5, center wavelength 1325 nm, Thorlabs GmbH, Dachau, Germany) under seven observation angles perpendicular to the B-scan plane (0° , $\pm 10^\circ$, $\pm 20^\circ$, $\pm 30^\circ$). At each ROI the discoloration intensity was scored visually (0–3) and according to the following characteristics: extent of interfacial adhesive defect (0–5), marginal gap (0–3), overhang (0 / 1), shortage of filling material (0 / 1), surface

signal disturbance adjacent to the filling (SSD) (0 / 1), other defects (0 / 1), cohesive defects in the filling (CDF) (0 / 1). The specimens' scores of discoloration and each parameter were defined as the average of the worst scores (comparing all observation angles) of all ROIs. Correlations between discoloration and each of the other characteristics were calculated (Spearman-Rho). **Results:** Discolorations and SSD showed an average correlation ($r = 0.5$, $p = 0.032$) whereas a high correlation ($r = 0.628$) was found to CDF ($p = 0.012$). All other correlations (e.g. marginal gap: $r = 0.450$) were not statistically significant. **Conclusion:** Discoloration at margins of composite restorations does not represent an indicator for internal gap formation at the adhesive interface. OCT provides support in identifying and differentiating potential weak spots of composite restorations with marginal discolorations.

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Visualizing and Correlating Fluorescence and Microfocus Computed Tomography (μ CT) Images of White-Spot Lesions

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Objective of this study was whether multiple severity levels (thresholds) of white-spot lesion determined by fluorescence technique would be corresponding to those of μ CT images. Nine extracted human premolars that had approximal white-spot lesions were used. Fluorescence images were acquired and analyzed by Quantitative Light-induced Fluorescence (QLF) technique with multiple thresholds. Visualization was performed by 5% increment from 95% to 70% of threshold level. Pixel values less than threshold levels from sound values were considered as caries lesions. μ CT images were acquired by Skyscan instrument with multiple thresholds and 3D images were reconstructed accordingly. Pixel values less than 95%, 75%, or 35% from sound values both in enamel and dentin were considered as caries lesions. Visual comparisons with μ CT images were performed in two ways: 1) with original fluorescence images; and 2) with multiple threshold fluorescence images. In general, shape of lesions in original fluorescence images corresponded well with μ CT images. In one lesion, although shape/outline of lesion was corresponding with μ CT images, the area of lesion did not match well. Darkness in the original fluorescence images was corresponding well with μ CT images; however, two lesions did not seem to match. Darkest area in original fluorescence images were not corresponding with μ CT images. As for the comparison with multiple threshold levels, similar trend was observed. Generally shape and severity of lesions that were lower threshold level were corresponding well, although the two specimens mentioned earlier did not match well. Within the limitations of this study, multiple severity levels (thresholds) of white-spot lesion determined by fluorescence technique were corresponding to those of μ CT images.

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Professional Tooth Cleaning: Effect of a Novel Air Polishing Powder on Bovine Enamel Surface Roughness

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Patients complain that the water-powder-air-stream of air-polishing is unpleasant at teeth with hypersensitivity pain sensation. For that purpose a new glycine/fumaric acid coated tricalciumphosphate air-polishing powder is under development to allow gentle professional tooth cleaning with immediate hypersensitivity pain relief by occlusion of dentinal tubules. Aim of the study was to evaluate the effect of a new glycine-based air-polishing-powder which contains fumaric acid coated tricalciumphosphate (<10%) on bovine enamel surface in comparison to other air-polishing-powders and prophy-pastes. Experimental Powder, 3M ESPE, Clinpro Prophy PowderTM, 3M ESPE; Cavitron Prophy-Jet[®] Powder, Dentsply were applied with a Cavitron Prophy-Jet[®] (Dentsply) air-polishing-device. Prophy-pastes Enamel Pro[®] Fine/Coarse (Premier) and Clinic[®] Prophy Paste (Kerr) were applied with a MED Dental Filling Unit (W&H) at 4000 rpm. All treatments were applied for 5 sec on bovine enamel samples with starting Ra-values between 0.022 μ m and 0.029 μ m. Surface roughness changes were measured with a Perthometer S2 (Mahr); ($n = 9$). Enamel surface roughness increase between $\Delta Ra = 0.035 \pm 0.021 \mu$ m (Clinpro Prophy PowderTM) and $\Delta Ra = 0.167 \pm 0.05 \mu$ m (Enamel Pro[®] Coarse) have been observed. The new experimental powder caused roughness increase of $\Delta Ra = 0.091 \pm 0.034 \mu$ m. In comparison to the starting enamel roughness all prophy-powders and prophy-pastes increased enamel surface statistically significant (One-way ANOVA, $p < 0.05$). Statistically pair wise comparison between the used materials revealed that only Enamel Pro[®] Coarse caused a statistically significant higher increase in enamel surface roughness. In conclusion the addition of fumaric acid coated tricalciumphosphate to the glycine-based new air-polishing-powder allows professional tooth cleaning and occlusion of dentinal tubules for hypersensitivity treatment without elevated surface roughness increase at bovine enamel samples in vitro.

The study was funded by 3M ESPE.

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3D Profilometry Analysis of In Vitro NCCLS Development by Toothbrushing Abrasion

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This in vitro study investigated the effect of dentifrices/abrasives slurries on the development of non-carious cervical lesions (NCCLS) using 3D optical profilometry. Upper first premolars were selected and randomly allocated to 7 groups ($n = 16$) of den-

tifrice/abrasive slurries: A-Zeodent113/5% (mean Radioactive Dentin Abrasivity: 69), B-Zeodent124/10% (147), C-Zeodent103/15% (208), D-Sensodyne Pronamel (30), E-Crest Cavity Protection (99), F-Crest Pro-Health Whitening (220), G-Deionized water, as negative control. Teeth were mounted on acrylic blocks, and their root surfaces covered with acrylic resin to simulate gingiva, except for the 2-mm area near the cemento-enamel junction that was exposed to toothbrushing. Specimens were brushed in an automated brushing machine (200 g load) with the test slurries for 5/15/35/65 k-strokes. Impressions of specimens were taken at baseline and after each brushing time, and were scanned by a 3D optical profilometer. Tooth loss (mm^3) was calculated by image subtraction using dedicated software. Data were analyzed using mixed-model ANOVA followed by multiple comparison tests ($\alpha = 0.05$). No significant differences among slurries were observed at 5 k and 15 k. At 35 k, F showed higher loss than all other groups except C, which did not differ from the others. At 65 k, F (mean \pm SD: 4.19 \pm 3.29) showed the highest loss, followed by C (2.33 \pm 1.47), which differed from all the other groups except B (1.85 \pm 0.91). Groups B, A (1.35 \pm 0.65), D (1.17 \pm 0.48), E (1.40 \pm 0.68) and G (1.12 \pm 0.73) did not differ from each other. Groups F and C showed significant increase of tooth loss starting at 35 k, while B, A, D and E only at 65 k; no increase was observed for G. 3D-image subtraction analysis was able to quantify and differentiate tooth loss, but only at advanced stages. The development of NCCLs was more evident and faster for highly abrasive slurries.

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Influence of Digital Filters on the Detection of Proximal Caries Lesions in Primary Molars

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Early detection of proximal caries lesions increases the chance of arresting caries through minimally invasive strategies. Digital filters are supposed to enhance the quality of digital radiographs, therefore improving caries diagnosis. The aim of this study was to evaluate the influence of digital filters on the detection of proximal caries lesions in primary molars. Twenty pairs of bitewing radiographs of patients aged 5 to 8 years, with 320 proximal surfaces, were analyzed by 3 calibrated examiners in 3 conditions: with no filter, using sharpen 1 filter, and using sharpen 2 filter under stan-

dardized conditions. Interval between examinations was 2 weeks. The Kavio Express digital system was used to take the radiographs, which were analyzed using the CliniView software. Percentage of agreement and kappa test were used to analyze the inter-examiners agreement and z test to compare the proportion of proximal caries detected using two cut-off points: enamel caries (EC) and dentin caries (DC). The % agreement and kappa values ranged from 83% to 90% and from 89% to 96%; and from 0.6 to 0.8 and from 0.7 to 0.9 for EC and DC, respectively, showing good to very good agreement independently of the use of digital filters. The proportion of proximal caries did not differ significantly independently of the use of digital filters, ranging from 25 to 32%, 28 to 33%, and 26 to 32% for EC and from 13 to 19%, 18 to 21%, and 17 to 32% for DC. Although it was not statistically significant it tended to be lower for the 3 examiners when no filter was used. Digital filters did not increase either the inter-examiner agreement or the proportion of proximal caries detected in primary molars.

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In Vitro Detection of Caries Around Composite-Resin Restorations with The Canary System

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This study evaluated the performance of The Canary System (CS), DIAGNOdent (DD), Spectra (SP), ICDAS II, and radiographic examination (RE) in detecting natural decay around the margins of composite restorations (MOR). 'Sound walls' were created by removing visible caries from one-half of cavitated caries in extracted teeth using round bur in a slow speed handpiece. In the other half of the cavity preparation, caries were left on the axial and gingival walls ('cariou walls'). Teeth were restored with 3M Filtek™ composite with etching and bonding. Visually, all MORs appeared sound with no sign of micro-leakage or marginal defects. 220 sites with three repeat measurements per site at MOR, 0.5-mm and 1-mm away from the MOR into tooth tissue and into restoration were scanned with CS, SP, and DD. Two blinded dentists ranked MOR using ICDAS II and ranked radiographs of tooth samples as 'caries' or 'no caries'. Consensus scores were recorded. Sensitivity and specificity of each caries detection method were calculated. Canary Number (CN) of sound walls at MOR, 0.5-mm and 1-mm into tooth and into restoration were significantly lower ($p < 0.05$) than CN at corresponding distances of cariou walls. DD readings of sound walls were not significantly different from cariou walls. All SP values were within healthy tissue range (≤ 1). The sensitivities/specificities of CS, DD, SP, ICDAS II, and RE were 0.83/0.88, 0.72/0.34, 0.11/0.99, 0.35/0.95, and 0.29/1.00, respectively. CS showed high sensitivity and specificity in detecting caries at and around MOR. DD showed poor specificity. SP, ICDAS II and RE showed poor sensitivity. The present in vitro study demonstrated that The Canary System can

detect secondary caries at and around 3M Filtek composite-resin restorations.

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Microbiochemical Characterization of Carious Dentine of Primary Teeth after Partial Caries Removal using RAMAN Spectroscopy

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The aim of this study was to correlate microbiochemical components (phosphate and calcium) of infected carious dentine with the microhardness of a primary molar after caries partial removal technique. An extracted human carious primary molar was submitted to a partial caries removal technique, where carious dentin on the lateral walls was totally removed and infected dentin was left on the pulpal wall. The sample was sectioned in the centre of the lesion with left dentin and submitted to RAMAN analysis across the lesion in a central line with 27 points (100 µm distance) with a laser of 785 nm, 2 co-additions and 5 s of integration time. The corresponding high peak rate of Ca/PO₄ were recorded and plotted. The same central line was submitted to Knoop microhardness test with 10 g and 10 s. Data were analysed with linear regression analysis. As the depth of the lesion increased, the Ca/PO₄ rate and microhardness number also increased, $r = 0.51$ ($P < 0.05$) and $r = 0.63$ ($p < 0.001$), respectively. The Ca/PO₄ rate and microhardness showed a moderate and significant correlation, $r = 0.45$ ($p < 0.05$), demonstrating that the left tissue area with high mineral content presented higher microhardness values. It can be concluded that there is a correlation between lesion depth, phosphate and calcium RAMAN peak rate and microhardness values. RAMAN spectroscopy proved to be a non-invasive and objective tool for the characterization of carious tissue, showing a potential for the in vitro evaluation of the infected dentin after partial caries removal technique.

This study was supported by Federal University of Rio Grande do Sul.

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Detecting Approximal Caries from Bitewing Radiographs – Do Ipad give Similar Results to an LCD Monitor?

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A variety of devices exist now for the reading of radiographs, and these may influence the diagnostic accuracy. We aimed to compare the diagnostic yield of the iPad-2, iPad-3 and conventional LCD monitor in detecting interproximal caries on digital bitewing radiographs, across bright and dark lighting environments. Six dental students each reviewed 140 bitewing radiographs using three devices in two lighting conditions, analysing standardized radiographic images collected by the Melbourne Dental School in a previous clinical trial. 20 radiographs were scored twice to assess reviewer reliability. All approximal surfaces were graded for caries presence using a five-point scale (1 = definitively no caries, 2 = probably no caries, 3 = questionable caries, 4 = probably caries, 5 = definitively caries). Surfaces with substantial overlap were excluded from the analyses. Receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic performance of each device under the two lighting conditions. The caries status for each surface had been determined by three experienced dentists (consensus diagnosis). Inter- and intra-reviewer kappas ranged from 0.58 to 0.87 and 0.33 to 0.91, respectively. The area under the ROC curves ranged from 0.735 (95% CI 0.717–0.751) for the LCD monitor, 0.721 (0.703–0.739) for the iPad-2 and 0.734 (0.718–0.752) for the iPad-3. For the two lighting conditions, the area under the ROC curves were 0.722 (0.707–0.736) for dark lighting and 0.738 (0.724–0.753) for bright lighting. There were no statistically significant differences between devices or between lighting conditions. No significant interaction between devices and lighting conditions was observed. In conclusion, all devices, in either the dark and light environments, produced more-than-adequate sensitivity and specificity, and any differences were minimal.

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Session 8 Fluoride and Hard Tissues

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Effect of Stannous Fluoride Containing Toothpastes on Artificial Enamel Caries Lesions In Vitro

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Stannous fluoride toothpastes commonly show lower remineralization and demineralization inhibition due to lower free fluoride concentrations, which is counteracted by the antibacterial properties of stannous ions. New formulas are supposed to have improved mineralizing effects. Thus, the aim of this *in vitro* study was to compare three toothpastes differing in fluoride compounds on pre-demineralized bovine enamel specimens in a pH-cycling model. In each of 125 specimens two artificial lesions were created. Halves of both lesions were cut before pH-cycling for analyses of baseline demineralization. Specimens with lesions between 3,000 and 11,000 vol% $\times\mu\text{m}$ were randomly allocated to five groups (n = 13/15). Treatments during pH-cycling (2 \times 20 minutes demineralization/day) were: no brushing (NB), brushing with SnF₂/NaF (1,100 ppm F⁻ + 350 ppm F; Blend-a-med Pro Expert), NaF (1,450 ppm F; Blend-a-med classic), AmF (1,400 ppm F; Elmex Kariesschutz) or fluoride-free (FF; Lavera Zahncreme) toothpaste for 28 days twice daily. Subsequently, from each specimen one lesion was cut, while the remaining lesion was demineralized for another 14 days. Differences in integrated mineral loss were calculated between values before and after pH-cycling ($\Delta\Delta Z_{E1}$) and after pH-cycling and second demineralization ($\Delta\Delta Z_{E2}$) using transversal microradiography. Mean (SD) baseline mineral loss was 6,296 (1,251) vol% $\times\mu\text{m}$. Except for AmF and NaF toothpastes all other treatments induced no significant reduction in mineral loss after pH-cycling [$\Delta\Delta Z_{E1}$: AmF: 2,891 (1,418); NaF: 2,260 (2,305); SnF₂/NaF: 478 (2,329);

FF: -96 (2,262); NB: -280 (1,544)]. After subsequent demineralization only for AmF no significantly increased mineral loss was observed. Significant differences (indicated by <; p < 0.05; ANOVA-test) were NB = FF < NaF = AmF; SnF₂/NaF = NaF; SnF₂/NaF < AmF for $\Delta\Delta Z_{E1}$ and NB = FF; NB < SnF₂/NaF < NaF = AmF for $\Delta\Delta Z_{E2}$. In conclusion, both toothpastes with either amine or sodium fluoride promoted remineralization, whereas SnF₂/NaF toothpaste could not promote remineralization appreciably in a bacteria-free pH-cycling model.

This study was supported by Department of Operative Dentistry, Periodontology and Preventive Dentistry, RWTH Aachen University, D – 52074 Aachen.

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The Effect of Acidic Phosphatase for Availability of Free Fluoride from NaMFP Containing Toothpastes

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At the ORCA congress in 2013 concentrations of free fluoride measured in 23 different toothpastes from Uzbekistan were presented. Very low concentrations of free fluoride had been found in the 15 toothpastes containing NaMFP, probably because no hydrolysing agents (HyA) were used. The aim of this study was to measure *in vitro* to what extent the use of acidic phosphatase increases the availability of free fluoride in these NaMFP toothpastes. Two lots of thirteen different brands of NaMFP containing toothpastes purchased in supermarkets from Uzbekistan were included in this study. For the F⁻ measurements standardised amounts of toothpaste were diluted in artificial saliva, followed by adding acidic phosphatase and a buffer solution [van Loveren et al.: Caries Res 2005;39:224–230]. Concentration of soluble, ionisable fluoride was measured with the aid of a fluoride specific electrode. Accord-

ing to the manufacturers in 10 toothpastes the mean soluble F- concentration was 972.0 ppm, SD 546.0. In these toothpastes mean F- concentrations of 165.0 ppm, SD 98.9 (lot 1) and of 157.3, SD 131.5 (lot 2) were measured without adding HyA. After using HyA the respective values were 901.7 ppm, SD 537.3 (lot 1) and 913.7, SD 608.9 (lot 2). For 3 toothpastes manufacturers did not indicate the F- content. Here mean concentration of free F was 75.3 ppm, SD 94.9 (lot 1) and 112.0 ppm, SD 157.7 (lot 2) without HyA. After adding HyA the respective values were 595.7 ppm, SD 489.6 (lot 1) and 545.7 ppm, SD 465.4 (lot 2). The differences between values obtained with HyA and without HyA were statistically significant ($p < 0.001$). Conclusion: Although in vitro availability of free F from NaMFP toothpastes can be increased by about 5-fold with the aid of acidic phosphatase it remains unclear whether in the clinical situation sufficient phosphatase is available in the oral cavity to cause this reaction in the same extent.

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Micro-Filled Resin Infiltration of Non-Cavitated and Cavitated Occlusal Caries Lesions In Vitro

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Infiltrants with incorporated micro-fillers might be used instead, for the treatment of non-cavitated and cavitated fissure caries lesions, whereby advantages of both resin infiltration and fissure sealing are employed. Therefore, the aim of this in vitro study was to evaluate an Infiltrant resin (IR), Micro-filled-infiltrating-resin (MFIR) and Infiltrant-sealant-combination (ISC) for their abilities to penetrate lesions differing in ICDAS-codes as well as to fill fissures and cavities. Extracted molars showing fissures with cavitated lesions (ICDAS-codes 3/5) and additional fissures without cavitated lesions (ICDAS-code 2) were etched with 15% hydrochloric acid (Icon, DMG) that was mixed with abrasives and 15% HCl-solution (1:1). Etching gel was rubbed for 30 s within the fissure pattern by using a special brush. After this pre-treatment either an infiltrant (Group IR; Icon, DMG) or an experimental infiltrant (Group MFIR: 45% organic fillers, 55% infiltrant resin [Icon]) was applied. ISC included application of an infiltrant followed by a fissure sealant. From each tooth slices showing a non-cavitated (ICDAS 2; $n = 69$) or cavitated lesion area (ICDAS 3/5; $n = 40/38$) were prepared. Lesion (LA) and penetration areas (PA) were analyzed using dual staining and confocal microscopy. Dimensions of resin-filled fissures and cavities were measured. Percentage infiltration (PI) was calculated as $100 \times PA/LA$. PI [median (Q25/Q75)] did not differ significantly between MFIR [93 (62/100)%], IR [95 (86/100)%] and ISC [89 (67/97)%] ($p > 0.05$; Mann-Whitney test). All three treatments filled about 90% of the dimensions of fissures and cavities ($p > 0.05$; Mann-Whitney test). It can be concluded that MFIR seems to be effective to fill up fissures and cavities sim-

ilarly to a fissure sealant and that it penetrates fissure caries like conventional infiltrants.

The study was supported by DMG, Hamburg the producer of the infiltration kit (Icon) which is hereby acknowledged. HML & SP receive royalties from Icon sales.

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Micromorphological Surface Analysis of Dentin and Enamel After Use of Different Tooth Paste Formulations In Situ

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Currently offered toothpastes in addition to cleaning and prevention of oral diseases promise special functions as whitening and/or repair of initial enamel defects.

The aim of this study was to investigate surface effects of different toothpaste formulations on tooth substances in comparison with a basic paste without functionality. Bovine enamel (H_3PO_4 etched 30 s, $n = 120$) and dentin ($n = 120$) slabs mounted on acrylic splints were worn by 5 volunteers in their upper jaws for 24 h. Specimens were brushed after 0.5 h, 12 and 23.5 h for 30 s each time. Toothpastes included were: basic paste (B-P: PEG-32, sorbitol, hydroxyethylcellulose, SiO_2 , TiO_2 , SLS), hydroxyapatite paste (HA-P: hydroxyapatite, Al-lactate, NaF 1360 ppm), abrasive paste (A-P: RDA: 100, Al-lactate, NaF 1360 ppm) chlorhexidine paste (CHX-P: chlorhexidine digluconate 0.05%, Al-lactate, AlF 1360 ppm), herb paste (H-P: carum petroselinum seed oil, curcuma xanthorrhiza root extract, NaF 900 ppm). Brushing with water (W) served as control. Surface morphology was investigated by scanning electron microscopy in plan view; alterations of etching patterns were assessed using fractured samples (prism tips eroded or prism bodies eroded). Deposits were investigated by energy-dispersive X-ray spectroscopy (EDX). Brushing led to alteration of the enamel prisms on etched surfaces (A-P>B-P = CHX-P = HA-P>H-P>W). HA-P left particle deposits in the enamel etching patterns, while H-P and W masked it. Particles were found present in the orifices of the dentinal tubules for HA-P. All inorganic elements of the above-mentioned compounds were detected by EDX on the enamel and dentin in all groups. Calcium and phosphate containing particles were detected in the dentinal tubules (ratios of elements referred to carbon (C), C/O/P/Ca-HA-P: C/O/P/Cs-W = 1/1/1.3/1.5) and on etched enamel (C/O/P/Ca-W: C/O/P/Cs-HA-P = 1/1.8//4.3/2.1) surfaces after use of HA-P.

Due to their abrasiveness as well as their composition, toothpastes have different effects on enamel and dentinal surfaces. In further studies, experimental pastes will be generated starting with the basic paste in order to explore quantitative and qualitative effects of specific components of toothpastes.

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Dentists' Caries Preventive Recommendations and Fluoride use in Volgograd Region

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Caries prevalence does not decline in Volgograd region (VR). Fluorides are commonly recommended for caries prevention but the data about fluoride use in VR are unclear. **Aim:** To study dentists' caries preventive recommendations for patients and fluoride use in VR. **Methods:** The study was approved by Regional Ethics Committee. 156 dentists from state and private clinics were questioned about caries preventive recommendations for their patients. Parents of children aged 1–3 years (596) and 4–6 years (330), schoolchildren aged 7–17 years (1244), non-medical students aged 18–25 years (831) and adults aged 26–65 years (929) were questioned about fluoride use. Mean (%) and 95% confidence intervals (CI) were calculated. **Results:** 87.8% (CI 82.7–92.9%) dentists considered toothpaste as the main measure for caries prevention. But only 50.6% (CI 42.7–58.4%) dentists recommended toothpaste with fluoride (TF) for all patients. Only 22.4% (CI 15.9–28.9%) dentists applied local fluorides and 10.2% (CI 5.4–14.9%) dentists prescribed fluoride supplements for their patients. Some dentists used non-fluorides for caries prevention: application of calcium preparations (7.0% answers, CI 3.0–11.0%) and consumption of calcium with D3-vitamin (25.0% answers, CI 18.2–31.8%). The frequency of TF use was the lowest among 1–3 year olds (11.6%, CI 9.0–14.2%), the highest among young adults aged of 17–25 years (81.2%, CI 78.5–83.9%) and medium in the other age groups (from 46.9% to 54.5%). The frequency of fluoride supplements use was low in all studied groups: from 6.7% (CI 5.3–8.1%) in 7–17 year olds to 16.1% (CI 12.1–20.1%) in 4–6 year olds. **Conclusions:** The study revealed low frequency of fluoride use in most age groups and low activity of dentists to provide fluorides for caries prevention as possible barriers for caries decline in VR.

This study was supported by Volgograd State Medical University.

Fluoride-Releasing Nanohybrid Composite: Surface Roughness and Anticaries Proprieties

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The current study investigated the anticaries action of a fluoride-releasing nano-hybrid composite resin and the effect of cariogenic biofilm on the material roughness through a randomized,

split mouth, double-blind in situ study. A fluoride-releasing nano hybrid composite resin (FCR) and non-fluoride-releasing nanohybrid composite resin (CR) were tested. Forty restored bovine enamel slabs and 20 specimens made with the studied materials were included in intra-oral palatal appliances that were used for 20 adult volunteers. For the simulated cariogenic challenge, each volunteer dropped a 20% sucrose solution onto all slabs 8x/day during 14 days. In the course of the experimental period, all subjects used nonfluoride-containing dentifrice 3x/day. The biofilm formed over the slabs was analyzed to determine fluoride (F) ion concentration. Demineralization (ΔS) was determined on enamel by cross-sectional microhardness at 30 and 80- μm from the restoration margin. Surface roughness of material samples was also determined. The F concentration in the whole biofilm related to the group FCR was higher than the group CR. At 30- μm distance, the ΔS was 2.579 ± 1.582 and 1.705 ± 1.292 , respectively, for the groups CR and FCR ($p = 0.039$), no statistical difference was found at 80 μm . Significant difference was found between baseline and post challenge surfaces roughness for both groups ($p = 0.03$ and 0.016), respectively for CR and FCR but this difference was not found between the ΔRa of these materials ($p = 0.49$). Concluding, under the cariogenic exposure condition of this study, the fluoride-releasing composite may have a small anticaries action without damages for its surface smoothness.

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Comparison of Fluoride Release from Several Fluoride Varnish Products In Vitro

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Fluoride varnish containing high level of fluoride is effective preventive measure for dental caries. Varnish adhered to the tooth surface prolongs the contact time between fluoride and enamel surface, thus it helps to remineralization process of demineralized tooth by fluoride ions. Recently, several new varnish products were released in the Korean market. The aim of this study was to compare fluoride release from several fluoride varnishes marketed in Korea. Five commercial products were used in this study as follows. Cavity shield (CS, 22600 ppm F, 3M ESPE, USA), Enamel pro varnish (EP, 22600 ppm F, Premier Dental Products, USA), V varnish (VV, 22600 ppm F, Vericom, Korea), Fluorine care (FC, 22600 ppm F, Hearim Dental, Korea), and Fluor protector N (FP, 7700 ppm F, Ivoclar Vivadent, Liechtenstein). Ten milligrams of each varnish were applied to the surface of acrylic discs (5 mm diam). Applied varnishes were immersed in 20 ml of distilled water at 37°C, and fluoride concentrations were measured at 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, and 4 days. Each varnish was tested in triplicate. Amounts of fluoride release at 30 min were followed by FC (5.98 ± 0.68), FP (0.77 ± 0.13), EP (0.31 ± 0.05), VV (0.15 ± 0.02), and CS (0.04 ± 0.01) in sequence. Amounts of fluoride release at 4 H

were followed by FC (7.49±1.62), EP (5.51±0.48), VV (5.19±0.14), FP (0.97±0.11), and CS (0.27±0.05), respectively. CS showed the lowest fluoride release, (2.3% of total fluoride) whereas FC showed the highest release (63.5% of total fluoride) during 4 hours in this experiment. These in vitro results suggest that fluoride release of varnishes is different in the products.

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Relative Caries Preventive Effect of Salt Fluoridation in Preschool Children in Gambia

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Aim: To investigate the relative caries preventive effect of fluoride table salt in the communal nutrition in preschool children.

Experimental Approach: This was a block-randomized, controlled, open-label trial conducted in two urban kindergartens in Gambia between 2011 and 2013. Children of the test kindergarten received a daily dose of 0.4 mg F⁻ on average with their meals for a period of 12 months, children of the control kindergarten received meals cooked without fluoride. Caries assessment was performed at baseline (t0) and 12 months later (t1) by 6 calibrated investigators. Primary end point was the incidence of cavitated carious lesions (D3/4). Secondary end points were (i) white spot lesions (Gorelik class III), (ii) the PUFA score, and (iii) extracted teeth. Independent data were analysed using Wilcoxon (continuous data) and Fisher (discrete data) tests, dependent data using signed tests with $p < 0.05$ for statistical significance. Preliminary results: 450 children, 4.1 years of age (t0) completed the study. Primary end point: at t0 D3/4 caries prevalence was 2.3 (test) vs. 2.2 (control) ($p = 0.8$); at t1 D3/4 was 2.8 (test) vs. 4.9 (control) ($p = 0.006$). Secondary end points (i): t0 Gorelik class III was 1.3 vs 1.6 ($p = 0.7$), t1 Gorelik class III was 0.7 vs 3.3 ($p < 0.001$); (ii): t0 PUFA score was 0.7 vs 0.6 ($p = 0.4$), t1 PUFA score was 1.7 vs 1.6 ($p = 0.9$); (iii): t0 extracted teeth was 0.1 vs 0.0 ($p = 0.1$), t1 extracted teeth was 0.08 vs 0.4 ($p = 0.4$). In conclusion the relative caries preventive effect of fluoridated table salt in the communal nutrition in preschool Gambian children was 57% after 12 months.

This was an IIT. Fluoride table salt was kindly granted by Südsalz GmbH, Heilbronn, Germany. Financially, the study was supported by an internal research grant from the Witten/Herdecke University Association for the Promotion of Dentistry (Fördergemeinschaft).

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Kinetics of Fluoride Release from *S. mutans* in the Presence of Calcium

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We have previously observed that fluoride release from fluoride-treated bacteria is fast in a low-fluoride environment. Nevertheless, the previous experiments were conducted in the absence of calcium, which can bridge bacterial fluoride binding. Here, we assessed the kinetics of fluoride release from *Streptococcus mutans* in the presence of calcium. Pellets obtained from cultures of *S. mutans* IB1600 were first treated with PIPES buffer, pH 7.0 containing 0 or 1 mM Ca and 1 or 10 ppm F⁻ at 37°C for 10 minutes. The bacteria were separated from the treatment solution by centrifugation and the release was assessed to a low fluoride (0.019 ppm F⁻) PIPES buffer, containing 0 or 1 mM Ca²⁺, pH 7.0, at 37°C, for 10, 30 or 60 min ($n = 3$ experiments). Fluoride remaining in the pellet after release was acid-extracted and determined using an inverted fluoride electrode. The amount of bound fluoride (nmol F/g) was lower ($p < 0.0001$) in the bacteria treated with 1 than with 10 ppm F (147.1±31.1 and 396.3±64.4, respectively), but treatment with calcium had no effect on binding. Calcium did not affect the kinetics of fluoride release, which was fast in all groups (86% of bound fluoride released after 10 min). Remaining fluoride after 10-min release was, for groups pretreated with 1 ppm F⁻, 94.6±26.2 and 98.3±39.2 in the absence or presence of calcium in the treatment/release solutions, respectively (no difference between groups, $p > 0.05$). For the 10 ppm F⁻ groups, these figures were 123.5±28.4 and 139.8±43.6, respectively ($p > 0.05$). The results suggest that, at the concentrations used in this study (no calcium fluoride precipitation), the presence of calcium does not affect the kinetics of fluoride release from biofilm bacteria.

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Dental Biofilm Fluoride Concentration of Subjects Exposed to Different Community-Based Fluoride Sources

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The aim of this study was to compare the fluoride concentration in dental biofilm of subjects living in fluoridated-salt (FS) or water (FW) areas and to determine the effect of fluoride dentifrice on the fluoride concentration [F] in dental biofilm of these sub-

jects. Sixteen individuals residing in Montevideo (FS) (aged 24 ± 2 years) and sixteen individuals living in Porto Alegre (FW) (aged 27 ± 10 years) participated in this randomized, double-blind, crossover trial. Dental prophylaxis and scaling were performed prior to each experimental phase. During the experimental phases (14 d/each) subjects brushed their teeth, 2x/day, with fluoridated dentifrice (FD) or non-fluoridated fluoride (NFD). Dental biofilm samples were collected 8 hours after the last toothbrushing. Analyses were performed with a fluoride ion-specific electrode and the reading was transformed into $\mu\text{gF}^-/\text{g}$ biofilm. Generalized estimating equations were used to analyze the variance for repeated measures and the differences in each outcome. Higher biofilm [F] was found in FS (2.69 ± 0.10 CI 2.48–2.89) compared to FW (2.44 ± 0.06 CI 2.32–2.57) areas under regular use of NFD ($p = 0.047$). However, no significant differences were found on dental biofilm [F] between FW (2.60 ± 0.12 CI 2.37–2.83) and FS (2.81 ± 0.10 CI 2.62–3.01) areas under FD use ($p = 0.153$). Furthermore, no significant difference was observed in the biofilm [F] when FD or NFD were used in the FS ($p = 0.294$) and FW ($p = 0.320$) areas. The higher dental biofilm [F] was found in FS areas under NFD use may not have clinical significance once it disappeared when FD was used.

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Fluoride in the Fluid of Biofilm Formed on Sound and Carious Dentine Under High F-Toothpaste

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The use of high fluoride toothpastes ($5,000 \mu\text{g F/g}$) has been advocated to control root caries. Nevertheless, the ability of these toothpastes to maintain elevated fluoride concentrations in the biofilm fluid, the target site of topical fluoride treatments, has not been assessed. Moreover, since the biofilm fluid is the dynamic interface between the biofilm and the root substrate, the fluoride concentration in the biofilm fluid over sound or carious dentine may differ. Here we tested these effects in a double-blind, crossover, in situ study. Eighteen volunteers wore a palatal appliance containing sound or carious dentine blocks, covered by a plastic mesh, and exposed to 20% sucrose 8x/day and 3x/day (to induce mineral loss and mineral gain), respectively. Non-fluoride, 1,100 and $5,000 \mu\text{g F/g}$ toothpastes were used twice/day to brush volunteers' teeth and the appliance. After 14 days, dental biofilm formed on the blocks was collected in the morning (~ 10 hours after last brushing), the fluid extracted and analyzed for fluoride concentration using an inverted fluoride electrode. Fluoride concentration in the biofilm fluid was linearly correlated ($p < 0.01$) to the fluoride concentration of the toothpastes. No significant difference was found between fluoride concentration (μM) in the fluid of biofilms formed on both dentine substrates in the no fluoride (3.6 ± 2.2) and $1,100 \mu\text{g F/g}$ groups (4.7 ± 2.3), but was significantly lower in the biofilms formed on carious dentine in the $5,000 \mu\text{g F/g}$ group (sound = 14.3 ± 15.7 , carious = 7.5 ± 6.5 ; paired test, $p < 0.01$). The results suggest that residual fluoride concentration in the biofilm fluid under exposure to fluoride toothpastes is concentration-dependent and seem to indi-

cate that, under high fluoride toothpaste use, carious dentine is able to take up fluoride from the fluid for remineralization.

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Fluoride in Saliva and Plasma after Ingestion of Fluoridated Meal

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We have shown that chewing foods cooked with fluoridated water immediately increases salivary fluoride concentration but it is unknown for how long this increase lasts considering residual fluoride in saliva and the systemic contribution of plasma fluoride. Here, salivary and plasma fluoride concentration were assessed after ingestion of food prepared with fluoridated or non-fluoridated water. In a crossover, double blind, in vivo study, 20 volunteers ingested a Brazilian meal (rice, beans, meat, carrots, French beans, gelatin and juice) prepared with non-fluoridated ($0.02 \pm 0.01 \mu\text{g F/mL}$) or fluoridated water ($0.95 \pm 0.04 \mu\text{g F/mL}$) to deliver fluoride doses of 0.8 (from fluoride in raw food) and $12 \mu\text{g F/kg}$ body weight, respectively. Immediately before meal intake (lasting 15 min) and 5, 10, 15, 30, 45, 60, 120 and 180 min after ingestion, non-stimulated saliva and micro-blood (from finger digital puncture) samples were collected. F concentration in plasma and centrifuged saliva were determined by microanalysis and HMDS-facilitated diffusion, respectively, using F electrode. Baseline salivary fluoride concentration was $0.15 \pm 0.12 \mu\text{M F}$. Fluoride concentration in saliva 5 minutes after meal was 1.88 ± 1.83 and $0.58 \pm 0.87 \mu\text{M F}$ in groups ingesting fluoridated and non-fluoridated food, with significant differences between them at all collection periods up to 120 min ($p < 0.01$). Salivary fluoride concentration remained significantly ($p < 0.05$) higher than baseline values for up to 120 min and 10 min after ingestion of the fluoridated and non-fluoridated food, respectively. Fluoride in plasma was increased only in the fluoridated group ($p < 0.05$), with an average increment throughout the test of $0.58 \pm 0.76 \mu\text{M F}$. The results suggest that increased fluoride concentrations in saliva after meal ingestion last for several minutes by local and systemic effects.

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Fluoride Content of Diet Consumed by Two-Year Old Children

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Excessive intake of fluoride during the first two years of life is associated with increased risk for enamel fluorosis. Food and beverages have been identified as significant sources of fluoride. Re-

sults of studies have suggested changes over time in the fluoride content of foods and beverages. The purpose of this study was to calculate current fluoride content of commonly consumed foods and beverages by two-year-olds utilizing market basket information for the US Midwest region. FDA Total Diet Study collection guides were cross-referenced with NHANES – What We Eat in America data to determine foods and beverages' inclusion criteria. Fluoride was determined using a modification of the microdiffusion technique. Means, standard deviations, ranges, and 95% confidence intervals were calculated for the fluoride level of each food or beverage group. Confidence intervals and t-tests were used to determine if the current fluoride concentrations have changed when compared to historical values used to determine public policy by federal US agencies. One hundred seventeen foods and beverages were assessed, fluoride content varied widely, ranging from non-detectable for some products to 3.0 µgF/g for some processed meats and fish, vegetables, and fruits. Low mean fluoride items included sweets (0.20 µF/g), vegetables (0.34 µF/g), and beverages (0.33 µF/g). High fluoride groups included grains (0.73 µF/g) and 'mixture' category (0.62 µF/g). Dairy, eggs, fats and oils, fruits and grains had some items with non-detectable fluoride levels. Current values were in general different than historical values, with significant differences for meats and grains. This study showed that foods and beverages' fluoride content varies widely and in some cases was significantly different than previously reported values, highlighting the need to obtain current and accurate values on an ongoing basis.

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Fluoride Content in Foods and Beverages Prepared with Non-Fluoridated Water and Fluoridated Salt

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Food and beverages make a significant contribution to fluoride intake. Their fluoride content is influenced by source and preparation. The current study aimed at comparing fluoride in samples of Mexican foods and beverages prepared with non-fluoridated water and fluoridated salt to those reported in the USDA National Fluoride Database, mostly prepared with fluoridated water. Foods and beverages reported as most frequently consumed by the Mexican National Health and Nutrition Survey were used to determine the sampling strategy. Samples were purchased from supermarket stores in Pachuca, Mexico, using a stratified random collection. Fluoride content was measured directly (for beverages) or using a modification of the microdiffusion method (for foods). Mean and standard deviations were calculated and compared to values reported in the USDA database using t-tests. 264 samples from 66 brands purchased in four supermarkets were obtained. Samples

were categorized as baked goods, beverages, cereal/grains/pastas, dairy/egg, fats/oils, finfish/shellfish, legume/legume products, snacks, and sweets according to the USDA categories. Baked goods had a mean 1.27±0.30 µg F/g; beverages had 1.21±2.26 µg F/ml; cereal/grains/pastas had 0.17±0.01 µg F/g; dairy/egg had 0.52±0.65 µg F/g; fats/oils had 0.006±0.002 µg F/g; finfish/shellfish had 0.27±0.04 µg F/g; legume/legume products had 0.34±0.32 µg F/g; snacks had 0.11±0.02 µg F/g; and sweets had 1.57±1.42 µg F/g. Legumes, beverages, and sweets had statistically significant higher mean fluoride concentration than the respective means reported by the USDA. Significant variation in fluoride concentration was observed for foods and beverages prepared with non-fluoridated water and fluoridated salt, highlighting the importance of developing local databases for food and beverages' fluoride concentration.

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Dose of Fluoride Intake from Diet by Children in Optimally Fluoridated Tropical Areas

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The optimal concentration of fluoride in water has been based on the maximum environmental temperatures of temperate countries. However, this concentration may not be suitable for cities that have a tropical climate. This study assessed the fluoride intake of 68 children, 3–4 years-old, from Teresina, PI, Brazil, who attended a full-time public daycare centres. Teresina has a tropical climate (minimum 21.8°C – maximum 34.4°C) and its water supply is optimally fluoridated (0.60 to 0.80 mg F/mL). Liquids and solids ingested by the children were collected separately during 2 days, using the duplicate-plate method. Fluoride concentration in the liquids and solids was determined separately with specific electrode after microdiffusion. All samples were analyzed in triplicate. The daily amount (mg) of fluoride intake from diet (liquids and solids) was divided by the children's body weight. The average concentration of fluoride in the nursery (used in food preparation and for drinking), in the days of data collection was 0.64 ppm F. And the average fluoride concentration in water collected from the homes of participants was 0.66 ppm F. The mean (± SD) total fluoride intake was 0.025±0.01 mg F/kg body weight/day, and 80% of the dose was due to the intake of liquids. Since this dose is 64% lower than the considered threshold upper limit dose of 0.07 mg F/kg/day (Burt, J Dent Res 1992;71(5):1228–1237.) for the risk of developing aesthetically undesirable fluorosis, the data suggest that the fluoride concentration in water of Teresina may be considered optimal in terms of the benefits and risks of the use of fluoride for caries control for the studied population. Criteria for determining optimal water F concentrations in temperate countries appear to be suitable for tropical cities.

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Enhanced Antimicrobial Activity and Penetration of Micelle-Stabilised Silver Nanoparticles into Dentine with Iontophoresis

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Monodisperse 6.7–9.2 nm sized silver nanoparticles formed by chemical reduction were stabilised with an anionic surfactant to form self-assembled molecular aggregate of micelles. Retaining an overall negative surface charge and suspended in aqueous colloid form, these micelles respond by moving towards the anode in an artificially induced electric field. Previous *in vitro* studies demonstrated these silver nanoparticles exhibit strong antimicrobial activity against a range of gram-positive and gram-negative bacteria found in the oral environment, vastly superior to the ionic form of silver present as silver diammine fluoride.

A further *in vitro* assay was developed to demonstrate that combinations of artificially applied current and voltage significantly enhanced the antimicrobial effects of silver nanoparticles (at silver concentrations up to 72 $\mu\text{m}/\text{mL}^{-1}$) when applied at the same time to diluted monoculture broths of bacteria (2×10^8 CFU mL^{-1}). Although the parameters for inducing an electric field were necessarily vastly different to those anticipated when applying silver nanoparticles to human teeth *in vivo* using iontophoresis, nevertheless reduced viability of bacteria was demonstrated in the presence of sublethal doses of electric current, changing to profound synergistic bacteriocidal effects when also conducted in the presence of silver nanoparticles. The silver nanoparticles were antimicrobial at up to 50 times lower silver concentrations ($1.3 \mu\text{g mL}^{-1}$) than when current was not applied. Standard sized cavities were prepared in the crowns of extracted human teeth, consistently exposing dentine. Following smear removal, 10 μl of 42 $\mu\text{g mL}^{-1}$ silver colloid suspensions were applied to each cavity for up to 120 sec, with varying levels of current applied, before thorough washing with distilled water. SEM examination demonstrated silver nanoparticles penetration up to 72 μm beneath the cavity surface without current, with enhanced penetration and retention of micelle aggregates carrying silver nanoparticle up to 400 μm into dentine tubules in the presence of an electric field generated over the tooth. Superior antimicrobial effectiveness of micelle-stabilised silver nanoparticles was demonstrated when applied in the presence of an electric field. Furthermore, iontophoresis was found to provide a useful delivery mechanism for enhancing the penetration and retention of silver nanoparticles into human dentine *in vitro*.

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Modified Resin Infiltration of Natural Caries Lesions Varying in ICDAS-Codes In Vitro

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Caries infiltration is currently indicated for non-cavitated proximal lesions. Micro-filled-infiltrating-resins (MFIRs) might be useful to expand the indication spectrum to micro-cavitated lesions and increase the efficacy of the method. Therefore, it was the aim of the present *in vitro* study to evaluate the ability of a newly developed MFIR to fill cavities and infiltrate porous enamel in natural lesions of different ICDAS-stages. Proximal lesions in 120 extracted human teeth with ICDAS-codes 2 (n = 30), 3 (n = 45) and 5 (n = 45) were etched with 15% hydrochloric acid gel for 2 min and then allocated to one of the following treatment groups: A) lesions (ICDAS-codes 2, 3 and 5; each n = 15) were treated with infiltrant (Icon; DMG) for 3 min (n = 15/each code). B) experimental MFIR [45% organic fillers, 55% infiltrant resin (Icon)] was applied to lesions (ICDAS-codes: 2, 3 and 5; each n = 15) for 3 min. C (positive control): infiltrant resin (Icon) was first applied for 3 min. After light curing cavities (ICDAS codes 3, 5; each n = 15) were filled with flowable composite (EcuSphere; DMG). In cavitated lesions transparent matrices were used to restore the proximal contour. Lesions were cut and percentage infiltration of demineralized enamel (PI) and percentage filling of the cavity (PF) were analyzed using dual fluorescence staining and confocal microscopy. No significant differences in PI (range of medians: 57%–100%) were observed between the different groups ($p > 0.05$; Kruskal-Wallis) for all ICDAS-codes. PF of cavities (median in ICDAS-codes 3/5) was significantly higher in groups B (100%/100%) and C (100%/100%) compared with group A (25%/38%) ($p < 0.05$). It can be concluded that the MFIR showed similar penetration into natural lesions as the commercial infiltrant but better ability to fill small cavities.

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