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### Abstracts

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## Session 1 Microbiology

1

### Enhanced Antimicrobial Activity of Silver Nanoparticle Micelle Structures over Traditional Ionic Silver Diamine Fluoride Formulations

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Silver nanoparticles are well known for their unique optical and antimicrobial properties but are inherently unstable in suspension, aggregating rapidly, reducing their effectiveness. Micelle structures offer a convenient way to stabilise small silver nanoparticles (<10 nm), thus retaining optimal potential prior to application. Micelle-stabilised silver nanoparticles synthesised under optimal reagent conditions in aqueous suspension, offer vastly enhanced antimicrobial activity based on equivalent silver mass content, remaining colourless after application on teeth. Surfactants selected for creating the supporting micelle structures can generate an overall surface charge, allowing transportation of these structures in an electric field to assist delivery deep into tooth structure. Silver diamine fluoride is an effective antimicrobial agent for treating dental caries but causes significant black discolouration of teeth, unless followed up by application of potassium iodide resulting in a white precipitate forming. The effectiveness of silver diamine fluoride and various forms of micelle-stabilised silver nanoparticles against a range of Gram-positive and Gram-negative bacteria found in the oral environment was tested in vitro. Monoculture lawns of bacteria were grown on TSB agar plates at 37°C for 24 h, exposed to 30 µl of colloidal silver nanoparticles at various concentrations (1.0–65 µg/ml). Zones of inhibition were measured using the agar well diffusion assay method. Minimum inhibitory concentrations were determined by exposing TSB broth cultures to silver, incubating for 24 h, and sub-culturing onto TSB plates. Silver nanoparticles were capable of effectively inhibiting bacterial growth at over 8,000 times lower silver concentrations (as low as 30 µg/ml) than a commercial silver diamine fluoride preparation.

The silver nanoparticles we produced demonstrated strong affinity for both synthetic gelatin (partially hydrolysed collagen) and hydroxyapatite, avidly binding to natural tooth surfaces, as determined by scanning electron microscopy.

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2

### Carolacton Incorporated into Dental Composite Materials Inhibits Biofilm Growth of *Streptococcus mutans*

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Caries adjacent to restorations is one of the main causes for restoration replacement. The presence of antibacterials in dental filling materials would potentially be able to affect the initiation and progression of secondary caries. This study investigated the antibacterial effect of experimental composite materials containing 25 µg/ml carolacton, a biofilm-inhibiting biomolecule. Biofilm formation was induced by culturing *Streptococcus mutans* UA159 onto material surfaces. The biofilm inhibition was monitored by counting colony forming units (CFU), viability staining (Life/

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Dead), and real-time quantitative PCR. All tested materials containing carolacton showed a strong biofilm inhibiting effect. Mean CFU per ml biofilm were for composite without antibacterials (negative control)  $7.42 \times 10^6$ , for composite containing CHX  $4.22 \times 10^6$ , for triclosan  $6.59 \times 10^6$  (positive controls, both antibacterials at a concentration of 0.02%), and for composite containing 0.002% carolacton  $1.13 \times 10^5$ . The calculated biofilm inhibition for carolacton was therefore 98% (about two log values). In contrast, filling materials containing CHX and triclosan just reached an inhibition of 43 and 11%, respectively, although a 100-fold higher concentration was used. Life/Dead staining and qPCR confirmed the biofilm inhibition for composite containing carolacton (64 and 94% respectively), whereas the additives CHX and triclosan again showed only an insignificant effect. Carolacton incorporated into dental filling material has a strong biofilm-inhibiting effect and is therefore potentially able to prevent secondary caries formation in clinical outcome.

This study was supported by the BMBF grant 'Medical Systems Biology-MedSys' (FK 0315411A).

### 3

#### In situ ex vivo Oral Biofilms Analyzed by Different Viability Assays

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**Aim:** Analysis of oral biofilms is often conducted with respect to bacterial quantification or visualization using fluorescence-based, two-color viability assays. Oral biofilm models have to be simple to produce and should closely resemble the oral in vivo situation. The aim of this study was the development and viability analysis of a simple in situ initiated ex vivo matured biofilm. **Experimental Approach:** Initial bacterial colonization was performed in situ for 8 h on bovine enamel specimens ( $n = 36$ ) attached to oral splints of two volunteers in three cycles. The initial oral biofilms were transferred into artificial saliva enriched with 0.5% sucrose and raised in vitro for another 5 days. Colony forming units (cfu) grown at aerobic and anaerobic conditions were counted ( $n = 6$ ). Bacteria in the biofilms were visualized using clsm (confocal laser scanning microscopy) following fluorescence-based live/dead assays (BacLight viability assay and fluorescein diacetate/propidium-iodide [FDA/PI] staining;  $n = 14$ ). Metabolic activity was analyzed by fluorimetry in resazurin solution ( $n = 6$ ). Negative controls were carried out without incubation in situ ( $n = 4$ ) and by rinsing with 0.2% chlorhexidine (CHX) for  $t_1 = 5$  min and  $t_2 = 10$  min for each live/dead assay ( $n = 6$ ). **Main Results:** Visualization showed a higher number of avital than vital bacteria. Following CHX treatment BacLight revealed both viable and avital bacteria, while FDA/PI-staining showed avital bacteria only. Quantification with cfu resulted in  $2.8 \pm 2.7 \times 10^7$  cfu/ml for aerobic and  $2.6 \pm 3.1 \times 10^7$  cfu/ml for anaerobic conditions. Resazurin metabolism revealed a linear time correlation for bacterial suspensions of quantities between  $10^6$  to  $10^7$  per well ( $r = 0.999$ ). Undisturbed biofilms always showed metabolic activity using resazurin

assay. Biofilms formed reliably on all specimens, but with inter- and intraindividual differences. Matured biofilms were sensitive to antibacterial treatment. FDA/PI assay seems to be more specific with respect to live/dead differentiation after antibacterial treatment than BacLight.

### 4

#### A Tri-Species Biofilm Model to Evaluate Cariogenic Potential of Dietary Products

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The cariogenic potential of dietary carbohydrates can be evaluated using biofilm models and a *S. mutans* biofilm has been used to evaluate the cariogenic potential of lactose, but to evaluate the cariogenicity of starchy products a more complex biofilm should be developed. Thus, the aim of this study was to develop a biofilm model composed of *A. naeslundii* (ATCC12104), *S. gordonii* (ATCC35105) and *S. mutans* (UA159), bacteria that respectively metabolize starch, express amylase-binding protein and use starchy hydrolysates. At present, data of this biofilm grown under sucrose exposure are shown. Biofilms of each species and of the three species were formed on saliva-coated bovine enamel slabs in ultrafiltered tryptone-yeast extract broth at 37°C, 10% CO<sub>2</sub>, during 4 days. Eight times a day, the biofilms were exposed to 10% sucrose. The pH of the media (indicator of biofilm acidogenicity) was determined 2x/day. At 5th day the biofilms were collected for: (a) biofilm biomass determination (bacterial viability, wet weight), (b) extracellular soluble and insoluble polysaccharide concentration, (c) intracellular polysaccharide concentration. On the dental blocks were measured the percentage of change in surface microhardness (%SMC). Also, the structure of the biofilms formed was evaluated by SEM and confocal laser microscopy. *S. mutans* biofilm was the most acidogenic (pH<sub>82h</sub>:  $4.41 \pm 0.10$ ;  $n = 3$ ), presented greater concentration of insoluble extracellular polysaccharide ( $15.8 \pm 6.1$ ;  $n = 3$ ) and provoked higher enamel %SMC ( $45.3 \pm 4.8$ ;  $n = 3$ ) than the other mono-species biofilms. The three species grew up together in the biofilm formed, which showed unique properties if compared with the mono-species biofilms. The findings show that it is possible to grow a complex biofilm formed by *A. naeslundii*, *S. gordonii* and *S. mutans*, which may be a more appropriate method to evaluate the cariogenic potential of starchy products.

The study was funded by CNPq Proc. 475178/2011-4 and 305310/2011-9.

## Effect of Starch Hydrolysates on Enamel and Dentine Demineralization

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Byproducts of starch hydrolysis of different dextrose equivalents (DE), which is a measure of their reducing power, have been widely employed by food industry. With regard to acidogenicity, starch hydrolysates are able to lead to a substantial drop in biofilm pH, suggesting a cariogenic potential. Thus, the present in situ study aimed to evaluate the effect of starch hydrolysates with different DEs on enamel and dentin demineralization. A crossover, split-mouth with regard to treatments and blind in situ study was conducted in 3 phases of 14 days each, during which 14 adult volunteers wore palatal appliances containing slabs of bovine enamel and dentine, with pre-determined surface hardness (SH). The slabs were extra-orally subjected 8 times a day to six groups of treatment: T1: purified water (negative control), T2: 2% starch, T3: 10% sucrose, T4: 10% starch hydrolysate DE 5, T5: 10% starch hydrolysate DE 20, and T6: 10% starch hydrolysate DE 40. On the 15th day, the slabs were collected, SH was determined again and its percentage of loss (%SHL) was calculated. The data for enamel and dentine slabs were independently analyzed. All data were analyzed by ANOVA followed by Tukey's test, with the volunteers considered as statistical blocks. The %SHL (mean  $\pm$  SD) for groups T1 to T6 were respectively: enamel:  $-1.1 \pm 4.9^a$ ;  $6.1 \pm 11.9^{ab}$ ;  $43.7 \pm 16.1^c$ ;  $8.3 \pm 7.8^{ab}$ ;  $13.4 \pm 8.7^{bd}$ ;  $26.6 \pm 20.1^d$ ; dentine:  $-2.9 \pm 6.7^a$ ;  $20.1 \pm 15.4^b$ ;  $63.9 \pm 14.4^c$ ;  $22.3 \pm 18.8^b$ ;  $41.9 \pm 14.3^d$ ;  $48.9 \pm 14.5^{cd}$ . Distinct letters show statistically significant differences among the treatments. In enamel, a significantly higher %SHL was found for starch hydrolysate DE 40 group when compared to negative control and starch groups ( $p < 0.05$ ), but statistically lower than sucrose ( $p < 0.05$ ). In dentine, %SHL was higher in starch hydrolysates DE 20 and 40 groups when compared to water and starch groups ( $p < 0.05$ ). In conclusion, the results suggest that the starch hydrolysates are capable of inducing enamel and dentine demineralization, which may depend on their dextrose equivalent.

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## Free Fatty Acids Modulate Sucrose Cariogenicity on the Oral Biofilm Formed on Enamel in situ

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We previously showed that free fatty acids decrease cariogenicity of sucrose in a caries/biofilm in vitro model. The aim was to test whether sucrose effect on enamel is hampered when free fatty acids

are exposed to the oral biofilm following a cariogenic challenge in situ. Eleven volunteers wore palatal appliances containing triplicate bovine enamel slabs at each side. The experiment had two phases of 15 days each, using a split-mouth, crossing-over and double-blind design. Volunteers dripped 20% sucrose to the slabs 8x/day for 5 min. After four out of eight sucrose exposures, slabs were immediately treated for five additional min with either suspension of free 18-carbon monounsaturated, polyunsaturated or saturated fatty acids (10 mM); oleic, linoleic or stearic acid, respectively. A fourth triplicate was dripped with 20% sucrose as caries-positive control. Biofilms were recovered to analyze biomass, *S. mutans*, *Lactobacillus*, *S. sanguinis*, total streptococci and intra- and extracellular polysaccharide formation. Enamel demineralization was estimated by percentage of surface microhardness loss (%SHL). Biofilms exposed to oleic and linoleic acids showed significantly lower biomass than the positive control. Linoleic acid exposure decreased *S. mutans* counts, but not the other bacteria ( $p < 0.05$ ). Although all free fatty acids tested reduced insoluble extracellular polysaccharide formation by the oral biofilm ( $p < 0.05$ ), only linoleic acid showed less soluble extracellular and intracellular polysaccharides ( $p < 0.05$ ). Slabs exposed to 20% sucrose followed by fatty acids had significantly lower demineralization than those exposed to sucrose ( $52.8 \pm 5.6$ ). Linoleic acid exposure showed the lowest %SHL values ( $35.1 \pm 6.7$ ), followed by oleic ( $38.6 \pm 4.1$ ) and stearic acids ( $42.3 \pm 4.1$ ) ( $p < 0.05$ ). In conclusion, free fatty acids, particularly polyunsaturated linoleic acid, exposed after sucrose appear to reduce caries formation in enamel, affecting cariogenicity of the oral biofilm.

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## Dental and Periodontal Phenotype in Sclerostin Knockout Mice

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**Objectives:** SOST encodes for sclerostin, a negative key regulator of bone mass. Here we determined the influence of sclerostin on dental structures and dimensions. **Methods:** Histomorphometric and microCT analyses were performed on the first left lower molar and its surrounding structures in mice that lack a functional SOST gene and in wild-type controls. **Results:** The size of the basal body of the mandible in SOST knockout mice was ap-

proximately double that of wild-type animals. The increase was less pronounced in the alveolar part. Lack of sclerostin did not change the sizes of the tooth, enamel or dentin. The pulp chamber and the periodontal ligament space of SOST knockout mice were moderately decreased in size, however without reaching significance. Dimensions of the lingual cementum were increased in SOST knockout mice, while buccal cementum thickness were comparable between genotypes. Distances from the cemento-enamel junction to the apex and to the alveolar crest were also unaltered. **Conclusion:** Taken together these results suggest that sclerostin is implicated in the development of the periodontium but not of the tooth.

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### In vitro Toxicity of Tin Chloride in a L929 Fibroblast Bioassay

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A highly concentrated tin chloride solution (SnCl<sub>2</sub>, 35%, pH ~0) might increase the microtensile bond strength ( $\mu$ TBS) between a self-etching bonding system and enamel or dentine if used as an etching or pre-treatment agent instead of or in combination with phosphoric acid (H<sub>3</sub>PO<sub>4</sub>; 35%). Only little is known about the potential toxicity of SnCl<sub>2</sub>, whereas the toxicity of H<sub>3</sub>PO<sub>4</sub> is widely recognized. The aim of the present study was to determine the in vitro toxicity of SnCl<sub>2</sub> and H<sub>3</sub>PO<sub>4</sub> with a L929 fibroblast bioassay. Cytotoxicity of various dilutions of SnCl<sub>2</sub> and H<sub>3</sub>PO<sub>4</sub> were investigated. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (MTT) assay (2 h at 37°C humidified incubator, 5% CO<sub>2</sub>). Cells were analyzed light microscopically after incubation time; absorbance (A) of MTT staining was determined spectrophotometrically at 550 nm. Cell morphology and their permeability for trypan blue were analyzed by light microscopy. Both SnCl<sub>2</sub> and H<sub>3</sub>PO<sub>4</sub> at 0.1–0.2% caused a half maximal reduction of cell viability; absorption was significantly decreased by incubation with SnCl<sub>2</sub> from 1.64 ± 0.74 (mean absorption ± standard deviation at concentrations lower than 0.1–0.2%) to 0.24 ± 0.12 (mean absorption ± standard deviation at concentrations higher than 0.1–0.2%;  $p \leq 0.05$ ) and by incubation with H<sub>3</sub>PO<sub>4</sub> from 0.42 ± 0.12 to 0.06 ± 0.02, respectively ( $p \leq 0.01$ ). Under these conditions, though, only SnCl<sub>2</sub> but not H<sub>3</sub>PO<sub>4</sub> affected the integrity of the cell membranes. These data suggest that SnCl<sub>2</sub> and H<sub>3</sub>PO<sub>4</sub> have a comparable toxicity in the indicated bioassay, though the increase in cell membrane permeability needs further investigation. The data provide the basis for clinical studies to evaluate the effect of SnCl<sub>2</sub> in the oral cavity.

The study was funded by the Department of Preventive, Restorative and Pediatric Dentistry, University of Berne.

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### Porphyrin Involvement in Redshift Fluorescence of Decayed Dentine

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The aim of this study is to understand the involvement of porphyrins in the origin of the red fluorescence observed with intra-oral cameras SoproLife<sup>®</sup> and VistaCam<sup>®</sup>. Fluorescence and micro-Raman spectra of sound and carious dentin were analyzed and compared to assess the involvement of porphyrins in this redshift of the fluorescence emission. Porphyrin I and Protoporphyrin IX powders, sound and 6 carious dentin samples, ranked according the ICDAS classification, were respectively observed with SoproLife<sup>®</sup> and VistaCam<sup>®</sup>. Fluorescence emission spectra were recorded using an epifluorescence microscope (Nikon Eclipse TE) with an excitation wavelength range of 380–420 nm and a spectrometer (Acton SP215i-CCD PIXI400B). The radiance shown was computed with image-analyzing software Win roof<sup>®</sup>. Micro-Raman spectra of the same samples were measured with LabRAM ARAMIS IR<sup>2</sup> confocal micro-Raman spectrometer equipped with BX41 Olympus microscope and a CCD detector. The Raman spectra were normalized using Peakfit v4.12 software. Porphyrin I and Protoporphyrin IX look red-brown dark with SoproLife<sup>®</sup> and VistaCam<sup>®</sup> signals varied according the distance of the sample, background color of support and focus of the picture. Fluorescence emission spectra of protoporphyrins revealed a maximum peak at 715 nm and is red shifted, compared to the carious dentine spectra with three peaks between 600 and 700 nm. Raman spectra revealed a high level of fluorescence in dentine decay but a very low relative intensities ratio of Porphyrin and Protoporphyrin. Fluorescence devices provide significant benefits to clinicians toward more preventive dental care. Understanding the specific role of porphyrins in the caries process brings additional elements to this complex biological reaction in spite of the weakness of this signal in the dentin decay. Further work is ongoing to establish the biochemical characteristics of porphyrins involved in the dental caries process.

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## Influence of Sodium Hypochlorite Pre-Treatment on Resin Infiltration of Molar-Incisor-Hypomineralized Enamel

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**Aim:** Resin infiltration has been developed to arrest non-cavitated caries lesions but might also be used to stabilize developmental enamel defects such as molar-incisor hypomineralization (MIH). These defects have a high organic content mainly proteins, representing a challenge for deep resin penetration. Thus, enamel deproteinization prior to resin application was proposed to improve resin penetration. Therefore, the aim of the present study was to evaluate the effect of sodium hypochlorite (NaOCl) pre-treatment of MIH lesions on resin infiltrant penetration. **Methodology:** Sixteen extracted first permanent molars with MIH were randomly allocated to two treatment groups (A, B). In both groups hypomineralized enamel lesions were etched with 15% HCl (Icon etch, DMG) for 120 s. In group B teeth were immersed in 5% NaOCl for 30 min (37°C). In group A no further pre-treatment was performed. In both groups lesions were desiccated and a resin infiltrant (Icon Infiltrant) was applied for 3 min and subsequently light cured. Lesion and resin penetration depths were measured using dual fluorescence staining technique for confocal microscopy. Percentage penetration was calculated for non-cavitated as well as cavitated lesion parts. **Results:** Median [median (Q25/Q75)] lesion depths of non-cavitated and cavitated enamel were 1,669 (1,520/1,883) µm and 1,237 (1,139/1,490) µm, respectively. Percentage penetration did not differ significantly between groups A [28 (9/45)%] and B [30 (25/43)%] ( $p > 0.05$ ; Mann Whitney test). However, probably due to thick remineralized surface layers, penetration was significantly lower in non-cavitated [9 (0/24)%] compared with cavitated [49 (38/68)%] areas of MIH lesions ( $p < 0.05$ ; Wilcoxon). **Conclusion:** Pre-treatment with 5% NaOCl did not significantly improve resin infiltration into MIH enamel lesions. Deeper penetration is more likely to occur in cavitated areas of MIH lesions than in non-cavitated ones.

The study was kindly supported by the Deutsche Forschungsgemeinschaft (PA 1508/1-3). Conflict of interest: S.P. and H.M.L. receive royalties and funding from DMG, the manufacturer of Icon.

## Light-Triggered Preparation of Nano-Silver Composite Materials: Toward New Antimicrobial Agents for Treating Dental Caries

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Silver nanoparticles have received renewed focus recently for potential healthcare applications, due in part to increasing bacterial resistance to classical antibiotics. Silver nanoparticles offer novel modes of action, targeting different cellular structures compared with existing antibacterial agents, and have vastly increased reactivity over ionic silver, based on equivalent silver mass content. Consequently, there is significant interest in designing improved strategies for preparation of biocompatible silver nanocomposite materials with high nanoparticle loading and enhanced antimicrobial effects. The aim of our work is to develop biocompatible silver nanoparticle-based materials using non-toxic formulations for the treatment of dental caries. Toward this goal, we have developed a method of preparing aqueous suspensions of nano-silver composite materials, stabilized by sodium dodecyl sulfate (SDS), with high antimicrobial activity. Our approach utilizes an LED dental curing light (380–515 nm wavelength) to photoreduce silver nitrate, therefore eliminating the need for strong and/or toxic reducing agents in the preparation. Our method forms stable aqueous suspensions of spherical silver nanoparticles with diameters of 2–6 nm (as determined by electron microscopy), for samples irradiated for 3 min. The antimicrobial activity of nano-silver is known to depend critically on particle size, with smaller particles (<10 nm) imparting greater antimicrobial activity. The antimicrobial activity of our nano-silver suspensions was tested in vitro. Monoculture lawns of Gram-positive and Gram-negative bacteria found in the oral environment were grown on TSB agar plates at 37°C for 24 h and exposed to 30 µl of SDS-stabilized nano-silver suspensions at various concentrations (1.0–65 µg/ml). Zones of inhibition were measured using the agar well diffusion assay method. Restreaked zones indicated bactericidal action for neat nano-silver suspension, remaining at least bacteriostatic to concentrations as low as 30 µg/ml.

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### Modeling of Invasion into Hard Tissue – Generalists (*S. sanguinis*) versus Specialists (*S. sobrinus*)

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The aim of the study was to model plaque formation and invasion of *S. sanguinis* and *S. sobrinus* into dental hard tissue. 30 caries-free extracted teeth (18, 28, 38, 48) from 11- to 16-year-olds were cleaned ultrasonically for one minute with de-ionized water and, after air-drying, embedded in epoxy resin (Epofix, Struers, Germany). After 8 h of setting at room temperature, the specimens were ground on both sides with SiC paper 1200 (particle size 13–16 µm) (Exakt-Mikroschleifsystem 400CS, Exakt Apparatebau, Norderstedt, Germany). Means of enamel removed from areas sized 3 × 3 mm were 0.23 ± 0.06 mm or 0.25 ± 0.08 mm; the amounts did not differ significantly ( $p = 0.181$ ). 15 specimens each were incubated anaerobically under standardized conditions with 24 h cultures of *S. sanguinis* 9S or *S. sobrinus* OMZ 176 in Balmelli bouillon at 37 ± 2°C. After 2, 4, 6, 8, and 10 weeks 3 teeth each were fixed in 2.5% glutaraldehyde in cacodylate buffer for 24 h, washed 3× in cacodylate buffer and dehydrated 30–60 min by sequential washes through a series of 30–100% graded ethanol. The teeth were cut in half longitudinally (Exakt-Trennschleifsystem 300CP, Exakt Apparatebau Norderstedt, Germany); the halves received multiple slits to obtain break surfaces in the infected area. After critical point drying the fragments were sputtered with gold and viewed in a scanning electron microscope (LEO 1450VP, Zeiss, Oberkochen, Germany) at magnifications of ×20–20,000. After 10 weeks of incubation, invasion of *S. sanguinis* (generalist) of up to 16 ± 26.6 µm below the break edges into the enamel was observed. The invasion of *S. sobrinus* (specialist) reached depths of up to 181 ± 73 µm. The difference was statistically significant (paired t test:  $p = 0.04$ ). The experimental invasion depths emphasize the importance of *S. sanguinis* ('generalist') versus *S. sobrinus* ('specialist') in the context of the extended ecological plaque hypothesis.

The project was funded by Jena University.

### 13

#### Effect of Scandium on *Streptococcus mutans* NCTC10449

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Novel scandium doped phosphate-based glasses (Sc-PBGs) are controlled delivery materials of scandium ions which may significantly impact on *S. mutans*, a caries-associated bacterium. Scandi-

um doped and control scandium free PBG rods (5 × 2 mm) were produced using a conventional melt quenching method, at 1,100°C for 1 h. Degradation studies (0–96 h) were carried out in triplicate using a weight loss method in dH<sub>2</sub>O at 37 ± 1°C and starting pH of 7 ± 0.1. The distribution of scandium in PBGs was analysed using Bruker Quantax70 EDX chemical microanalysis system attached to a Hitachi Tabletop SEM TM3000 using various magnifications at an operating voltage of 15 kV. For antibacterial assay, the glass discs were placed on brain heart infusion agar plates previously inoculated with standardised cultures of *S. mutans* NCTC10449 and incubated for 24 h aerobically at 37°C. The diameters of any zones that had formed around the disks were measured in triplicate using calipers and unpaired t-test was used to compare values. The degradation rates and corresponding ion release rates of the Sc-PBGs were found to decrease with increasing calcium concentration in the glasses (34.8, 28.0 and 18.5 µg mm<sup>-2</sup> · h<sup>-1</sup> respectively for 11, 12 and 13 mol% of calcium). Zones of inhibition were also found to decrease (from 16 ± 1.0, 14 ± 0.5 to 8.0 ± 0.5 mm) significantly ( $p < 0.03$ ) as the calcium concentration in the glasses increase. SEM-EDX analyses revealed uniform distribution of scandium ions in Sc-PBGs. Sc-PBGs were found to inhibit *S. mutans* growth by controlled delivery of Sc<sup>3+</sup> ions that has the potential to disrupt Fe<sup>3+</sup> metabolism of *S. mutans*. Increasing calcium concentration in the Sc-PBGs resulted in decreasing ion release rates and corresponding antibacterial effect and may have potential use as an ant-caries agent.

R.S. was funded internally by the University of Liverpool, School of Life Sciences.

### 14

#### Involvement of Cell Surface Protein Expression Patterns in Interaction of *Streptococcus mutans* with Vein Endothelial Cells

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*Streptococcus mutans*, a major pathogen of dental caries, is considered to cause infective endocarditis (IE). One of the important steps in the pathogenesis of that disease is bacterial interaction with heart tissue endothelial cells. Two types of 120-kDa cell surface collagen-binding proteins (CBPs) of *S. mutans* have been characterized and CBP-positive strains have been shown to possess collagen-binding activity, while they also adhere to and invade human umbilical vein endothelial cells (HUVEC). Recently, we found that *S. mutans* strains with CBPs frequently lack expression of the cell surface 190-kDa protein antigen (PA) known to be associated with sucrose-independent initial adhesion to tooth surfaces. Here, we analyzed the mechanisms of *S. mutans* strains with various expression patterns for PA and CBP in regard to interaction with HUVEC. We used the oral isolate MT8148 (PA+/CBP-), and blood isolates TW871 (PA+/CBP+) and TW295 (PA-/CBP+). TW295 showed a significantly higher rate of invasion to HUVEC than TW871 and MT8148 ( $p < 0.001$ ). Confocal scanning laser microscopy observation of TW295 organisms interacting with HUVEC revealed that prominent masses of aggregated bacteria adhered to and invaded HUVEC, whereas

a drastic reduction in invasion rate was observed when HUVEC were cultured without fetal bovine serum. Furthermore, bacterial aggregation of TW295 was observed following addition of serum obtained from human volunteers. Analysis of several *S. mutans* clinical isolates revealed that aggregation of PA-/CBP+ strains was significantly higher than that of PA+/CBP+ and PA+/CBP- strains in the presence of human serum ( $p < 0.001$ ). These results suggest that PA-/CBP+ *S. mutans* strains demonstrate intensified bacterial aggregation and increased invasion of HUVEC in the presence of serum, which may be associated with virulence for IE.

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## 15

### **Virulence of *Streptococcus mutans* Strains for the Development of Non-Alcoholic Fatty Liver Disease**

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The association of oral bacteria with systemic diseases has received attention, while infection with the periodontopathic pathogen *Porphyromonas gingivalis* was recently shown to be a possible risk factor for development of non-alcoholic steatohepatitis (NASH). The purpose of the present study was to analyze the effects of systemic administration of *Streptococcus mutans*, a pathogen of dental caries, on development of NASH in a mouse model. *S. mutans* oral strain MT8148 (serotype *c*) as well as blood isolates TW295 (*k*) and TW871 (*k*) were used. C57BL/6J mice (6 weeks old) were fed a high fat diet for 4 weeks, and a test strain or phosphate-buffered saline (PBS) was intravenously administered. Mice were euthanized at 8 or 12 weeks after administration. Measurements of whole body, extirpated liver, and visceral fat weights, as well as histopathological evaluations of liver specimens were performed. Mice infected with TW871 showed significantly greater values for body and liver weight than those administered MT8148, TW295, or PBS at both 8 and 12 weeks after infection ( $p < 0.05$ ). Furthermore, the mean values for body and liver weights at 12 weeks after infection with TW871 were greater than those at 8 weeks ( $p < 0.05$ ). Histopathological analyses revealed prominent infiltration of inflammatory cells and adipocellular deposition in livers extirpated 8 weeks after infection with TW871, while fibrosis was also observed in those extirpated after 12 weeks. In contrast, no obvious histopathological appearance related to steatohepatitis was seen in mice infected with MT8148 or TW295, similar to the PBS group. These results suggest that specific strains of *S. mutans* have potential to induce NASH conditions following bloodstream invasion.

This study was supported by the Grants-in Aid for Scientific Research (B) 23390472 from the Ministry of Education, Culture, Sports, Scientific and Technology of Japan.

## 16

### **Antimicrobial Activity of *Croton doctoris* Fractions against Mutans Streptococci and Lactobacilli Clinical Isolates**

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The activity of dichloromethane fractions obtained from ethanolic (ED) and hydroalcoholic (HD) extracts of *C. doctoris* S. Moore (Euphorbiaceae) against cariogenic microorganisms was recently found [Borges et al.: Caries Res 2012;46:282]. The aim of this study was to evaluate the antibacterial potential of these fractions against mutans streptococci and lactobacilli isolated from carious dentine (Ethical Board 087/2011-PH/CEP). Carious dentine was collected from ten children (mean age  $8 \pm 2$  years), transferred to PBS, sonicated (40 kHz, 50 W, 10 min) and diluted. Mutans streptococci were isolated in MSBS Agar and lactobacilli were isolated in Rogosa agar. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was assessed by microdilution method (concentration range 0.01–50 mg/ml). Chlorhexidine was used as positive control. For lactobacilli, MIC varied from 0.1 mg/ml (for at least 50% of the isolates) to 12.5 mg/ml in both fractions. MBC ranged from 0.2 to 50 mg/ml for up to 90% of isolates. For mutans streptococci, MIC varied from 0.8 (25% of isolates) to 25 mg/ml and MBC varied from 3.1 up to 50 mg/ml (25–63% of isolates). In conclusion, both fractions were active against clinical isolates. The fractions were more active against lactobacilli isolates. ED fraction showed slightly better performance for MBC against mutans streptococci.

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## 17

### **pH Is a Significant Determinant of Red Fluorescence Intensity in *Prevotella intermedia***

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Dental plaque, particularly that near the gingival margin, has the capacity to fluoresce red due to the accumulation of protoporphyrin IX by oral anaerobes such as *Prevotella* spp. Plaque pH can range from below 5.5 in caries to over pH 9 within the gingival crevice. The aim of this study was to determine the effects of this pH range upon red fluorescence in an oral bacterium known to produce protoporphyrin IX. *Prevotella intermedia* (ATCC 25611) was grown on anaerobic basal agar containing horse blood under anaerobic conditions (80% N<sub>2</sub>, 10% CO<sub>2</sub>, 10% H<sub>2</sub>). After 24 h incubation, colonial growth was swabbed from the plate and trans-



ferred to saline solution. The planktonic suspension was adjusted to a cell density of  $\sim 4.5 \times 10^6$  cfu/ml with further saline before being split to produce three aliquots of 10 ml. One of these aliquots was left untouched, whilst 10  $\mu$ l of 0.05 M or 0.1 M NaOH was added to the remaining two aliquots; the pHs of the aliquots was 5.74, 7.14 and 8.63 respectively. The emission spectra ( $\lambda_{em}$  550–750 nm) of the three bacterial suspensions were measured in a fluorescence spectrophotometer ( $\lambda_{ex}$  405 nm), being commensurate with the operational parameters of QLF-D. The peak fluorescence emission was at 634 nm for all three samples. The maximum fluorescence reading ( $\lambda_{em}$  634) was 71.2 (arbitrary units) at pH 5.74, which increased to 141.0 at pH 7.14 and peaked at 228.6 at pH 8.63. Increasing pH from 5.74 to 8.63 caused a 3.2-fold increase in peak fluorescence. This suggests that the normal physiological pH range within dental plaque could have a significant effect upon plaque assessment measurements based upon red fluorescence, such as QLF-D.

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## 18

### A Tissue-Dependent Hypothesis of Dental Caries

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Current understanding of dental caries considers this disease a demineralization of tooth tissues due to the acid produced by sugar-fermenting microorganisms. We present here the first metagenomic analysis of the bacterial communities present at different stages of caries development, with the aim of determining whether the bacterial composition and biochemical profile are specific to the tissue affected. We have performed pyrosequencing of DNA extracted from cavities at different stages of caries progression followed by bioinformatic analysis to determine the taxonomic and functional composition of those bacterial communities. The data show that microbial composition at the initial, enamel-affecting stage of caries, is different from that found at subsequent stages ( $p < 0.002$ , Unifrac distance test), and presents five times lower diversity than dental plaque of sound teeth surfaces. Although the relative proportion of *Streptococcus mutans* increased from 0.12% in dental plaque to 0.72% in enamel caries, *S. mitis* and *S. sanguinis* were the dominant streptococci in these lesions. *Prevotella* increased in dentin cavities between two and three times relative to its proportion in enamel lesions and *Lactobacillus* was found only at deep dentin cavities. The functional profile of caries-associated bacterial communities indicate that genes involved in acid-stress tolerance and dietary sugar fermentation are over-represented only at the initial stage (enamel caries), whereas other genes coding for osmotic stress tolerance as well as different proteases (including collagenases, sprT family metalloproteases, and omega peptidases) enabling dentin degradation are significantly over-represented in dentin cavities ( $p < 0.01$ , FDR test). The results support a scenario in which pH and diet are determinants of the disease

only during the degradation of enamel, when fermentable carbohydrates are available to acidogenic bacteria. We propose that caries disease is a process of varying etiology, in which acid-producing bacteria are the vehicle to penetrate enamel and allow dentin-degrading microorganisms to expand the cavity.

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## 19

### New Methodology for Studying the Structural Ecology of Occlusal Caries

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Microbiological studies of occlusal dental biofilms have hitherto been hampered by inaccessibility to the sampling site and demolition of the original biofilm architecture. The aim of the present study was to explore the spatial distribution of bacterial taxa in vivo at various stages of occlusal caries, applying a new methodology involving preparation of embedded hard dental tissue slices for fluorescence in situ hybridization (FISH) and confocal microscopy. 11 extracted teeth were included in the study and classified according to their occlusal caries status (active/inactive/sound; cavitated/non-cavitated). The teeth were fixed (paraformaldehyde 3%), embedded (Technovit 8100), sectioned and decalcified (EDTA 17%, pH 7.0) before FISH was performed using oligonucleotide probes for the most abundant species/genera associated with occlusal caries including *Streptococcus* and *Actinomyces*. The findings were related to histological features of lesion penetration. The sites showed distinct differences in the bacterial composition and fluorescence intensity between different ecological niches in occlusal caries. Biofilm observed along the entrance of fissures showed an inner layer of microorganisms organized in palisades often identified as *Actinomyces*, covered by a more loosely structured bacterial layer consisting of diverse genera, similar to supra-gingival biofilm. Biofilm within the fissure proper seemed less metabolically active, as judged by the low fluorescence signal intensity and the presence of material of non-bacterial origin resembling developmental protein, calculus and/or dead bacteria. Bacterial invasion with penetration into the dentinal tubules was seen only at advanced stages of the caries process with manifest cavity formation. It is concluded that the new methodology represents a valuable supplement to previous methods for the study of microbial ecology in caries by allowing analysis of the structural composition of the undisturbed biofilm in caries lesions in vivo.

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### Cariogenicity of *Candida albicans* Biofilms in the Presence of Fructose, Maltose and Galactose

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The cariogenicity of *C. albicans* biofilm in the presence of sucrose and glucose was evaluated previously [Pereira et al.: Caries Res 2012;46:282]. The aim of this study was to evaluate these features in the presence of other dietary sugars – fructose, maltose or galactose. The reference strain *C. albicans* ATCC 18804 and seven clinical isolates from caries lesions were included. Standardized suspensions containing 10<sup>7</sup> cells/ml were prepared in YNB (yeast nitrogen base medium, supplemented with 10, 50, 100, 200, 500 mmol · l<sup>-1</sup> fructose, maltose or galactose. Biofilms were developed on polystyrene, flat-bottomed 96-well plates. The medium was refreshed daily. After incubation at 37°C for 24, 48 and 72 h, the following evaluations were performed: (a) assessment of viable fungal cells by plating method; (b) analysis of culture medium pH by microelectrode pHmeter; (c) assessment of metabolic activity by XTT-menadione methodology. Data were analyzed by ANOVA and post-hoc Dunn (5%). Counts of viable cells (cfu/ml) were higher in presence of fructose after 24 h (1.6 × 10<sup>6</sup> ± 0.5 × 10<sup>6</sup>). After 48 h, the highest counts were observed for maltose (1.8 × 10<sup>6</sup> ± 0.4 × 10<sup>6</sup>). After 24 and 48 h of incubation, the lowest pHs were observed in biofilms formed in the presence of fructose. The highest metabolic activity was observed for maltose and fructose in the periods of 24 and 48 h, respectively. Dietary carbohydrates can modulate *C. albicans* biofilm and affect its cariogenicity. The results suggest that fructose may enhance cariogenic features in *C. albicans* biofilms.

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### Regulation of Ammonium Transport by Nitrogen Regulatory Protein in *Streptococcus mutans*

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Although ammonia is an excellent nitrogen source for many bacteria including *Streptococcus mutans*, its bioavailability is often a growth-limiting factor in natural environments. In Gram-positive bacteria, NrgA protein, encoded by the *nrgA* gene, controls the ammonium transporter that is required for the transport and utilization of ammonium at a low concentration. Analysis of the com-

plete genome of *S. mutans* UA159 in the Oralgen database (<http://oralgen.lanl.gov/oralgen-tng/>) indicated the *nrgA* gene as a possible *S. mutans* ammonium transporter and *glnB*, located upstream of the *nrgA* gene, as nitrogen regulatory proteins. In the present study, we analyzed the function of those genes in *S. mutans*. To specify the functioning unit or operon of *nrgA*, Northern blotting and PCR analyses were performed, while expression of the *nrgA* and *glnB* genes at a transcriptional level was examined under the influence of 2 mM glutamine using real-time quantitative reverse transcription (RT)-PCR. In addition, expression of the *glnB* gene was determined using isogenic mutant strains with inactivation of the *nrgA* gene (NRGD). Northern blot analysis for determination of transcriptional organization of the *nrgA* gene locus revealed a positive band, estimated to be 1,600 bp, PCR analysis confirmed that the band was located in the intergenic region between the *nrgA* and *glnB* genes. RT-PCR results also revealed that expressions of both *nrgA* and *glnB* were elevated approximately 4-fold in the presence of 2 mM glutamine as compared to in its absence. Furthermore, RT-PCR revealed that *glnB* gene expression was significantly reduced in NRGD. These results suggest that the *nrgA* gene is transcribed with the *glnB* gene and may have a relationship with ammonium transport.

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### Correlation between Cariogenicity and Bacteriocin Production in *Streptococcus mutans* Clinical Isolates

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*Streptococcus mutans*, known as a primary causative agent of dental caries, produces bacteriocins that inhibit the growth of similar or closely related oral bacteria. The purpose of the present study was to examine the distribution of 2 peptide bacteriocin genes, *smbA* and *nlmA* encoding SmbA and NlmA, respectively, and the correlation between cariogenicity and bacteriocin production in clinical isolates. Dental plaque samples were collected from either carious or non-carious sites in 20 Japanese children (age range: 3–14 years old). *S. mutans* organisms were grown in mitis salivarius agar plates containing bacitracin (100 U/ml), then selected based on their rough colony morphology. Chromosomal DNA was extracted from the strains, and *smbA* and *nlmA* were amplified by PCR using specific primers for each. A total of 273 strains from 20 carious sites and 190 from 20 non-carious sites were isolated. The *nlmA* gene was detected in many of the *S. mutans* strains isolated from both carious (30.8%) and non-carious sites (22.6%), whereas *smbA* was found in only a few strains and both were rarely detected in the same specimen. In addition, the odds ratio for detection of strains with either gene from carious sites was 1.57 (95% confidence interval 1.06–2.33) as compared to

the presence of either in strains from non-cariou sites. On the other hand, the *smbA* gene was detected at a higher rate in strains from cariou sites. These results suggest that bacteriocin has a relationship with cariogenicity and NlmA is a major type of bacteriocin in *S. mutans* strains. In addition, *SmbA* may be associated with the advance of dental caries of *S. mutans*.

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### Initial Bacterial Adhesion and Biofilm Formation on Resin-Infiltrant Compared to Sound and Cariou Enamel

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The infiltration of non-cavitated cariou lesions has become popular and effective over the last years. While this method is clinically superior to an improved oral hygiene regimen only, it has not been investigated whether the infiltrated surface might be a preferential site for biofilm formation. The aim of this in situ study was to compare the initial (30 and 120 min) bacterial adhesion and the long-term (72 h) biofilm formation of oral bacteria on the surfaces of pure resin-infiltrant, sound enamel, cariou enamel, and infiltrated enamel. For all investigations, bovine enamel specimens were used (diameter 5 mm) and also specimens of purely resin-infiltrant (diameter 5 mm). The enamel was left sound for group 1, demineralized via Buskes' solution for group 2, infiltrated after the demineralization for group 3, infiltrated although sound for group 4, and made up of purely resin-infiltrant for group 5. These specimens were placed into intra-oral splints and worn by healthy volunteers for 30 min, 120 min, or 72 h, respectively. The splints were removed after the respective period and all specimens were immediately prepared for evaluation. To detect bacterial adhesion, the following methods were used: fluorescence in situ hybridization (FISH), DAPI staining, live/dead staining, colony forming units (CFU), and/or confocal laser scanning microscopy (CLSM). Initially, there were no statistically significant differences between the five groups ( $p > 0.05$ ) but in each group bacterial adhesion was significantly higher after 120 min ( $p < 0.01$ ). The biofilm formation after 72 h was significantly increased in half of the volunteers ( $n = 3$ ,  $p < 0.05$ ) on the infiltrated specimens compared to the demineralized specimens (24.00  $\mu\text{m}$  vs. 31.71  $\mu\text{m}$ ). While the surface integrity did not play a crucial role for the initial adhesion of oral bacteria, it has not been investigated whether the infiltrated surface might be a site for preferential biofilm formation. But as only half of the specimens were affected from this phenomenon after 72 h, the results need to be confirmed for major biofilm formation in further studies. Good oral hygiene remains an important factor for the efficacy of the resin infiltration in the long run.

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### Effect of a Toothpaste Containing S-PRG Filler on the Metabolism of Polymicrobial Biofilms

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Surface pre-reacted glass ionomer (S-PRG) filler is a novel particle that can be incorporated into dental materials and releases various ions such as F, B, Sr, Si. This study investigated the effect of the toothpaste containing S-PRG filler on biofilms formed on dentin using a polymicrobial biofilm model [Exterkate et al.: Caries Res 2010;44:372–379]. Finely finished bovine root dentin specimens were used as substrata. Stimulated whole saliva obtained from a single donor was inoculated anaerobically in McBain medium supplemented with 0.2% sucrose. The medium was refreshed twice daily. The specimens received a single treatment after 72 h and biofilm formation continued for 5 days. Treatment groups were: deionized water (D), toothpaste containing 950 ppm F (NaF) (950F) and toothpaste containing S-PRG filler (SPRG). The toothpastes were diluted three times with deionized water and then, specimens with biofilms were immersed in toothpaste supernatants for 5 min. Output parameters were pH of spend medium (pH), CFU counts of bacteria in biofilms (CFU) and integrated mineral loss (IML: vol%  $\times$   $\mu\text{m}$ ). The differences among the groups were tested by one-way ANOVA followed by Tukey's multiple range test ( $p < 0.05$ ). This experiment was repeated. The experimental groups showed different mineral profiles. Vol% mineral of surface and lesion body were higher for SPRG (surface: 33.6, lesion body: 35.2) compared to the 950F (surface: 23.0, lesion body: 22.8). Also, there were significant differences between the IML of SPRG and other groups (D: 4,221.3, 950F: 4,283.1, SPRG: 1,809.8). However, there were no significant differences regarding pH (D: 5.29, 950F: 5.17, SPRG: 5.14) and CFU (D:  $3.93 \times 10^8$ , 950F:  $4.58 \times 10^8$ , SPRG:  $1.96 \times 10^8$ ), between 950F and SPRG at 192 h.

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## Active Microbiota and Gene Expression Dynamics in the Human Oral Biofilm

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Micro-organisms inhabiting teeth surfaces grow on biofilms where a specific and complex succession of bacteria has been described by co-aggregation tests and DNA-based studies. Although the composition of oral biofilms is well established, the active portion of the bacterial community and the patterns of gene expression in vivo have not been studied. Using RNA-sequencing technologies, we present the first metatranscriptomic study of human dental plaque during biofilm formation and after ingestion of a meal. Sequence analysis of more than 398 million reads revealed that microbial communities are individual-specific and none bacterial species was detected as key player at any time during biofilm formation. However, *Streptococcus* and *Actinomyces* were found on average at 12–19% and 3–12% respectively, in different biofilm time samples. Multiple putative synergies and antagonistic interactions were depicted that need further considerations for future studies and their role in oral biofilm formation. For instance, several genera appear to have an antagonistic effect on Actinobacteria, particularly *Clostridium*, *Veillonella*, *Haemophilus* and above all *Aggregatibacter*, which showed the most significant negative correlation with *Actinomyces* (Spearman's rho parameter = -0.76). Finally, we detected the expression of 19,500 genes on average per sample, and could discern some gene expression patterns characteristic for early and mature oral biofilms. Specifically, 35 functional categories were found to be over-represented in early biofilm samples and 236 in mature biofilms. We confirmed by qPCR the over-expression pattern of several adhesion genes in early biofilm including *sspA* and *sspB* in *Streptococcus* and the type 2 fimbriae-associated genes *srtA* and *fimA* from *A. naeslundii*. Once the biofilm was formed, specific changes were observed when comparing the metatranscriptome of the same individual before and after a meal, which can narrow down the list of organisms responsible for acid production and therefore potentially involved in dental caries.

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### In vitro Biofilm Formation: NaF Exposure within a Sucrose Pulsing Strategy

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Frequent fermentation events within oral biofilms result in the production of organic acids which alter their immediate environment and, in turn, may trigger self-induced species selection to-

wards a community with a greater cariogenic potential. Further, fluoride inhibits dental decay chemically but may also have some influence on microbial selection. Thus, twice-daily NaF exposures (300 ppm F<sup>-</sup>) vs. dH<sub>2</sub>O were investigated in conjunction with a sucrose pulsing strategy (50 mM for 15 min every 2 h over 16 h of a 24 h cycle) using a dual constant-depth film fermenter (dCFFF) model (shared microcosm inoculum) with the purpose of investigating the capacity of fluoride to augment the selective process. 200 µm biofilms, produced 5 mm on hydroxyapatite, were sampled in triplicate every 48 h and bacterial composition quantified per unit volume (mm<sup>-3</sup>) and compared by ANOVA for mutans streptococci (MS; TYCSB agar), total streptococci (TS; MSA agar), lactobacilli (LB; Rogosa agar) and volatile sulphur compound producers (VSCP; OOPSII agar). TS, LB and VSCP showed exponential growth trends in both conditions although approximately 60% of CFU were recovered under NaF exposure. In the latter stages (144–192 h) these trends did not achieve significance for VSCP ( $p = 0.1559$  and  $p = 0.2907$ ). In the absence of NaF, MS growth reached a climax at 144 h ( $7.55 \log \text{CFU} \cdot \text{mm}^{-3} \pm 0.085$ ). In its presence MS growth was suppressed in comparison by condition ( $p < 0.05$ ); however differences were no longer significant by the end of the experiment (192 h;  $p = 0.2128$ ). In conclusion, results demonstrate the adaptive capacity of multispecies biofilms and that NaF exposures have a transient albeit limited effect on community structure. However, further work is needed in order to establish the degree of NaF sensitivity over longer periods of time.

G.J.O. is the recipient of a GlaxoSmithKline PhD Studentship awarded to the University of Liverpool.

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### Metatranscriptomic Analysis of *Veillonella* spp. in Carious Dentine

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To better understand the basis for the differential intra-oral distribution of *V. parvula*, *V. atypica* and *V. dispar* we used a metatranscriptomic. Eleven independent samples (cariou dentine and wax stimulated saliva) were obtained for each group and total RNA was extracted (>100 ng per sample). RNA was processed using the Illumina<sup>®</sup> TruSeq<sup>™</sup> RNA Sample Preparation Kit and 76 bp paired end sequencing was carried out. FASTQ files were imported into CLC Genomic Workbench and the reads mapped to individual genes in 150 annotated bacterial genomes. Reads mapping to *V. parvula* DSM2008, *V. dispar* ATCC 17748 and *V. atypica* ACS-0049-V-Sch6 were extracted, rRNA genes reads were removed, the level of expression genes was calculated as (number of transcript reads count/gene length [in kbases]/total reads count [in millions]) and compared using t-tests and a FDR correction (Benjamini and Hochberg) applied with  $p < 0.01$  regarded as significant. In the carious lesions  $16.62 \pm 11.17\%$ ,  $2.18 \pm 1.13\%$  and  $0.91 \pm 0.43\%$  of the mapped reads were assigned to *V. parvula*, *V. dispar* and *V. atypica*, respectively compared to  $4.76 \pm 7.21\%$ ,  $7.08 \pm$

5.07% and  $4.09 \pm 3.47\%$  in the stimulated saliva samples (all  $p < 0.05$ ). 148 genes were significantly up-regulated in *V. parvula* in the carious lesions while only 45 were up-regulated in *V. dispar* and 37 in *V. atypica*. Most genes involved in histidine biosynthesis pathway were significantly up-regulated in *V. parvula* but not in the other two species. Of particular importance is the up-regulation of ATP phosphoribosyltransferase (EC 2.4.2.17) which catalyses the condensation of ATP and 5-phosphoribose 1-diphosphate to form N'-(5'-phosphoribosyl)-ATP which has a central role in the His pathway. The His operon was significantly up-regulated in *Lactobacillus casei*, in response to acid adaptation (20 min at pH 4.5), increased intra-cellular levels of His contributes to intracellular buffering as the imidazole groups of His have a  $pK_a$  around 6.0. Metatranscriptomic analysis of *Veillonella* spp. was achieved with oral samples and provided some evidence for the intra-oral distribution of members of this genus.

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### Phenotyping of *Streptococcus mutans* from Saliva, Plaque and Carious Dentine by MALDI-TOF-MS

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The pheno-/genotypic adaptation of *Streptococcus mutans* to planktonic or biofilm environment is discussed. Samples of stimulated saliva, plaque and carious dentin from 30 adult patients were plated on mitis salivarius agar with bacitracin, and *S. mutans* colonies were selected. Isolates were cultured for 24 h at 37°C in 10 ml Balmelli's broth, harvested by centrifugation, and classification was confirmed by species-specific polymerase chain reactions (gtf B, gtf S, 16S rRNA genes). Finally,  $10^7$  *S. mutans* cells were prepared for matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) by washing in ethanol and resuspension in formic acid/acetonitrile. After centrifugation, 1 µl of supernatant was placed on a stainless steel target and overlaid with matrix (HCCA in acetonitrile/2.5% TFA). Acquired MALDI-TOF peptide-spectra (mass range: 2–20 kDa) were compared by hierarchical clustering and support vector machines for intra- and interindividual heterogeneity of phenotypes. Overall, 449 *S. mutans* strains (saliva: 167, plaque: 172, dentin: 110) were isolated from 27 patients. Eleven patients harbored *S. mutans* in all three, 9 patients in two and 7 patients in one of the compartments. While identified phenotypes differed between the patients, one to seven *S. mutans* phenotypes were detected in each patient. In patients harboring *S. mutans* in saliva, plaque and carious dentin compartment-dominating phenotypes were found in seven of eleven patients. Phenotypes were evenly distributed in four patients. The species *S. mutans* is able to form different phenotypes in plaque, saliva and carious dentin. However, no specific phenotypes were found for any of the compartments. Intraindividual differences of

phenotypes were smaller than interindividual ones. Further investigation will be necessary to understand mechanisms of *S. mutans* phenotype differentiation in relation to caries process.

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### Competition between *Streptococcus oligofermentans* and *Streptococcus mutans* on Biofilm Formation and Biofilm Acidogenicity

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Biofilm acidogenicity is one of the key factors in caries formation. Previous studies showed that *Streptococcus oligofermentans*, isolated from caries-free subjects, could inhibit the growth of cariogenic *Streptococcus mutans* by hydrogen peroxide production in both suspensions and biofilms. The aim of this study was to examine the overall biofilm formation and biofilm acidogenicity when the two species were growing together. To this end, single and dual-species biofilms of *S. oligofermentans* LMG22279 and *S. mutans* UA159 were formed in nutrient-poor buffered media (pH 7.0), supplemented with 0.2% sucrose, in an active attachment biofilm model for 48 h. Biomass of the biofilms was examined by a crystal violet staining method and the metabolic activity was evaluated by a resazurin assay. Biofilm acidogenicity was determined by measuring the lactic acid production of 48 h old biofilms. Lactate concentration was measured with an enzymatic method after 2 h incubation in a pH 7.0 buffer containing 1% glucose. Data was analyzed by one-way ANOVA. The biomass of the dual-species biofilms ( $0.3 \pm 0.1$ ) was significantly lower than that of single *S. mutans* biofilms ( $1.1 \pm 0.1$ ) ( $p < 0.01$ ), but similar to the single *S. oligofermentans* biofilms ( $0.2 \pm 0.1$ ). The resazurin assay showed that all three biofilms were metabolically active. Again, single *S. mutans* biofilms had the highest metabolic activity. Lactate production was only found in single *S. mutans* biofilms ( $2.1 \pm 0.4$  mM/biofilm), but undetectable in other two types of biofilms. In conclusion, the competition between *S. oligofermentans* and *S. mutans* in biofilms resulted in lower biomass and reduced lactate production.

### A Pilot Cohort Study of Caries Progression, Bacterial Diversity and the Immune Response in Children

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**Aim:** To investigate the relationships between the progression of caries, the oral microbiome and immune responses in young children. **Experimental Approach:** Children received dental examinations when aged 4 years, then again aged 5–6 years. Children were grouped according to presence of caries at neither time point (NN; n = 20); only at second time point (NY; n = 10); or at both time points (YY; n = 20). Saliva was collected at the second time point. Bacterial DNA was extracted and bacterial diversity determined by 16S PCR and GS-FLX pyrosequencing. Salivary concentrations of antimicrobial peptides and anti-bacterial IgA were assessed by ELISA. **Results:** There was no significant difference in bacterial diversity between the groups, NN, NY or YY, which had a Shannon diversity index of 4.28, 4.43 and 4.26 respectively. Streptococci were the most abundant genera (70%), and species were identified using a curated database of all complete 16S rRNA sequences. *S. mitis* was the most abundant species (70.4–66.1%, greatest in the NY group,  $p < 0.05$ ). There were similar and low levels of *S. mutans* in all groups (0.3–0.1%). Lactoferrin was greatest, and calprotectin and LL37 lowest in YY participants compared with NN and NY ( $p < 0.05$ ). The NY group demonstrated the lowest titres of anti-*S. mutans* salivary IgA ( $p < 0.001$  vs. YY or NN) and *S. mitis* (ns). Principal component analysis demonstrated some grouping of the NN/NY and YY groups, with the NY participants most commonly placed between the NN and YY groups. **Conclusion:** These data suggest a dominance of *S. mitis* associated with caries, and that salivary lactoferrin may be elevated and calprotectin and LL37 lower in children with caries.

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### *Streptococcus mutans* and *Streptococcus sanguinis* Levels according to ICDAS II Criteria in Chilean Preschool Children

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The aim of this study was to examine the colonization of *S. mutans* and *S. sanguinis* in the oral cavity and its relation to caries prevalence according to ICDAS II criteria in Chilean preschool children. A cross-sectional study with a total of 269 randomly selected patients aged 2–3 years that belonged to nursery schools in the Metropolitan Region, Santiago, Chile, was carried out. After informed consent was obtained, caries prevalence was determined by clinical examination according to ICDAS II criteria, code 1 was excluded as exams were performed at nursery schools. Non-stimulated whole saliva samples were collected and after serial dilution were seeded on agar plates to culture *S. mutans* (selective mitis salivarius-bacitracin agar) and *S. sanguinis* (MM10 sucrose blood agar). The plates were incubated anaerobically at 37°C in 5% CO<sub>2</sub> for 48 h (*S. mutans*) and 72 h (*S. sanguinis*) and then examined under magnification. Colonies were phenotypically identified as described in Little et al. [J Clin Microbiol 1977;5:578–583] and Syed and Loesche [Appl Microbiol 1972;24:638–644] and quantified to estimate the number of colony forming units (cfu/ml). As part of the descriptive phase of this study, data was analyzed by calculating total mean bacteria using STATA 11.0 software. The mean cfu/ml counts of *S. mutans* and *S. sanguinis* was  $2.08 \times 10^7$  (95% CI:  $1.28 \times 10^7$  to  $2.89 \times 10^7$ ) and  $3.33 \times 10^7$  (95% CI:  $2.65 \times 10^7$  to  $4.00 \times 10^7$ ) respectively in the caries-free group (ICDAS = 0; n = 140). The mean cfu/ml counts of *S. mutans* and *S. sanguinis* was  $2.57 \times 10^7$  (95% CI:  $1.75 \times 10^7$  to  $3.39 \times 10^7$ ) and  $3.08 \times 10^7$  (95% CI:  $2.42 \times 10^7$  to  $3.75 \times 10^7$ ) respectively in the moderate caries group (ICDAS = 2–4; n = 67). The mean cfu/ml counts of *S. mutans* and *S. sanguinis* was  $4.35 \times 10^7$  (95% CI:  $3.01 \times 10^7$  to  $5.70 \times 10^7$ ) and  $3.27 \times 10^7$  (95% CI:  $2.60 \times 10^7$  to  $3.95 \times 10^7$ ) in the severe caries group (ICDAS = 5–6; n = 62). All groups presented high levels of *S. mutans* and *S. sanguinis* counts. The lowest cfu/ml counts for *S. mutans* were found in caries-free patients (ICDAS = 0) and the highest cfu/ml counts for the same species were found in the severe caries group (ICDAS = 5–6).

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### Acid Production by *Streptococcus mutans* and Bifidobacteria Associated Biofilms Exposed to Different Carbohydrates

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The aim of this study was to evaluate the acid production by *Streptococcus mutans* and bifidobacteria associated biofilms exposed to different carbohydrates. Biofilms were grown on glass discs from a standardized suspension (OD<sub>620</sub> = 0.7, diluted 1:50) of *S. mutans* UA159 for 24 h in a 24-well plate. Then the discs were transferred to a new plate with a standardized suspension (as mentioned above) of one of the following bifidobacteria: *B. dentium* DSM 20436, *P. denticolens* DMS 10105 or *S. inopinata* DMS 10107 and incubated for 24 h. The biofilms were washed twice and depleted of endogenous reserves of carbohydrates by incubation for 30 min at 37°C. The biofilms were incubated for 3 h in a plate containing SDM medium + 10 mM of carbohydrate (glucose, lactose, raffinose, sucrose) or water. Concentrations (mM) of organic acids (acetic, butyric, formic, lactic, propionic) were determined by capillary electrophoresis. More expressive concentrations of acetic acid were produced in presence of glucose, raffinose and sucrose. Higher concentrations of lactic acid were produced with glucose and sucrose. Concentrations of acetic acid for glucose, raffinose and sucrose were respectively: *S. mutans* (0; 0; 0), *S. mutans* + *L. acidophilus* (0.38 ± 0.1; 0.53 ± 0.07; 0.22 ± 0.06), *S. mutans* + *B. dentium* (0.11 ± 0.04; 0.22 ± 0.05; 0.11 ± 0.03), *S. mutans* + *P. denticolens* (0.34 ± 0.04; 1.24 ± 0.15; 0.34 ± 0.05), *S. mutans* + *S. inopinata* (0.55 ± 0.01; 1.46 ± 0.10; 0.38 ± 0.08). Concentrations of lactic acid for glucose and sucrose were respectively: *S. mutans* (2.96 ± 0.20; 2.25 ± 0.46), *S. mutans* + *L. acidophilus* (2.15 ± 0.46; 3.23 ± 1.27), *S. mutans* + *B. dentium* (2.51 ± 0.14; 2.71 ± 0.12), *S. mutans* + *P. denticolens* (9.50 ± 0.96; 12.60 ± 1.05), *S. mutans* + *S. inopinata* (9.23 ± 0.28; 11.79 ± 0.70). The association between *S. mutans* and *P. denticolens* or *S. inopinata* produced increased concentrations of lactic acid in presence of glucose or sucrose.

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### In situ Evaluation of Antibacterial Dental Materials Containing Nanosilver and Quaternary Ammonium Monomer

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Cariogenic bacteria from biofilms around restorations are the main reason for dental restoration failure and replacement. The objective of this in situ study was to investigate antimicrobial effects of the incorporation of silver (NAg) and quaternary ammonium monomer (DMADM) nanoparticles into direct restorative dental materials. NAg and DMADM were incorporated into primer and adhesive components as well in nanocomposite at 0.1% and DMADM at 10% by mass. Sixteen subjects wore an acrylic device containing four bovine enamel discs with drilled holes restored with control or NAg+DMADM-containing restorative materials. Using a split-mouth design, the enamel discs were subjected to 20% sucrose solution (8×/day) to induce a cariogenic challenge. Biofilms accumulated over the discs for 7 and 14 days were collected and microbiologically analyzed by culture for total viable microorganisms, total streptococci, mutans streptococci, and lactobacilli. A viability fluorescence stain was performed to biofilm accumulated for 1, 3, 7 and 14 days. The two sample independent Student's t-test was used to evaluate the results ( $\alpha = 5\%$ ). The tested restorative materials resulted in a significant reduction of numbers of mutans streptococci at 7 day evaluation as compared to control (1.91 ± 1.70 and 4.36 ± 3.42, respectively, mean ± SD expressed by 10<sup>5</sup> CFU/mg biofilm). In contrast, similar bacterial viability and total bacterial numbers were observed at 14 day evaluation with regard to the total number of microorganisms, total streptococci, mutans streptococci, and lactobacilli ( $p > 0.05$ ). It can be concluded that NAg+DMADM incorporation in restorative materials has potential to inhibit caries-associated bacteria around restored tissue, however their effects on biofilm ecology are related to biofilm degree of maturation. The novel method of combining antibacterial agents (NAg and DMADM) may enable new strategies for caries control by reduction of mutans streptococci around restorations.

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### CovR and VicRK Regulate Cell Surface Biogenesis Genes Required for Biofilm Formation in *Streptococcus mutans*

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**Aim:** The two-component system VicRK and the regulator CovR of *S. mutans* co-regulate a group of virulence genes associated with the synthesis of and interaction with extracellular polysaccharides of the biofilm matrix. Here we investigated the contribution of five genes controlled by CovR or VicR in biofilm formation. Single gene nonpolar deletions were performed in genes SMU.609, SMU.1090, SMU1437c, SMU.2146c and SMU.2147c of strain UA159 (WT). Putative lytic and autolytic activities of genes were evaluated in WT and mutants. Gene expressions were evaluated in biofilm developed in polystyrene plates containing BHI (w/out sucrose) during 4 h. Extracellular DNA released during biofilm formation was measured by qPCR. **Results:** The five novel proteins analyzed in this study have domains suggestive of functions linked to murein biogenesis or cell surface structure. The loss of genes promoted significant alterations in biofilm initiation, including increased fragility, defects in microcolony formation, atypical cell morphology and/or chaining. Expression of vicR, covR and targets (gbpB, wapE, smaA, lysM, SMU.2146c) are up-regulated up to 4-fold during biofilm initiation, in a sucrose-dependent manner. Mutants with impaired lytic activity on cell wall (SMU.1090- and SMU.2147c-) showed significantly lower amounts (2- and 3-fold, respectively) of eDNA compared to parent. SMU.609- and SMU.2146c- mutants released more eDNA (6- and 18-fold, respectively) during biofilm formation. **Conclusions:** These data support a model in which VicRK and CovR coordinate cell division and surface biogenesis, a process apparently linked with formation and structuring of stable biofilms in the presence of sucrose.

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### Caries Prevalence and *Streptococcus mutans* in Saliva of Vietnamese Schoolchildren

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The aim of this study was to quantify oral streptococci and *Streptococcus mutans* in saliva and to evaluate the association of the amounts of bacteria with caries experience. 94 6-year-old schoolchildren in Ho Chi Minh City, Vietnam were examined for caries status (dmf-t, WHO 1997) and stimulated saliva samples were collected. Genomic bacterial DNA was extracted using the QIAamp DNA Mini Kit (Qiagen). The DNA level of total bacteria, oral streptococci and *S. mutans* were quantified using TaqMan real-time PCR (7900HT, Applied Biosystems) with absolute standard curve method and converted to bacterial cells/ml saliva. The results showed that the levels of bacteria varied widely among subjects. There was no significant difference in the number of total bacteria, oral streptococci and the proportion of oral streptococci between caries free (n = 28, dmf-t = 0) and caries group (n = 66, dmf-t ≥ 1). *S. mutans* was not detected in saliva of 5 subjects with a detection limit of approximately 20 bacterial cells in the PCR reaction mixture. The mean amount of *S. mutans* (cells/ml saliva) in caries-free and caries group was  $8.5 \times 10^5$  (SD  $13.2 \times 10^5$ ) and  $1.28 \times 10^7$  (SD  $2.61 \times 10^7$ ), respectively. Significant difference was found between caries-free and caries subjects in both the number and the proportion of *S. mutans* to total bacteria and to oral streptococci (Mann-Whitney test, p < 0.01). There was a positive correlation of dmf-t with both the number of *S. mutans* (r = 0.517) and the proportion of *S. mutans* to total bacteria (r = 0.550) and to oral streptococci (r = 0.589) (Spearman's rank test, p < 0.01). This suggested that *S. mutans* seems to be associated with caries experience in the studied population.

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### Pilot Longitudinal Analysis of Caries-Associated Biological Risk Factors in Children Aged One to Three Years

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**Aim:** To develop methodologies to longitudinally investigate the relationship between the development of the cariogenic oral biofilm coincident with development of salivary immune responses. **Experimental Approach:** Children aged 1 year were recruited to the study from Childsmile, a Scottish dental public health improvement programme. Dental plaque and unstimulated saliva were collected from children at baseline and at follow-up aged 3 years, at which time a dental examination was also performed. Dental plaque was investigated by TaqMan<sup>®</sup> qPCR for the presence of *S. mutans* and saliva was investigated by ELISA for the presence of lactoferrin, LL37, HNPs 1–3, calprotectin and sIgA antibodies specific for a panel of oral streptococci. **Results:** At baseline 63 children were recruited to the study, of whom 23 were successfully followed up. The proportion of *S. mutans* in dental plaque increased from 0.002% in 1-year-olds to 2.66% in 3-year-olds ( $p < 0.001$ ). Coincidentally, mean salivary concentrations of HNPs 1–3 increased from 108.8 to 499.0 ng/ml ( $p = 0.008$ ) and calprotectin from 504.2 to 676.4 ng/ml ( $p = 0.030$ ). Furthermore, mean concentrations of sIgA antibodies specific for oral streptococci increased over time: *S. mutans* (246 to 382 EU,  $p = 0.049$ ), *S. mitis* (265 to 604 EU,  $p = 0.001$ ) and *S. sanguinis* (249 to 484 EU,  $p = 0.009$ ). Ninety-one percent ( $n = 21/23$ ) of children at age three were found to be caries-free according to BASCD criteria. **Conclusions:** In this pilot study successful methodologies were developed which allowed for detection of cariogenic bacteria and salivary antimicrobial proteins in young children. *S. mutans* colonises the oral cavity of young children at a time when they are relatively immunologically immature. As colonisation increases, salivary immune responses undergo maturation as indicated by increases in salivary antimicrobial peptides and sIgA antibodies specific for oral streptococci.

This work was funded by the Scottish Government Health Department.

### The Effect of 1% Chlorhexidine Varnish on the Prevention of Caries in Erupting Permanent Molars

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The aim of this clinical study was to evaluate the effect of 1% chlorhexidine thymol varnish (Cervitec Plus<sup>®</sup>, Ivoclar Vivadent) on (a) oral health and (b) levels of cariogenic bacteria, in patients with  $\geq 1$  erupting first (1<sup>st</sup>M) or second permanent molars (2<sup>nd</sup>M), 1 year after full occlusion. A control group of similar age participated in a standard preventive program. Subjects were allocated randomly to receive chlorhexidine varnish every 3 months (CHX group) or fluoride and placebo varnish, alternatively, every 3 months (F group). Plaque accumulation (Hygiene Index [Lindhe: Nor Tannlaegeforen Tid 1981;91:357–358]), caries [Carvalho et al.: J Dent Res 1989;68:773–779, modified] and levels of mutans streptococci (MS) (CRT test, Ivoclar Vivadent<sup>®</sup>) were recorded, at baseline and 12 months. The sample consisted of 184 children with mean age of 8.7 years (SD 3.0 years) (110 with 1<sup>st</sup>M and 74 with 2<sup>nd</sup>M). At baseline, approximately 90% of tooth surfaces were plaque-free and remained so during the program. From baseline to 1 year, the number of children with no detectable MS ( $<10^3$  cfu/ml) increased from 22.8% to 56.5% in the CHX group but by significantly less ( $p < 0.001$ ), from 18.5% to 28.3%, in the F group. The dmfs scores for children with 1<sup>st</sup>M were: CHX group, mean = 6.93 (SD = 11.73) at baseline vs. 7.14 (11.90) at 1 year, and F group, mean = 12.27 (15.82) vs. 12.31 (15.79), respectively. DMFS scores for the older group of children were: CHX, 1.53 (3.89) vs. 1.59 (3.87) and F group, 1.75 (3.75) vs. 2.0 (3.77). The 3-monthly application of CHX varnish could significantly reduce MS levels in saliva. However, no significant differences between F and CHX groups were seen in the caries experience at 1 year vs. baseline, for either molar.

The study was partially funded by Ivoclar-Vivadent.

## Session 2

# De- and Re-Mineralization and Caries Risk

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### Caries Risk in Children Aged 12–23 Months in Connection with Toothpaste Active Ingredients

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The aim was to assess caries risk in young children in connection with toothpaste active ingredients. The study was approved by Regional Ethics Committee. 45 caries-free children aged 12–23 months (mean 16.6) who had not had oral hygiene were enrolled. The mothers' informed consents were obtained. The mothers were trained in tooth brushing for their children. The children were randomly assigned to 3 groups (15 children in each) for home tooth brushing using toothpastes with different active ingredients: fluoride 250 ppm F<sup>-</sup> (F2), fluoride 500 ppm F<sup>-</sup> (F5), xylitol (X). Simplified biofilm index was used to reveal dental plaque. Dentocult LB and Dentocult SM kits (Orion Diagnostica, Finland) were used to reveal salivary Lactobacilli (LB) and *Streptococcus mutans* (SM) levels, colony counts of >10,000 CFU/ml were considered high. Children with visible dental biofilm and high level of LB and SM were considered high caries risk (HCR) children. Dental biofilm and the levels of LB and SM were estimated at baseline and after 3 months. Statistical analysis was performed using STATISTICA-6; mean, 95% confidence interval (CI), and relative risk (RR) were calculated, comparisons were assessed by Student t-test and McNemar test. At baseline percentage of HCR children was 20.0% in F2, 40.0% in F5, 26.7% in X, after 3 months 20.0%, 13.3% and 33.3% respectively. In the F5 amount of HCR children decreased by 26.7% (CI 15.2–38.0), RR = 0.69 (CI 0.43–1.09); in the F2 amount of HCR children remained the same, 20.0% (CI 9.7–30.3), RR = 1 (CI 0.75–1.32); in the X amount of HCR children increased by 6.7% (CI 0.3–13.1), RR = 1.3 (CI 0.86–1.95). The differences between the groups were not statistically significant,  $p > 0.05$ . Toothpaste with 500 ppm F<sup>-</sup> was superior over toothpaste

with 250 ppm F<sup>-</sup> or xylitol in decreasing high caries risk connected with dental biofilm, high salivary LB and SM levels in young children, but the differences were not significant.

The study was supported by Volgograd State Medical University.

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### Influence of White Spot Lesions Treatment and Dental Bleaching on Opacity and Fluorescence of Enamel

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**Aim:** The aim of this study was to evaluate the effects of different white spot lesions treatments associated with dental bleaching on opacity and fluorescence of dental enamel. **Methods:** Eighty flat enamel disks (3 mm diameter and 1 mm thick) were obtained from bovine incisors. The initial (INI) opacity (translucency parameter or TP units) and fluorescence (fluorescent emission or DeltaE\*ab-FL units) of the samples were measured using a spectrophotometer (CM-2600d, Konica-Minolta). Artificial caries (CAR) were created in all samples and the measurement was repeated. The specimens were divided into four groups, according to the treatment applied ( $n = 20$ ): CON (control) – immersion in ultrapure water for 8 weeks; SAL immersion in artificial saliva for 8 weeks; FL daily application of 0.05% sodium fluoride for 1 min/artificial saliva for 8 weeks; and ICON-resin infiltration (Icon<sup>®</sup>, DMG). After the treatments (TRE), the assessment was repeated. Dental bleaching (BLE) using 35% hydrogen peroxide gel was performed in all samples for 30 min and the measurements were performed after

1 week. The data were submitted to ANOVA and Tukey's tests across the treatments for each moment of evaluation. **Results:** Opacity: For INI ( $p = 0.876$ ) and after CAR ( $p = 0.373$ ) there were no significant differences. After TRE ( $p = 0.000$ ): ICON-13.76a, FL-17.21a, CON-23.50b, SAL-24.25b. After BLE ( $p = 0.012$ ): ICON-12.45a, FL-13.13ab, SAL-14.12ab, CON-14.57b. Fluorescence: For INI ( $p = 0.143$ ) and after CAR ( $p = 0.134$ ) there were no significant differences. After TRE ( $p = 0.000$ ): CON-0.90a, SAL-1.01a, FL-1.14a, ICON-2.13b. After BLE ( $p = 0.280$ ) there were not significant differences among the groups: CON-0.73a, SAL-0.80a, FL-1.01a, ICON-0.76a. **Conclusions:** The treatments with ICON and FL produced a higher opacity than the control group. After the BLE, just the ICON group showed a higher opacity in relation to the control. Only the treatment with ICON showed higher fluorescence than the control group, but this difference disappear after the bleaching.

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### Anti-Cariou Effect of Cerium Chloride on Initially Demineralised Enamel

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This in vitro study aimed to determine the potential of cerium chloride to reduce artificial caries mineral loss and lesion depth progression on pre-demineralised enamel. Eighty enamel samples were prepared from 20 bovine lower central incisors. Crowns were sectioned in four pieces, embedded in acrylic resin, ground flat from the labial aspect and each one piece per tooth was randomly allocated to one out of four groups (A–D;  $n = 20$  per group). Specimens were stored in a demineralizing buffer solution [Buskes et al.: Caries Res 1985;19:490–496] to induce artificial caries lesions. After 7 days, samples were treated for 30 s with one of the following solutions: placebo (water; A), amine fluoride (Elmex fluid; B), cerium chloride (10%; C) and combined fluoride/cerium chloride application (D). After another 7 days in demineralizing buffer solution, the integrated mineral loss ( $\Delta Z$ ) and lesion depth (LD) was determined by transversal microradiography and compared by Scheffé's post hoc tests ( $p \leq 0.05$ ). The statistically highest mineral loss ( $\Delta Z$ ) and lesion depth (LD) was observed for the placebo group A ( $4,098 \pm 346$  vol% $\mu\text{m}$  and  $181 \pm 9$   $\mu\text{m}$ , mean  $\pm$  SD). The significantly lowest  $\Delta Z$  and LD were observed for samples treated with the combined fluoride/cerium chloride (group D) ( $2,993 \pm 209$  vol% $\mu\text{m}$  and  $103 \pm 9$   $\mu\text{m}$ ) and cerium chloride ( $3,068 \pm 209$  vol% $\mu\text{m}$  and  $109 \pm 7$   $\mu\text{m}$ ). Following treatment with amine fluoride  $\Delta Z$  and LD ( $3,384 \pm 260$  vol% $\mu\text{m}$  and  $140 \pm 9$   $\mu\text{m}$ ) were significantly lower as compared to the placebo group but significantly higher compared to groups C and D. Cerium chloride and its combination with amine fluoride showed a higher potential to significantly reduce mineral loss and lesion depth development of demineralised enamel than fluoride alone.

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### Caries Lesion Rehardening as a Function of Stannous, Fluoride and pH

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The aim was to investigate the effects of stannous and fluoride over a broad pH range on surface hardness changes in early caries lesions under plaque fluid-like conditions. Bovine enamel specimens were demineralized at 37°C for 24 h using a solution containing 50 mM lactic acid and 0.2% Carbopol 907 which was 50% saturated with respect to hydroxyapatite. Lesions were assigned to 20 treatment groups ( $n = 14$ ) and exposed at 37°C for 24 h to a gel containing 50 mM acetic acid, 4.1 mM  $\text{CaCl}_2$ , 8 mM  $\text{KH}_2\text{PO}_4$ , 63 mM KCl, 1% carboxymethylcellulose and 3 mM  $\text{NaN}_3$ . These gels were supplemented with stannous (0 or 0.55 mM/65 ppm; as stannous chloride) and fluoride (0 or 10.5  $\mu\text{M}$ /0.2 ppm; as sodium fluoride) and pH adjusted (KOH) to 4.5, 5.0, 5.5, 6.0 or 6.5 in a  $2 \times 2 \times 5$  factorial design. Knoop surface microhardness was used throughout the experiment to determine the average specimen indentation length from five indentations (50 g load) of specimens before ( $\text{IL}_{\text{base}}$ ), after lesion creation ( $\text{IL}_{\text{lesion}}$ ) and after gel exposure ( $\text{IL}_{\text{post}}$ ). The percent surface microhardness recovery (%SMHr) was calculated as follows:  $\% \text{SMHr} = (\text{IL}_{\text{lesion}} - \text{IL}_{\text{post}}) / (\text{IL}_{\text{lesion}} - \text{IL}_{\text{base}}) \times 100$ . Data were analyzed using three-way ANOVA ( $p < 0.05$ ). The stannous  $\times$  fluoride  $\times$  pH interaction was not significant ( $p = 0.48$ ), however all two-way interactions were (stannous  $\times$  fluoride  $p < 0.0001$ ; stannous  $\times$  pH  $p < 0.0001$ ; fluoride  $\times$  pH  $p = 0.0206$ ). Stannous and fluoride ions each had a significant effect on %SMHr compared to no stannous or no fluoride at all pH values apart from 6.0 with higher pH values resulting in higher %SMHr for fluoride. Stannous enhanced %SMHr at pH 4.5, 5.0 and 5.5 but diminished %SMHr at pH 6.5. There were no synergistic effects between stannous and fluoride. While interpretation of the present data is complex and not straightforward, it can be concluded that fluoride, as expected, enhanced %SMHr while the effect of stannous was pH dependent.

This study was solely funded by the Oral Health Research Institute Remineralization Research Program.

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### Evaluation of a Selenium-Containing Antimicrobial Pit and Fissures Sealant

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The purpose of this study was to evaluate a new selenium-containing antimicrobial pit and fissure sealant, SeLECT Defense<sup>®</sup>, for microleakage, comparing it to the commonly used UltraSeal XT<sup>®</sup> sealant. 40 teeth, consisting of molars and premolars were collected. Teeth with pits and fissures suitable for sealing were se-

lected and sealed (20 teeth/sealant type), following the current recommended clinical procedure. Following sealing, the teeth were thermocycled 300 times in hot and cold water baths (5 min in each temperature) to simulate 3.5 months of exposure in oral cavity and influence of hot and cold foods. The samples were then soaked for 8 h in silver nitrate for possible dye penetration and indication of leakage. Then a tooth section approximately 100 µm in thickness was cut from each tooth through the sealant. The section was then soaked for 8 h in photo development fluid. The degree of microleakage was then analyzed by an evaluator who was blind to the sealant used in each tooth. The evaluator rated the degree of microleakage by scoring method, 0–4, with 0 indicating no leakage and 4 indicating leakage extending to the base of the sealant. An unpaired t-test analysis (n = 20; α = 0.05) indicated that UltraSeal XT (mean = 3.6; 95% CI = 3.24–3.96) displayed significantly (p < 0.01) more microleakage than SeLECT Defense (mean = 2.55; 95% CI = 1.8–3.3). In conclusion, after simulated 3.5 months oral exposure, UltraSeal XT showed marginal leakage exceeding that of SeLECT Defense, thus indicating that UltraSeal XT sealant has a higher chance of failing due to microleakage than SeLECT Defense sealant.

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### Identification of Carious Lesions Using Optical Coherence Tomography

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**Background:** Whilst prevention is better than cure, early diagnosis is important in the prompt management of a disease process. Dental caries is one of the most common chronic childhood diseases, and deterioration in oral health can be detrimental to whole body wellbeing. Thus early identification of caries is vital. Optical coherence tomography (OCT) may be able to identify lesions in the clinic, and therefore promote the early initiation of treatment strategies. **Technique/Methods:** Six extracted teeth with no visible carious lesions were selected. All teeth were initially scanned by X-ray microtomography to locate a carious lesion. Next this region was scanned using an OCT microscope. Scattering Attenuation Microscopy software was used to obtain an image. Short-term demineralization of the enamel in acetic acid (pH 4) was performed to determine whether OCT could quantitatively differentiate between the depth of light penetration in sound and partially demineralised enamel. **Results:** OCT was able to detect the carious lesion invisible to the eye (enamel cracks were also identifiable). Depth of light penetration was influenced by the degree of mineralisation; pre-demineralisation:  $10.65 \pm 1.55 \mu\text{m}$ , partial demineralisation:  $23.29 \pm 3.65 \mu\text{m}$ ,  $p = 0.03$  Student's paired t-test. OCT can detect carious lesions that are invisible to the eye and assess mineral quality and structural integrity of the enamel. Development of the technique to allow chairside diagnosis will permit early intervention to maintain oral, and preserve whole body, health.

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### Effect of Fluoride-Releasing Restorative Materials on Artificial Proximal Caries: An in vitro Study

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This in vitro study investigated the remineralisation of artificial caries when in proximal contact with different fluoride-releasing restorative materials. One hundred and twenty primary enamel blocks were covered with varnish, except for a  $3 \times 2 \text{ mm}$  area, and submitted to an artificial caries induction pH cycling model (demineralizing solution, pH 4.6, 8 h; remineralising, pH 7, 16 h, 10 days). Blocks (n = 20) of composite resin, glass ionomer cement (GIC), encapsulated GIC, resin modified GIC and compomer were prepared and attached to an enamel block, simulating the contact point with a proximal restoration. These sets were submitted to a subsequent caries challenge (demineralizing solution, pH 4.5, 8 h; remineralising, pH 7, 16 h), for 7 or 14 days (n = 10). After these periods, cross-sectional microhardness (CSMH) analysis was carried out to quantify mineral gain/loss.  $\Delta\text{CSMH}$  was calculated comparing the measurements performed within the white spot lesion area and those performed on the sound surface of the same enamel slab (covered with acid-resistance varnish). Kruskal-Wallis test demonstrated that there was no significant difference among groups for the baseline values ( $p = 0.0982$ ) or those obtained after lesion development ( $p = 0.148$ ). Distribution of data and equality of variances were confirmed using Kolmogorov-Smirnov and Levene tests. Two-way ANOVA followed by Tukey test were used to compare the periods of challenge and the materials, respectively. CSMH analysis demonstrated that significant higher mineral loss was observed for lesions in contact with the composite resin, when compared to all other groups ( $p < 0.0001$ ). The mean (SD) integrated area of mineral loss for contact with composite resin was 32,090 (6,545) and 29,524 (6,166)  $\text{kg}/\text{mm}^2 \cdot \mu\text{m}$ , for 7 out 14 days of challenge, respectively. After both periods, conventional GIC was the most efficient material to prevent lesion progression. Mineral loss for specimens in contact with conventional and encapsulated GIC after 7 and 14 days was 8,426 (6,219); 7,630 (2,689) and 10,272 (4,023); 10,791 (4,766)  $\text{kg}/\text{mm}^2 \cdot \mu\text{m}$ , respectively. In conclusion, different fluoride-releasing materials can prevent the progression of adjacent enamel lesions when in contact with them, however the conventional GIC is the most efficient.

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### Enamel Mineral Measurements with EPMA and TMR

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Transverse microradiography (TMR) and electron probe microanalysis (EPMA) are commonly used for characterizing dental tissues. TMR utilizes an approximately monochromatic X-ray beam to determine the mass attenuation of the sample which is converted to volume percent mineral (vol%min). An EPMA stimulates the emission of characteristic X-rays from a variable volume of sample (dependent on density) to provide compositional information. The aim of this study was to compare the assessment of sound, demineralized and remineralized enamel using both techniques. Human enamel samples were demineralized, part was subsequently remineralized and a further part was acid challenged. The demineralization and remineralization solutions used were those described by Tanaka and Iijima [J Dent 2001;29:421–426]. The same line profile through each of the lesions (n = 12) was analyzed using TMR and EPMA to determine vol%min and wt% elemental composition and ratio information respectively. EPMA data were transformed into vol%min data to test the agreement between the two techniques. The pattern of parameters determined by each technique correlated well (correlation coefficients > 0.76, p < 0.001), however, the absolute values were not similar. The mean difference between the measurements at the lesion maxima and minima were 10.2 ± 6.4 vol%min (p < 0.001) and 21.0 ± 15.1 vol%min (p < 0.001) respectively with EPMA always providing the higher values. This is likely to be attributable to the complex ultra-structural composition of the samples, their variable density as well as charge buildup and non-linear interaction of the EPMA generated X-rays. In conclusion, EPMA remains an important technique for obtaining chemical and elemental ratios from dental tissues but its limitations must be appreciated and it should not be used in place of TMR for determining mineral content.

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### Bio-Inspired Enamel Repair via Cooperation of Calcium Release Agent and Glutamate

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Over recent decades, many attempts at enamel remineralisation or synthesis of enamel-like apatite crystals in the presence of organic additives such as gels and surfactant/polymer have been undertaken. However, the harsh experimental conditions (e.g. the high reaction temperature) and the involvement of non-biocom-

patible materials prevent their biomedical application. The purpose of this study was to remodel enamel-like hydroxyapatite structural features directly on the enamel substrate by crystal growth in the presence of calcium salt and glutamate under physiological conditions. 60 mg calcium salt was added into 30 ml simulated oral fluid (SOF) which contained 100 mM glutamate (Glu) and then the human enamel specimens were incubated with the reaction solution for 24 h at 37°C. Scanning electron microscope (SEM), transmission electron microscope (TEM) and nano-indentation techniques were used to reveal the structure and mechanical properties of the newly formed hydroxyapatite layer on enamel surface. The results revealed that the preferential orientation of the newly formed hydroxyapatite crystals on enamel surface was similar to natural enamel. We noted that the enamel surface treated with the calcium salt and glutamate had a hardness of 4.12 ± 0.53 GPa and elastic modulus of 95.16 ± 6.23 GPa. These values were similar to that of natural enamel (hardness of natural enamel: 4.56 ± 0.68 GPa, elastic modulus: 98.04 ± 7.28 GPa). Moreover, we also found that glutamate promoted the kinetics of the formation and crystallisation of hydroxyapatite from 5 to 2 h. In contrast, SOF or the calcium salt without Glu could not provide a permanent repair, which showed no obvious promotion effect on the formation of ordered enamel-like hydroxyapatite layer on enamel surface. The cooperation of calcium salt and Glu can result in the regeneration of enamel-like structure under physiological conditions. Importantly, the mechanical characteristics of the repaired enamel are maintained well by using this enamel model.

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### Effect of Cu<sup>2+</sup> on Hydroxyapatite Demineralisation Relevant to Dental Caries Studied Using Scanning Microradiography

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Copper has been reported to be associated with low caries prevalence in animals and in human beings. This has been attributed mainly to its antimicrobial effect against oral bacteria associated with caries. However, it is plausible that the copper ion itself interacts with hydroxyapatite (HAp) to alter the resultant salt's solubility. The aim of this study was to investigate the effect of Cu<sup>2+</sup> directly on the dissolution kinetics of HAp under artificial caries-like conditions using scanning microradiography (SMR). HAp discs (Plasma-Biotol, UK) with 20 wt% porosity, sintered, 1 mm thick, were used as enamel analogues and coated with acid-resistant varnish leaving one surface exposed and located in an SMR cell. Demineralising solutions of 0.1% acetic acid pH 4.0 simulating caries conditions were circulated through the SMR cells. The rate of HAp discs demineralisation (RD<sub>HAp</sub>) was measured using SMR. SMR

measurements were carried out using identical demineralising conditions, but with increasing  $\text{Cu}^{2+}$  concentrations of 0.00, 11.25, 22.50, 45.00, 90.00, 150.00, and 180.00 mg/l and SMR measurements were continued for each case. The SMR measurements were then repeated at decreasing  $\text{Cu}^{2+}$  concentrations (180.00, 150.00, 90.00, 45.00, 22.50, 11.25, and 0.00 mg/l). Each experiment was duplicated.  $\text{RD}_{\text{HAP}}$  decreased significantly ( $p < 0.05$ ) from  $3.52 \times 10^{-4}$  to  $2.46 \times 10^{-4}$ ,  $1.65 \times 10^{-4}$ ,  $1.33 \times 10^{-4}$ ,  $1.19 \times 10^{-4}$ ,  $1.03 \times 10^{-4}$  and  $8.43 \times 10^{-4} \text{ g} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$  at increasing  $\text{Cu}^{2+}$  concentrations (0.00–180.00 mg/l respectively) and increased significantly ( $p < 0.05$ ) from  $8.28 \times 10^{-5}$  to  $1.44 \times 10^{-4}$ ,  $1.62 \times 10^{-4}$ ,  $1.84 \times 10^{-4}$ ,  $2.04 \times 10^{-4}$ ,  $2.47 \times 10^{-4}$  and  $3.11 \times 10^{-4} \text{ g} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$  at decreasing  $\text{Cu}^{2+}$  concentrations (180.00–0.00 mg/l). The results showed direct inhibitory effect of  $\text{Cu}^{2+}$  on HAP dissolution kinetics. The reversibility of the effect suggests a surface controlled action rather than change in the bulk composition. It demonstrates the potential usefulness of  $\text{Cu}^{2+}$  in prevention against caries.

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### Reduction of Enamel Demineralisation by a Fluoride Toothpaste Containing Enzymes and Proteins

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The objective of this study was to evaluate the enamel protective effects of a fluoride toothpaste formulation (1,450 ppm fluoride as NaF) containing enzymes and proteins (Zendium Enamel Protect), versus control formulations, from acid erosion in vitro. The Zendium Enamel Protect toothpaste contains the following enzymes and proteins at a total concentration of 0.48%: amylo glucosidase, glucose oxidase, lactoperoxidase, lactoferrin, lysozyme, colostrum and sodium caseinate. Bovine enamel specimens were prepared and baseline surface microhardness (SMH) measurements taken. Specimens were stratified into three groups ( $n = 9$ ) and mounted in impression material. Each group underwent a pH cycling protocol as follows: toothpaste slurry (1:2, toothpaste:water) (1 min); followed by 3 cycles of: remin, pH 7 (1 h) and 1% w/w citric acid, pH 2.3 (2 min); then remin, pH 7 (1 h); toothpaste slurry (1:2, toothpaste:water) (1 min). This whole cycle was repeated before incubating samples in remin solution, pH 7 overnight. The cycling protocol was performed for 2 days and then the SMH was re-measured. The mean percentage change in SMH was calculated for each group. The toothpaste slurry treatments were either Zendium Enamel Protect toothpaste (A), 1,450 ppm NaF control toothpaste (B) or a non-fluoride placebo toothpaste (C). The mean percentage reduction in SMH (SD) for each treatment group was: A 38.41 (4.96); B 44.6 (4.38), and C 64.61 (5.61). Statistical analysis (ANOVA, Tukey-Kramer) showed that all treatments were significantly different ( $p < 0.05$ ) to each other. It can be concluded that the fluoride toothpaste containing enzymes and proteins can give additional protection to enamel from acid erosion over a standard fluoride formulation.

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### Comparing Two Methods in the Early Detection of in vitro Chemical Demineralisation: A Pilot Study

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The purpose of this study was to compare the ability of the QLF-D Biluminator™ (Inspektor, Amsterdam, The Netherlands) and Nuance™ multispectral imaging system (CRI, Woburn, USA) to detect and quantify early in vitro chemical demineralisation. Ten specimens were prepared from bovine teeth and immersed in acid buffer demineralising solution (2.2 mM  $\text{KH}_2\text{PO}_4$ , 50 mM acetic acid, 2.2 mM  $\text{CaCl}_2$ , 0.05 ppm F, pH 4.5). Each sample was illuminated with a 405 nm exciting light. Four different sets of fluorescence images were captured using QLF-D and Nuance™ device fitted with a 460 high-pass filter at 500, 520 and 550 nm emission filters. Measurements were taken at 1, 3, 6, 9, 12, 24, 48 and 72 h. The percentage of fluorescence loss ( $\Delta F$ ) was calculated using QA2 image analysis software (Inspektor, Amsterdam, The Netherlands), and tested for correlation using Pearson correlation coefficients ( $r$ ). QLF-D  $\Delta F$  values were compared with those obtained with Nuance 500, Nuance 520, Nuance 550 respectively using paired sample t-tests. The earliest time points where the two methods detected demineralisation were compared using the non parametric Mann-Whitney test. Both methods detected and quantified demineralisation. The Pearson coefficients ( $r$ ) were QLF-D/Nuance 500: 0.910 (95% CI 0.863–0.941), QLF-D/Nuance 520: 0.905 (95% CI 0.855–0.938), QLF-D/Nuance 550: 0.885 (95% CI 0.826–0.925). Nuance™ detected higher  $\Delta F$  values than QLF-D ( $p < 0.001$ ) for each wavelength. At 72 h  $\Delta F$  values for QLF-D are: mean:  $-18.2$ , SD 5.2; for Nuance 500, mean:  $-29.4$ , SD 9.5; for Nuance 520: mean:  $-27.6$ , SD: 8.9; for Nuance 550: mean:  $-23.9$ , SD: 8.9. Nuance™ detected fluorescence loss significantly earlier than QLF-D. The median time point for the first detection was 6 h for QLF-D and 3 h for Nuance all wavelengths ( $p < 0.005$ ). These preliminary results indicate that Nuance™ showed a high correlation with QLF-D as a method for detecting fluorescence loss. It was able to detect this significantly earlier and with larger margins compared with QLF-D. Further work is required to determine the specificity and sensitivity of these methods.

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### Restoration of Demineralised Enamel by Synthesis of Ordered Apatite Crystals

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Acid attack due to dental caries results in disruption of the highly organised enamel microstructure. Remineralisation studies focus on the restoration of mineral density lost during deminer-

alisation of enamel, rather than the regrowth of ordered mineral. The objective was to understand the process of crystal growth to show possible repair of the ordered structure. We aimed to synthesise thin films of highly ordered hydroxyl (fluoro) apatite nanorods directly onto demineralised enamel sections, using three different physiochemical methods. Scanning electron microscopy (SEM) and synchrotron X-ray diffraction (S-XRD) were applied to identify the microstructural morphology and preferred crystallite orientation respectively of synthetic crystals. Three healthy molars were sectioned and artificially demineralised, using 0.1% acetic acid at pH 4 for 3, 6 or 7 days. Each section was placed under one of three conditions: ambient pressure at 70°C [Wang et al.: *J Nanosci Nanotechnol* 2009;2:1361–1364], ambient pressure at 37°C [Yin et al.: *Chem Commun* 2009;39:5892–5894] and mild hydrothermal conditions [Chen et al.: *Adv Mater* 2006;14:1846–1851]. Growth was observed using SEM. The preferred orientation of the crystallites formed by ambient condition was characterised using S-XRD. SEM images show the presence of synthetic crystals on all enamel specimens, with different degrees of crystallite ordering for each method. Hydrothermal treatment produced evenly distributed growth, with the most well-defined and aligned crystallites in the form of hexagonal rods extending to approximately 10 µm along the c-axis. Ambient –70°C and ambient –37°C methods had moderate coverage with variation in size and shape of the crystals, the former showed small hexagonal-like cross-sectional structures with some clusters of spherical particles, whereas the latter was dominated with regions of aggregated nano-spheres, lacking order and organisation. We have successfully grown ordered apatite crystals directly on demineralised surfaces, with crystallite organisation similar to natural enamel, indicating that with optimum conditions it is possible to regain lost apatite structure.

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### Remineralization of Incipient Enamel Lesions Using Two Fluoride Dentifrices Containing Tri-Calcium Phosphate (TCP)

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The aim of this study was to compare the surface-microhardness and F uptake from incipient lesions in human enamel using two dentifrices containing fluoride (F) and TCP in an in vitro pH cycling study. Human enamel specimens were cut into 4.5 mm square pieces. Each specimen was cast in an acrylic resin cylindrical block, ground, polished, rinsed, and stored in a hydrated environment. Specimens were immersed into a pH 4.9 Carbopol demineralization solution and stored for 96 h at 37°C to establish incipient lesions. After demineralization, Vickers indentations were made on each specimen (4 × 200 g load for 15 s, averaged). Specimens were placed into stratified treatment groups (n = 3) so average initial surface microhardness (SMH) of the groups were statistically not different. Dentifrices 3M™ ESPE™ Clinpro™ Tooth Crème (950 ppm F, TCP) and 3M™ ESPE™ Clinpro™ 5000 (5,000 ppm F, TCP) and an artificial saliva control were evaluated

using a daily pH cycling regimen (4 one-minute treatments and one 4-hour acid challenge per day) for a total of 10 days. After cycling, the specimens were subjected to Vickers surface microhardness (200 g force, 15 s dwell time). The specimens were then biopsied (100 µm deep × 1 mm diameter) with a microdrill in order to determine enamel fluoride uptake (EFU). After pH cycling, a statistically significant difference between artificial saliva control (a), Clinpro™ Tooth Crème (b), and Clinpro™ 5000 (c) groups was observed for both ΔSMH (a = 5.14 ± 5.82, b = 71.42 ± 16.61, and c = 133.17 ± 55.73; p = 0.00) as well as for EFU in µg/cm<sup>2</sup> (a = 29.20 ± 20.46, b = 83.60 ± 14.34, and c = 142.68 ± 6.12; p = 0.00) using one-way ANOVA. Dentifrices containing TCP and 950 or 5,000 ppm F improve human enamel hardness independent of F level. A clear dose response was observed for both endpoints.

The study was funded by 3M ESPE Dental Products Laboratory.

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### Polygamma Glutamic Acid Reduces Kinetics of Hydroxyapatite Demineralization under Caries-Simulating Conditions

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Statherin's enamel dissolution inhibition function during carious challenges is associated with interaction of glutamic acid residues with hydroxyapatite (HAp) surfaces. The traditional Japanese foodstuff (Natto) is a naturally occurring inexpensive polypeptide polygamma glutamic acid (PGGA), and may perform similar caries inhibitory function. The aim was to determine if PGGA also reduces dissolution kinetics of HAp discs used as enamel analogues when subjected to simulated cariogenic challenges. A high molecular weight sodium salt of PGGA was obtained from Natto Biosciences (Montreal, Canada). HAp discs (porosity 20%) were located in scanning microradiography (SMR) cells through which demineralizing solutions were circulated. Experiment 1: concentrations (0.1%, 0.2%, 0.5%, 1% and 2%) of PGGA were prepared in acetic acid (pH 4.0) demineralizing solution. Experiment 2: concentrations (0.1%, 0.2%, 0.5%, 1% and 2%) of PGGA were dissolved in water. Initially, the discs were subjected to acetic acid (pH = 4.0) for 24 h, and SMR used to determine the rate of HAp demineralization (RD<sub>HAp</sub>). Experiment 1: discs treated with demineralizing solutions containing increasing concentrations of PGGA, each for 72 h. Experiment 2: discs pre-treated with PGGA solution for 24 h, then demineralized for a further 48 h, repeated for each PGGA concentration. SMR was used to determine RD<sub>HAp</sub>. For PGGA dissolved in acetic acid, RD<sub>HAp</sub> at 0.1, 0.2, 0.5, 1.0, 2.0% PGGA was 10.5 × 10<sup>-5</sup> g · cm<sup>-2</sup> · h<sup>-1</sup>, 9.7 × 10<sup>-5</sup> g · cm<sup>-2</sup> · h<sup>-1</sup>, 6.0 × 10<sup>-5</sup> g · cm<sup>-2</sup> · h<sup>-1</sup>, 4.7 × 10<sup>-5</sup> g · cm<sup>-2</sup> · h<sup>-1</sup>, 4.0 × 10<sup>-5</sup> g · cm<sup>-2</sup> · h<sup>-1</sup> respectively. For PGGA dissolved in water, RD<sub>HAp</sub> at 0.1, 0.2, 0.5, 1.0, 2.0% PGGA was 19.0 × 10<sup>-5</sup> g · cm<sup>-2</sup> · h<sup>-1</sup>, 15.1 × 10<sup>-5</sup> g · cm<sup>-2</sup> · h<sup>-1</sup>, 10.0 × 10<sup>-5</sup> g · cm<sup>-2</sup> · h<sup>-1</sup>, 9.5 × 10<sup>-5</sup> g · cm<sup>-2</sup> · h<sup>-1</sup>, 5.3 × 10<sup>-5</sup> g · cm<sup>-2</sup> · h<sup>-1</sup> respectively. All values were significantly lower than the control (p < 0.001). In conclusion, PGGA reduces HAp demineralization kinetics under acidic conditions relevant to caries.

### Physical and Chemical Influences of Zinc on Surface Enamel Dissolution

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Zinc compounds are formulated into dentifrices for its ability to reduce oral malodor. Considerably less work is reported on zinc's interaction with dental hard tissues and its putative role in demineralization and remineralization. This study assesses zinc as a potential inhibitor of enamel demineralization. An in vitro model was used to measure real-time quantitative changes in enamel mineral mass during exposure to caries-simulating conditions as a function of  $[Zn^{2+}]$ . Scanning microradiography (SMR) was used to measure demineralization kinetics and scanning electron microscopy-energy dispersive X-ray (SEM-EDX) for elemental analysis. Enamel blocks cut from molars were placed in SMR environmental cells and demineralizing solutions (0.1% acetic acid, pH 4.0) were circulated (~48 h). Subsequently,  $[Zn^{2+}]$ -containing demineralizing solution ( $[Zn^{2+}] = 0, 25, 100, 300, 5,000, 10,000$  ppm) were circulated (~48 h). Rate of enamel mineral loss ( $RML_{enamel}$ ) was measured at 3 fixed positions on each sample as a function of  $[Zn^{2+}]$ . Further, SEM-EDX was performed on enamel surfaces.  $RML_{enamel}$  was linear with time for all  $[Zn^{2+}]$ . Control (0 ppm  $Zn^{2+}$ )  $RML_{enamel}$  was  $6.89 \times 10^{-4} \text{ g cm}^{-2} \cdot \text{s}^{-1}$ . An approximate 20% reduction in  $RML_{enamel}$  was observed with 25 ppm, 23% with 100 ppm and ~90% with 10,000 ppm  $Zn^{2+}$ . SEX-EDX showed increasing zinc on enamel surfaces with  $[Zn^{2+}]$  up to 5,000 ppm, correlating with the dissolution kinetics data. However at 10,000 ppm, a decrease in zinc uptake was observed. A quantitative in vitro model measuring the anti-demineralization potential of zinc was observed using SMR and SEM-EDX. A linear logarithmic relationship between % reduction in  $RML_{enamel}$  (y) and  $[Zn^{2+}]$  (x) was observed in the concentration range used [ $y = 7.27\ln(x) + 19.3$  ( $R^2 = 0.87$ )]. These results indicate that zinc is an inhibitor of enamel demineralization.

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### Contact with Fluoride-Releasing Restorative Materials Can Arrest Approximal Caries Lesion

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This study investigated the possibility of caries lesions arrest when in approximal contact with fluoride-releasing materials. White spot lesions formed in 120 primary enamel specimens via a pH cycling regimen (pH = 4.6 – 8 h; pH = 7 – 16 h, 10 days) were

put in contact with cylindrical blocks of 6 different materials ( $n = 20$ ) – composite resin, glass ionomer (GIC), encapsulated GIC, resin-modified GIC, nano-ionomer and compomer. These settings were designed to simulate the contact point between the restoration and the lesion. A subsequent in situ cariogenic challenge was conducted in two phases (7/14 days). Ten volunteers wore palatal devices containing 6 sets (caries lesion + restorative), using fluoride dentifrice 3×/day and dripping 20% sucrose solution 8×/day. QLF images were made of each slab at the different phases of the study to assess lesions mineral loss/gain. Cross-sectional microhardness (CSMH) was also measured for validation. Indentations were as well carried out on the caries-free part of the enamel slab to serve as control ( $\Delta$ CSMH). Distribution of data was determined using Anderson-Darling and Levene tests. Multilevel analysis compared  $\Delta Q$  (%mm<sup>2</sup>) values and CSMH data regarding duration of challenge and materials ( $\alpha = 5\%$ ). After 7/14 days, mean (SD) mineral loss was: resin 29,468 (7,638)/30,999 (7,738); GIC 8,879 (6,899)/11,146 (7,744); encapsulated GIC 11,672 (7,593)/9,134 (4,682); resin-modified GIC 25,956 (5,698)/21,029 (3,597), nano-ionomer 29,043 (6,558)/35,603 (6,136) and compomer 26,563 (4,468)/27,459 (6,286) kg/mm<sup>2</sup> ·  $\mu\text{m}$ . Considering CSMH analysis, higher mineral loss was observed for lesions in contact with resin ( $p < 0.0001$ ), with mean (SD) mineral loss. Conventional GICs were the most efficient in preventing mineral loss, whereas the others materials presented an intermediate behavior. Similar results were obtained using QLF, except that all the fluoride-releasing were almost equally able to prevent mineral loss. Compomer was as inefficient as resin to avoid lesion progression at the 14-day period. All fluoride-releasing materials are capable of arresting enamel lesion when in approximal contact with them; however conventional GIC represents the best choice.

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### Synthesis of Nanocomposite Resins with Remineralizing Capability

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Nanoparticles of hydroxyapatite (F-HA) and bioactive glass (F-BG) were synthesized via the sol-gel method incorporating fluorine. These particles were characterized by X-ray diffraction (XRD) and transmission electron microscopy (TEM), and then used as filler in the synthesis of photocured nanocomposite resin. The ability of the nanocomposite to induce mineralization was evaluated in simulated body fluid (composition in mM: Na<sup>+</sup>: 144, K<sup>+</sup>: 5, Mg<sup>2+</sup>: 1.5, Ca<sup>2+</sup>: 2.5, Cl<sup>-</sup>: 103, HCO<sub>3</sub><sup>-</sup>: 10, HPO<sub>4</sub><sup>2-</sup>: 1.0, SO<sub>4</sub><sup>2-</sup>: 0.5) at 37°C for 3 and 7 days. The apatite formation was observed by scanning electron microscopy (SEM). Fluoride release was eval-



uated by using an ion-selective electrode in solutions at pH 4 and 5.5 every 7 days. Both synthesized nanocomposite resins showed capacity for fluoride liberation. However, F-BG released more fluoride than F-HA. On the other hand, the ability of inducing mineralization was observed as deposits of apatite crystals on the surface of both types of resins. The higher mineralization density was observed on F-BG resin. The incorporation of BG (bioactive glass without fluoride) and BH (hydroxyapatite without fluoride) nanoparticles improved the releasing properties of the resin and induced fluorine mineralization suggesting an ability to prevent caries. Resin nanocomposite with either F-HA or F-BG nanoparticles incorporated acquired the ability to induce mineralization and to release fluoride, both aimed at preventing new caries lesions.

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### Effects of Tea Polyphenol-Modified Amorphous Calcium Phosphate on Remineralization of Initial Enamel Lesion

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Tea polyphenols (TP), natural extracts from green tea, are potent antimicrobial and antioxidant agents. Recently, they were also found to be effective modifiers for the crystallization of nano-sized particles. Since nano-sized calcium phosphate, particularly nano-hydroxyapatite, is known to enhance remineralization of dental hard tissue, our aims are to synthesize TP-modified calcium phosphate nanoparticles and to test their potential as caries preventive agent. To this end, an ammonia water diffusion method was used to synthesize the nanoparticles. The resultant products were characterized by three methods: scanning electron microscopy (SEM), X-ray diffraction (XRD) and thermogravimetric analysis (TGA). Their remineralization potential was investigated in a 12-day pH-cycling study with preformed enamel lesions. The products were applied onto enamel lesions 4 × 3 min per day at pH 7.0 and pH 5.5. Nano-hydroxyapatite (nHA) was used as positive control, and deionized water (DDW) served as negative control. Remineralization was evaluated by surface microhardness analysis, and the data were analysed by one-way ANOVA. The SEM images clearly showed nano-sized particles were formed under 27 mg/ml TP. The particle contains amorphous calcium phosphate (ACP) and 35% TP. Application of this TP-ACP on enamel lesions resulted in a significantly enhanced remineralization at pH 5.5 (147.7 ± 23.6 KHN) compared to the DDW treatment group (105.9 ± 15.6 KHN) ( $p < 0.05$ ). The pH 7.0 group did not differ from the control (103.3 ± 20.1 KHN) ( $p > 0.05$ ). Enhanced remineralization was also observed for the nHA groups (151.8 ±

41.7 KHN at pH 7.0 and 147.3 ± 40.7 KHN at pH 5.5) ( $p < 0.05$ ), irrespective of the pH value. In conclusion, with the modifier TP, nano-sized ACP could be synthesized. The TP-ACP promoted remineralization of initial enamel lesion under an acidic condition. Further work is needed to explore the antimicrobial activity of this potential anticaries agent.

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### Validation of QLF<sup>TM</sup> for Caries Assessment in an in vitro Biologically Relevant Caries Biofilm Model

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Quantitative light induced fluorescence (QLF<sup>TM</sup>) is a non-destructive diagnostic tool for the assessment and quantification of caries. This study seeks to evaluate the in vitro application of the QLF<sup>TM</sup>D system in comparison with the long-standing gold standard method in assessing changes in enamel mineral content; transverse microradiography (TMR). Caries lesion formation, in a biologically relevant caries biofilm model, was investigated when subjected to high-frequency sucrose-pulsing. Microcosm oral biofilm representative of interproximal plaque was produced on 5 mm bovine enamel discs using a constant-depth film fermenter (CDFF) and pulsed 8 times daily for 15 min with either 20 or 100 mM sucrose solution. Samples were extracted over a 14 day period; bacterial composition of biofilm was enumerated using traditional microbiological culture techniques and discs were imaged using QLF<sup>TM</sup>D before analysis to obtain  $\Delta F$  values. TMR provided integrated mineral loss ( $\Delta Z$ , vol% $\mu$ m) data. TMR and QLF indicate caries proceeded much more rapidly in 100 mM sucrose treatment than in 20 mM pulsed biofilm. At 20 mM  $\Delta Z$  of 202 ± 103 SD ( $n = 3$ ) was observed at day 4 reaching maximum  $\Delta Z$  (486 ± 217 SD,  $n = 3$ ) by day 12 whereas in 100 mM treatment  $\Delta Z$  was 488 ± 298 SD ( $n = 3$ ) at day 4 and maximum  $\Delta Z$  of 1,637 ± 248 SD ( $n = 3$ ) was detected by day 12. Separate ANOVA tests of TMR  $\Delta Z$  and QLF<sup>TM</sup>D  $\Delta F$  data revealed statistically significant differences between sucrose concentrations, time and the interaction between the two factors ( $p < 0.05$ ). Two-tailed Pearson's correlation coefficient of  $\Delta Z$  versus absolute transformed  $\Delta F$  was found to be 0.76 ( $p < 0.01$ ). To summarise, the laboratory based biological model system can discriminate sucrose dose response on biofilm-mediated enamel demineralisation as determined by both QLF<sup>TM</sup>D and TMR whilst demonstrating strong correlation between assessment techniques.

The study was funded by a BBSRC/GSK CASE award.

### Long-Term Effect on Enamel Surface Microhardness of Wi-Fi Systems in Albino Rats

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**Aim:** The number of sources that emit electromagnetic fields especially radiofrequencies are growing rapidly in the world. Oral tissues are one of the important parts of the head/body that absorb the radiation from Wi-Fi systems; however, scientists did not take this field into consideration. There exists no data in the literature regarding the long-term efficacy of Wi-Fi on enamel surface microhardness (SMH) of teeth. Therefore, in this study we aimed to investigate the effect of long-term exposure to Wi-Fi systems on microhardness of enamel on Albino rats. **Experimental Approach:** Sixteen Wistar Albino adult male rats (final average weight of the animals was  $348 \pm 28.8$  g) were separated equally into two groups such as sham ( $n = 8$ ), and exposure ( $n = 8$ ), and kept on a 14/10 h light/dark schedule. Rats were exposed to 2.4 GHz radiofrequency radiation 24 h/day during 12 months by a generator. Same experimental circumstances were applied to the rats in the sham group, except the generator was turned off. Eight enamel were prepared to two study groups from the buccal surface of each extracted rat teeth. Digital Micro-Vickers Hardness Tester fitted with a Vickers diamond and a 25 g/10 s load was used to make indentations in the buccal side of 8 enamel pieces. The mean values of all three measurements at each group were then compared. The data were statistically analysed by t-test, with significance level  $<0.05$ . **Results:** Mean enamel surface microhardness of rats in the exposure group ( $212.67 \pm 43.11$  N) was found lower than the control groups ( $316.87 \pm 16.06$  N). The difference between sham and exposure groups was found statistically significant ( $p < 0.001$ ). **Conclusion:** Despite the result of this preliminary study, it can be considered that long-term exposure of radiation 24 h/day during 12 months emitted from Wi-Fi decrease the enamel surface microhardness of rats. However, more experimental study is necessary to clarify the effect of Wi-Fi systems on teeth.

### In situ Kinetics of Fluoride Release from *Streptococcus mutans* and Enamel Demineralization

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Despite the abundant evidence on the anticaries effect of fluoride, its retention in dental biofilm after using fluoride treatments and its release to the biofilm fluid to interfere with the caries process are poorly understood. In the present study, fluoride-treated bacteria were tested in a short-term in situ model to evaluate the kinetics of fluoride concentration in the fluid before and after a cariogenic challenge and the subsequent enamel demineralization. In a double-blind, crossover design, 15 volunteers wore palatal appliances containing enamel blocks which were kept in contact with *S. mutans* IB1600 cells (test plaque) pre-treated with 0, 1 or 225 ppm F PIPES buffer, pH 7.0. After 30 min, test plaque was collected for determination of fluoride concentration in its fluid phase, using a fluoride electrode adapted for microanalysis. Volunteers rinsed (1 min) with 20% sucrose solution as a cariogenic challenge, and after 45 min, the test plaque was collected for fluid analysis, and the blocks for determination of surface hardness loss (demineralization). Fluoride concentration in the fluid phase of the test plaque decreased significantly ( $p < 0.05$ ) in the groups pre-treated with 1 and 225 ppm F, reaching  $0.32 \pm 0.21$  and  $57.5 \pm 22.9$  ppm F after 30 min in situ. At the end of the experiment, the concentrations decreased further, reaching  $0.06 \pm 0.02$ ,  $0.07 \pm 0.03$  and  $12.8 \pm 11.8$  ppm F for groups 0, 1 and 225 ppm F, respectively. Significantly lower ( $p < 0.05$ ) demineralization was observed in the 225 ppm F group ( $-4.2\% \pm 6.0$ ), but no significant difference was observed between the 0 and 1 ppm F groups ( $-30.3\% \pm 13.3$  and  $-27.5\% \pm 11.2$ , respectively). The data suggest that fluoride bound to bacterial reservoirs after toothbrushing or rinsing is able to reduce enamel demineralization.

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### Maturation and Ageing in Biominerals with Application to Enamel Maturation

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The aims of this work were to study the kinetics of simulated enamel transformation in the oral environment and to monitor changes in enamel physicochemical properties due to ageing.

Ground and polished bovine incisors were randomly divided into 6 groups (A–F, 12 blocks/group) and pH-cycled (10 days). Demineralization time was 2 min/cycle (40 min/day in total) and 5 min/cycle (1 h/day in total), for groups A–C and D–F respectively. Groups A, D and B, E were demineralized using solutions of different pH (4.1, 4.9), while C, F served as controls and were immersed into deionized water. All solutions contained a small amount of NaF (10.5  $\mu\text{mol/l}$ ). Between demineralization, overnight and during weekends enamel blocks were immersed inside plaque fluid proxy (pH  $\sim$ 6.58). Solutions were refreshed daily. Three blocks were randomly selected from each group every 3 days for surface-microhardness analysis (Vickers). For control groups C and F surface hardness remained stable with time. Group C was found to have in average  $311.934 \pm 11.262$  VHN and F  $238.076 \pm 14.473$  VHN. Groups' A and B hardness decreased until the 4th day ( $171.415 \pm 14.323$  VHN and  $167.418 \pm 3.515$  VHN respectively) but remained stable thereafter. Hardness of groups C and E declined continuously. Group C presented bulk tissue loss and after the 10th day hardness was down to dentine levels ( $85.784 \pm 3.121$  VHN). For group E the phenomenon seems to diminish with time probably due to the acquisition of fluoride. In fact while there was a 25.79% drop in hardness between days 1 and 4 the drop between the 7th and 10th day was 11.19%. ANOVA ( $p < 0.05$ ) was used for analysis. After an initial softening, surface hardness seemed to stabilize. Extension of cycling period could lead to further rehardening. Further modification of parameters is needed to acquire a model of enamel maturation.

The study was funded by the University of Birmingham, School of Geography, Earth and Environmental Sciences.

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### Microspectral Assessment of Biodentine/Dentine Interface

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Recently it has been shown that very deep lesions, extending through the enamel into the dentin, can still be remineralized when brought into contact with a mineralizing agent. Biodentine is a bioactive calcium silicate-based dentin substitute recommended to use in dentistry for direct and indirect capping, perforations, apexification and retrograde root filling. The aim of the study was to examine the chemical composition of Biodentine and the reaction between Biodentine and dentine comparing chemical composition of both of them in microspectral analysis. Standardized class I cavities according to Black were prepared in human extracted molars and filled with Biodentine. The 1 mm specimens were sectioned longitudinally. The samples were analyzed by scanning electron microscopy (SEM) and the elemental composition/distribution in the material-adjacent dentine was tested using an electron probe microanalyser (EDS) with image observation function. On the basis of images from scanning electron microscope it was observed that Biodentine is inhomogeneous. It contains light and dark crystals with different amount of carbon, oxygen, silicon, calcium and zirconium. Biodentine released also some of its compo-

nents into dentin specimens and caused the uptake of Ca and Si in the adjacent dentine. The formation of an interfacial layer at the Biodentine-dentin interface was identified as a 'transition zone'. It can be concluded that Biodentine has crystalline structure. Microspectral analysis used in the experiment is suitable technique to investigate this bioactive material. The chemical process can lead to the formation of an interfacial layer at the Biodentine-dentin interface what can open up possibilities for a non-operative approach to deep caries cavities.

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### Frequency of Root Caries in Teeth with Sound Crowns: An in vitro Study

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Root caries occurs when root surface is exposed to bacteria. It is diagnosed in patients with periodontal diseases and gingival recessions. The aim of the present study is to register the frequency of root caries in teeth extracted due to periodontal reasons with sound crowns, higher resistance, which have overcome demineralization and caries. All teeth ( $n = 593$ ) were mature, from the same geographical region and dental practice area. 111 of them are included in a pilot study. After this study 482 teeth are separated in groups: 239 upper and 210 lower, 391 molars, 123 front and 33 premolars. The groups are observed under magnification  $\times 5$ , treated with 2% methylene blue, rinsed, dried and observed again. Root caries is registered according to the margins (2/3) in dentine and cementum. The lesions varied from white spot lesions of  $1 \text{ mm}^2$  to massive tissue losses with pulp chamber involvement. The frequency of root caries was 20.7% in molars without restorations and 17.6% in front teeth. This was symptomatic for all groups of teeth with generalized and pathological periodontal changes. In conclusion, early prevention of root caries is essential. Dietary restriction of erosive beverages and sticky sweeteners are part of a general treatment and preventive plan. The combination of mouthrinses, electric toothbrushes, vitamin C, and intradental brushes included in standard periodontal treatment schemes may be carefully revised in relation to patients' dental caries experience.

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### Caries Risk Assessment in Adults Using Classification and Regression Trees

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Preventive treatments for dental caries based on an individual's risk have long been accepted in dental practice. The purpose of this study was to identify the factors which affected the frequency of

primary and secondary caries in adults, and to identify those patients with high or low risk of caries by using classification and regression trees (CART). A clinical data set of 442 patients aged between 20 and 64 in a general practice was analyzed. Multiple regression analyses were performed to identify factors which affected the accumulated numbers of caries needed restorations per year. The following parameters according to each patient were considered: age, DMFT, numbers of mutans streptococci (SM) and lactobacilli (LB), the secretion rate and buffer capacity of saliva, compliance to a preventive programme. CART was performed by using the factors which were identified as statistically significant by the multiple regression analyses. The incidence of primary caries in adults was affected by SM ( $p = 0.0373$ ) and LB ( $p = 0.0633$ ), while that of secondary caries was affected by DMFT ( $p = 0.0003$ ), SM ( $p = 0.0014$ ), LB ( $p = 0.0131$ ). CART was able to identify the low-risk patients against primary caries by the levels of SM ( $<10^6$  CFU/ml) and LB ( $<10^5$  CFU/ml), while those against secondary caries by DMFT ( $<22$ ) and SM ( $<5 \times 10^5$  CFU/ml). In conclusion, cariogenic bacteria were proved to be the most influential factors for the incidence of frequent caries. CART is an effective method to predict the future caries risk of an individual patient.

This study was supported by Grants-in-Aid for Scientific Research (23659888) from the Japan Society for the Promotion of Science.

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### Longitudinal Evaluation of Inactive Occlusal Enamel Lesions in Children and Adolescents

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This study was undertaken to gather evidence about the long-term outcome of inactive occlusal enamel lesions in children and adolescents with regular access to fluoride. The aims were to compare caries incidence and progression on sound occlusal surfaces and on surfaces presenting inactive enamel lesions and to estimate the risk of caries incidence and progression on these surfaces. The study was designed as a prospective cohort study and included 258 7–15-year-old children attending one public school in the municipality of Santa Maria, Brazil. The clinical assessments made on permanent molars were: (1) stage of eruption, (2) occurrence and distribution of occlusal plaque, (3) occurrence and localization of occlusal caries. Reliability was assessed at baseline examination for stage of eruption ( $k = 0.86$ ), occlusal plaque index ( $k = 0.78$ ) and occlusal caries ( $k = 0.78$ ) as well as at 1 year examination for occlusal caries ( $k = 0.76$ ). A total of 200 children were examined after 1 year and 22 (11%) were assigned to the category 'caries progression' characterized by the presence of at least one active lesion on molar teeth. At site level, no significant difference after 1 year was

observed in caries incidence and progression between sites classified either sound or with inactive enamel lesion at the baseline examination (chi square test,  $p = 0.48$ ). After controlling for the number of molar teeth within the children's dentitions included in the study (generalized estimating equations), the presence of easily detectable plaque on occlusal sites was the only predictor for caries incidence and progression after 1 year (OR = 2.73, 95% CI = 1.01–7.41,  $p < 0.05$ ). The null hypothesis that the risk of caries incidence and progression on occlusal surfaces presenting inactive enamel lesions was equivalent to those with sound occlusal surfaces was accepted.

The study was funded by CAPES.

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### Evaluation of Salivary *Streptococcus mutans* Test 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. Saliva samples were collected from 328 school students (age 12–16). 0.25 ml of collected saliva was treated with 0.05 ml of reagent #1 vigorously in order to reduce the viscosity of saliva. Then 0.1 ml of reagent #2 was added to adjust the pH of treated saliva. 0.3 ml of the treated saliva was applied to a test device of the system. After 15 min, when a positive line appeared in the test window, it was classified as high *S. mutans* level. When a positive line did not appear, it was classified as low *S. mutans* level. The remained saliva samples were subjected to the real-time PCR methods and salivary *S. mutans* levels were enumerated. At the same time, caries prevalence was examined and DMFT was calculated. Real-time PCR methods revealed that the subject with positive result of the system had significantly higher salivary *S. mutans* (median  $8.9 \times 10^4$  cells/ml) than the subject with negative result (median  $3.7 \times 10^3$  cells/ml) (Wilcoxon:  $p < 0.01$ ). Additionally, the median DMFT score in the low *S. mutans* detected group was 0.0 and in the high *S. mutans* detected group, it was 2.0 (Wilcoxon:  $p < 0.01$ ). The detection system was a simple and reliable tool to detect salivary *S. mutans* which showed positive correlation with DMFT in oral cavity. The study suggested that this detection system could also be useful for caries risk screening at group examination.

### Study regarding the Caries Occurrence and Its Correlation with the Glycemic Control for Patients with Diabetes Mellitus

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The aims of this study were to compare the rate of caries occurrence for patients with diabetes mellitus (DM) type 1 and type 2 and to establish the existence of any correlation between the degree of caries occurrence and the control of glycemia levels for the diabetic patients. This study involved 156 patients with DM type 2 and 124 patients with DM type 1. These patients have been clinically examined and we calculated the DMFT value for them. Also, we measured their glycemia level and glycosylated hemoglobin (HbA1c) using Glycomat500. The data were statistically analyzed using the following tests: t-test, Pearson correlation coefficient, chi square and odds ratios. The mean DMFT value for DM type 2 was 11.4, statistically significant higher compared to the mean DMFT value for DM type 1 which was 9.6 ( $p = 0.02 < 0.05$ ). Glycemic values were slightly higher for patients with DM type 2 ( $132.3 \pm 2.7$ ) compared to DM type 1 ( $121.7 \pm 3.2$ ),  $p = 0.0008 < 0.05$ . HbA1c values have been similar for all patients ( $9.4 \pm 1.7$  type 2, respectively  $9.6 \pm 1.2$  type 1,  $p = 0.0001 < 0.05$ ). For all the patients we established a strong correlation between the DMFT values and the HbA1c values (OR = 0.773, chi square = 0.553, trust range 95%). The Pearson correlation test showed a stronger correlations between the DMFT value and HbA1c values for the patients with DM type 2 compared to the patients with DM type 1 ( $R^2 = 0.820$  to  $R^2 = 0.702$  respectively). As a strong correlation has been established between the diabetic control (expressed by HbA1c values) and the caries occurrence, with a stronger value for patients with DM type 2 ( $R^2 = 0.820$ ), the conclusion of the study is that less glycemia control leads to a higher caries occurrence for diabetic patients, those affected by DM type 2 being more susceptible to develop caries.

### Comparison of Saliva-Check Mutans and Saliva-Check IgA Mutans with the Cariogram for Caries Risk Assessment

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The purpose of the present study was to evaluate the combined use of two rapid semi-quantitative detection kits (Saliva-Check Mutans and Saliva-Check IgA Mutans, GC, Japan) to assess the caries risk using the computer-based program the Cariogram as a reference method. Stimulated saliva samples were collected from 71 subjects aged 20–73 years and tested using Saliva-Check

Mutans to determine the number of *S. mutans* per ml saliva. After 15 min, a red line in the test detection window indicated *S. mutans* numbers of  $>5 \times 10^5$  cfu/ml of saliva. The level of salivary immunoglobulin A (IgA) in relation to mutans streptococci was examined using the Saliva-Check IgA Mutans test kit. Portions of 0.1 ml saliva were mixed with buffer and applied to the test device. The presence or absence of a red line after 15 min indicated a high or low level of IgA, respectively. By combining these two test systems, each patient was classified into one of four groups from low to high caries risk. The groups were compared with the results from the Cariogram which used 10 caries-related variables to produce and display the percent chance of an individual avoiding caries on a scale from 0–20% (low chance) to 60–100% (high chance) in the immediate future. Compared with the Cariogram data, the combined detection kits showed that 39 of 41 subjects (95%) had a high chance of avoiding caries and 20 of 30 subjects (67%) had a low chance. The data suggest that the combination of the two test systems could be used for caries risk assessment.

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### Caries Risk Assessment in Children under an Oral Health Program: A Two-Year Follow-Up Study

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The aim of this study was to evaluate dietary habits, oral hygiene and oral health of children undergoing an infant oral health program and their mothers. Seventy-four pairs of babies and mothers were evaluated at 6, 12, 18 and 24 months of age. Diet and oral hygiene were evaluated using a questionnaire. Gingival conditions and dental caries prevalence were assessed by a single examiner. Data were analyzed with McNemar test with significance level of 5%. At 6 months of age, 67% of babies were already using baby bottles and at 24 months 10% were still breastfeeding. The percentage of babies who slept while nursing ( $p = 0.850$ ) and woke up to nurse ( $p < 0.000$ ) significantly reduced along the time. The consumption of foods with high content of starch and sugar was little reported at 6 months (9%). However, at 24 months, the consumption increased significantly (81%,  $p < 0.000$ ). Sweetened milk and dairy products were already part of the diet of 16% of the babies at 6 months. After 24 months, this number increased to 91% ( $p < 0.000$ ). At 24 months, sweetened beverages were consumed by 83% of babies in a mean frequency of 7 times a week ( $p < 0.000$ ). Oral hygiene compliance increased 41% during the evaluation period, reaching 100% of the babies after 24 months ( $p < 0.000$ ). Nocturnal hygiene procedures [the oral cavity and the teeth were cleaned with a solution of hydrogen peroxide and boiled/filtered water, or toothbrush without fluoride dentifrice followed by topical applica-

tion of fluoride (0.05% NaF); this solution was applied using a cotton-tipped swab with four drops of fluoride solution on each tip, one of which is applied to the upper teeth and the other to the lower teeth at night before the child goes to sleep] increased by 32%, reaching a total of 90% after 24 months ( $p < 0.000$ ). Only one child (1.51%) had carious lesions at 18 months of age. There were no changes in gingival condition. At the end of the study caries prevalence among mothers (DMFT) was 10.06 and 67% have no changes in gingival condition. In conclusion, although there was a considerable increase in high sugar content food consumption, caries prevalence was low due to a greater concern with oral hygiene.

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### Risk Indicators of Severe Dental Caries in Low-Income Brazilian Children

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**Aim:** Previous studies have shown an important social gradient in early childhood caries (ECC), with greater severity in specific population groups that reflect the phenomenon of polarization. The objective of this research was to identify determinants of ECC polarization in children with living in a low-income area following a hierarchical approach. **Methods:** This was a retrospective cohort study with 248 low-income children. The Significant Caries Index (SCI) was used for identification of the polarized group (mean dmft of group with fewer caries = 1.38, mean dmft of the 'polarization' group = 3.82). The hierarchical model consisted of 8 blocks: (1) child's age, (2) social-economic determinants, (3) mother's caries experience, (4) child's health, (5) child's behavior, (6) oral hygiene behaviors, (7) dental plaque accumulation (VPI) and (8) mutans streptococci levels in saliva. Statistical analysis was performed using multinomial logistic regression and the risk relative rate (RRR) calculated. **Results:** Age ( $p < 0.04$ , RRR = 3.3, CI 1–10.86) and frequency of sucrose intake between the main meals ( $p < 0.01$ , RRR = 1.8–2.1, CI 1.37–2.85) were associated with the polarization of the ECC. **Conclusion:** Sucrose consumption between main meals was a risk factor to ECC in polarized group, meaning that its consumption may explain why in a group of underprivileged children, some have even more caries than other (i.e. the group of mild/moderate caries).

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### Frequency of Caries Lesions in Patients with Moebius Syndrome

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Moebius syndrome (MS) is a rare congenital disorder characterized by total or partial paralysis of the VI and VII cranial nerves, associated with other malformations. Oro facial aspects include micrognathia, hypodontia, overbite, teeth hypomineralization, dental crowding, ogival palate, and morphological alterations of the tongue. Sporadic cases showed high incidence of caries. The aim of this study is to compare the frequency of caries lesions among individuals with MS and healthy nonsyndromic children (control group, C). The study group consisted of 30 subjects with MS with a median age of 8.5 years and a control group was composed by 30 individuals matched for gender and age. The International Caries Detection and Assessment (ICDAS) was used by a single examiner, trained and calibrated ( $\kappa = 0.87$ ). For analyses different cut-off were used. Then, DMFT+dmft and prevalence were calculate for each cut-off point and compared using the Student t test and chi square test, respectively. The DMFT+dmft average was higher in MS group than in control for the cut-offs 0–1 (MS:  $22.4 \pm 19.0$ ; C:  $8.6 \pm 5.8$ ), 1–2 (MS:  $20.4 \pm 18.7$ ; C:  $3.8 \pm 3.5$ ) and 2–3 (MS:  $6.7 \pm 9.3$ ; C:  $2.3 \pm 2.8$ ). There were no differences between the groups in number of restored surfaces. On the threshold 1–2, individuals with MS showed prevalence of lesions about 20% higher than the control group (MS: 0.97; 95% CI: 0.9–1.0; C: 0.79; 95% IC: 0.6–0.9). Patients with MS have a higher frequency of caries in the early stages when compared to control group. Prevention is the key to these patients thus avoiding the evolution of these carious lesions.

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### Bonding Longevity of Flowable GIC Layer in Caries-Affected Dentin: One-Year Storage

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High-viscosity glass ionomer cements (GIC) developed for atraumatic restorative treatment (ART) present difficulties regarding their hand-mixing and insertion into the cavity, leading to inaccurate adaptation to tooth surface. The use of a thin layer of GIC with more fluid consistency before the insertion of a regular layer could minimize this adverse effect. Thus, this study evaluated the GIC bonding longevity in sound and artificial dentinal caries,

using a conventional and a flowable GIC, after one year of water storage. Thirty bovine incisors were polished to obtain flat buccal dentin and standardized smear layer. Teeth were then randomly allocated in 4 groups, according to substrate – sound and artificial dentinal caries (pH cycling for 14 days), and powder/liquid ratio of high-viscosity GIC – conventional (manufacturer's instruction) and flowable GIC (mixing two liquid drops with one powder portion). Polyethylene tubes with internal diameter of 0.76 mm were placed over the pre-treated dentin and filled up with GIC. GIC restorations had their surfaces protected with a layer of petroleum jelly. Half of specimens were evaluated by microshear bond strength test after 24 h of water storage at 37°C, and the other half twelve months later. Data (MPa) were analyzed with 3-way repeated measures ANOVA and Tukey's tests ( $\alpha = 0.05$ ). Statistical analysis revealed reduction of GIC bonding effectiveness after one year of water storage ( $5.4 \pm 1.4$  to  $3.4 \pm 1.3$ ) ( $p < 0.001$ ). No significant differences were observed ( $p = 0.126$ ) between conventional ( $4.2 \pm 1.8$ ) and flowable ( $4.6 \pm 1.5$ ) GIC. Moreover, GIC showed better bonding effectiveness on sound ( $5.1 \pm 1.7$ ) than artificial dentinal caries ( $3.7 \pm 1.3$ ) ( $p < 0.001$ ). Two-layer GIC using a flowable GIC can be an alternative to ART without jeopardizing the bonding to dentin, since both GIC show similar reduction of bonding effectiveness in water storage.

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### Association between Oral Motor Performance and Caries Severity in Subjects with Moebius Syndrome: A Pilot Study

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Moebius syndrome (MS) is a genetic disease (OMIN #157900) characterized mainly by the palsy of facial nerve (cranial nerve VII) and abducens nerve (CN VI). The affected children show facial palsy and dysphagia that could influence on oral motricity and consequently, make these subjects more susceptible to dental caries. We aimed to test the association between the oral motor performance and caries severity in children/adolescents. Twenty-eight normal subjects and 19 subjects with a medical diagnostic of MS, aged between 3 and 19 years, were selected. Two calibrated researchers performed independently examinations using a validated scale OMAS (Oral Motor Assessment Scale – scores from 0 to 3) for assessing orofacial motricity and ICDAS (International Caries Detection and Assessment System) used for caries lesions assessment. We considered two outcomes: total number of lesions in surfaces of primary and permanent teeth and simply, presence or absence of dental caries. Different cut-off points (sound-cari-ous) were adopted in each outcome considering ICDAS scores. Univariate and multiple regression analyses were used to verify association between oral motor impairment and presence of dental caries or number of dental caries lesions. Age was used as an inde-

pendent variable for adjustment. Children/adolescents who presented better oral functions (OMAS score 3) had lower number of lesions scored as ICDAS 2 or higher (mean  $\pm$  SD =  $7.3 \pm 8.5$ ) than those who presented lower OMAS scores (mean  $\pm$  SD =  $18.7 \pm 12.2$ ; regression coefficient =  $-9.9$ ; SE = 0.35). The same was not valid for other cut-off points used. Therefore, the higher number of initial lesions observed are associated to children/adolescents with MS, despite being this kind of impairment an important aspect of this syndrome.

The study was funded by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo – 'São Paulo Research Foundation') (#2010/18931-2), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – 'Coordination for the Improvement of Higher Level or Education Personnel') and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico – 'National Counsel of Technological and Scientific Development').

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### Three-Year Outcomes of a RCT on the Nexø Model in the Netherlands

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A randomized controlled trial on caries-preventive strategies was conducted in a regular dental practice with patient population of mixed socioeconomic background. A total of 179 children (6.0 years  $\pm$  3 months of age) were randomly assigned to two experimental groups (1 or 2) or a control group (3). These subjects were followed for a period of 3 years. The first experimental group ( $n = 54$ ) followed a non-operative caries treatment and prevention protocol program (NOCTP), adopted from the Nexø studies. The second experimental group ( $n = 62$ ) followed an intensified professional fluoride applications regime (four times a year) (IPFA). Children in the control group ( $n = 63$ ) received regular dental care consisting of check-ups twice a year with professional fluoride applications and pit and fissure sealants in all permanent molars. Three years after the start of the trial, mean caries increment scores (using  $\Delta D_3MFS$ ) were respectively 0.13 (NOCTP), 0.35 (IPFA) and 0.39 (control). The difference between the control group and the NOCTP group was statistically significant ( $t = -2.17$ ;  $p = 0.03$ ). The results of this study suggest that a self-care based tailor-made prevention strategy (NOCTP) can be effective in a regular dental practice in a mixed socio-economic status population with low caries incidence.

### Value for Money in Caries-Preventive Strategies

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This study aimed to investigate the cost-effectiveness of two different caries prevention strategies compared with standard dental care in the Netherlands. A cost-effectiveness analysis was conducted alongside a 3-year randomized controlled clinical trial in which a total of 179 six-year-old children ( $\pm 3$  months) were assigned to regular dental care, an increased professional fluoride application program (IPFA) or a non-operative caries treatment and prevention (NOCTP) programme. Regular dental care consisted of dental check-ups twice a year; these dental check-ups included oral hygiene instruction, dietary counseling, professional 1.23% fluoride-gel application and routine placement of pit-and fissure sealants once the occlusal surfaces of permanent molars were fully erupted. The IPFA programme followed this same regime but the IPFA programme included two additional professional fluoride treatments (a total of four per year). The NOCTP programme, adopted from the Nexø studies, followed a non-invasive caries treatment and prevention protocol where the check-up interval was assessed individually at each visit using predetermined risk factors. Professionally applied prevention tools, such as local application of fluoride varnish and pit and fissure sealants, were only used under strict indications in this programme. Caries increment scores (at D3MFS level) were used to assess effectiveness. Incremental cost-effectiveness ratios (ICERs) were expressed as additional costs per prevented DMFS. The ICERs compared with regular dental care from a societal perspective and health care perspective were EUR 733 and EUR 977 per prevented DMFS in the IPFA programme, and EUR 111 and EUR 108 in the NOCTP programme, respectively. The largest investments for the NOCTP group were made in the first year of the study, and decreased in the second and third year of the study. From both dental and economic points of view, the NOCTP strategy may be more ideal than the IPFA strategy. Compared with regular care, the NOCTP strategy appears to be reasonably cost-effective.

### An in situ Study Investigating Sub-Surface Dentine Tubule Occlusion of Dentifrices following an Acidic Challenge

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This in situ study aimed to evaluate the depth of penetration and constituents of deposits formed within dentine treated with desensitising dentifrices designed to occlude dentine tubules; one containing 8% strontium acetate, 1 040 ppm sodium fluoride (Sensodyne<sup>®</sup> Rapid Relief) and the other containing 8% arginine, calcium carbonate, 1,450 ppm sodium monofluorophosphate (Colgate Sensitive Pro-Relief<sup>®</sup>) to negative control paste and water. This clinical study was a single-centre, randomised, crossover, single blind design. Twenty-eight healthy participants wore two intra-oral appliances each retaining four human dentine samples for two periods of four days. Samples were power brushed twice daily with test products and subjected to an agitated grapefruit juice acid challenge on days three and four. Eighteen dentine samples were randomly selected from those used in the study and were dry fractured. One half of each fractured sample was coated with carbon for EDX analysis and the remaining half gold coated for SEM imaging. Imaging and analysis took place consistently from the centre of each sample. Samples treated with 8% strontium on days one and two (pre-acid) and day four (post-acid) and samples treated with 8% arginine on day four (post-acid) revealed sub-surface deposits covering 80% (SD 10%) of the dentine surface. These were statistically significant versus controls ( $p < 0.01$ ). Tubule occlusion from tubule occlusion dentifrices occurred within 5  $\mu\text{m}$  (SD 2  $\mu\text{m}$ ) of the dentine surface. The 8% arginine on days one and two and water and control paste on days one and four revealed no sub-surface deposits. Within the limitations of this study, cross-sectional SEM analysis suggests that the positive control dentifrices more likely demonstrated occlusion of tubules sub-surface in dentine compared to negative controls. EDX analysis was insufficiently sensitive to detect the nature of deposits.

The clinical component of this study was funded by Glaxo-SmithKline.



## Session 3 Diagnostics and Fluoride

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### In vivo Performance of CarieScan Pro for Detection of Occlusal Caries

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**Aim:** To evaluate the in vivo performance of the CarieScan Pro on occlusal surfaces. **Methods:** The study was approved by the ethics committee and informed consent was given by the participants. 306 unrestored permanent molars of 26 patients were available for the study. The teeth were cleaned, isolated with cotton rolls and dried for 5 s. The occlusal surfaces of the teeth were performed using (1) ICDAS-II criteria and (2) CarieScan Pro (CS), based on the AC Impedance Spectroscopy technique (ACIST). The actual depth of the lesions (clinical gold standard) were assessed using radiographs and/or clinically by opening the lesion when appropriate. Correlation between all methods were assessed using Spearman's rank correlation coefficient (rs). Sensitivity (SE) and specificity (SP) were calculated at D1 (enamel lesions) and D3 (dentine caries) level and area under the ROC curve (AUC) were assessed. **Results:** Significant positive correlation was found between ICDAS-II, CS measurements and the clinical gold standard (rs 0.41–0.66,  $p < 0.01$ ). SE and SP were: D1: SE = 100%, Sp = 7.5%; D3: SE = 66.7%, Sp = 95.3%. The diagnostic performance (AUC) was 0.70 at D1 level and 0.88 at D3 level. A combination of CS measurements with the visual examinations showed the following SE values: D1: 100%, D3: 92.6%. **Conclusion:** The CS showed good diagnostic performance. The combination of CS with ICDAS-II improved the SE in detecting enamel and dentine lesions.

The device CarieScan Pro was provided by CarieScan Ltd., Dundee, UK.

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### Correlation with Caries Lesion Depth of the Canary System, DIAGNOdent and ICDAS II

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The Canary System™ (CS) is a laser-based detection system for caries. The aim of this study was to correlate lesion depth of natural caries to Canary Numbers (CN) derived from CS, numerical readings from DIAGNOdent (DD), and lesion scores from ICDAS II. A total of 20 examination sites from 10 extracted human molars and premolars were selected. The selected examination sites consisted of healthy to enamel caries on smooth and occlusal surfaces of each tooth. Two blinded dentists ranked each examination site using ICDAS II and the consensus score for each examined site was recorded. The same examination sites were scanned with CS and DD, and the CN and DD readings were recorded. After all the measurements were completed, the readings of the three caries detection methods were validated with a histological method, polarized light microscopy (PLM). PLM performed by blinded scorers was used as the 'gold standard' to confirm the presence or absence of a caries lesion within each examined site and to determine caries lesion depth in  $\mu\text{m}$ . Pearson's coefficient of correlation with caries lesion depth of CNs, DD readings and ICDAS scores were 0.84, 0.21 and 0.77 respectively. Mean  $\pm$  SD CN for sound sites ( $n = 3$ ), caries lesion depths  $<800 \mu\text{m}$  ( $n = 11$ ), and caries lesion depths  $>800 \mu\text{m}$  ( $n = 6$ ) were  $11 \pm 1$ ,  $55 \pm 15$  and  $75 \pm 22$ , respectively. Mean  $\pm$  SD DD readings for sound sites, caries lesion depths  $<800 \mu\text{m}$ , and caries lesion depths  $>800 \mu\text{m}$  were  $1 \pm 1$ ,  $7 \pm 11$  and  $8 \pm 9$ , respectively. Mean  $\pm$  SD ICDAS II scores for sound sites, caries lesion depths  $<800 \mu\text{m}$ , and caries lesion depths  $>800 \mu\text{m}$  were  $0 \pm 0$ ,  $2 \pm 1$  and  $2 \pm 1$ , respectively. This study demonstrated that CS exhibits much higher correlation with caries lesion depth compared to ICDAS II and DD.

This study was supported by Quantum Dental Technologies Inc., Toronto, Ontario, Canada.

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### Association between Two Visual Criteria in Assessing Non-Cavitated Caries Lesions Activity on Permanent Molars

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The aim of this clinical study was to evaluate the association between two visual criteria in assessing non-cavitated caries lesions activity on permanent molars. For this, 39 patients aged from 5 to 13 with at least one permanent molar with non-cavitated caries lesion on its occlusal surface were selected. Molars were classified according to the presence of caries lesions using ICDAS as well as to their activity status following two criteria: ICDAS-LAA and a standard criterion based on clinical characteristics which are consensus in the literature (brightness, color and texture). In standard criterion, an opaque, whitish and rough surface were defined as active caries lesion while a white or brownish lesion with smooth, shiny and hard surface were classified as inactive caries lesion. In both criteria the caries lesions were dichotomized in active and inactive. The results showed a weak association between both criteria in assessing caries lesions activity (Cramer's V coefficient 0.39,  $p < 0.001$ ) and very low specificity for ICDAS-LAA (0.35). When all caries lesions ( $n = 124$ ) were considered, there was agreement between the two criteria in 71 lesions (57.3%). In the 53 lesions (42.7%) in which there were disagreement between the criteria, the ICDAS-LAA classified the caries lesions as active and the standard criterion classified them as inactive. From these results, it can be concluded that ICDAS-LAA criterion was not appropriate for activity assessment of non-cavitated caries lesions on occlusal surfaces of permanent molars, since this criterion seems to overestimate caries lesions activity.

The study was conducted with the authors' own sources of funding.

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### Associated Factors with Non-Cavitated Caries Lesions Activity after Non-Operative Treatment: A Clinical Study

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The aims of this study were (1) to evaluate the effectiveness of a treatment for patients with non-cavitated caries lesions on per-

manent molars in different eruption stages and (2) to evaluate if the eruption stage and biofilm accumulation are associated to caries lesions activity in patients that received a non-operative treatment. For this, 48 patients aged from 5 to 13 years old were selected. Those patients should have presented at least one permanent molar with an active non-cavitated caries lesion on its occlusal surface. Molars were classified according to type (first or second), eruption stage, biofilm accumulation and presence of lesions as well as their activity status. All patients received a weekly non-operative treatment for 4 weeks based on oral health instructions and topical fluoride application. After 3 weeks, 39 patients were re-assessed. A logistic regression model was used to evaluate the association between the eruption stage, biofilm accumulation, type of molar and caries lesions activity after a non-operative treatment. Since data were clustered, odds ratios (ORs) were obtained using generalized estimating equations with a logistic link function. Of the 103 re-assessed teeth, 42 remained with active caries lesion. After adjustment the data for the effect of biofilm accumulation and type of molar, molars with occlusal surfaces partially exposed to the oral cavity were 301.1 times more susceptible to caries activity than molars with full occlusion (OR = 301.1;  $p = 0.011$ ). Likewise, high biofilm accumulation was more associated to the presence of active caries lesions when compared to the absence of visible biofilm (OR = 123.5;  $p = 0.007$ ) after adjustment for eruption stage and type of molar. After adjustment for eruption stage and biofilm accumulation, there was no statistically significant association between type of molar and caries lesions activity after the treatment ( $p = 0.411$ ). Based on these results, it can be concluded that (1) treatment was not effective for patients with active lesions on partially erupted molars and (2) a strong association between partially erupted molars, high biofilm accumulation and presence of active lesions was observed even after the treatment.

The study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES).

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### Effect of a Digital Learning Tool on the Detection of Dental Caries Using ICDAS

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This study aimed to evaluate the effect of a digital learning tool on dental students' ability in detecting dental caries using ICDAS. Digital photographs of sound and carious primary and permanent teeth were selected and a digital learning tool was created. An ICDAS expert scored the selected sites and 60 questions were formulated. To test the effect of this digital learning tool, 39 fourth year dental students, who never had any contact with ICDAS, conducted full-mouth assessments of 15 patients aged 6–12. For this first evaluation, ICDAS scores were provided in a table. Then, the students were divided into 3 groups ( $n = 13$ ) as follows: G1, students

went through the ICDAS e-learning program (<http://www.icdas.org>); G2, ICDAS e-learning program followed by the digital learning tool; G3 (control), any learning strategy. After two weeks, patients were reassessed by 32 students (G1 = 7; G2 = 12; G3 = 13) and by two ICDAS experts (gold standard). When compared before and after the learning strategies, the McNemar test did not show any difference in the values of specificity and area under the ROC curve (AUC) for all groups. Sensitivity was statistically significantly higher for G1 and G2. When the groups were compared, G2 showed a significant increase in sensitivity at D2 and D3 thresholds. Rank correlation (Spearman coefficients) with gold standard before and after learning strategies were, respectively, 0.60 and 0.61 (G1), 0.57 and 0.63 (G2) and 0.54 and 0.54 (G3). The Wilcoxon test showed a statistically significant difference between the assessments for G1 and G2. In conclusion, both strategies showed a positive learning effect for ICDAS. The digital learning tool used after the ICDAS e-learning program tended to increase the sensitivity of ICDAS used by inexperienced dental students.

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### Correlation between ICDAS and ERK Histological Classification System Applied to Micro-CT Images of Occlusal Surfaces

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ICDAS is a reproducible and accurate visual caries diagnosis/detection system. The ERK histological criteria were originally developed for use with standard histological preparations [Ekstrand et al.: *Caries Res* 1997;31:224–231]. Micro-CT is a 3D non-destructive imaging system which can be used to visualize the extent of lesion penetration. The overall aim of this study was to establish the use of Micro-CT as a gold standard for the validation of ICDAS. In this particular study, the aim was to investigate the use of ERK to classify the extent of lesion penetration as visualized in Micro-CT imaging and to compare with ICDAS examination scores of same teeth. Fourteen extracted teeth were selected with varying appearance from sound to non-cavitated lesions on the occlusal surface. Three trained examiners independently examined one to two investigation sites per tooth ( $n = 18$ ) according to the ICDAS criteria ([www.icdas.org](http://www.icdas.org)). This was repeated at a second sitting. Teeth were scanned using a desktop  $\mu$ CT 40 Scanco system with an isotropic resolution of 20  $\mu$ m. Two examiners evaluated lesions depths of reconstructed images, if present, using the ERK criteria. Consensus ERK scores were used as the 'gold standard'. From the Micro-CT images 3 sites gave a score 0, 3 scored 1, 8 scored 2, and 4 scored 3. The relationship between ICDAS scores and the histological depth determined from Micro-CT images were moderate to strong ( $r_s = 0.481, 0.605, 0.668, p < 0.05$ ). Inter-examiner reproducibility for ICDAS was fair-moderate (un-

weighted kappa = 0.25–0.8; mean = 0.45), while intra-examiner reproducibility was substantial (unweighted kappa = 0.65–0.8; mean = 0.72). In conclusion, application of subjective histological criteria to Micro-CT images may be an acceptable alternative to histological examination for the ICDAS system on occlusal surfaces.

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### Evaluation of Fluorescence-Based Methods to Detect in situ Demineralization and Remineralization on Smooth Surfaces

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The aim was to evaluate the effectiveness of fluorescence-based methods (DIAGNOdent [LF], DIAGNOdent pen [LFpen] and VistaProof fluorescence camera [FC]) in detecting demineralization and remineralization on smooth surfaces in situ. In this study, 10 volunteers wore acrylic palatal appliances, each containing 6 enamel blocks that were demineralized for 14 days by exposure to a 20% sucrose solution. Then, the remineralization phase was performed for 7 days with fluoride dentifrice (1,450  $\mu$ g F/g). Each enamel block was evaluated at baseline, after demineralization and after remineralization by two independent examiners using LF, LFpen and FC. Superficial microhardness (SMH) was measured in all phases. Furthermore, the enamel blocks were submitted to a cross-sectional microhardness (CSMH) analysis and integrated loss of surface hardness ( $\Delta$ KHN). The intraclass correlation coefficient for interexaminer reproducibility ranged from 0.21 (FC, baseline) to 0.86 (LFpen, demineralized). SMH values and LF and LFpen fluorescence values presented statistically significant differences among the three phases ( $p < 0.05$ ). However, FC fluorescence values showed no significant differences between the demineralization and remineralization phases ( $p > 0.05$ ). Fluorescence values for baseline, demineralized and remineralized enamel were, respectively,  $5.4 \pm 1.0, 9.2 \pm 2.2$  and  $7.0 \pm 1.5$  for LF;  $10.5 \pm 2.0, 15.0 \pm 3.2$  and  $12.5 \pm 2.9$  for LFpen;  $1.0 \pm 0.0, 1.0 \pm 0.1$  and  $1.0 \pm 0.1$  for FC. CSMH and  $\Delta$ KHN were significantly different between demineralization and remineralization phases. There was a positive correlation between SMH and fluorescence values in all phases, with a significant difference in the remineralization phase ( $p < 0.05$ ). However, a negative correlation was found between  $\Delta$ KHN and fluorescence values. In conclusion, LF and LFpen devices were capable of monitoring incipient lesions on smooth surfaces in situ, mainly during the remineralization phase.

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### Magnetic Resonance Imaging and Quantification of Dental Pulp Decay in Carious Teeth

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Diffusion-weighted imaging with its mapping of the apparent diffusion coefficient (ADC) is an advanced MRI technique highly sensitive to the microscopic incoherent motion of water, which allows the quantification of water ADC in tissue. In the present study, high-resolution morphological MRI sequence at 2.35T was used to assess caries lesions. The influence of demineralization in tissues to adjacent pulps was then assessed by using ADC maps in order to evaluate the potential of ADC mapping for quantifying the inflammatory status of the pulp. 33 extracted human teeth were reviewed in accord with the ICDAS and subsequently analyzed by MRI. Volume-rendered images of teeth were reconstructed from high-resolution 3D multi-slice T<sub>1</sub>-weighted images, while ADC maps of the same teeth were calculated from corresponding diffusion-weighted images. The distributions of ADC along dental pulp were obtained from ADC maps by calculating the average ADC of all pixels having equal radial distance from the deepest point of the caries lesion. Morphological MRI sequence enabled high-resolution detection of the dental pulp along with a reliable volume-rendered assessment of the relation between the caries lesions and the pulp in all clinically relevant orientations. ADC maps enabled the assessment of water mobility at caries-pulp interface. The distributions of ADC along dental pulps of intact teeth (ICDAS 0) were relatively uniform with an average of ADC  $\approx 1.3 \cdot 10^{-9}$  m<sup>2</sup>/s. A noticeable shift towards lower ADC values (ADC  $< 0.6 \cdot 10^{-9}$  m<sup>2</sup>/s) was found at the caries site in the pulps of teeth with ICDAS 3 and 6. We conclude that two complementary MRI methods, 3D T<sub>1</sub>-weighted MRI and ADC mapping, may have potential for the morphological and functional assessment of dental tissues comparable to that of ICDAS scoring.

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### Optical Coherence Tomography to Assess Non-Cavitated Occlusal Carious Lesions

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The objective was to evaluate the ability of spectral domain optical coherence tomography (SD-OCT) to assess non-cavitated occlusal carious lesions. 17 extracted human molars with 33 occlusal lesions (ICDAS code 2) were visually selected and examined

radiographically (80 ms; VistaScan Mini Plus, Dürr Dental, Germany). 33 regions of interest (ROI) were marked by 2 drill holes each in mesio-distal direction and the lesions were imaged with SD-OCT (3D image stacks; Telesto SP5, center wavelength 1,325 nm, Thorlabs GmbH, Dachau, Germany). The radiographs and OCT signals were categorized according to the lesion extent (score 1–3): 1 – sound, 2 – lesion limited to enamel, 3 – lesion into dentin. Light microscopy (AxioVision, Carl Zeiss, Germany) was used as validation standard. Statistics: Cohen's kappa coefficient ( $\kappa$ ). Agreement between methods was slight (OCT/histology:  $\kappa = 0.276$ ) or none (X-ray/histology:  $\kappa = 0.093$ ; OCT/X-ray:  $\kappa = 0.048$ ). 22 of 33 ROI were histologically confirmed to have a carious lesion penetrating into dentin. 91% (20/22) of these could be detected by radiography and 41% (9/22) by OCT. 63% of enamel carious lesions were erroneously diagnosed as dentin caries (7/11) by radiography, 0% by OCT (0/11). In 71% of specimens the dentino-enamel junction was displayed by OCT. Within enamel differences in mineralization as well as cracks and Retzius striae could be distinguished. Even though the present OCT provides additional information about the extent of occlusal carious lesions it is currently not suited to serve as a diagnostic tool for the assessment of non-cavitated occlusal carious lesions unless the signal to noise ratio is improved.

Supported by Thorlabs GmbH (provision of OCT).

### Visualisation of Pulp Chamber Roof by Optical Coherence Tomography

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**Objective:** To evaluate the ability of optical coherence tomography (OCT) to display the roof of the pulp chamber. **Methods:** The roots of 15 extracted human molars were embedded in epoxy resin and the crowns were sectioned mesio-distally exposing the pulp chamber. Coronal parts of the crowns were removed up to a remaining dentine thickness to the pulp of 2 mm. Samples were imaged by spectral domain coherence tomography (3D image stacks; SD OCT, Telesto SP5, center wavelength 1,325 nm; Thorlabs, Germany) from coronal view and by light microscopy (Axio-plan 2, Carl Zeiss, Germany). Dentine was removed from occlusal by a microtome in steps of approximately 250  $\mu$ m until pulp exposure. Each intermediate step was documented by both methods. Once the pulp chamber roof could be displayed by OCT residual dentine thickness was measured using light microscopy (AxioVision, Carl Zeiss). These data were compared to the measurements based on OCT images and statistically analysed (Spearman-Rho correlation, Wilcoxon test). **Results:** Using OCT, margins of the pulp chamber were displayed as of a mean residual dentine thickness of  $0.85 \pm 0.3$  mm (min: 0.46 mm, max: 1.94 mm). Values of both methods were strongly correlated (0.88–1.00,  $p < 0.0005$ –0.02) and significantly different ( $p < 0.01$ , large distance)/not different ( $p > 0.4$ , minor distance). **Conclusions:** The roof of the pulp chamber could be visualised by SD OCT. Therefore it could be a

diagnostic tool during the treatment especially of deep cavities and might help to among others avoid an iatrogenic opening of the pulp chamber by estimating the residual dentine thickness.

Supported by Thorlabs GmbH, Dachau, Germany (provision of OCT).

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### Detection of Caries around Ceramic Crown Restorations with the Canary System and DIAGNOdent

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The aim of this study was to evaluate the ability of The Canary System (CS) and DIAGNOdent (DD) to detect secondary caries around margins of crown restorations. Carious and sound teeth were prepared for a 3/4 ceramic crown, ensuring that margins remained on enamel. For the carious teeth, a dentist removed visible caries from the walls of the cavity ('sound walls' of carious teeth) while leaving some caries on the wall of one section of the cavity preparation ('cariou walls' of carious teeth). Three groups of margins were examined: 'cariou walls' in carious teeth; 'sound walls' in carious teeth; and sound walls in sound teeth. A blinded operator scanned 48 examination sites with CS and DD at the margins around crown, 0.5 and 1 mm away from the margins into tooth tissue. Canary Numbers (CN) and DIAGNOdent readings (DDR) were recorded. Previous studies established CN ranges of 0–20 and 21–100, and DDR of 0–13 and 14–99 to correspond to sound and carious tissue, respectively. For 'cariou walls' of carious teeth, the mean  $\pm$  SD CN/DDR at the margins, 0.5, 1 mm away from margin into tooth were:  $31 \pm 15 / 9 \pm 7$ ;  $33 \pm 19 / 8 \pm 7$ ;  $23 \pm 12 / 7 \pm 5$ . For 'sound walls' of carious teeth, the corresponding data were:  $26 \pm 10 / 10 \pm 3$ ;  $24 \pm 9 / 12 \pm 4$ ;  $19 \pm 10 / 10 \pm 4$ . DDR were at healthy tissue range for all examination sites. CN were within carious tissue range from the margins to 1 mm into tooth in all 'cariou walls' and from the margins to 0.5 mm into tooth in all 'sound walls' of carious teeth, indicating presence of caries, but at healthy tissue range in all walls in sound teeth. This study demonstrated that, unlike DD, CS has the potential to detect secondary caries around crown margins.

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### Influence of Different Magnification Devices on Visual Caries Detection with ICDAS

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In visual caries diagnostics 'optimum' vision is often recommended. Several devices are available to enlarge the vision: Galilean loupes, Keplerian loupes and surgical microscopes. We therefore wanted to test the influence of these magnification devices on validity and reliability of the ICDAS system. The influence of the clinical experience of the examiners was also assessed. There were three groups of examiners: (A) 5 3rd-year students without clinical experience; (B) 5 5th-year students with little clinical experience; (C) 4 dentists (2–16 years after graduation). Each of them examined 100 extracted molars with no obvious cavitations two times by naked eye, or by using a Galilean loupe (2.5 $\times$ ), a Keplerian loupe (4.5 $\times$ ), or a surgical microscope (10 $\times$ ) with at least one day between each examination. Histology served as gold standard. Bayesian analysis, kappa statistics and a chi-square test were applied. As a principal finding, the number of score '0' (healthy) decreased with magnification, while the number of score '3' (enamel breakdown, small cavity) significantly increased, especially with the surgical microscope. Sensitivity generally increased with magnification, while specificity decreased. For instance, in dentists, for D3 lesions the sensitivity (range) was 0.47 (0.17–0.79) by naked eye and 0.91 (0.83–1) using a surgical microscope, but the specificity (range) dropped from 0.78 (0.58–0.95) to 0.3 (0.07–0.55), respectively. The use of the surgical microscope had a negative effect on inter-examiner reliability in groups B and C (Fleiss' generalized kappa;  $p < 0.05$ ). Intra-examiner reliability was not influenced by different magnifications. Experienced dentists showed the highest levels of specificities on all caries levels with all magnification devices. ICDAS is better used without the additional use of magnification devices, because their use might result in more and unnecessarily invasive treatment decisions.

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### Caries Assessment of Occlusal Surfaces on QLF and White Light Photographs

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The aim of this in vitro study was to compare different assessment methods of occlusal surfaces: ICDAS – score on photographs, modified ICDAS and fluorescence loss assessed on QLF photographs and lesion depth assessed on photographs of hemisected teeth. Forty-six permanent molars (freshly extracted, without restorations, stored in tap water) were autoclaved and then the occlusal sides of the crowns were photographed with a QLF-D

SLR-camera ( $\lambda_{\text{exc}}$  405 nm; Inspektor Research Systems, Amsterdam, The Netherlands). White light photos were assessed according to the ICDAS score. QLF photos were assessed using a modified ICDAS score (F-ICDAS) and fluorescence loss ( $\Delta F$ ) was determined using dedicated software (QA2v1.20; Inspektor Research Systems). The molars were then hemisected and photographed with a stereomicroscope (Stemi-SV6, AxioCam KL-2500-LCD, Zeiss, Weesp) to assess lesion depths (the highest score of the two fractions was used). The level of agreement between the different methods was assessed using Spearman rank correlation ( $r_s$ ). There was a strong correlation between the ICDAS on the white light photographs (range 0–5; median 2) and F-ICDAS on QLF photographs (range 0–5; median 2) ( $r_s = 0.74$ ,  $p < 0.001$ ). The ICDAS correlated strongly with the lesion depths on the sections (range 0–4; median 2) ( $r_s = 0.55$ ,  $p < 0.001$ ). The F-ICDAS correlated moderately with the lesion depths ( $r_s = 0.40$ ,  $p < 0.01$ ). There was a strong correlation between  $\Delta F$  (range 7.5%–66.6%; median 31.8%) and the lesion depth ( $r_s = -0.63$ ,  $p < 0.001$ ) and a moderate correlation between  $\Delta F$  and ICDAS respectively F-ICDAS ( $r_s = -0.39$ ,  $p < 0.01$  and  $r_s = -0.56$ ,  $p < 0.001$ , respectively). We conclude that in vitro caries assessment on QLF photographs has a good correlation with ICDAS score on white light photographs and a moderate correlation with the lesion depth on sections. The fluorescence loss has moderate to good correlation with the visual scores ICDAS, F-ICDAS and lesion depths obtained from the sections.

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### Determination of Light Output from a Luminescent Assay for the Assessment of Caries Lesion Activity

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**Background:** Previously ORCA abstracts and posters have shown the potential for a luminescent assay in assessing caries lesion activity and erosive challenges (ORCA 2008 – posters 87 & 129, ORCA 2009 – poster 107, ORCA 2010 – poster 182). The present study seeks to help to optimise the luminescence assay with a view to potential in vivo application. **Aim:** To determine the light output in the time domain of a known quantity of a luminescent calcium ion marker to be used to capture released calcium ions from active caries lesions [patent: WO2008075081 GB2445460 (A)]. **Methods:** A bespoke stopped-flow luminometer system was designed to test the luminescent calcium marker with a view to determining the light output from the marker over time. The stopped flow process uses fixed syringes to rapidly (within a few nanoseconds) dispense set volumes of known concentration of luminescent marker and 1 mM calcium chloride to a mixing chamber, from which light output is collected and assessed. **Results:** Results from 5 runs (using 5 duplicates per run) on 2 separate batches of marker consistently show that the luminescence peaked

at approximately 10 ms, with a fall-off to 50% of that peak value at 70 ms and 10% at 300 ms. The speed of development of the light flash will allow for the short exposure times required of a handheld intraoral stills camera. **Conclusions:** The stopped-flow methodology allowed characterisation of the luminescence output over time, indicating the potential suitability of the marker for use in vivo.

The study was funded by Calcivis Ltd., Edinburgh, UK.

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### The Visual Assessment of Early in vitro Chemical Demineralisation Using a Multispectral Imaging Method

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The purpose of this study was to determine whether Nuance™ multispectral imaging system (CRi, Woburn, USA) was an appropriate method for use when compared with QLF-D Biluminator™ (Inspektor, Amsterdam, The Netherlands) and white light in the visual detection of early in vitro chemical demineralisation. Ten specimens were prepared from extracted bovine teeth and immersed in acid-buffer demineralising solution (2.2 mM  $\text{KH}_2\text{PO}_4$ , 50 mM acetic acid, 2.2 mM  $\text{CaCl}_2$ , 0.05 ppm F, pH 4.5). Samples were illuminated with a 405 nm exciting light source. Fluorescence and white light images were captured with Nuance™ fitted with a 460 nm highpass colour filter and QLF-D Biluminator™ devices respectively at baseline, 1, 3, 6, 9, 12, 24, 48 and 72 h. Images were randomised and visually assessed by four examiners for presence or absence of demineralisation, all blinded to the demineralisation stage for all images viewed on two occasions. Kappa statistics for inter and intra-examiner reliability were calculated for each method. The ability of all methods to detect demineralisation was compared using McNemar's test. All examiners were unable to detect demineralisation at baseline. The earliest detection was at 1 h with Nuance™ and 3 h with QLF-D and white light images. Intra-examiner reliability scores for Nuance ranged between 0.803–0.934, QLF-D (0.765–0.94) and white light (0.641–0.857). Inter-examiner reliability for Nuance™ ranged from 0.860–1, QLF-D 0.692–0.895 and for white light ranged from 0.578–0.607. Nuance™ and QLF-D both detected more demineralised lesions compared with white light but these differences were not statistically significant. Nuance™ also detected slightly more lesions when compared with QLF-D however this was not significant ( $p \geq 0.05$ ). Nuance™ appears a reliable and promising method for detecting demineralisation. However more work is needed to determine its accuracy in detecting and quantifying demineralised lesions when compared with established methods.

### Caesium Fluoride Radiographically Camouflages Residual Caries after Incomplete Caries Removal

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**Objectives:** One-step-incomplete caries removal was shown advantageous for treating deep caries lesions. However, it is impossible to radiographically distinguish intentionally from accidentally left residual caries (RC) or recurrent caries. Dentists familiar with both the concept of incomplete excavation and the patient's dental history may not find this problematic. In contrast, radiographic detection of RC by other dentists may lead to unnecessary re-treatment. Therefore, this study investigated radiopaque tagging of RC. **Methods:** 36 extracted, deeply carious teeth (ICDAS code 5/6) were excavated incompletely (leathery RC remaining). The cavity was filled using composite resin without adhesive and a standardized radiograph was obtained. Dentinal fluid flow was simulated using Ringer's solution (3,000 Pa). After removal of composite, ethanol-dissolved caesium fluoride (15%; CF) was applied onto RC twice. Subsequently, the cavity was restored using an etch and rinse adhesive and composite resin. Ageing and possible leaching were simulated by 10,000 thermal cycles (TC) of 5–55°C. Digital radiographs were obtained before and after TC. Radiographs were evaluated using repeated linear plot analysis. Calculated grey value differences ( $\Delta$ GV) between sound dentin and the area under the restoration were compared between consecutive radiographs. Eventually, six blinded dentists assessed eight radiographs (before and after CF application) for RC detection. **Results:**  $\Delta$ GV [Q25/Q75] at baseline was 1.4 [0.8/2.4]%. After CF treatment,  $\Delta$ GV was -0.04 [-0.34/0.26]%, but increased to 0.97 [0.51/1.48] after thermal cycling. Differences between all treatment steps were significant ( $p < 0.001$ , Wilcoxon), and measurements reproducible (ICC 0.74–0.93). Dentists diagnosed 85 ± 13% of untreated lesions but only 15 ± 5% of tagged lesions as RC. **Conclusions:** CF successfully tagged natural RC in vitro, but was leached from the lesion during TC. Stable radiopaque materials might be suitable to radiographically tag RC, thus avoiding unnecessary re-treatment of uninformed dentists.

### Comparison of Occlusal Caries Detection Using the ICDAS Criteria on Extracted Teeth or Their Photographs

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Using photographs of occlusal surfaces instead of extracted teeth for the detection of caries using the ICDAS criteria can be useful in multicenter studies or education. Using a panel of observ-

ers, ICDAS scores on teeth or photographs were evaluated against the histological gold standard via ROC curves. The working hypothesis was that both outcomes were equivalent. A panel of 4 observers scored high-resolution photographs of the occlusal surfaces of 110 extracted permanent molar teeth projected on a computer screen. The criteria of ICDAS-II were used. The results of an earlier study of ICDAS scores on the original extracted teeth (with 2 of the observers of the panel) served as comparison. The histological slides prepared earlier were re-evaluated by the panelists in consensus and served as gold standard. Sensitivity and specificity were calculated (cutoff: D3 caries) and ROC curves were established. Statistical comparison between ROC-AUC values of both scoring techniques were performed using the tooth surface as unit [Lee and Rosner: Appl Stat 2001;50:337–344]. Further evaluation was performed using kappa statistics and Spearman correlation coefficients. AUC values of ROC curves of ICDAS-II scores of extracted teeth (0.794) and photographs (0.769) were not statistically significant ( $p = 0.88$ ). Intra-rater scores for ICDAS on teeth were 0.74–0.86, on photographs they ranged from 0.78 to 0.84. Inter-rater kappa was 0.74 for extracted teeth and ranged from 0.55 to 0.74 for the photographs. One observer not trained for ICDAS had a significant lower correlation with the other trained observers (0.62) than these among each other (0.73) and with histology (0.48 vs. 0.58). Scoring occlusal surfaces using ICDAS-II on the tooth or on its photograph yielded comparable results. This allows its use in training, education and multi-center studies even after histological procedures.

### Characterizing Fluorosed Teeth by Optical Coherence Tomography and Polarized Raman Spectroscopy

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Chalky white surfaces of mildly fluorosed teeth resemble carious white spot lesions (WSL) yet warrant different clinical interpretation. This study's aim was to determine whether the optical methods of optical coherence tomography (OCT) imaging and polarized Raman spectroscopy (PRS) can help characterize and distinguish fluorosed regions from WSL. Facial and approximal enamel surfaces of extracted human teeth were examined by two clinicians. Using Dean's index for classifying fluorosis, samples were grouped as sound ( $n = 10$ ), WSL ( $n = 9$ ), mild ( $n = 6$ ), moderate ( $n = 18$ ) or severe fluorosis ( $n = 14$ ). OCT images showed increased light back-scattering intensity at surfaces/subsurfaces of carious and fluorotic regions compared to sound enamel. Subtle visual changes in the light back-scattering pattern between WSL and fluorosed regions were observed. However, initial attempts at deriving non-subjective classification parameters have not yielded reproducible results. Using PRS, spectral band position of the dominant hydroxyapatite phosphate vibration revealed statistical-

ly significant (one-way ANOVA, followed by Tukey HSD post-hoc comparison,  $p < 0.05$ ) differences in the mean band position of moderate ( $960.58 \pm 0.12$  SE) and severe fluorosed ( $960.58 \pm 0.18$ ) sites compared to sound ( $959.24 \pm 0.19$ ), WSL ( $959.11 \pm 0.22$ ) and mild fluorosed ( $959.44 \pm 0.29$ ) enamel. Mean Raman depolarization ratios were significantly different for discriminating WSL ( $0.41 \pm 0.02$ ) from mildly fluorosis regions ( $0.23 \pm 0.03$ ). Shifts to higher peak position indicate more highly mineralized/crystalline enamel for moderate and severe fluorosis samples. Depolarization ratio changes suggest orientational differences in enamel crystal-line structure between mild fluorosis and WSL. Overall, OCT is useful as a screening tool for rapidly detecting regions of anomaly due to demineralization and fluorosis. Thereafter, PRS can be used to further characterize and confirm the identity of suspect enamel regions as caries or fluorosis.

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### Aspects of $\Delta F$ by QLF-D in Screening for Remineralization Potential of Enamel Lesions in vitro

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Clinically, it is important to evaluate the remineralization potential of caries lesions as well as risk factors. The aim of this study was to examine the aspects of initial fluorescence [ $\Delta F$ ] to screen enamel lesions to be remineralized effectively by fluoride in vitro. A total of 100 bovine enamel specimens were demineralized by 0.1 M lactic acid with 1% carbopol gel (pH 4.8) for 3 to 40 days. Subsequently, according to the  $\Delta F$  values after demineralization ( $\Delta F_{\text{base}}$ , %), all the specimens were divided into 6 groups. The specimens were remineralized by combined exposures to a 2% NaF for 4 min and artificial saliva for 24 h. After this cycle, the  $\Delta F$  recovery rate ( $R_{\Delta F}$ , %) was calculated from the  $\Delta F_{\text{base}}$  and  $\Delta F$  values after remineralization ( $\Delta F_{\text{Tx}}$ , %) using the quantitative light-induced fluorescence digital (QLF-D). The lesion depth values were also digitally measured by a polarized light microscope (PLM) before and after remineralization. For all over samples, mean  $\Delta F$  ( $\pm$  SD) after remineralization ( $-17.41 \pm 12.11\%$ ) were significantly recovered from baseline ( $-25.24 \pm 13.83\%$ ;  $p < 0.05$  by paired t-test) with averaged  $R_{\Delta F}$  of  $38.23 \pm 20.83\%$ . Due to ROC analysis, the suggested  $\Delta F_{\text{base}}$  cutoff value was  $-14.60\%$  for the threshold  $R_{\Delta F}$  of 50% (95% CI: 0.88–0.98;  $p < 0.0001$ ) with sensitivity of 1.00 and specificity of 0.81, whereas  $\Delta F_{\text{base}}$  cutoff value of  $-19.15\%$  for the threshold  $R_{\Delta F}$  of 40% (0.84–0.96;  $p < 0.0001$ ). According to PLM analysis, histological lesion depth values were significantly correlated with  $\Delta F$  values at  $r = 0.94$  (Spearman rank correlation,  $p < 0.0001$ ). In

conclusion,  $\Delta F$  assessment by QLF-D was confirmed to be reliable and it was suggested that incipient enamel lesions within initial  $\Delta F$  of  $-15\%$  could have mineral recovery over 50% by fluoride treatment.

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### Reliability of Nyvad Caries Diagnostic Criteria in a Community with a High Incidence of Mild Fluorosis

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The aim of this study was to evaluate the intra- and inter-examiner reliability of the Nyvad Caries Diagnostic Criteria, based on caries lesion activity assessment in a Brazilian child population with high prevalence of dental caries and dental fluorosis (TF 1–4) in permanent teeth. The caries diagnostic codes used were: 0 = sound; 1 = active (intact); 2 = active (surface discontinuity); 3 = active (cavity); 4 = inactive (intact); 5 = inactive (surface discontinuity); 6 = inactive (cavity); 7 = filling; 8 = filling with active caries; 9 = filling with inactive caries. Distinctions between active and inactive caries lesions and fluorotic lesions were made on the basis of a combination of visual and tactile criteria. Reliability was assessed after 2 days of practical training, through repeated examinations by four recorders, of fifty 12-year-old children, from Nova Friburgo, Brazil. Examinations were carried out in a dental office under standardized conditions. Analyses were based on three caries diagnostic thresholds, one distinguishing between sound and carious; one distinguishing between sound/inactive and active caries lesions; and one distinguishing between sound/non-cavitated and cavitated lesions. Thirty-four (68%) patients were identified as having mild dental fluorosis. The caries kappa values varied between 0.39 and 0.90 for intra-examiner and between 0.38 and 0.71 for inter-examiner recordings. The lowest kappa values were observed in one recorder, who had never used the criteria before, particularly when the cut-off points were cavitated versus non-cavitated and active versus inactive. We conclude that examiners knowledge and prior training with lesion activity assessment significantly influences the reliability of caries recordings, mainly in patients with mild-fluorotic lesions. More intensive training is needed for some recorders, especially in populations with mild dental fluorosis.

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### Association between Cariogenicity of Biofilm and Its Red Fluorescence Detected by Quantitative Light-Induced Fluorescence-Digital

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The aim of this study was to evaluate whether Quantitative Light-Induced Fluorescence-Digital (QLF-D) can detect levels of cariogenicity of dental biofilm by assessing its red fluorescence intensity. Microcosm plaque biofilms were initiated from human saliva on bovine enamel discs. Biofilms were grown in artificial saliva supplemented with sucrose of different concentrations (0.05, 0.1, 0.2, 0.5, 1, 2%) in 24-well microplates. After 10 days, fluorescent images of the biofilm formed on the enamel discs were captured by QLF-D (Inspektor Research Systems BV, The Netherlands) to analyze red fluorescence intensity, which was calculated by red/green ratio (R/G value). After that, supernatant pH was measured, and the biofilms were collected for microbiological analyses. Mineral loss in enamel discs was also evaluated by calculating the percentage of surface hardness change (SHC). The results showed that the R/G values and all cariogenic properties of the biofilms formed under the condition of different sucrose concentrations statistically differed from each other ( $p < 0.0001$ ). The R/G value increased consistently with increasing sucrose concentration up to the 0.5% sucrose, at which the mean R/G value was highest, 2.56. The R/G value showed a significant correlation with every cariogenic property ( $p < 0.05$ ). A strong correlation was identified between the R/G value and number of aciduric bacteria ( $r = 0.70$ ,  $p < 0.0001$ ), the supernatant pH ( $r = -0.73$ ,  $p < 0.0001$ ), and %SHC ( $r = 0.79$ ,  $p < 0.0001$ ). In conclusion, the red fluorescence detected by QLF-D was correlated with cariogenic properties of biofilm. Therefore, assessing red fluorescence intensity using QLF-D may enable the cariogenic potential of individual dental biofilms, *in vivo*, to be predicted.

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### Merits and Limitations of ICDAS at Japanese Public Dental Center for Patients with Disabilities

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Discussed in the present study are merits and some limitations with ICDAS at Kobe Dental Center, founded by Kobe City and run by Kobe Dental Association. Staff members at the center include 3 full-time dentists, 5 full-time dental hygienists, 19 private practitioners, 3 dentists from universities, 10 part time dental hygienists and 4 nurses. Initially implemented at the center in April 2010, the

ICDAS criteria were employed as reference for monitoring incipient carious lesions of patients between 6 and 12 years of age. Staff members seem to agree on the utility of ICDAS, but actual application of ICDAS detection is voluntary and its use depends on dentists and hygienists in charge. Of 1,078 patients who have visited the center from April to October 2012, ICDAS detection was limited to 92 patients (8.5%), and in the age group of 6–12 year old, 42 of 206 (20.4%). Some employed ICDAS detection because it (1) helps keeping objective records for follow-ups, (2) leads to more careful cleaning and closer observation of the tooth surface, and (3) reduces invasive treatments – hence general anesthesia. Limitations pointed out by staff members include: (1) in case patients' disability hampers air-blow process ICDAS detection may not be applied; (2) for patients with difficulties in keeping their mouths open, detection with ICDAS criteria is not feasible. Though dental professionals have to take these limitations into consideration, ICDAS still brings benefits by providing reference standard for caries detection to the center where many dental professionals work by rotation and the examiner changes from appointment to appointment. Integration of ICDAS criteria into caries management, together with other non-invasive efforts may lead to reduction of general anesthesia and sedation often required for invasive procedures for patients with disabilities.

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

### In vitro Quantification of Occlusal Caries Lesion Using QLF-D, ICDAS and Diagnodent

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The QLF is the well-known caries detection system based on the measurement of fluorescence loss of demineralized enamel. Recently, the new QLF system (QLF-D) was introduced, which consists of QLF light source, filters, and digital camera. The aim of this study was to compare the QLF-D with ICDAS, Diagnodent, and histologic feature for *in vitro* quantification of occlusal caries. 122 extracted permanent teeth were selected and site of interest of occlusal surface examined with each detection method. The occlusal sites were classified according to ICDAS II criteria by consensus of two investigators who trained with ICDAS E-learning course. Then, the investigation site was measured by the Diagnodent and the peak value was recorded. Also, the occlusal site was taken pictured by the QLF-D to obtain the  $\Delta F_{\max}$  value. After completion of all the assessments, the occlusal sites were sectioned vertically to assess histological features as a gold standard. The histological criteria were graded by 4 point scale: S = sound ( $n = 21$ ), E1 = caries limited enamel ( $n = 27$ ), E2 = caries extending to dento-enamel junction ( $n = 49$ ), D = caries involving dentine ( $n = 25$ ). All occlusal sites were assigned to ICDAS code between 0 and 4, and showed

QLF-D value from -95 to 0, and Diagnodent value from 8 to 99. The correlations with histology were 0.68 (QLF-D), 0.58 (ICDAS), 0.46 (Diagnodent), respectively ( $p < 0.01$ ). QLF-D also showed an acceptable correlation with ICDAS ( $r = 0.75$ ,  $p < 0.01$ ). The average QLF-D values of each histological grade were statistically different, which were -28.5 (S), -53.7 (E1), -68.1 (E2), -84.4 (D), respectively. Visual inspection with QLF-D would improve accuracy of early caries detection and diagnosis.

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### Evaluation of Thermal Images under Pulse Heating for Quantification of Natural Non-Cavitated Lesions

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The objective was to evaluate whether pulse heating thermal images could quantify natural sound enamel and non-cavitated lesions. The approximal surfaces of premolar teeth were visually assessed. Simulated bitewing X-rays were taken to evaluate lesion extension. The lesions were divided into three groups: lesions within outer half of enamel (D1), lesions within inner half of enamel (D2), and lesions that reached to the dentin-enamel junction (DEJ) (D3). Among these lesions, total of nine non-cavitated lesions that were surrounded by sound enamel was selected (three for each group). The distribution of infrared radiation energy was captured via infrared camera (113 images/s). A xenon flash-lamp was employed as the heat source (1,600 × 4 J and excitation time: 1/1,200 s). Sound and non-cavitated lesion areas were analyzed. The temperature descent curve between  $I_r$  (intensity of infrared radiation energy) and  $t$  (elapsed time after pulse heating) was fitted on the logarithmic curve expressed by the following equation:  $I_r = -A \ln(t) + B$ . One-way ANOVA was used to compare the coefficient  $A$  among the groups. There was no difference ( $p > 0.05$ ) in coefficient  $A$  for the sound areas among the groups: D1 ( $23.1 \pm 5.0$ ), D2 ( $18.7 \pm 2.7$ ), and D3 ( $18.6 \pm 6.2$ ). Lesion areas in D2 ( $39.7 \pm 7.4$ ) and D3 ( $34.4 \pm 4.6$ ) groups showed higher value than sound areas ( $p < 0.05$ ); however there was no difference for D1 ( $34.5 \pm 7.3$ ) group ( $p = 0.08$ ). Although there was no significant difference in lesion areas among the groups ( $p > 0.05$ ), lesion area in D2 group presented highest value. Thermal images under pulse heating have the potential to quantify non-cavitated enamel caries from sound enamel.

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### Quantitative Light-Induced Fluorescence to Measure Enamel Remineralisation in vitro

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The aim of this study was to compare the ability of quantitative light-induced fluorescence (QLF) and surface microhardness (SMH) to measure the remineralisation of enamel subsurface lesions, using a pH-cycling model including treatment with either 0 ppm, 550 ppm or 1,100 ppm NaF dentifrices. Subsurface lesions were created in human enamel specimens ( $n = 36$ ) and exposed to a remineralisation pH-cycling model for 14 days. Lesion baseline values were  $93.9 \pm 8$ ,  $92.5 \pm 7.5$  and  $92.1 \pm 6$  for SMH and fluorescence values were  $90.72 \pm 4.9$ ,  $95.4 \pm 5.9$  and  $90 \pm 4.8$  for the 0, 550 and 1,100 ppm F treatments, respectively. pH cycling was performed in a robot system where specimens were subjected to a demineralising for 20 min, treatment for 1 min and remineralised for 7:39 h, three times daily. The treatments consisted of three water-based sodium fluoride (NaF), silica-containing dentifrices (0 ppm F, 550 ppm F, 1,100 ppm F). The outcome variables were: change from baseline in surface hardness ( $\Delta Z$ ) and percentage change from baseline in fluorescence from baseline ( $\Delta F\%$ ), calculated as:  $\%F = (\Delta F \text{ reference area} - \Delta F \text{ lesion area}) / \Delta F \text{ reference area}$ . An analysis of covariance (ANCOVA) explored differences between different treatment groups (at the  $p < 0.05$  level). Associations between QLF and SMH were evaluated using Spearman's correlation coefficient. The percentage SMH changes were  $14.9 \pm 2.1\%$ ,  $56.6 \pm 9.6\%$  and  $103.9 \pm 14.6\%$  for the 0, 550 and 1,100 ppm F dentifrices, respectively. The percentage fluorescence changes were  $15.6 \pm 7.1\%$ ,  $59.8 \pm 11.9\%$  and  $85 \pm 13.2\%$  respectively. The differences between all pairwise comparisons were statistically significant for both methods ( $p < 0.05$ ). The QLF changes were correlated with the SMH ( $r = 0.67$ ,  $p = 0.01$ ). Both the SMH and QLF methods demonstrated a significant F dose response for toothpaste in this 14-day in vitro remineralisation model.

This study was supported by Colgate-Palmolive and The University of Manchester.

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### Human-Based Interactive Laboratorial Training on ICDAS as Teaching Strategy to Undergraduate Students

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We evaluated the impact of a human-based interactive practical training on use of ICDAS for teaching caries detection for undergraduate students. Sixty-two 4th year undergraduate dental

students were included in the sample. For evaluating the impact of interactive training, we assessed: cognitive skills and reasoning-contextualization (scored from 0 to 5); practical ability to detecting caries in extracted teeth and perception of and reactivity to the learning experience. Teeth used in practical test were examined histologically to validate caries depth. Results obtained before and after the activity were compared. Cognitive evaluation was compared using Student's t-test. Friedman's test was used for testing differences between students' perception. Students' performances in detecting caries lesions in practical test were compared by multilevel analysis. Regression analyses were used to associate cognitive skills, performance on practical tests and students' perception about the laboratorial activity. The mean score in theoretical test increased after interactive training with images (before:  $3.5 \pm 0.9$ ; after:  $4.2 \pm 0.7$ ;  $p < 0.001$ ). The baseline specificity for detecting initial lesions in enamel (0.33) and dentine lesions (0.33) was also improved by this kind of training (enamel: 0.53,  $p < 0.001$ ; dentine: 0.63,  $p = 0.001$ ). Higher false-positive results in practical tests were associated with lower initial scores in theoretical tests (OR = 0.58; 0.56–0.91). Most students were motivated (60%), judged to be prepared (92%) and expected a good result (67%) when participating into the practical activity. However, the students' perception did not change during the study. Students who reported to be curious or bored with activity classified more false negatives than others did ( $p < 0.05$ ). Thus, human-based interactive laboratory training is a well-accepted activity by students that can contribute to undergraduate students' learning about caries detection using ICDAS in conceptual and practical skills, despite students' recognition of this improvement.

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### Comparison of Interproximal Caries Lesion Detection by Clinical and Bitewing Radiographic Method: A Pilot Study

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**Aim:** The purpose of this study was to determine the correlation between the bitewing radiographic and clinical detection methods of interproximal caries in cavitated and non-cavitated lesions in posterior teeth. **Methods:** A cross-sectional study was conducted in 58 adult subjects. All interproximal posterior surfaces with radiographic images different from R0 were included and analyzed by a trained examiner. The surfaces were also clinically assessed using immediate separation technique with orthodontic bands to determine the presence or absence of cavitation. 268 surfaces were selected for analysis according to inclusion criteria. **Results:** 85.4% of the evaluated surfaces were sound and only 14.6% of them presented clinical cavitation. A low percentage of enamel (R2, 11.2%)

and dentin lesions (R3, 25.4%) detected radiographically presented clinical cavitation. **Conclusion:** The results of this study suggest that the presence of a radiographic image of an interproximal caries lesion does not always correspond with the presence of a clinical cavitation. Temporary separation of permanent posterior teeth is a useful tool for detecting cavitated caries lesions when direct visual inspection raises diagnosis doubts, allowing its use in research and clinical practice.

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### Can Caries Status in Primary Teeth Be Useful in Predicting Active Caries in Permanent Successors?

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This study aimed to investigate if caries lesions activity status in primary molars could predict active caries in permanent successors and the impact of using this type of evaluation. For this, 50 patients (mean age  $\pm$  SD =  $12 \pm 1.9$ ) who had participated in a previous study were reexamined four years later. In the baseline, occlusal surfaces of primary molars had been examined when they were, on average, 7-year-old children (mean age  $\pm$  SD =  $7.3 \pm 2.5$ ). In the reevaluation, occlusal surfaces of permanent successors were assessed. For both examinations, two calibrated examiners examined surfaces using Nyvad's system and International Caries Detection and Assessment System (ICDAS) in conjunction with a system of points for Lesion Activity Assessment (LAA). When assessing caries activity using LAA, two cut-off points were adopted, as proposed by Braga et al. [Caries Res 2010;44:300–308]. Univariate and multiple logistic regression analyses were performed to verify association between activity status in primary and permanent occlusal surfaces. For analyses, sites were dichotomized into sound and inactive caries vs. active caries. Relative risk (RR) and numbers need to treat (NNT) with 95% confidence intervals (95% CI) were calculated considering different indices used for caries activity assessment. The risk of presenting active caries in premolars was around 70% higher when active caries had been detected in primary molars (RR: 1.7; 1.2–2.4). These results were found independently of presence of mature plaque. Using this variable as predictor, about 4 assessments of caries activity in primary teeth should be done to prevent one case of active caries in permanent successors (NNT: 4.4; 2.5–13.2). Slightly higher NNT was observed when LAA cut-off was set as  $>7$ . In conclusion, caries activity status in primary teeth is a predictor of active caries lesions in permanent successors in young adolescents.

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### Intra- and Inter-Examiner Reliability of Red-Fluorescence Analyses of in vivo Carious Lesions

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The aim of this study was to assess the intra- and inter-examiner reliability of the analysis of red-fluorescence seen from carious lesions in vivo. Ethical approval was given by North West 2 Research Ethics Committee (11/NW/0018). Patients attending Liverpool University Dental Hospital with smooth surface dental caries lesions were invited to participate in this single-centred clinical trial and informed written consent was given by all subjects. Participants' teeth were cleaned, air dried and imaged using a digital single-lens reflex (DSLR) camera adapted to take successive images consisting of a white light image and a quantitative light-induced fluorescence digital image (QLF-D Biluminator™). Twenty teeth with carious lesions showing a range of severities were selected for analysis by three different examiners. Instructions for examiners were provided, and examiners were asked to independently examine their own set of images using the software program QA2 v1.20. Images were analysed on the same laptop computer with a 1,366 × 768 pixel resolution HD screen under non-standardised lighting conditions. Following an interim period of two weeks, examiners were provided with a second set of images for repeat analyses. Data recorded was percentage of tooth area with an intensity level of red fluorescence ( $\Delta R$ )  $\geq 30\%$ . Reliability was calculated using intra-class correlation coefficients (ICCs). Mean % tooth areas with  $\Delta R \geq 30\%$  for Examiners 1, 2 and 3 were  $9.5 \pm 11.4$ ,  $9.4 \pm 11.4$  and  $9.4 \pm 11.4$  respectively. Inter-examiner agreement for percentage of tooth area with red fluorescence (at  $\Delta R \geq 30\%$ ) was found to be excellent with ICCs ranging from 0.997 to 0.999. ICCs for intra-examiner reproducibility ranged from 0.993 to 1.000. In conclusion, the analysis of red-fluorescence seen from carious lesions in vivo is reliable between examiners and following re-analysis by the same examiner using the above methodology.

This study was supported by School of Dentistry research funds at the University of Liverpool.

### The Reproducibility of the CAST Instrument in Primary Dentitions

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**Background:** The Caries Assessment Spectrum and Treatment (CAST) instrument consists of codes that cover the total spectrum of carious lesion progression: from no carious lesion (code 0), sealants and restorations (codes 1, 2), to lesions in enamel (code 3) and dentine (codes 4, 5), and advanced stages in pulpal and tooth-sur-

rounding tissue (codes 6, 7). **Aim:** To determine the reproducibility of the CAST instrument in primary dentitions. **Methods:** An epidemiological survey was carried out among 6–9-year-old children in Paranoá, Brazil. Three trained and calibrated examiners performed the examinations at school premises. Reproducibility was calculated for intra- and inter-examiner consistency at a tooth level and expressed as percentage of agreement (Pa) and unweighted kappa coefficient ( $\kappa$ ) value. The cut-off points used were healthy (codes 0–2) versus diseased (codes 3–7) and non-cavitated (codes 0–3) versus cavitated (codes 4–7) teeth. **Results:** 170 children constituted the sample resulting in 349 duplicate examinations. The prevalence of dental caries and that of cavitated teeth was 64.1 and 54.7%, respectively. The intra-examiner  $\kappa$  for healthy versus diseased teeth ranged from 0.81 to 0.89 and the Pa from 93.0 to 97.2%, while the inter-examiner  $\kappa$  ranged from 0.54 to 0.86 and the Pa from 88.5 to 96.6%. The intra-examiner  $\kappa$  for non-cavitated versus cavitated teeth ranged from 0.84 to 0.93 and the Pa from 96.9 to 98.1%, while the inter-examiner  $\kappa$  ranged from 0.78 to 0.88 and Pa from 96.8 to 97.9%. **Conclusions:** The CAST instrument showed an almost perfect intra-examiner reproducibility and at least a moderate inter-examiner reproducibility for detecting healthy versus diseased and non-cavitated versus cavitated teeth in primary dentitions.

The study was funded by Capes, University of Brasília and Radboud University Nijmegen institutional funds.

### Detection Criteria of Caries Lesion over Caries Prevalence and dmft Index

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**Aim:** To compare two caries lesion detection systems, International Caries Detection and Assessment System II (ICDAS II) and World Health Organization (WHO) criteria, in the estimation of dental caries prevalence and dmft index in preschool children. In this transversal observational study approved by the ethical committee of the University of Chile 371 dental exams were carried out in 371 preschool children (2–3 years old) from the Integra foundation (preschool national net) at the Metropolitan Region of Chile in the context of Probiotic Clinical Trial project. The exams were assessed by four calibrated dentists according to ICDAS II detection criteria, using artificial illumination and the WHO periodontal probe. WHO dental caries detection criterion was applied to the data collected and the indicators were built depending on four different combinations of ICDAS's codes (WHOC1, WHOC2, WHOC3, WHOC4). For data analysis logistic regression was used. **Results:** Dental caries prevalence: 47.2% (ICDAS II), 23.7% (WHOC1), 25.1% (WHOC2), 31.3% (WHOC3), 32% (WHOC4). For dmft index the percentile 50 and the percentile 25 were 0 for both methods. The maximum score was 14 for the ICDAS II criteria and 9 for the WHO caries detection criteria. WHO dental car-

ies detection criteria under estimate the dental caries prevalence and dmft index.

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### Fluoride Release from Different Materials Used as Fissure Sealants

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The aim of the study was to evaluate the fluoride (F) release from different materials used as fissure sealants mainly in non-western countries. Furthermore the ability of these materials to be recharged with fluoride was also investigated. Four different fissure sealant materials which the producers claim to be F-releasing were tested and a non F-releasing sealer served as control. The materials were Argecem<sup>®</sup> (group A; a glass ionomer cement), Fisskhim<sup>®</sup> (group B; a composite resin), Fissulight<sup>®</sup> (group C; a composite resin), Helioseal F<sup>®</sup> (group D; a composite resin) and Helioseal<sup>®</sup> (group E; a composite resin). Materials A to C are produced by Vladmiva JSC (Belgorod, Russia) and materials D and E are produced by Vivadent (Schaan, Liechtenstein). From each material 10 discs (1.8 mm thick and 8.0 mm in diameter) were produced. Each disc was stored in a tube which contained 5 ml of distilled water. Within the test period of 6 weeks the discs were removed on 18 days and the F concentration was measured in the distilled water using a fluoride-ion specific electrode. Three weeks after the start of the study the samples were dipped for 5 min in a slurry consisting of F toothpaste and distilled water. The cumulated F release values (in  $\mu\text{g}/\text{cm}^2$ ) of the third storage week were 15.8 (A), 0.1 (B), 0.1 (C), 0.4 (D) and 0.1 (E). One week after the F recharge procedure the respective F release values were 30.6 (A), 0.7 (B), 0.3 (C), 0.8 (D) and 0.3 (E). The increase was significant ( $p < 0.05$ ) for groups A, C and D but not for groups B and E. It is concluded that fissure sealing material A and to a lesser extent material D have a potential to deliver an additional caries preventive effect due to their F release without and with F recharge.

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### Effect of Sodium Lauryl Sulfate on Plaque and Saliva Fluoride One Hour after a Fluoride Rinse

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This study examined the effect of sodium lauryl sulfate (SLS), a common dentifrice detergent, on total saliva, total plaque, saliva fluid and plaque fluid fluoride 1 h after a fluoride rinse. Ten sub-

jects accumulated plaque for two days, and then fasted overnight. In the morning the subjects rinsed with 12 mmol/l fluoride (228  $\mu\text{g}/\text{g}$  fluoride as NaF) or a 12 mmol/l fluoride rinse containing 0.5% SLS (both rinses 20 ml in volume and 1 min in duration). One hour later plaque and saliva samples were collected, the fluid phases separated by centrifugation from the plaque sample and from an aliquot of the saliva. The remaining plaque and the remaining saliva samples were overnight digested with 1 M HClO<sub>4</sub> and then neutralized with NaOH. All samples were then diluted with TISAB and analyzed using microchemical methods [Vogel et al.: Caries Res 2000;34:404–411]. The data is given below as the geometric mean and {standard error factor}. Total saliva/plaque fluoride was nearly unaffected by SLS (total saliva [ $\mu\text{mol}/\text{l}$ ]: no SLS = 62 {1.6}, with SLS = 81 {2.5}; total plaque [ $\mu\text{mol}/\text{g}$ ]: no SLS = 1.64 {1.8}, with SLS 1.65 {3.6}). Saliva/plaque fluid fluoride however, was increased ( $p < 0.05$ ) by SLS (salivary fluid [ $\mu\text{mol}/\text{l}$ ]: no SLS 15 {2.2}, with SLS 42 {2.9}; plaque fluid [ $\mu\text{mol}/\text{l}$ ]: no SLS = 16 {2.0}, with SLS 46 {3.3}). These results are consistent with an SLS mediated increase in the rate of fluoride release from the type of calcium mediated fluoride deposits that appear to be the major source of fluoride ions in oral fluids. Such results suggest that the non-fluoride components of fluoride dentifrices and rinses can increase the caries preventive effect of this ion.

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### An Electron Microscopic Study Comparing Effects of Human Enamel Exposure to SnF<sub>2</sub>, TiF<sub>4</sub> and NaF Solutions

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This in vitro study aimed to investigate the surface characteristics of dental enamel exposed to acidic SnF<sub>2</sub> and TiF<sub>4</sub> solutions in comparison to a NaF reference. Human teeth ( $n = 4$ ) were each divided into 4 enamel specimens. One specimen from each tooth was exposed for 10 min to 0.2 mol/l F solutions of SnF<sub>2</sub> (pH 2.9), TiF<sub>4</sub> (pH 1.6) and NaF (pH 4.5). One untreated specimen from each tooth functioned as a control. The microstructure and elemental composition of the surface zone was studied by SEM/EDX, TEM and nanospot-EDX following cross-sectional preparation using FIB technology. TEM/EDX analyses of NaF-exposed specimens showed a 500-nm-thick closed surface film containing 20–40 at% F, as expected. TiF<sub>4</sub>-exposed specimens showed a surface film of 200–300 nm thickness containing 8–11 at% Ti. In contrast to NaF, no fluoride was detectable. TEM of SnF<sub>2</sub>-exposed specimens showed formation of a 60–100-nm-thick surface film above a 200–300-nm-thick modified surface enamel layer. The fluoride content was close to or below the EDX detection limit (1.0 at%). Although significant surface incorporation of Sn was detected by SEM/EDX, the Sn concentration analyzed by comparative TEM/EDX local cross-sectional analyses was only close to the detection

limit. It may be concluded that the reaction mechanisms of the SnF<sub>2</sub> and TiF<sub>4</sub> solutions with the dental enamel differed completely from that occurring after exposure to acidulated NaF solutions. While the NaF treatments resulted in formation of CaF<sub>2</sub>-like material, no significant surface fluoridation was found for SnF<sub>2</sub> and TiF<sub>4</sub> solutions within the TEM/EDX detection limits. This study indicates that the erosion-protective mechanisms of these latter compounds probably relates to the formation of hardly soluble and acid-resistant reaction surface films, as suggested previously, and less to surface fluoride incorporation.

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### One-Year Control of Fluoride in Public Water Supply in the State of Rio de Janeiro

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Water fluoridation in Brazil is obliged by law since 1974, being essential for preventing dental caries. According to the National Survey of Oral Health (SB-Brazil 2010) the State of Rio de Janeiro is located in the region of the country with the highest prevalence of fluorosis in twelve-year-old children (19.1%). DMF-T is 1.4 in the same age group. The aim of this study was to analyze samples of drinking water in 17 municipalities of the State of Rio de Janeiro during 12 months (2010–2011), while considering the benefit to prevent caries and the risk of fluorosis development. The best risk-benefit combination is around 0.55–0.84 mg F/l. 408 samples were collected and analyzed using the ion-sensitive electrode (Orion 9609) coupled to a potentiometer (Procyon, model 720), adding 1 ml of TISAB II to 1 ml of sample. The average of fluoride concentration in the period was 0.35 mg F/l. The results showed that 78.9% of samples (n = 316) were classified as insignificant to prevent dental caries or at risk to cause fluorosis; 9.4% (n = 38) had minimal benefit in preventing caries and a low risk to cause fluorosis; 7% (n = 28) showed maximum benefit to prevent dental caries and 4.8% (n = 20) had high risk to produce moderate fluorosis. These results indicate that despite the obligation in law, there are large fluctuations in the levels of fluoride found in water supply in the State of Rio de Janeiro. It is necessary to implement and maintain the external control of water fluoride levels in the State of Rio de Janeiro, aiming at maximizing the benefits to prevent dental caries while minimizing the risk of the occurrence of dental fluorosis.

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### Assessment of Direct and Diffusion Methods for Fluoride Analysis of Soya Milks

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There has been a rapid growth in the consumption of soya milk in western countries due to its claimed health benefits. Several studies have reported that soya milk has a higher fluoride concentration than cow's milk. However, the analysis method used could impact the fluoride concentrations determined in samples. The aim of this pilot study was to investigate the most appropriate analytical method for measuring the endogenous fluoride concentration of soya milks. The objectives were to compare fluoride concentrations of (i) European and Chinese-produced samples and (ii) regular and light soya milk samples. Samples of a UK-produced (Alpro-light and Alpro-regular) and a Chinese-produced (Vitasoy-light and Vitasoy-regular) soya milk were purchased. Each sample was analysed for fluoride concentration, in triplicate, using a Fluoride-Ion-Selective Electrode (Orion, 9609) and a 720A pH/ion meter by (a) a direct method after sample buffering with TISAB III and (b) a silicon-facilitated acid diffusion method. Deionised-distilled water was used as a blank and analysed alongside samples. The fluoride-blank was 0.03 mg/l for direct and <0.1 mg/l for diffusion methods. The overall mean (SD) repeatability of the fluoride readings based on the triplicate samples was 95 (3)% for direct and 93 (4.5)% for diffusion methods. Fluoride concentrations of Alpro-light, Alpro-regular, Vitasoy-light and Vitasoy-regular, when analysed by the direct method, were 0.14, 0.29, 0.37 and 0.43 mg/l, respectively. When the samples were analysed using the diffusion method, the corresponding values were 0.47, 0.29, 0.39 and 0.35 mg/l, respectively. The results showed higher concentrations of fluoride measured in Alpro-light with diffusion analysis, however, this between-method difference in fluoride concentration was not observed for the other 3 products. The sodium content of Alpro-light (0.04 g/100 ml) was twice that of Alpro-original (0.02 g/100 ml), while it also contained 1.2 g/100 ml fibre compared with 0.5 g/100 ml in Alpro-original. A possible complex formation between fluoride and other elements present in Alpro-light which could potentially interfere with fluoride analysis might explain the higher recovery of fluoride seen with the diffusion method. In conclusion, it is recommended to use a diffusion method for measuring the fluoride concentration of soya milks.

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### Deposition of CaF-Like Material on and Fluoride Uptake into Demineralized Enamel after pH Cycling

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The in vitro fluoride uptake in demineralized enamel of an acidic fluoride varnish 43,000 ppm F<sup>-</sup> combined with a Ca(OH)<sub>2</sub> suspension (group 1, Humanchemie, Alfeld, Germany) was compared with a standard neutral fluoride varnish (group 2, 23,500 ppm F<sup>-</sup>, Duraphat, Colgate, Hamburg, Germany). Demineralized bovine enamel specimens (2,500 vol.%×µm) were randomly assigned to 4 groups (n = 50). Specimens of groups 1 and 2 were treated with a defined amount of the respective study product. After storing all specimens in pooled human saliva for 3 h the specimens were brushed until no more remnants of the applied product were found. Specimens of all groups were pH-cycled for 10 days.

In the morning and in the evening specimens of groups 1, 2 and 3 (fluoride control) were stored in toothpaste slurry for 3 min. Group 4 served as negative control. CaF<sub>2</sub>-like deposit on the enamel surface and structurally bound fluoride (three layers of 40 µm each) were determined using an ion-selective electrode and statistically analyzed using ANOVA and Tukey-B test. The highest amount of CaF<sub>2</sub>-like material was found in group 1 (9.79 µg/cm<sup>2</sup>) followed by group 2 (4.64 µg/cm<sup>2</sup>), and 3 (3.39 µg/cm<sup>2</sup>), being significantly different from each other. In all three layers (outer/middle/inner) the highest fluoride concentration was found in group 1 (4,089.45 µg/cm<sup>3</sup>; 3,903.50 µg/cm<sup>3</sup>, 2,904.19 µg/cm<sup>3</sup>). Significantly less fluoride content was found in group 2 (1,771.84 µg/cm<sup>3</sup>; 1,534.49 µg/cm<sup>3</sup>; 936.27 µg/cm<sup>3</sup>) and group 3 (1,670.55 µg/cm<sup>3</sup>; 1,469.48 µg/cm<sup>3</sup>; 774.05 µg/cm<sup>3</sup>) being not significantly different from each other. The fluoride content of the specimens in group 4 was below the detection level. The combination of an acidic fluoride varnish and a Ca(OH)<sub>2</sub> suspension leads to a higher fluoride uptake after a pH challenge.

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## Session 4 Epidemiology

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### Prevalence of Dental Caries and Its Treatment among Children with Mental Retardation in Baku, Azerbaijan

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**Background:** In Azerbaijan, there have not been comprehensive epidemiological studies of dental health status of children with mental retardation. **Aim:** The aim of this study was to assess the prevalence of dental caries and its treatment among the schoolchildren with mental retardation in Baku. **Methods:** Oral health examinations were performed on 236 children with mental retardation (169 boys and 67 girls) aged 6–16 years (on average  $12.1 \pm 5.31$  years old) were held among all of whom studied in 4 Special Secondary School in Baku city. Calibrated specialists used the WHO 1997 criteria for diagnosis and recording of DMFT index. Dental caries were diagnosed at the caries into dentine at the (D3) threshold, using a visual method with mouth mirrors, dental probes and daylight illumination. Ethical approval was obtained from the ethical committee of Azerbaijan Medical University. **Results:** Results demonstrated an overall average caries prevalence of 97.9%. The mean DMFT for all ages was 5.31 of which the D element was 5.02 and the F element only 0.02, resulting in a very low Care Index of 0.38%. There was a mean of 1.19 teeth per subject, which manifested symptoms of either pulpitis or periapical infection and a mean of 0.99 teeth per subject present as retained roots. No gender differences were observed. In general the subjects exhibited very poor level of hygiene. **Conclusions:** The study demonstrated a very high prevalence of caries among the schoolchildren with mental retardation in Baku, a very high proportion of decayed teeth and a very low proportion of filled teeth, resulting in an extremely low Care Index. There is a need to develop a program aimed at improving the dental health service provided for of these group children.

The study was funded by Azerbaijan Medical University.

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### Decline of Dental Caries in Six-Year-Old Children in Greifswald, Germany

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In 2003, the community-based prophylaxis programme in the federal state of Mecklenburg-Western Pomerania (MWP) was amplified by an additional risk-specific component for preschool and school children. Among others, this programme concentrated on regular brushing with fluoridated toothpaste or gels in the institutions with the highest baseline caries levels. The aim of this study was to assess the benefit of this programme for the oral health status of 6-year-old children in Greifswald and to compare this to caries data from MWP and Germany. The data was extracted from the results of the compulsory school entry examinations in Greifswald involving about 380 6–7-year-olds each year (>90% of the 6–7-year-olds population). Caries reduction was estimated by the comparison of the caries scores (deft) between 2003 and 2009 among study children with the data of representative surveys for MWP and Germany (Deutsche Arbeitsgemeinschaft für Jugendzahnpflege, 2010; calibrated dentists >85% agreement). Compared to the 4.1 deft in 1995 before the implementation of any community programme, the baseline caries levels had already dropped in Greifswald moderately until 2003 with 41% caries-free children and a mean deft of  $3.2 (\pm 3.8)$ . The d component corresponded to 55% of the index. In 2012, 55% of children were caries-free; the mean deft was  $1.6 (\pm 2.7)$ . The SiC Index deft decreased from  $7.9 (\pm 2.3)$  in 2003 to  $4.5 (\pm 2.7)$  in 2012 ( $p < 0.001$ ). Caries reductions in Greifswald between 2003 and 2009 were calculated to be 38%, 12% in MWP and 13% in Germany (2004–2009). In conclusion, the enforced public oral health programme in Greifswald has achieved considerable caries reductions compared to MWP and Germany. Nevertheless, additional attention to preventive actions should be focused on children with the highest caries scores as is reflected by the SiC Index.



### Failure Rates of Class V Restorations in the Management of Root Caries in Adults: A Systematic Review and Meta-Analysis

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**Aim:** The aim of this systematic review was to compare cumulative failure rates and recurrent caries rates of different restorative materials in carious class V lesions on the root surfaces of adult patients, to enable practitioners to select the most appropriate material for use in these lesions. **Experimental Approach:** PubMed, Embase, CENTRAL and OpenSIGLE were searched using specific MeSH terms and RCTs and CCTs were included or excluded according to predefined criteria. Clinical trials with adult participants (over 18 years) which reported cumulative failure rates and/or failure due to marginal caries of each restorative material assessed were eligible for inclusion. Those that solely compared different techniques of placing the same material instead of comparing different materials were excluded. **Main Results:** Five studies met the predefined inclusion criteria. In total, 629 carious lesions on root surfaces were restored. The pooled risk ratios (RR) were determined for cumulative failure rates after 12 and 24 months of glass ionomer cement (GIC), resin-modified glass ionomer cement (RMGIC), resin composite, and amalgam. The pooled risk ratios of failure due to marginal caries were also determined. Resin composite showed statistically significant lower cumulative failure rates at 24 months. GIC restorations displayed fewer incidences of marginal caries at 24 months, and this result is statistically significant at 5% significance levels. The pooled RR for marginal caries at 24 months was 0.26 (95% CI = 0.08–0.86), which indicates that teeth restored with resin composite would be nearly four times (1/0.26) as likely to develop marginal caries within a two-year period as those restored with glass ionomer cement. **Conclusion:** There is a need for more research in this area as many of the studies identified in this systematic review treated post-radiation, xerostomic patients which are not typical of the general population. Increased adherence to CONSORT guidelines for reporting is also advised to facilitate future systematic review in this area.

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### Prevalence and Risk Indicators for Dental Erosion among 12-Year-Old Schoolchildren from South Brazil

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**Objective:** To assess the prevalence and risk indicators for dental erosion among 12-year-old schoolchildren from South Brazil.

**Methods:** A cross-sectional survey was carried out in Porto Alegre, Southern Brazil, from September 2009 to December 2010. A multistage probability sampling strategy was used to select a representative sample of 12-year-old schoolchildren attending public and private schools. One examiner recorded the Gingival Bleeding Index and another recorded the presence and severity of dental erosion in permanent incisors and first molars using the Basic Erosive Wear Examination (BEWE). A questionnaire was sent to parents/legal guardians to gather information on socioeconomic characteristics, brushing habits and general health (presence of gastroesophageal or respiratory disorders). Students answered another questionnaire on dietary habits (consumption of acid beverages and fruits). The association between predictor variables and dental erosion was assessed using Poisson regression models. Prevalence ratios (PR) and 95% confidence intervals (CI) were estimated. **Results:** 1,528 schoolchildren participated (response rate of 83.17%). 229 schoolchildren presented at least one tooth with dental erosion, yielding a prevalence rate of 15% (95% CI = 13.6–16.5). Regarding severity, mild erosion (BEWE score 1) was observed in the majority of cases (n = 207) while severe erosion (BEWE scores 2 and 3) was detected in 22 individuals. Males (PR = 1.47, 95% CI = 1.19–1.82), private school attendees (PR = 1.36, 95% CI = 1.17–1.59), schoolchildren with gastroesophageal disorders (PR = 1.24, 95% CI = 1.09–1.41), and those who reported the daily consumption of lemon (PR = 1.32, 95% CI = 1.01–1.73) and canned tea (PR = 1.71, 95% CI = 1.17–2.51) were significantly more likely to have dental erosion. **Conclusion:** Although 15% of the schoolchildren presented dental erosion, its severity was low in the majority of cases. Gender, type of school, the presence of gastroesophageal disorders, and dietary habits were associated with dental erosion in this population.

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### A Consensus Curriculum for Undergraduate Cariology Teaching in Colombia

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**Aim:** This study aimed to achieve a national consensus for an undergraduate cariology teaching curriculum between Colombian dental schools in line with the 2015 Global Alliance for a Cavity-Free Future goal of '90% of dental schools adopting the current caries paradigm'. **Materials and Methods:** 1st phase: 4-region 2-day workshops were conducted (1st semester 2012) with 4 representative teachers (cariology, clinics, basic sciences, public health) from each of the 24 ACFO schools (Colombian Dental Schools Association). Presentations were delivered on main cariology teaching barriers in Colombia and the current caries paradigm, together with poster discussions on cariology-teaching. The European cariology curriculum [Schulte et al.: Eur J Dent Educ 2011;15(suppl 1):32–39] was presented with sub-group discussions on the 5 main domains: (1) the knowledge base; (2) risk assessment, diagnosis and synthe-

sis; (3) decision-making and preventive non-surgical therapy; (4) decision-making and surgical therapy; and (5) evidence-based cariology in clinical/public health practice. The domains, objectives and contents were adapted to meet Colombian curriculum needs, public health situation and national health system. Subsequently, each school was given one month to review the consensus documentation. All 4-region and 24-school consensuses were amalgamated into one national preliminary document. 2nd phase: document peer review. Initially by 10 cariology faculty experts, via teleconference, achieving agreement in document content and structure. Then, 10 faculty peer experts in curriculum, basic sciences, research, clinical management and public health and the 24 school deans reviewed (via e-mail) the document with suggestions discussed and adopted into a final consensus document, which was officially presented to the academic community at the ACFO National Research Meeting (September, 2012). **Results:** The Colombian cariology curriculum included six domains, positioning public health into one main domain. Other relevant modifications were the focus on social determination and the decision to give more importance to dental fluorosis over dental erosion.

This study was partially funded by the Colombian Chapter of the Global Alliance for a Cavity-Free Future, Colgate Palmolive Colombia.

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### Caries, Caries Risk and Nutritional Status of Young Children in the Municipality of Anapoima, Colombia

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**Aim:** Following the 2026 Global Alliance for a Cavity-Free Future's goal 'controlling caries in children born from 2026' the Colombian Chapter started a feasibility study in the municipality of Anapoima, Cundinamarca. The aim of this study was to describe the caries prevalence, severity, risk and nutritional status in young children, and to establish if caries risk classification or nutritional status correlates with caries experience. **Methods:** Ethical approval and informed consent were obtained. 265 2–59-month-old children were clinically assessed by 4 calibrated examiners for caries using the epidemiological ICDAS A merging codes recording system with scores at different cut-off points:  $d_{4-6}mf-s$  (ICDAS codes 4–6, dentine caries according to WHO criteria);  $d_{1-6}mf-s$  (including all lesions; codes 1 and 2 combined) and caries risk (Cariogram). Four trained pediatricians assessed height, weight and cephalic perimeter to calculate children's nutritional weight-age and height-age relations (WHO standards). The mean dmf-t was classified according to national age figures in worse than normal, normal, and better than normal, and correlated with individual caries risk and with height- and weight-age relation ( $\chi^2$  test). **Results:** Children ( $n = 265$ ) were distributed by age and gender as follows: <1 year:  $n = 26$ ; <2 years:  $n = 39$ ; <3 years:  $n = 55$ ; <4 years:  $n = 74$ ; <5 years:  $n = 71$ ; female:  $n = 145$ ; male:  $n = 120$ . Prevalence and

mean caries experience were:  $d_{4-6}mf-t$ : 30.6%, mean  $d_{4-6}mf-t$ :  $1.11 \pm 2.26$ ;  $d_{4-6}mf-s$ : 31%, mean  $d_{4-6}mf-s$ :  $1.92 \pm 4.69$ ;  $d_{1-6}mf-s$ : 51.7%, mean  $d_{1-6}mf-s$ :  $3.75 \pm 6.40$ , respectively. Percentage distributions for individual caries risk were: low: 12.8%; moderate: 7.2%; high: 12.4%; very high: 67.6%. The distribution for weight-age relation was: malnutrition: 1.9%; malnutrition risk: 15.1%; normal: 72.5%; obesity risk: 9.8%; obesity: 0.7%. Corresponding data for height-age relation were: normal: 68.3%; low height risk: 23.0%; low height: 8.3%; pathological low height: 0.4%. There was a high correlation between caries risk and caries experience ( $\chi^2$ ;  $p = 0.001$ ), but no statistically significant association between nutritional status and caries experience ( $\chi^2$ ;  $p > 0.05$ ). **Conclusion:** The population caries experience was commensurate with national figures, despite an apparent high caries risk.

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### Dental Caries Trends among Preschool Children in Arkhangelsk, 1989–2010

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The aim of the study was to analyze dental caries trends from epidemiological studies conducted among preschool children over the period 1989–2010 in Arkhangelsk, Northwest Russia. Between 1989 and 2010, eight cross-sectional surveys were conducted by the Department of Pediatric Dentistry, Northern State Medical University. Sample selection and approaches to examiner training were similar for each survey. The total sample comprised 1,090 children (53% boys, 47% girls) with mean age 4.6 (SD 1.1) years. The caries experience status of primary teeth was assessed by senior dentists in accordance with the WHO (1987, 1997) criteria at the  $d_3$  level and  $d(3)ft/d(3)fs$  values (presented as mean  $\pm$  SD); Care Index ( $ft/d(3)ft \times 100$ ) was calculated. Dichotomous and continuous data were analyzed using chi-squared and Mann-Whitney tests, respectively. Altogether, significant overall trends of increasing caries prevalence and caries experience were observed from 64% (95% CI: 56–71) with mean  $d(3)ft/d(3)fs$  values  $3.1 \pm 3.5/3.6 \pm 4.4$  in 1989 to 76% (95% CI: 68–83) with  $4.0 \pm 3.7/6.3 \pm 7.6$  in 2010 ( $p < 0.05$ ). The predominant components  $dt/ds$  increased from  $1.1 \pm 1.6/1.2 \pm 1.6$  in 2001 to  $2.7 \pm 3.1/4.2 \pm 6.4$  in 2010, respectively ( $p < 0.01$ ). There was a significant increase in  $ft/fs$  components from  $0.6 \pm 1.3/0.8 \pm 1.5$  to  $1.2 \pm 1.9/2.0 \pm 3.3$  between 1989 and 2010 ( $p < 0.01$ ). It was observed that since 1989 there had been a significant increase in the Care Index from 19% to 53% ( $p < 0.05$ ) by 2000 and then a gradual decrease to 30% in 2010 ( $p < 0.05$ ). This study identified a significant increase in caries prevalence in the samples investigated particularly since the early 2000s. Caries experience and the level of untreated caries are still high and tend to be rising during the last decade. Preventive measures are urgently needed among the preschool population of Arkhangelsk.

### Sensitivity and Specificity of dmfs/DMFS to ICDAS II in Riga's Children with Baseline Age 6 and 12 Years: A Follow-Up Study

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**Aim:** To perform sensitivity and specificity tests of dmfs/DMFS (old version) to ICDAS II (gold standard) in Riga's children initially aged 6 or 12 years. **Materials and Methods:** 38 children initially aged 6 years and 39 children initially aged 12 years were examined using ICDAS II and dmfs/DMFS by turns accordingly. All children were examined at baseline and after 3 years. Total dmfs/DMFS were recorded using bitewing radiographs. ICDAS II was measured on all participants. The data were analyzed using t-test, chi-square test and sensitivity and specificity tests. **Results:** The mean (SD) dmfs was 6.32 (5.33) in 6 initially aged; DMFS was 3.13 (3.14) and 14.77 (9.85) in the original 6- and 12-year-olds accordingly. The percentage of cariously affected surfaces to all side surfaces (approximal ( $n = 3,830$ ), occlusal ( $n = 1,026$ ), buccal/lingual ( $n = 3,830$ ) and to total number ( $n = 8,700$ ) of surfaces using dmfs/DMFS vs. ICDAS II was: approximal – 5.5% and 2.4% ( $n = 210$ ) vs. 51.3% and 22.6% ( $n = 1,965$ ); occlusal – 7.8% and 1% ( $n = 80$ ) vs. 34.2% and 4.03% ( $n = 351$ ); buccal/lingual – 0.6% and 0.3% ( $n = 22$ ) vs. 37.6% and 16.6% ( $n = 1,440$ ). The percentage of cariously affected and filled surfaces to all side surfaces (approximal ( $n = 3,830$ ), occlusal ( $n = 1,026$ ), buccal/lingual ( $n = 3,830$ ) and to total number ( $n = 8,700$ ) of surfaces using dmfs/DMFS vs. ICDAS II was: approximal – 9.1% and 4% ( $n = 351$ ) vs. 55.14% and 24.2% ( $n = 2,112$ ); occlusal – 34.9% and 4.1% ( $n = 358$ ) vs. 61.7% and 7% ( $n = 633$ ); buccal/lingual – 3.7% and 1.6% ( $n = 142$ ) vs. 40.8% and 17.95% ( $n = 1,565$ ). The sensitivity vs. specificity in approximal surfaces was 0.57 vs. 0.98, in occlusal – 0.83 vs. 0.98, in buccal/lingual – 0.43 vs. 0.99. **Conclusions:** Gained results give possibility to suggest that ICDAS II instead of dmfs/DMFS (old version) should be used to detect dental caries in new caries risk-based preventive programs in Riga's 6- and 12-year-olds.

### Do Contextual Factors Influence the Impact of Dental Caries on Quality of Life of Children?

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Studies have suggested associations between social capital, contextual factors and subjective measures of oral health in el-

derly population. These associations in children, however, are still unclear. Therefore, this study assessed the effect of individual and contextual variables on the Oral Health-Related Quality of Life (OHRQoL) in preschool children. This cross-sectional study was performed with a multistage random sample of 478 preschool aged 1–5 years, representative of Santa Maria, a southern city in Brazil. Caregivers completed the Brazilian version of the Early childhood oral health impact scale (ECOHIS) and answered questions about the socioeconomic status and social capital. The dental examination provided information on the prevalence of dental caries, dental trauma and occlusion of preschoolers. Data were analyzed using multilevel linear regression model. The mean ECOHIS was 1.8 (SD  $\pm 3.9$ ) and the functional domain of the session was the most affected children (mean = 0.5, SD  $\pm 1.4$ ). The multilevel analysis identified the presence of dental caries, dental trauma, anterior open bite, low household income and low features of social capital as significant individual determinants of lower child OHRQoL. When the contextual-level covariates were included in the model, the association between subjects' level characteristics and child OHRQoL still persisted, being those who lived in areas without community cultural centers more likely to reported higher impacts on their quality of life. In conclusion, dental caries negatively impacts OHRQoL, but for children living in regions with unfavorable social conditions and poor oral health status and poor socioeconomic status have a more negative OHRQoL.

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### Evaluation of a Milk Fluoridation Program in Chilean Rural School Children

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The aim of this study was to evaluate the efficacy of a fluoridated milk program that is currently underway in Chilean, rural 12-year-old school children compared with rural, neighboring areas, socio-economic matched samples of children in water-fluoridated areas and a negative control group. Clinical dental examinations, following WHO guidelines, were carried out on 257 adolescents ( $n = 82-90$ , in each group). Enamel fluorosis (Dean's index), tooth brushing habits and pits and fissure sealants were assessed as well. The DMFT index scores were in the range 1.38–1.48 for the three groups (Kruskal-Wallis  $p = 0.87$ ). Proportion of adolescents free from caries were in the range 40–49% ( $\chi^2$ ;  $p > 0.5$ ) in the three groups. The proportion of adolescents that brush their teeth daily was  $\geq 97.6\%$  and the proportion of school children presenting with pits and fissure sealants was in the range 41–49% ( $\chi^2$ ;  $p = 0.5$ ). Enamel fluorosis prevalence in the three groups was  $< 25\%$  (including 'questionable' score). The DMFT index scores found in this study are the lowest ever reported in Chilean rural areas. The DMFT index for the fluoridated milk group was as low as the cor-

responding index for adolescents residing in a water fluoridated area. The DMFT index for the negative control group was unexpectedly low. Further studies, using a different experimental design, will be necessary to elucidate this latter value.

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### Dental Caries Experience, Frequency Pattern and Individual Caries Risk: Baseline Data in Young Children from Bogotá, Colombia

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**Aim:** This study aimed to report caries baseline data from a longitudinal study in young children from Bogotá, Colombia. **Materials and Methods:** After ethical approval, informed consent was obtained from the parents of 600 2- to 6-year-olds in six Bogota schools. Clinical examinations using the epidemiological ICDAS A merging codes recording system with scores at different cut-off points:  $d_{4-6}mf-s$  (ICDAS codes 4–6, dentine caries according to WHO criteria);  $d_{1-6}mf-s$  (including all lesions, codes 1 and 2 combined) were conducted by four trained examiners (inter-/intra-reproducibility kappa values  $>0.70$ ). Caries risk classification was determined using the Cariogram program. **Results:** The sample corresponded to 290 girls and 310 boys;  $n = 200$  in each age cohort of 2-, 4- and 6-year-olds. Caries prevalence ( $d_{4-6}mf-t \geq 1$ ) was: 2 years: 23.5%; 4 years: 51.5%; 6 years: 56.0%. The mean  $d_{4-6}mf-t$  was: 2 years:  $0.9 \pm 1.8$ ; 4 years:  $2.0 \pm 2.7$ ; 6 years:  $2.1 \pm 2.4$ . On average the  $d$  and  $f$  components made up about 50% each of the  $d_{4-6}mf-t$  in all groups, leaving very few teeth extracted due to caries. Corresponding  $d_{4-6}mf-s$  means were: 2 years:  $1.5 \pm 3.7$ ; 4 years:  $3.3 \pm 6.7$ ; 6 years:  $3.2 \pm 4.8$ . When initial lesions were included the prevalence increased to: 2 years: 72.5%; 4 years: 84%; 6 years: 85.5%. The mean  $d_{1-6}mf-s$  was: 2 years:  $4.4 \pm 5.9$ ; 4 years:  $7.4 \pm 8.5$ ; 6 years:  $7.1 \pm 6.1$ . The occlusal surfaces of the 2nd primary molar teeth were the most frequently caries-affected surfaces. Individual caries risk was classified in 2 year-olds as: very high (22%); high (1%); moderate (4%); low (24%), and very low (50%); 4 years: very high (7%); high (3%); moderate (9%); low (33%), and very low (49%); 6 years: very high (8%); high (1%); moderate (4%); low (24%), and very low (63%). **Conclusion:** In this population the prevalence of caries at age 4 fails to meet the WHO year 2000 goal. There is a very high prevalence of initial lesions and the occlusal surfaces of primary molar teeth remain the most caries affected surface in this sample.

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### Prevalence of Dental Caries in Childhood: Polarization, Trends and Inequality in the New Century

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The decline of caries rates has been accompanied by a process of imbalance in the distribution of the disease. Thus, it is important to summarize the data of recent primary studies published and update concepts. Thus, we aimed to perform a systematic review and meta-analysis of prevalence of caries in Latin America and the Caribbean in 5–6- and 11–13-year-old children. Two independent reviewers searched PubMed and Embase through October 2011 to identify papers published in English, Portuguese or Spanish. The inclusion criteria were studies: (1) with subjects with 5–6 or 11–13 years old, (2) conducted in Latin America and the Caribbean, (3) that utilized the WHO criteria or some other index (cavitated or non-cavitated data separately), (4) with population basis, (5) that provided caries prevalence data, and (6) that performed separate analysis of primary and permanent teeth. We performed a descriptive analysis of studies, meta-analysis and analyze the trends of prevalence of caries in both primary and permanent teeth. Fifty-one studies were included from the 542 articles initially searched. The meta-analysis of caries prevalence grouped for Latin American and the Caribbean countries found a prevalence of around 56% at 5–6 years (primary teeth) and 64% at 11–12 years (permanent teeth). For studies conducted only in Brazil, the prevalence was 54% (5–6 years) and 67% (11–12 years) while in other countries were 66% (5–6 years) and 49% (11–12 years). In conclusion, dental caries still affects many children in the 21st century.

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### The Prevalence of Molar Incisor Hypomineralisation in 16-Year-Old Adolescents in Northern Norway

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**Background:** A wide variation from 2.4% to 40.2% in prevalence of molar incisor hypomineralisation (MIH) has been reported. The reported prevalence in Scandinavia varies from 17.0% to 37.5%. No published prevalence data exists from Norway. **Aims:** The aim of the study was to determine the prevalence of MIH in 16-year-olds in Northern Norway and to report the distribution of affected teeth. **Materials and Methods:** This study was part of a

health survey in Tromsø, Norway including 16 year old adolescents born in 1994. 795 participants were included in the study. Clinical photos of all teeth were taken and the previous dental history was available. Three clinically experienced, trained and calibrated dentists examined the photos. Opacities (>1 mm), defects, atypical restorations and previous extractions caused by MIH were registered based on a joint decision. Index teeth were permanent first molars and incisors (12 teeth). **Results:** The prevalence of MIH was found to be 13.9% and significantly higher for girls than for boys (16.8% vs. 11.1%;  $p < 0.05$ ). The upper first molars were 1.8 times ( $p < 0.001$ ) more frequently affected than the lower ones. 50.9% of the patients with MIH had 1 or 2 teeth affected, 27.3% had 3 or 4, while 21.8% had more than 4 teeth affected. It was found that 1.4% of the affected molars had been extracted due to MIH while 18.9% had restorations. **Conclusion:** The prevalence of MIH (13.9%) in our study was lower than in previously published Scandinavian data. Girls had higher prevalence of MIH than boys, in contrast to most other published data. MIH affected first molars were almost twice as common in the maxilla as in the mandible.

The study was funded by Tromsø fylkeskommune, Norway.

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### Oral Health Inequalities in Italian Schoolchildren: A Cross-Sectional Evaluation

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**Background:** Caries remains one of the most common diseases of childhood worldwide. Thus, there is still need to fully understand the role of the different risk factors for disease to occur. The hypothesis behind this report is that the socio-economical level might be a risk factor for caries in schoolchildren. A cross-sectional study was designed and carried out in two areas of Sardinia Region (Italy), with different median household income (medium income and low income). **Materials and Methods:** 2,040 schoolchildren (42.5% boys, 57.5% girls), aged 6–7 years, were examined. Dental caries was assessed using dmfs/DMFS at d3/D3 level. Parents answered a standardized questionnaire regarding the level of education, the nationality, child's oral health habits, the frequency of their dental check-up and their perception of child's oral health. Multinomial logistic regression analysis, through a backward procedure, was performed to determine statistically significant associations between dmfs/DMFS and the different variables, using dmfs categorized as 0 = healthy (no lesions), 1 = moderate (<3 lesions), 2 = severe (>3 and <5 lesions), and 3 = high (>5 lesions). **Result:** Overall caries prevalence was 35.7% (95% CI = 33.6%–

37.8%) with a prevalence of 33.9% (95% CI = 31.9%–36.1%) in the deciduous dentition. The mean dmfs index was  $2.04 \pm 0.12$  (95% CI = 1.8–2.3). Multinomial logistic regression analysis revealed that the low level of maternal education ( $p < 0.01$ ), no dental check-up ( $p = 0.03$ ), and parental bad perception of child's oral health ( $p < 0.01$ ) regarding caries prevalence. The median household income was not associated with caries prevalence. **Conclusions:** The present report reveals that caries experience in Sardinian schoolchildren, from medium and low household income, is moderate and mainly influenced by socio-behavioural variables including parents' level of education, the frequency of parents' dental check-up and their perception of child's oral health.

The study was funded by the WHO Collaborating Centre for Epidemiology and Community Dentistry (University of Milan, Italy).

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### Reducing the 'Glasgow Effect' over Time in Relation to 5-Year-Old Dental Health in Scotland

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**Aim:** The health of residents in the West of Scotland is poor and the literature shows that, within Glasgow, a negative effect on health persists, even after controlling for deprivation using standard socio-economic status (SES) variables (the 'Glasgow Effect'). The aim was to determine whether this pattern was also seen in relation to child dental health and whether it was reduced by the introduction of 'early years' health improvement initiatives. These were rolled out in an incremental manner across Scotland from 2000/1. **Materials and Methods:** Successive cross-sectional national caries epidemiology datasets for 5-year-old children, from eight time points 1993/94 to 2007/08, collected by trained and calibrated examiners, were obtained. Caries d3mft outcomes for the Greater Glasgow Health Board (GGHB) area and for the remainder of Scotland (RoS) were calculated and odds ratios (ORs) used to quantify differences in caries morbidity between GGHB children and those from RoS, after controlling for age, gender and SES. **Results:** Data from 18,174 (GGHB) and 50,224 (RoS) children indicate that at baseline the weighted mean d3mft and %d3mft = 0 for GGHB children and those in RoS were 3.8, 31.8% and 2.9, 42.9% respectively. The OR [95% CI] for %d3mft >0 was 1.34 [1.10–1.64] (controlling for the above variables) comparing GGHB to RoS. By 2005/6 and 2007/8, the OR [95% CI] had decreased to 1.0 [0.93–1.12] and 0.9 [0.94–1.04], respectively. **Conclusion:** In 1993/94 the 'Glasgow Effect' was seen in relation to the dental health of 5-year-olds, with those in GGHB having poorer dental health than their peers in RoS, even after controlling for standard SES variables. However, by 2005/6, following the introduction of health improvement initiatives using a proportionate universalism approach, the 'Glasgow Effect' was no longer evident.

We acknowledge funding from the Scottish Government Health Department.

## Knowledge Translation in Oral Health Promotion

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Although the UK national target set in 1994, for improving dental health, was >70% caries-free 5-year-olds by 2003, only 57% was achieved. This, with 40% of 5-year olds with dental caries experience in North-East England, indicates a need for more effective oral health promotion strategies for children. In order to investigate the most effective method(s) for delivery of oral hygiene and nutritional interventions to children and/or parents, the study explored the process of evidence translation in the implementation of oral health promotion interventions (OHPI) towards reducing dental caries in children. Mixed methods approach was utilised to explore (i) evidence of the most effective intervention method(s) for improving oral hygiene and diet in children and (ii) existing barriers to implementation of OHPI that are effective in reducing dental caries. A descriptive synthesis approach was employed in the systematic review which assessed effectiveness of a range of OHPIs in children. Regular fluoride use, involvement of parents and OHP specialists were found to have contributed to reduction in dmft. A major gap observed in the literature was the lack of understanding of dynamics and complexities of processes involved in workability and effective implementation of OHPIs. This was investigated in 2nd phase of the study which employed a qualitative research approach. Thematic analysis of data collected revealed patterns of commissioning, leadership, cohesive working, adequate fidelity and monitoring of interventions delivered in schools influenced effectiveness of OHPIs in reducing dental caries. Although clinical effectiveness of OHPI on dmft was clearly demonstrated in several studies, barriers to implementation of OHPI in communities for optimal effectiveness remain. Appropriate commissioning process, leadership and cohesive working are to be considered when developing strategies for OHPI.

The study was funded by a Research Scholarship provided by Teesside University.

## Dental Caries Experience in Children under Long-Term Liquid Oral Medication Therapy

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Sucrose added as sweetening agent in liquid oral medication (LOM) to mask the objectional taste of medicines is potentially cariogenic. To assess the dental caries experience and its relation-

ship with long-term usage of LOM among 2–12-year-old children with various chronic diseases in India. A cross-sectional study was carried out at a government paediatric hospital in India for a period of 6 months in 2012. A total of 455 children aged 2–12 years who were suffering from various chronic diseases receiving liquid oral medication for more than 6 months were selected (study group) and for comparison 531 children of similar age group and diseases but on other forms of medication were included as control group. Dental caries was the outcome and was measured by DMFT/DMFS, dmft/dft and dmfs/dfs. The data was analyzed with SPSS using statistical tests such as t-test and one-way ANOVA. Univariable logistic regression were used with p value was fixed at 0.001. Children who were on liquid oral medication are at the increased risk of dental caries than those with other forms of medications (OR: 3.142, 95% CI: 2.37–4.15,  $p < 0.001$ ). The prevalence of dental caries in the study group was more (77.8%) when compared to control group (52.7%). The mean DMFS was significantly higher in the study group ( $0.60 \pm 2.1$ ) when compared to control group ( $0.23 \pm 1.16$ ) ( $p = 0.001$ ). The mean dmfs/dfs ( $7.66 \pm 7.98$ ) in the study group was also higher when compared to control group ( $2.12 \pm 3.52$ ) ( $p < 0.000$ ). Long-term usage of liquid medicines containing sucrose is a risk factor for dental caries among children with various chronic diseases.

The study was funded by the Department of Science of Technology, Government of Rajasthan, India.

## Longitudinal Progression of Caries in Children Aged Three to Five Years by Socioeconomic Status: A Cohort Study

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The aim of the study was to track longitudinally changes in dental caries from baseline in children at aged three years in nursery schools to the first year of primary education nearly two years later, and to determine if this differed by type of tooth and/or by socioeconomic status. Longitudinal follow-up is rare outside of clinical trials. The study children had participated in routine dental inspection programmes. We linked the inspections from nursery schools (March–June 2008) and primary schools (November 2009–March 2010) in the same children to achieve a cohort study. d3mfs was calculated for each tooth separately, and then totalled for each child's overall decay experience. The change from baseline in each metric was calculated (also d3mft). The primary endpoint was an increase in overall d3mfs from baseline. The Scottish Index of Multiple Deprivation (SIMD) was used, and analyses were by odds ratio (OR) from exact logistic regression. We linked and calculated SIMD for 494 children. Mean follow-up was 1.77 years (SD 41 days). Mean d3mft/d3mfs was 0.9/2.2 at age 3 and 2.1/6.0 at age 5. The mean increase in d3mfs was 3.8 (SD 8.2). Overall 42% of children experienced an increase in d3mfs, ranging from 25% for the least deprived children to 47% for the most deprived children,

OR = 2.64 (1.30, 5.65),  $p = 0.005$ . Progression of caries (worsening d3mfs) was not evident in the lower anterior teeth (0–1%), was 2–6% in the upper anteriors, and very high in both the upper posteriors (16–19%) and lower posteriors (18–23%). Social inequalities in progression were greatest in the upper first primary molars (OR = 6.57–6.72). We found that worsening of decay experience over time from the age of 3 years was common and varied by type of tooth and socioeconomic status.

The data collection was funded by NHS Greater Glasgow and Clyde.

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#### Exploring the Social Patterning of Dental Caries in Scottish Children: A Pilot Cohort Study

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**Aim:** The social patterning of dental caries in children and the contribution of diet and oral hygiene-related behaviours to these inequalities have not been well quantified. This pilot cohort study aims to explore the relative contribution of these factors in explaining the observed socio-economic inequalities in dental caries. **Experimental Approach:** A pilot longitudinal cohort study of 219 Scottish nursery school children aged 4 years followed up 1 year later at school ( $n = 175$ , 80%). A detailed dental examination was carried out by a calibrated dentist following the BASCD criteria. Questionnaires completed by parents provided detailed information on habitual diet (non-milk extrinsic sugar [NMES] intake, sugared drinks, parental restriction of sugar), oral hygiene behaviours (brushing frequency; age brushing commenced; brushing before bed) and socio-economic position (SEP) of the family (area-based; household income; parental education; parental occupation). Binary logistic regression was used to estimate the relative index of inequality (RII) for caries experience by SEP and caries experience attenuation by the aforementioned factors. Prevalence of caries experience at 5 years was 47% (82/175), and was socially patterned irrespective of the measure of SEP. Using household income, the RII odds ratio (OR) [95% confidence interval (CI)] for caries experience was (OR = 6.1 [1.9–19.2]). Mutually adjusting for all diet- and oral hygiene-related behaviours reduced the RII by 10% (OR = 5.6 [1.5–21.3]). Similar results were obtained using other measures of SEP. **Conclusions:** All measures of SEP demonstrated similar RIIs for caries experience. Despite some attenuation in the RII after adjustment for diet- and oral hygiene-related behaviours, clear social gradients in caries experience persisted, suggesting that perhaps other more distal factors may be important.

The study was funded by Childsmile (Scottish Government-funded oral health improvement programme for children).

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#### Association among Health-Promoting Schools, Socioeconomic Factors and Tooth Wear in Brazilian Adolescents

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**Objective:** Prevalence tooth wear has attracted increasing attention in the dental community; however, population-based studies that assessed the association between health-promoting schools and this condition are scarce. This investigation assessed relationship among the prevalence and severity of tooth wear, socioeconomic factors and health-promoting schools' policies.

**Method:** A cross-sectional study in a random sample of 201 15-year-old Brazilian schoolchildren was conducted in Rio Grande da Serra, São Paulo, Brazil. The prevalence and severity of tooth wear were evaluated using a modified version of the Tooth Wear Index [TWI], by two calibrated examiners ( $\kappa > 0.85$ ). Socioeconomic factors and health-promoting schools' policies data were collected using a structured questionnaire. Data were analyzed using Poisson multivariate regression with the Stata 12.0 software. **Results:** In total, 16,629 dental surfaces were evaluated. Among buccal surfaces, 62.2% did not present dental wear, 37.6% had incipient lesions and 0.1% had moderate lesions. Among the incisal/occlusal surfaces, 1.0% did not present dental wear, 93.6% had incipient lesions, 5.3% had moderate lesions, and 0.1% had severe ones. In the lingual surfaces, absence of dental wear was observed in 61.6% of the sample, 37.9% had incipient lesions and 0.5% had moderate ones. A positive association was found between the prevalence and severity of tooth wear and socioeconomic factors ( $p < 0.05$ ), and an inverse association was observed between these conditions and health-promoting schools' policies ( $p < 0.05$ ). **Conclusion:** Tooth wear prevalence was high, with low severity. Socioeconomic condition was associated with a higher prevalence of tooth wear, and health-promoting schools' policies resulted in lower prevalence of toothwear.

The study was funded by CNPq Brazil.

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#### Early Childhood Caries: The Last Cariologic Problem?

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A caries decline can be detected over many decades in many countries in children, adolescents and even adults. The data on early childhood caries (ECC) are scarce and seem less promising than for other age groups. Thus, the aim of this study was to ana-

lyse the caries development in Mecklenburg-Vorpommern (MV) in nursery children from 1997 to 2012 and to compare these data to the general caries decline. The data were extracted from the regular community examinations in nurseries, kindergartens and schools in the 16 cities and districts in MV including about 7,000 3-year-olds and 10,000 6- and 12-year-olds each year (60–85% of the cohort). In Greifswald, the dmft data were supplemented for 0–3-year-olds ( $n = 682$ ) by the diagnosis of ECC 0 (no caries), 1 (initial caries lesions) and 2 (cariou defects, fillings or missing teeth) in upper incisors. Caries in 3-year-olds dropped in MV continuously from 0.80 dmft (1997) to 0.53 (–34%, 2012), while caries in 6- and 12-year-olds was reduced from 2.9 dmft/2.8 DMFT (1997) to 1.62/0.71 in 2012 (–44% and –75%, resp.) which was equivalent to the national 15-year caries decline of –33%/–70% in 6–7-/12-year-olds (Deutsche Arbeitsgemeinschaft für Jugendzahnpflege, 2010). Two percent of the 0–3-year-olds in Greifswald ( $n = 682$ ) presented with ECC 1 and 2 each. In 3-year-olds ( $n = 293$ , mean: 0.26 dmft), 10 children (3%) with ECC 2 (mean 4.7 dmft, 10.0 dmfs) exhibited 62% of all carious lesions at this age. Thus, the caries reduction in early childhood was less pronounced and an extremely small group of children exhibits the vast majority of all lesions calling for a risk group approach in prevention.

The study was funded by the counties and cities of Mecklenburg-Vorpommern.

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### Factors That Influence the Retention of Pre-School Children within Dental Practices Participating in Childsmile

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**Aim:** Childsmile, Scotland's national child oral health programme, was established in 2006. Children aged 0–5 years are facilitated to attend Childsmile dental practices by specially trained dental health support workers (DHSW). Preliminary analysis indicated that 47% of children who first attended a Childsmile practice appointment between 2006 and 2009 returned within 12 months. Our aim was to identify factors associated with retention and to develop a model to predict those children who had the highest probability of being 'retained' (returning within 12 months). **Methods:** The cohort of 14,213 children had 34 covariates. These covariates were grouped into 'patient', 'practice' or 'intervention' factors. Univariate significant variables ( $p < 0.05$ ) were considered for the stepwise selection algorithm and a model was created for each group. Variables were added in order of significance until no further variable could add to the models predictability. These models' variables were regrouped and the stepwise process repeated to produce an overall retention model. **Results:** Nineteen variables remained within the retention model which had a moderate level of predictability ( $c$  index = 0.66). The age of the child was the most predictive variable ( $c$  index = 0.55,  $p < 0.001$ ) with children aged <6 months most likely to be retained (OR = 1.38). Key findings indicated that children from affluent areas were more likely

to be retained than those from the most deprived area (OR = 1.33), children that resided with a smoker were significantly less likely to be retained ( $p < 0.0002$ ), as were those whose parent last attended a dentist for pain relief. **Conclusions:** Inequality in retention of children attending Childsmile practices was observed in this cohort. From 2010/11, changes to the programme have been made to attempt to address this including changes to the DHSW role and the dental practice payment/contract.

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### Sequential DMF-T in Family Health Program of Duque de Caxias, Brazil

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**Background:** The Family Health Program (FHP) is the Brazilian public health strategy that has been gradually implemented in the cities. In this program, the dentists combine preventive and restorative care, specifically to a registered population that lives inside the health unit demographical area. The aim of this study was to evaluate the DMFT index of 12-year-old children registered in the FHP of Duque de Caxias city, the third population of Rio de Janeiro state, located in a region of poor social development. The FHP was introduced in this city in 1997, but only in 2008 there were sufficient dentists appointed to occupy all health units. The period of study was from 2009 to 2012. **Material and Methods:** Sixty-nine dentists, trained and calibrated, using artificial lights, dental mirrors and ball ended probes, examined 7,031 12-year-old children. Data were analysed using Kruskal-Wallis test followed by multiple comparisons. **Results:** Data showed reduction in the DMFT average during the years:  $1.81 \pm 0.78$  (2009);  $1.68 \pm 0.65$  (2010);  $1.67 \pm 0.86$  (2011);  $1.51 \pm 0.74$  (2012). From 2009 to 2012 the DMFT decreased significantly ( $p < 0.05$ ). Consequently, the percentage of caries-free children has gradually increased: 32.69% (2009); 42.26% (2010); 43.35% (2011); 45.39% (2012). When we analysed the index distributed in the four sanitary districts, the DMFT showed a slight reduction, but it decreased significantly ( $p < 0.05$ ) only in district I, from  $1.56 \pm 0.73$  (2009) to  $1.11 \pm 0.39$  (2012). These results indicated that, despite the social problems in Duque de Caxias, FHP's dentists were able to improve the oral health conditions of the analysed population. However, systematic actions of oral health promotion must be reinforced mainly in districts II, III and IV.

The research was conducted with support from Family Health Program of Duque de Caxias, RJ complemented by authors' resources.



### Prevalence and Severity of Dental Erosion in Brazilian Adults and Elderly: A Population-Based Study

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The aim of this population-based study was to assess the prevalence and severity of dental erosion in adults and elderly from Porto Alegre, Southern Brazil. A total of 826 subjects (aged 35–95 years, mean 52.8 ± 11.9) were selected by multi-stage probability cluster sampling strategy based on the monthly income of heads of families. A single calibrated examiner performed the clinical examinations in the residences and recorded the BEWE (basic erosive wear examination) index, which grades the severity of wear on the surface into four level score from no surface loss (0), initial loss of enamel surface texture (1), hard tissue loss <50% of the surface area (2) or hard tissue loss >50% of the surface area (3). Differences in the prevalence and severity of dental erosion according to gender and age were assessed by chi-square test. The prevalence of dental erosion was 32.2%. Male subjects were more likely to present at least one surface with dental erosion ( $p = 0.003$ ). Level 1, 2 and 3 of BEWE as the maximum hard tissue loss was found in 0.7%, 4.8% and 26.6% subjects, respectively. The severity of dental erosion was significantly associated with male gender ( $p = 0.010$ ). Regarding the affected surfaces, 54.4% of them suffered the most severe wear. In conclusion, dental erosion is highly prevalent in this Brazilian population. The greatest level of hard tissue loss, measured using BEWE, was present in approximately 1/3 of the subjects and on half of the affected surfaces. These results suggest the need to implement community programs for the prevention and treatment of dental erosion. This study was approved by Ethics Committee of Federal University of Rio Grande do Sul (protocol 19794).

This study was funded by the National Coordination of Post-Graduate Education (CAPES), Federal University of Rio Grande do Sul (PROPESQ) and Oral-B (Procter & Gamble, Brazil).

### Beta-Blockers Increase Dry Mouth and Caries Prevalence in Hypertensive Patients under Multi-Drug Treatment

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Anti-hypertensive therapy may usually require the prescription of multiple drugs, for long periods of time. Beta-blockers are one of the most frequently used medications among hypertensive

patients and may be associated to dry mouth and higher prevalence of caries, specially root decay. The present study aimed to evaluate the possible influence of beta-blockers on the prevalence and severity of caries in a population of severe refractory hypertensive patients. 170 hypertensive subjects, mean age 53.6 years, taking multi-drug anti-hypertensive treatment for at least 2 years (mean: 4.8 years) were examined and radiographs were taken, in order to evaluate the prevalence of caries and periodontal disease. Subjects were divided in 2 groups. Group 1 ( $n = 83$ ) included subjects taking beta-blockers and at least 2 other anti-hypertensive drugs, such as calcium channel blockers and diuretics. Patients not taking beta-blockers were allocated to group 2 ( $n = 87$ ). Caries was assessed by visual clinical examination and periapical radiographs and were scored according to its severity as follows: 0 = no caries; 1 = incipient caries/white spots; 2 = moderate caries/enamel and dentin; 3 = advanced caries reaching the pulp. Patients were divided according to the severity of carious lesions in 3 groups: no caries/incipient lesions ( $n = 102$ ); enamel/dentin caries ( $n = 36$ ); deep caries reaching the pulp ( $n = 32$ ). There was no difference between the groups regarding age, gender, smoking, blood pressure levels, number of teeth, plaque index and duration of anti-hypertensive treatment. Patients taking beta-blockers were almost three times more likely to have root caries (prevalence = 31.1 vs. 10.1%) and coronal caries (83.6 vs. 35.8%) (OR = 2.83 and OR = 2.7 respectively) and to report oral dryness (prevalence = 78.7 vs. 22.9%) (OR = 2.77). Patients taking beta-blockers also experienced higher but non-significant increases in the number of periapical lesions associated to severe caries and detected through radiographs (OR = 1.3). In conclusion, there were significant deleterious effects of beta-blockers use on caries prevalence. These data may suggest that hypertensive patients taking xerostomic medications should be enrolled in more intensive plaque control programs and receive fluoride supplementation in order to prevent carious lesions and tooth loss.

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### Association between Nutritional Status and Early Childhood Caries: A Hierarchical Approach

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No consensus exists regarding nutritional status and early childhood caries (ECC). Most previous studies had a cross-sectional design and few were guided by a theoretical framework. This study was conducted to evaluate the association between nutritional status and ECC, adjusted for confounding factors, through hierarchical approach. It was a retrospective cohort study, involving 625 children (24–71 months). Nutritional status was evaluated using anthropometric measures and biochemical serum parameters (hemoglobin, albumin and zinc). Weight and height were measured to calculate z score (Anthro software, WHO). The number of carious teeth (dft) plus assessment activity of enamel lesions (opaque and rough lesions) [Nyvad et al.: J Dent Res

2003;82:117–122] were measured as outcome. Following a theoretical framework the hierarchical model was built in a forward fashion, by adding the following levels in succession: level 1: age and sex; level 2: social variables; level 3: birth variables; level 4: variables from 1st year; level 5: current diet; level 6: anthropometric measures at present; level 7: biochemical parameters. Sequential forward multiple Poisson regression analysis was employed. Age ( $p = 0.000$ ), mother's educational level ( $p = 0.000$ ), high sucrose consumption (0.002), lower height for age at present ( $p = 0.006$ ) and lower levels of hemoglobin ( $p = 0.006$ ), albumin ( $p = 0.031$ ) and zinc ( $p = 0.012$ ) were associated with high number of ECC lesions. Lower nutritional status was associated with ECC in the population studied.

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### Identifying Dental Students' Training Needs to Improve Oral Health for Institutionalised Older People

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**Aim:** Though geriatric dentistry is a relatively new component of dental education programs in France, very few practical training hours have been utilized. To identify what programs are still needed, we conducted a learning needs analysis with prospective dental students. **Methods:** A sample of 20 undergraduate students (Nice Dental School) completed a questionnaire in two parts. To reveal the students' perceived educational needs, the first part concerned their level of understanding of and attitudes toward several items – including carious lesions – in dependent older people. For each of the listed oral pathologies, the student had to score his/her own perception of the frequency (0 = scarce, 1 = likely, 2 = common), the severity (0 = minor, 1 = moderate, 2 = serious), problems of knowledge/technical skills/interpersonal skills (0 = no problem, 1 = some difficulties, 2 = many difficulties). The second part of the form assessed the knowledge actually acquired by the students. The answers were analysed with a qualitative and quantitative grid. **Results:** The students admit that carious lesions for frail older people are frequent (score 2: 75%), but the severity is largely underestimated (score 1: 65% – score 0: 30%). They do not recognise the lack of knowledge (score 0: 70%). They feel able to provide quality care as far as both technical skills (score 0: 40% – score 1: 50%) and interpersonal skills (score 0: 60%) are concerned. Yet, in the second part of the questionnaire, 46.3% of the knowledge answers are correct. **Conclusion:** There is a clear discrepancy between actual training needs and felt needs. In order to challenge this problem, we decided to include practical courses – directly in the nursing homes – in the dental curriculum and develop a real partnership between students and nursing homes staffs.

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### The Impact of Community Water Fluoridation on Tooth Eruption and Dental Caries

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It has been suggested that observed caries reductions from community water fluoridation are a consequence of delayed tooth eruption. The aims of this study were to determine whether fluoridation impacts on tooth eruption and caries experience. Data were analysed from an observational epidemiological survey in matched fluoridated and non-fluoridated communities [results published in McGrady et al.: BMC Public Health 2012;12:1122] involving males and females aged 11–13 years, examined using ICDAS criteria. As eruption dates for teeth were not available, the data were analysed for the presence of selected teeth as a proxy measure (2nd permanent molars, premolars and canines) and caries experience at examination, whilst controlling for gender and age. Data for 1,783 subjects were available for analysis (910 fluoridated, 873 non-fluoridated). There were no significant differences in the mean age at examination (fluoridated 12.86 years (SD 0.49); non-fluoridated 12.79 years (SD 0.55):  $t$  test  $p > 0.05$ ). Logistic regression analyses for the presence of the selected teeth at examination (with fluoridation status, gender and age at examination as explanatory variables) revealed only age at examination as statistically significant ( $p < 0.001$ ). When considering initial carious lesions ( $D_{1-6}$ MFT) for the selected teeth, the caries experience and proportion of subjects 'caries-free' were 0.83 (SD 1.75), 64.9% in the fluoridated population and 1.82 (SD 2.61), 49.1% for the non-fluoridated population. For a threshold of caries into dentine ( $D_{4-6}$ MFT) results were 0.04 (SD 0.27), 96.5% in the fluoridated population and 0.10 (SD 0.40), 92.6% for the non-fluoridated population. The differences between fluoridated and non-fluoridated areas were statistically significant (chi-square;  $p < 0.001$ ). The results of this study suggest community water fluoridation does not significantly impact on tooth eruption. Data for key selected teeth confirms that community water fluoridation does reduce caries experience.

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### Distance-Learning Management System to Complement Education in Cariology for Undergraduate Students

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Distance-learning activities related to cariology have been performed with undergraduate students since 2010 in paediatric dentistry at USP in order to complement theoretical classes. We aimed to evaluate these activities performed in a learning management system after 2 years of implementation, in terms of contents and functionality. All the given tele-courses from 2010 to 2012 were included and statistics provided for them as well as all their contents were collected for analysis. Five tele-courses were proposed. 322 students participated in distance-learning activities (mean per course  $\pm$  SD = 64.4  $\pm$  17.2) and, on average, 15 tutors accompanied the process per course. Caries lesion detection and fluoride therapy were topics discussed in all modules, but different activities were focused on different aspects for each course. Other topics were also included in lessons: caries activity (60%) and caries risk assessment, oral hygiene education and clinical examination (40%). Two lecturers were responsible for preparing practical exercises and tutors helped them in creating, revising and correcting the proposed exercises. The main type of validation was open questions (83%). A high level of students' access of the platform has been observed to the platform during the 2-year period (mean per course  $\pm$  SD = 21,372  $\pm$  6,775.2). More than 32,000 external visitors also accessed the contents in the same period. On average, 14 tutor-student feedbacks were sent per student in each tele-course. In conclusion, the use of a distance-learning management system in pediatric dentistry has been used especially to complement teaching in caries detection and management using fluorides. Tele-courses have been highly accessed by undergraduate students and also, external participants.

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### A Proposed Method of Deriving ICDAS Scores Using Tooth Surfaces

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The aim was to derive ICDAS scores using tooth surfaces and compare them with DMFS scores in patients undergoing orthodontic treatment. A secondary objective was to study the associa-

tion of plaque with white spot lesion (WSL). A total of 157 patients undergoing fixed orthodontic therapy were screened for dental caries using ICDAS and DMFS. The proposed method of developing ICDAS was as follows: The surfaces with ICDAS scores 3–6 were counted and divided by the total number of surfaces scored for that individual. This gives the dental caries index for the individual. The same method was applied to calculate DMFS index: D+M+F surfaces divided by the total number of surfaces scored for that individual. The conventional method of calculating mean ICDAS and DMFS was also performed for the same patient. Pearson's correlation was estimated between conventional (-C) and proposed (-P) calculation of ICDAS and DMFS. Exposure to plaque (plaque index) was recorded for all surfaces and its association to presence of WSL was estimated. The mean surface per individual was 102.2  $\pm$  12.14. The mean score per individual for ICDAS-P was 2.8  $\pm$  2.7. The mean score for DMFS-P per individual was 3.1  $\pm$  4.1. Correlation was significant between ICDAS-C and ICDAS-P, and DMFS-C and DMFS-P (0.53 and 0.99 respectively). Correlation between DMFS-C and ICDAS-C was (0.04) which improved to 0.29 with ICDAS-P. Correlation between ICDAS-P and DMFS-P was 0.30. Presence of plaque was strongly associated with WSL (OR 3.72, CI 3.39–4.02). Using surfaces instead of teeth in denominator made no difference in the DMFS index, while the same improved the performance of ICDAS. The proposed method of using surfaces as denominator estimates proportions affected by dental caries instead of morbidity and correlates better with disease state.

### Nutritional Status and Dental Caries in Chilean Preschool Children

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The objective was to establish an association between the presence of caries lesions according to ICDAS II and nutritional status in preschool children. A cross-sectional study with a total of 342 randomly selected patients aged 2–3 years that belonged to nursery schools in the Metropolitan Region, Santiago, Chile was carried out. After informed consent was obtained, caries prevalence was determined by clinical examination according to ICDAS II criteria. Weight for height of the subjects was calculated using standard formula: weight for height (%) = weight of subjects/weight of normal child of same height  $\times$  100. Nutritional status of the subjects was classified as underweight, normal weight, overweight and obese, if their weight-for-height values were <89% (underweight), 90–110% (normal weight), 111–120% (overweight) and >120% (obese). The nutritional status of the total sample was 0% of the children were underweight, 43.27% were normal weight, 36.84% were overweight and 19.88% were obese. The mean of caries lesions (ICDAS = 2–6) in the normal weight group was 2.31 (95%

CI: 1.77–2.85) and in the overweight and obesity groups together was 1.63 (95% CI: 1.28–1.98), showing significant differences between children with normal weight and children with overweight and obesity ( $p < 0.05$ ). Preschool children of the Metropolitan Region examined present high levels of overweight and obesity. Those with normal weight present significantly more caries lesions according ICDAS II than children with overweight and obesity.

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### Contribution of Biological, Behavioral and Socioeconomic Factors to Dental Caries in Brazilian Schoolchildren: Multilevel Analysis

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This survey assessed the association between biological, behavioral and socioeconomic factors and the experience of dental caries in Brazilian schoolchildren. A population-based cross-sectional study was carried out with a multistage random sample of 589 12-year-old schoolchildren representative of a southern city in Brazil. Oral examinations for dental caries experience (DMFT index) and visible dental plaque were performed by a single calibrated examiner. Biological, behavioral and socioeconomic data were also assessed through specific tests and questionnaires applied to the children and their mothers. Data were analyzed using adjusted multilevel Poisson regression. This strategy allowed estimating rate ratios (ratio of arithmetic means) between comparison groups and their respective 95% confidence interval (RR: 95% CI). A total of 73% of the children had dental caries; the mean DMFT was 2.4 (95% CI: 1.9–2.8). Children with visible dental plaque (RR 1.48: 1.30–1.68), highest salivary levels of mutans streptococci (RR 1.23: 1.09–1.38), and lactobacilli (RR 1.29: 1.07–1.56) and those with low family income (RR 1.08: 1.02–1.15) presented a higher DMFT mean. Carbohydrates consumption was also significantly associated with dental caries experience, being those with daily consumption of more than 5 foods with sucrose presenting highest mean of DMFT (RR 1.15: 1.02–1.29). According to these results, the variables that better explained dental caries experience in 12-year-old schoolchildren in southern Brazil were presence of dental plaque, high levels of specific acidogenic and aciduric bacteria groups in saliva, sucrose-rich diet and low socioeconomic backgrounds.

The study received support from the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil.

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### Early Childhood Caries: Results from an Academic Town versus an Industrial Town

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The purpose of this cross-sectional study was to assess and compare the oral health status of children aged 6–36 months living in two German cities (Greifswald, university city vs. Schwedt, industrial city) and to determine the parental knowledge and reported behaviours toward preschool oral health and early childhood caries (ECC) to tailor a preventive programme. A total of 235 kindergarten children in Greifswald ( $n = 137$ , >20% of 0–3-year-old population, mean age  $28 \pm 7$ ) and Schwedt ( $n = 98$ , >30% of 0–3-year-olds,  $27 \pm 7.8$ ) and their parents participated. The kindergartens were randomly selected and informed consent was achieved. In addition to a validated questionnaire [Adair et al.: Community Dent Health 2004;21(suppl 1):102–111] on the parents' knowledge and their children's oral health behaviour, the children were examined by one calibrated dentist (>80% agreement) for plaque (QHI), dental caries (dmft/ECC) and gingival health (yes/no bleeding). 97.1% of children in Greifswald and 94.9% in Schwedt had  $\text{deft} \leq 1$  with an identical mean  $\text{deft}$  of  $0.14 (\pm 0.7)$ . The prevalence of ECC in Greifswald was 12.4% and 13.3% in Schwedt ( $p = 0.9$ ). Plaque ( $p = 0.16$ ) and gingival status ( $p = 0.77$ ) were also similar in both cities in spite of higher parental education level ( $p < 0.001$ ). The prevalence of ECC was highly correlated to prolonged and frequent use of feeding bottles with cariogenic drinks ( $p < 0.05$ ) as well as to the mother's education level ( $p = 0.01$ ). A tendency towards ECC was observed in children of unemployed parents ( $p = 0.12$ ). Parents' beliefs, attitudes and practices play a crucial role in the oral health status of young children. In this study the kind of city barely influenced the children oral health status. Strategies targeting parents, caregivers and children are required to reduce the knowledge gaps and alter deleterious oral health practices.

The study was funded by the University of Greifswald and Community Health Services Greifswald.

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### Caries Prevalence among Brazilian Indigenous Population

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**Aim:** To describe and analyze the caries prevalence among self-identified indigenous individuals and to compare the findings with the caries status within the non-indigenous population. Secondary

data from the Oral Health Brazilian survey (SB Brasil 2010) was used to address the null hypothesis that there were no differences between caries prevalence among the indigenous and non-indigenous population. The age groups analyzed were the following: 5, 12, 15–19, 35–44 and 65–74 years. Kruskal-Wallis and logistic regression were used for statistical analysis. Information of 308 indigenous was assessed in comparison to data of 37,211 non-indigenous persons. The percentage of caries-free individuals found among adults from both groups was around the same (31%). However, non-indigenous children presented a 46% caries-free rate while 30.8% were observed among 5-year-olds from the indigenous sample. The mean dmft score observed among indigenous children was  $4.10 \pm 4.01$  and among non-indigenous  $2.42 \pm 3.35$  ( $\chi^2 = 10.8$ , d.f. = 1,  $p = 0.001$ ). Decayed teeth are accounted for 96% of the overall indigenous dmft score. The mean DMFT observed among indigenous persons for all age groups was  $12.05 \pm 11.72$  and among non-indigenous  $10.92 \pm 11.58$  ( $\chi^2 = 0.37$ , d.f. = 1,  $p = 0.542$ ). Missing teeth are accounted for 73% of the overall indigenous DMFT score. Regarding the DMFT index, there was statistically significance difference between indigenous and non-indigenous individuals at the five age bracket ( $\chi^2 = 3.81$ , d.f. = 1,  $p = 0.05$ ). The results lend credence to the suspicions that in Brazil there are unequal differences in caries status between self-identified indigenous individuals and their respective national counterpart.

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### What Is the Optimal Age for Sealing 1st Molars in School-Based Programs?

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Recommendations for initial placement of sealants on 1st molars range from 6 to 9 years of age, many considering eruption status and children's cooperation. The current study investigated differences in caries presence, eruption status and children's cooperation as they relate to sealant placement decisions for school-based programs (SBP). Data from one SBP were assessed. Variables included children examined, not receiving a sealant and reasons for this (sealants, restorations or cavitation [ICDAS 4–6] presence, 1st molars not fully erupted [NFE], and uncooperative child). Comparisons between age groups were performed using ANOVA. From January 2009 to December 2012, 4,126 children were seen. 1,060 were 6 years old, 1,070 were 7, 1,069 were 8, and 927 were 9. 831 6-year-olds were not-sealed (NS); 666 (80%) had molars NFE; 76 (46%) had sealants; 43 (26%) had caries; 21 (12%) had restorations; and 25 (15%) were uncooperative. 489 7-year-olds were NS; 204 (42%) had molars NFE; 158 (55%) had sealants; 70 (25%) had caries; 38 (13%) had restorations; and 19 (7%) were uncooperative. 420 8-year-olds were NS; 1% (6) had molars NFE; 280 (67%) had sealants; 78 (22%) had caries; and 84 (20%) had restorations. 371 9-year-olds were NS; 3 (1%) had molars NFE; 231 (63%) had sealants; 75 (20%) had caries; and 84 (23%) had restora-

tions. No statistically significant difference was observed for caries or restorations as a reason for not sealing. Seven years of age may be the ideal age group for SBP as children have more teeth fully erupted and are more cooperative than younger ones.

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### Preliminary Survey on Education in Cariology for Undergraduate Dental Students in Brazil

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**Aim:** Dental caries prevalence is still high among special groups in Brazil. It continues to be responsible for significant health, social and economic impacts and imposes the necessity to deliver a profound education in cariology for dental students. The aim of this survey was to evaluate the undergraduate cariology education in Brazil. **Methodology:** The ORCA/ADEE cariology curriculum with 12 questions were translated to Portuguese and sent to 203 dental schools in Brazil. **Results and Discussion:** A preliminary sample of 73 schools answered and returned the questionnaire. Most of the schools (63%) have a specific curriculum on cariology, which is taught mainly by three units: operative dentistry (35%), preventive dentistry (34%) and pediatric dentistry (22%). Cariology was reported to be taught during the second (61%), third (63%) and fourth (50%) year of undergraduate course, being the second year when students mainly start with pre-clinical activities and the third when they start with clinical procedures in patients. Most of schools include the topic dental erosion (66%) and enamel defects (75%) on cariology education. The majority of schools taught most main teaching topics, except minimal intervention, caries lesions sealing, treatment decision based on caries activity, restorative procedures and ART. The majority of the responding dental schools (93%) would support the development of an international curriculum on cariology. **Conclusion:** Preliminary results showed that, although most of assessed dental schools give an special attention to aspects related to cariology curriculum, it is still necessary to assess the undergraduate teaching in the whole country prior to establish a national or international cariology curriculum, as recognized by the ORCA/ADEE cariology curriculum group.

## Effectiveness of an Oral Health Program for Mothers and Their Infants

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**Aim:** To assess the effectiveness of an oral health program developed to prevent dental caries in very young children. **Methods:** Between 2006 and 2009, 194 pregnant women were invited to participate in a program aimed at providing them and their newborns with oral care at regular check-ups. 87 mothers returned with their babies (G1) while 107 did not come back after delivering (G2). In 2011 all women were contacted, and 87 from G1 and 90 from G2

responded positively. Mothers and children were dentally examined by two calibrated examiners (kappa of 0.76 and 0.91 for primary and permanent dentition, respectively). A questionnaire about socioeconomic status (SES) and education level (EL) was completed. T-tests were used for statistical analysis. **Results:** Statistically significant differences were observed in mothers' mean age (G1 = 33.8 years; G2 = 35.6 years;  $p = 0.02$ ), and mean DMFS score (G1 = 24.71; G2 = 32.58;  $p < 0.001$ ), but not for SES ( $p = 0.76$ ) and EL ( $p = 0.1$ ). Children from G1 attended the program on average 2.8 times/year. 90.8% children from G1 and 18.8% from G2 were free of cavitated dentine lesions. Children's mean age and mean dmfs score from G1 and G2 were 50.4 and 53.1 months ( $p = 0.07$ ), and 0.25 and 4.12 ( $p < 0.001$ ), respectively. A multivariate logistic regression showed an OR of 44.3 for children in G2 to have cavitated dentine lesion as opposed to those in G1. **Conclusions:** The oral health program was effective in preventing cavitated dentine lesions for those children who attended the program at least once a year.

The authors are grateful to University of Brasília for financially supporting the study.

## Session 5 Clinical Studies

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### Association of Occlusal and Gingival Factors with Non-Carious Cervical Lesions: A Tooth-Paired Clinical Study

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The purpose of this clinical study was to evaluate the association of non-carious cervical lesions (NCCL) with disocclusion type, presence of protrusive contact, non-functional occlusal centric contacts, occlusal tooth wear and with some gingival characteristics. This study was approved by the local ethical committee (0027.0.203.000-11). The sample size was calculated ( $\alpha = 5\%$ ,  $\beta = 20\%$ , odds ratio = 6.47) and 37 subjects were selected from a university dental clinic, according the eligibility criteria. From each individual, a pair of premolars was selected, one with NCCL (case group) and another without NCCL (control group). A calibrated examiner ( $\kappa \geq 0.89$ ) performed the clinical examination. The Tooth Wear Index [Smith BG, Knight JK: Br Dent J 1984;156:435–438] was used to determine the cervical tooth loss. The subjects were instructed to perform the protrusive and lateral disocclusion movements to register the presence of contacts on premolars. The occlusal centric contacts were marked with articulating paper. The occlusal wear was evaluated according a five-point ordinal scale [Carlsson et al.: Acta Odontol Scand 1985;43:83–90]. The keratinized mucosa height, attached gingival height and gingival thickness were measured with a periodontal probe. The conditional odds ratio evaluated the association between NCCL and the categorical variables. Gingival variables were compared in control and case groups by Wilcoxon test ( $p \leq 0.05$ ). There was no significant difference between case and control groups according their pattern of disocclusion (OR = 1.57; CI = 0.62–3.99), protrusive contact (OR = 1.00; CI = 0.19–5.31), non-functional occlusal centric contacts (OR = 0.85; CI = 0.30–2.42), occlusal wear (OR = 0.00; CI = 0.00–0.00) and gingival thickness (OR = 2.01; CI = 0.77–5.23). The case group presented keratinized mucosa and attached gingiva sig-

nificantly lower than control group ( $p < 0.05$ ). The association of NCCL with occlusal factors was not confirmed; however some gingival characteristics seem to be associated with NCCL and need to be further evaluated.

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### Predictive Modeling of Root Caries Incidence

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X-ACT, a three-year randomized clinical trial, assessed the effectiveness of xylitol mints in reducing caries incidence in adults. This secondary analysis aimed to identify the set of risk indicators best able to predict root caries (RC) incidence in X-ACT control group participants. Utilizing all available data to improve model prediction performance, the analysis includes xylitol and control group participants with exposed root surfaces at baseline ( $n = 522$ ,  $50.3 \pm 12.4$  years, 61.3% females, 54.2% white/Caucasian, 22.7% black/African-American). Trained and calibrated examiners completed caries exams at baseline and yearly using modified ICDAS II criteria. Three logistic regression models were evaluated for the ability to predict incident RC. Explanatory variables in model M1 included treatment group, the number of follow-up years at risk (mean 2.59; range 0.84–3.11), and linear and quadratic terms both for the number of at-risk root surfaces and RC index at baseline. Additional variables in model M2 were gender, race, and age. Further additional variables in model M3 were high blood pressure, brushing and flossing frequency, dry mouth, self-rated oral health, and whether visited dentist in last year. Sensitivities, specificities and area under ROC curve were calculated for control group participants only. All analyses were performed in SAS. RC incidence

in the control group was 42.5%; area under the curve improved only slightly with increasing model complexity (areas: 0.77, 0.79, 0.78); for a cutpoint probability of 0.425, sensitivity was 65.5 and specificity was 76.7 for model M2, based on 500 bootstrap samples. Inclusion of health variables did not improve the predictive performance of a logistic regression model for three-year incident RC based on number of root surfaces at risk, RC index, gender, race and age.

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### Evaluation of Chair-Side Saliva and Caries Risk Tests: In vivo Study

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**Aim:** The comparative evaluation of various chair-side saliva and caries risk tests according to current tooth caries status in adult patients. **Study Design:** Teeth in 87 adult patients were examined for carious lesions according to ICDAS II criteria in order to calculate DMFT and DMFS indexes at D1 and D3 threshold (D<sub>1</sub>MFT, D<sub>3</sub>MFT, D<sub>1</sub>MFS, D<sub>3</sub>MFS) and the number of possible active lesions according to the Lesion Activity Assessment criteria. For each patient, the following saliva parameters were calculated by means of chair-side tests: (1) stimulated and non-stimulated saliva flow rate, (2) saliva consistency, (3) resting saliva pH, (4) stimulated saliva buffer capacity, (5) lactic acid production, (6) *S. mutans* and lactobacilli count. Parameters 1–4 were assessed by the Saliva Check Buffer Kit (GC Europe), 5 and 6 by the Clinpro Cario L-Pop (3M, ESPE) and CRT Bacteria (Ivoclar Vivadent) tests, respectively. The statistical analysis performed was Student's t test ( $\alpha = 0.05$ ) and Mann-Whitney U test ( $\alpha = 0.05$ ), respectively. The non-normal resting saliva pH was related with a high value in D<sub>1</sub>MFT index [17.89 (7.84) vs. 16.26 (5.67),  $p = 0.007$ ]. The high number of *S. mutans* and *Lactobacilli* count was related with a high number of possible active caries lesions [6.50 (8.86) vs. 3.42 (4.79),  $p = 0.012$  and 7.27 (9.66) vs. 3.03 (2.68),  $p = 0.009$ , respectively]. The examination of microbial count by means of CRT bacteria can be proven as helpful in the assessment of caries active patients. Among the other parameters examined, only non-normal resting pH is associated with increased DMFT value in D1 threshold, but not with the number of active lesions. None of the commercially available saliva tests evaluated can accurately indicate the tooth carious status.

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### Remineralization Potential of Herbal Medicaments and Assessment by a Recent Caries Detection Device

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The aim of this study was to evaluate the effectiveness of herbal medicaments; ginger and rosemary on remineralization of white spot enamel lesion and assessment by a recent detection device, FluoreCam. This in situ investigation was a single-site, randomized, four-way crossover study involving a total of 10 healthy participants with 3-week test periods and between these periods there were 1-week washout periods. Demineralized human enamel specimens were measured for baseline surface microhardness and light fluorescence with FluoreCam. 3 specimens were fixed with light curing temporary filling material (Clip, Voco) on each of the removable intra-oral appliances that were specially designed for participants. 30 specimens in each of four groups were used in this in situ study with the following treatments applied three times daily after brushing their teeth with sodium fluoride (NaF) toothpaste (Ipana): (1) ginger-honey-chocolate, (2) rosemary-ginger-honey-chocolate, (3) no treatment material only NaF toothpaste, control group, (4) hydroxyapatite and fluoride agent (Voco, Remin Pro). Each test period lasted 21 days. The post-treatment data were obtained by measurements of surface microhardness and FluoreCam. Statistical analyses of the data included ANOVA test with Student Newman-Keuls test. Significant differences between treatments (Ipana  $16.37 \pm 4.47$ , rosemary-ginger-honey-chocolate  $19.60 \pm 4.88$ , Remin Pro  $21.39 \pm 9.50$ , ginger-honey-chocolate  $23.73 \pm 8.27$ ) were observed by microhardness; compared to the positive control group (NaF toothpaste) significantly greater remineralization was observed with the Remin Pro and ginger-honey-chocolate treatment groups ( $p \leq 0.05$ ). With fluorescence assessments, although all treatments resulted in remineralization (rosemary-ginger-honey-chocolate  $\Delta F 0.17 \pm 1.10$ ;  $\Delta Q 5.88 \pm 2.88$ , ginger-honey-chocolate  $\Delta F 0.63 \pm 0.90$ ;  $\Delta Q 7.76 \pm 2.69$ , Ipana  $\Delta F 2.02 \pm 1.12$ ;  $\Delta Q 14.06 \pm 2.89$ , Remin Pro  $\Delta F 3.26 \pm 1.09$ ;  $\Delta Q 14.65 \pm 2.79$ ), no difference was found between the treatment groups. Remin Pro group was numerically more effective than the other groups ( $p > 0.05$ ). In conclusion, enhanced remineralization was observed with several of the treatment systems including ginger-honey-chocolate and Remin Pro the most.



### Changes of Environment in the Oral Cavity during the Early Phase of Orthodontic Treatment

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The aim of this study was to assess the influence of fixed orthodontic appliance on some caries risk factors in orthodontic patients during early phase of the orthodontic treatment. The research was conducted on 22 patients (mean age = 25.1) who satisfied the inclusion criteria: healthy systemic and periodontal condition, avoidance of antibiotics and antiseptic mouthwashes in the past three months. All clinical measurements took place at T1 (prior to fixed orthodontic appliance placement) and T2 (twelve weeks after placement of fixed orthodontic appliance) in the following order: (1) stimulated saliva flow (SS), (2) OHI-S index and (3) DMFT index. Polymerase chain reaction (PCR) was used to detect presence of *S. mutans* and *S. sobrinus*. Results indicated significant improvement in OHI-S index ( $p = 0.004$ ) from T1 to T2. Tests of correlation demonstrated no significant correlation between DMFT and other variables. Therefore, we divided patients according DMFT into two groups, with low (DMFT <11.44) and high past caries experience (DMFT >11.44). Non-paired t test did not show significant difference in the SS between those two groups ( $p = 0.452$  at T1,  $p = 0.888$  at T2). Mann-Whitney test also did not show significant difference in OHI-S index between groups ( $p = 0.173$  at T1,  $p = 0.669$  at T2). Using PCR method, *S. mutans* was detected in 2 patients at T1 in subjects with high past caries experience. At T2 two more patients from same group had *S. mutans*, statistical significance was not found ( $p = 0.157$ ). *S. sobrinus* was not detected at T1 and two patients from high past caries experience group were positive at T2. We can conclude that fixed orthodontic appliances may induce detrimental changes in composition of cariogenic microorganisms (increase of *S. mutans* from T1 to T2 was 100%), even in the presence of enhanced oral hygiene ( $Z = -2.08$ ,  $p = 0.004$ ).

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### Caries Pattern in 5-Year-Old Children with Congenital Heart Defects in Western Norway

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Children with congenital heart defects (CHD) are reported to have an increased risk of developing oral diseases and their systemic effects (e.g. infective endocarditis). The present study aimed to estimate the distribution, prevalence and severity of caries in a

group of 5-year-olds in need of life-long follow-ups due to CHD. Caries was defined as severe when it had involved and penetrated the dentin. 107 children were invited to participate in this cross-sectional clinical study of whom 72 responded. Caries registrations were done by two trained and calibrated dentists, using a detailed 5-graded caries diagnostic system (enamel caries included). Radiographic bitewings were taken in case of intramolar contact when possible. Inter- and intra-kappa values (weighted Cohen's kappa) were 0.77 and 0.85, respectively. The distribution of caries ( $d_{1-5}mfs$ ) in the group was heavily skewed, as 73% of all carious surfaces were found in 15% of the children. The prevalence of caries ( $d_{1-5}mfs$ ) was 37.5%. The mean  $d_{1-5}mfs$  in children with caries was 6.9 (SD 5.4). The teeth most frequently affected by caries were the second molars. The most common locations were the occlusal surfaces. Enamel caries ( $d_{1-2s}$ ) lesions exceeded dentin lesions ( $d_{3-5s}$ ), showing a prevalence of 60% to 40%. A quarter of the children experienced caries at dentin level ( $d_{3-5}mfs$ ). Nine children had 4 or more (up to 13) surfaces affected by dentin caries ( $d_{3-5}mfs$ ). However, few surfaces were filled ( $n = 9$ ) and few teeth extracted due to caries ( $n = 3$ ). In conclusion, caries was found to be severe in 25% of children with CHD. Enamel caries lesions were more common than dentin lesions. Almost all dental lesions were untreated.

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### Shade Improvement and Its Stability in Post-Orthodontic White Spot Lesions Using Noninvasive and Micro-Invasive Strategies

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The aim was to evaluate shade improvement and its stability in post-orthodontic WSLs when treated with different non-invasive (NIS) [ACP-CPP Toothpaste, Fluoride Varnish (FV), Fluoridated Dentifrice (FD)] and micro-invasive [Resin-Infiltration technique (RI)] strategies. 60 PO-WSLs on labial aspect of anterior teeth were randomly divided into four groups and treated with different agents. Standardized photographs were taken before, 'immediately after' and at each recall, scheduled every month for six months.  $L^*a^*b^*$  values were derived and  $\Delta E$  was calculated for different time intervals. On using Mann-Whitney U test, RI exhibited significantly better shade-improvement as compared to NIS, both immediately (mean  $\Delta E$ , RI = 10.62,  $p = 0.002$  and  $p < 0.001$ ,  $p = 0.009$ , when compared with ACP-CPP, FV and FD, respectively) and at all time intervals (6-month mean  $\Delta E$ , RI = 25.83,  $p < 0.001$ ,  $p = 0.028$ ,  $p < 0.001$  with ACP-CPP, FV, FD) while non-significant differences were seen among FV and ACP-CPP group for first 3 months ( $p = 0.149$ , 3rd month) but later FV exhibited significantly better results ( $p = 0.013$ , 6th month). Stability of shade change was evaluated by sequentially comparing differences in shade (comparing shade change from post-op to 1st month vs. shade change from post-op to 2nd month, then comparing change from

post-op to 2nd month vs. post-op to 3rd month and so on). Using Wilcoxon signed rank test, significant shade improvement was seen with RI till 2 months ( $p = 0.003$ ) after which, shade stabilized with non-significant changes till 6 months ( $p = 0.374$ ). Trend was same for RI and FV but at all time intervals  $\Delta E$  for FV was lesser than RI group. ACP-CP also exhibited significant shade improvement till 2 months ( $p = 0.008$ ) but beyond that decrease in  $\Delta E$  values was seen till the end of study ( $p = 0.695$ ) while FD exhibited non-significant differences in shade throughout study period. Conclusively RI showed both better immediate results and better shade stability over the period of 6 months.

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### In vivo Comparison of Three Materials Used for Indirect Pulp Treatment in Primary Molars

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Indirect pulp treatment (IPT) has proved to be an effective method for managing deep caries lesions in asymptomatic primary teeth to preserve the pulp vitality. The aim of this randomized clinical study was to compare the clinical and microbiological outcomes on the dentin-pulp complex of primary molars treated with two-step indirect pulp treatment (IPT) using three materials: Mineral Trioxide Agent (MTA), medical Portland cement (PC), and calcium hydroxide (CH). In 38 paediatric patients ( $6.5 \pm 1.9$ ), 38 deep carious lesions without radiographic or clinical signs or symptoms of pulp degeneration were treated with IPT using three pulp capping materials (MTA = 11, PC = 13, CH = 14). Caries was removed completely from the cavity-lateral-walls and dentino-enamel junction, while the necrotic dentin surrounding the pulp was left and then covered with one of the three materials. Carious dentin from the cavity floor was assessed clinically (color, humidity, and consistency) and a tissue sample was collected for microbiological analysis (*Lactobacillus/Streptococcus mutans*) immediately after cavity excavation and 6 months after the IPT and cavity sealing. After  $6 \pm 0.9$  months 28 teeth were re-assessed (MTA = 9, PC = 9, CH = 10). IPT had a high success rate [MTA: 88.9% (8), PC: 88.9% (8), CH: 90.0% (9),  $p = 0.99$ ]. After IPT, carious dentin was significantly harder ( $p = 0.04$ ), drier ( $p = 0.03$ ) and darker ( $p = 0.045$ ) with lower bacterial counts which did not reach the level of statistical significance ( $p = 0.35$  and  $p = 0.47$ , respectively). The findings of this study support the use of one-step IPT in deep carious lesions in primary molars to stimulate pulpal healing. Arrest of dentinal caries lesions was observed irrespectively of the material used. These findings encourage the use of non-resorbing capping materials such as PC or MTA.

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### Non-Dentists' Ability to Detect Enamel and Dentinal Caries after Brief Training: A Pilot Study

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**Aim:** To investigate the reliability and accuracy of student dental care professionals' (DCPs) detection of caries from images using the International Caries Detection and Assessment System (ICDAS 0–6). **Method:** Fourteen participants had 1 hour face-to-face training using projected images after 90 min of self-directed learning (ICDAS e-learning). Each independently scored 40 electronic images with decay at various levels. **Results:** The prevalence of caries at  $D_1$  (ICDAS 1–6) and  $D_3$  (ICDAS 4–6) thresholds in the 40 images was 85% and 37.5% respectively. Participants showed excellent sensitivity (1) at  $D_1$ , but poor specificity (0.33). At  $D_3$  participants showed good sensitivity (0.87) and specificity (0.95). The level of agreement between participants was high at both thresholds ( $\kappa = 0.98$  at  $D_1$  and  $0.92$  at  $D_3$ ). **Discussion:** The sensitivity, specificity and reliability varied depending on the diagnostic threshold. When enamel caries was included, the specificity of the participants reduced, largely due to the increased number of false positives. Ability to discriminate between code 0 (sound) and code 3 (cavitated enamel caries) was poorest. This may partly be explained by the limitations of assessing a two-dimensional image and having no tactile information. **Conclusions:** This brief training resulted in high levels of sensitivity and specificity at  $D_3$  threshold on electronic images, but not when enamel lesions were included ( $D_1$  level). In this small sample, it was feasible however to use brief training to achieve high levels of reliability at both thresholds. DCPs are not currently permitted to diagnose caries in the UK but these findings suggest further exploration of their role as epidemiological survey examiners may be warranted following further research into the levels of training required to achieve accuracy in enamel caries detection in vivo.

A very small amount was required to fund the DCPs participating for their time. This was provided from Prof. Douglas's endowment fund.

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### Caries Incidence in Older Patients Receiving Atraumatic Restorative Treatment

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**Background:** The ageing of the population together with a decrease in the prevalence of edentulism is changing the way dental treatment is provided to older age groups. The elderly present now

with a high caries prevalence, especially root caries. Little is known about the effect of a minimally invasive oral healthcare regime in the control of caries among senior citizens. **Objectives:** This study aimed to assess the caries incidence in a population of community-dwelling older adults receiving a minimally invasive oral healthcare regime. **Methods:** In a randomised clinical trial, patients over 65 years of age were recruited and randomly assigned to receive minimally invasive dental treatment ( $n = 99$ ). In order to detect a difference of 10% in restoration survival between groups, regarded as clinically significant, with a 5% significance level and a power of 80%, 129 restorations per group were necessary, allowing for a 30% dropout rate. The treatment protocol included standardised oral hygiene instructions, scaling and polishing and restoration of caries lesions with either atraumatic restorative treatment (ART) or a conventional restorative technique. After 1 year, they were examined and the restorations were assessed using the ART criteria. **Results:** 300 restorations were placed in 99 patients and after 1 year, 90 patients (270 restorations) were examined. Among these, 82% were caries-free. The root caries increment was 0.17 (SD: 0.48) and 0.09 (SD: 0.36) surface/year in the ART and conventional groups, respectively. The root+coronal caries increment in the ART and conventional groups were 0.26 and 0.27 surface/year, respectively (SD 0.57 and 0.75). The great majority (79.2%) were primary caries lesions and only 1 secondary caries lesion developed around a restoration placed in the study. **Conclusion:** A minimally invasive oral healthcare regime may have a positive impact on older patients' oral health, reducing the caries incidence in this age group.

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### Restorative Treatment Decision in Posterior Teeth: A Systematic Review

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The reduction on caries incidence and rate of progression lead to a shift in treatment philosophies towards a more conservative approach rather than restorative treatment. However, a wide disparity of diagnoses and treatment plans still has been observed. The aim of the study was to review the literature about the stage chosen by the dentist to perform a restoration in permanent teeth. A search of the MEDLINE-PubMed, EMBASE and Cochrane Library was conducted from January 1980 to January 2012. The main search terms used were: decision-making, restorative treatment, dental caries lesion, occlusal surface and approximal surface. The eligible studies should include graduate dentists only. Three reviewers independently screened title and abstracts for eligible papers. After that, full-text papers were read and included in the data extraction process. The studies were analyzed according to their methodological quality. The review encompassed 14 studies. The quality of the studies was considered moderate. Most dentists

would restore lesions confined to enamel and reaching dentine outer half irrespective the surface studied. On the occlusal surface, the percentage of dentists that would restore enamel lesion varied between 50 and 92%. In two studies, dark fissures would be restored according to 21.8–51% of the dentists. Regarding dentine lesions, 50.2–99.4% of the dentists would indicate operative treatment. In the approximal surface, the choice for operative treatment of enamel lesions varied between 42 and 81%. In dentine lesions, 42–96.9% of the dentists would restore lesions in the outer 1/3 of dentin and 52–99.7% would restore in the inner half of dentin. In conclusion, despite all the progress achieved in the understanding of the development and management of dental caries, dentists still recommend the restorative treatment in its early stages.

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### Long-Term Comparative Study on the Efficacy of One Self-Adhesive Composite in Dental Hypersensitivity

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The aim was to investigate, in a comparative clinical trial, the 12-week effectiveness of Vertise Flow™ (VF), self-adhering resin composite as desensitizing agent. A total of 116 teeth from 46 patients suffering from DH were included in the study. VF was compared to: (1) Universal Dentine Sealant (UDS), a desensitize sealant, (2) Clearfil Protect Bond (CPB), a self-etching adhesive, and (3) Flor-Opal® Varnish (FOV), a fluoride varnish. The pain reduction was evaluated in hypersensitive teeth according to the visual analogue scale (VAS), using a split-mouth randomized clinical trial design: before the application of the resin materials (PRE-1), immediately after (POST-1) and 12 week controls (POST-2). Clinical data was analyzed using one-way ANOVA. At PRE-1 VF mean was  $3.75 \pm 0.30$  (95% CI 3.14–4.36), median = 4, no statistically significant differences between the groups were observed. At POST-1 VF mean was  $0.85 \pm 0.17$  (95% CI 0.49–1.21), median = 1, and statistically significant differences ( $p = 0.02$ ) were recorded among VF and the other materials. At POST-2 VF mean was  $2.57 \pm 0.21$  (95% CI 2.13–3.01), median = 2, without statistical significance. The teeth treated with VF had the highest decrease in VAS scores at POST-1 (77.33%), however the biggest increase in scores was recorded in this group after the 12 weeks of oral exposure, with effectiveness of the material reduced by the 31.46%. The 12 weeks of oral environmental fluids affected significantly the sealing efficiency of VF in dental hypersensitivity probably due to water degradation of the composite with weakness of the bond in dentin.

### Caries Risk Assessment in Patients with Gastro-Oesophageal Reflux Disease

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The purpose of present study was to investigate the effect of gastro-oesophageal reflux disease (GERD) on caries risk. A total of 90 adult subjects were examined. The experimental study group represented 45 patients with GERD (mean age  $46.0 \pm 11.6$ ), diagnosed by 24-hour pH monitoring test as the gold standard for the diagnosis of GERD. The experimental group was compared with the age and gender match group of 45 healthy control subjects (mean age  $40.8 \pm 13.5$ ). Ethical approval and patients' written consent were obtained. Caries risk assessment was carried out by investigation of salivary risk factors: stimulated salivary flow rate, salivary pH and buffering capacity in subjects of both groups. Furthermore the influence of GERD on growth of salivary *Streptococcus mutans*, *Lactobacillus* and *Candida albicans* was investigated. Caries was assessed clinically according to ICDAS criteria. We observed significantly lower salivary pH value (t test,  $7.4 \pm 0.7$  vs.  $7.7 \pm 0.5$ ,  $p = 0.017$ ) and buffering capacity (t test,  $2.6 \pm 0.6$  vs.  $2.9 \pm 0.3$ ,  $p = 0.006$ ) and significantly higher stimulated salivary flow rate (t test,  $3.3 \pm 4.4$  vs.  $1.4 \pm 0.6$ ,  $p = 0.015$ ) in GERD patients compared to healthy controls. Patients with GERD also showed higher CFU/ml count of *Lactobacillus* (t test,  $1.3 \pm 1.1$  vs.  $0.6 \pm 0.8$ ,  $p = 0.001$ ) and *Candida albicans* (t test,  $0.4 \pm 0.7$  vs.  $0.1 \pm 0.3$ ,  $p = 0.009$ ). In contrast there was no significant difference in *Streptococcus mutans* CFU/ml count between groups. Among patient with GERD number of long reflux episodes ( $> 5$  min) correlated with *Lactobacillus* CFU/ml (Pearson correlation,  $R = 0.325$ ,  $p = 0.031$ ), *Candida albicans* CFU/ml (Pearson correlation,  $R = 0.346$ ,  $p = 0.021$ ) and number of filled teeth (Pearson correlation,  $R = -0.295$ ,  $p = 0.049$ ). We concluded that GERD may influence caries risk.

### Consistency of Toothbrushing Habits: A Pilot Video Analysis Study

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Video analyses from single observations before and after interventions have been used e.g. for comparing the efficacy of brushing techniques. However, as little is known about the consistency of toothbrushing habits, it is not clear how reliable single observations are. Besides methodological aspects, consistency could be important for oral hygiene instruction strategies as it might indicate the degree of rootedness of movement patterns. The descriptive pilot study aims to determine the consistency of toothbrushing habits at consecutive observations. Habitual toothbrushing of 12 uninstruced participants was filmed thrice at two-week intervals. Vid-

eos were analysed with INTERACT. Parameters of interest were 'brushing duration', 'brushing events' (= movements between sextants), and 'brushing sequence'. Basically, intra-individual consistency of brushing duration and events was described as maximum difference of three observations, which was additionally calculated as percentage of the mean of three observations. As a more exhaustive approach, brushing sequences occurring throughout the brushing time were compared. Agreement was determined by GSEQ 5.1 [Quera et al.: Behav Res Methods 2007;39:39-49]; the parameter was 'event based kappa' (EBK). Mean (Min; Max) inter-individual brushing duration was 136 s (61; 230). The intra-individual maximum difference was 42 s (3; 109) which was 31% (4%; 94%) of the brushing duration. Respective values for brushing events were 41 (19; 69), 16 (3; 34) and 39% (12%; 103%). Mean EBK was 0.44 (0.32; 0.63) with 0.32-0.35 for the least agreeing and 0.51-0.63 for the best agreeing 25% of participants. Intra-individually, variability was lower than inter-individually. Some participants showed considerably varying habits, but at least 25% of the participants reproduced their brushing sequence consistently. The data provide a first basis for reliability estimations of single observations and open perspectives for instruction strategies. Intra-individually, variability of toothbrushing habits was lower than inter-individually; some participants showed considerable variations, but 25% of the participants reproduced their brushing sequence consistently.

The study was funded by the German Society for Dental, Oral and Maxillofacial Surgery (DGZMK) and Justus-Liebig University of Giessen, Germany.

### Criteria for Replacing Dental Restorations in the Discipline of Clinical Integrated FOUERJ, Rio de Janeiro, Brazil

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**Aim:** The repetitive cycle of restoration has been maintained for decades because the quality of restoration depends on subjective criteria adopted by each professional. Furthermore recurrent caries is the main reason for replacement of restorations in traditional restorative dentistry. The aim of this study was to evaluate the criteria for replacement of dental restorations in a group of patients treated for 5, 10 and 20 years in the disciplines of Integrated Clinic, the School of Dentistry of FOUERJ, Rio de Janeiro. **Material and Methods:** 44 patients (age 25-56) were longitudinally evaluated during 20 years. Initially 111 restorations were made in the early 90s. 39 were made by amalgam, 68 by resin, 4 with other materials. Recurrent caries were diagnosed by X-rays and visual inspection. All teeth that needed restorations were included in this study; teeth that where extracted were excluded. After 5 years, 21 new restorations were made; 91 restorations were replaced due to recurrent caries, 64 were composite resin. The average (total) restorations

was  $2.07 \pm 1.78$  (SD) per patient. After 10 years, there was a reduction of 29% amalgam fillings and an increase of 75% resin and other materials. An average of  $1.86 \pm 1.80$  restorations per patient was found. 78 (57%) restorations were made due to recurrent caries. 41 restorations were not replaced. After 20 years there has been a replacement of 71 restorations (70 due to recurrent caries and 1 fracture), 62 remained acceptable, 28 restorations were restored with amalgam, 29 photo polymerizable resins; 8 with cast metal restorations and 4 teeth were extracted. An average of  $2.8 \pm 1.80$  restorations per patient were found. Recurrent caries were most often the reason for replacement of the fillings ( $p < 0.05$ , chi-square). Amalgam fillings were less frequently replaced than resin fillings ( $p < 0.05$ , chi-square). The criteria of renewal of dental restorations were mainly secondary caries. Restorations made by photo polymerizable resin showed more recurrent caries during their lifetime than amalgam. Since the initial treatment until the 20-year follow-up, there was a repetitive restoration cycle showing the need for a re-orientation to replace disease focus to oral health promotion both in teachers and dental students in UERJ.

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### Use of Conscious Sedation in the Management of Carious Lesions in Paediatric Dental Patients

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**Objective:** In paediatric dentistry, anxiety or behavioural impairments often challenge dental treatments. Sedation procedures can then be a helpful tool. The present study aimed to evaluate the success rate of dental procedures of a 50% nitrous oxide in oxygen premix for the management of dental caries and their complications on children scheduled for dental treatments under sedation in the Nice University hospital, in France. **Methods:** This one-year (2012) clinical study examined first-visit sedation performed in healthy children who were non-compliant with dental treatment. They were aged between 2 and 16 years, ASA I or II, had no mental or motor retardation. Parents gave their informed consent. The type of dental treatment performed was recorded. The session was considered successful if the planned dental treatment could be carried out whereas it was considered as a failure otherwise. Logistic regression analysis, conducted using SPSS 18.0 for Windows, using a stepwise selection of independent variables. The dependent variable was the success of sedation. **Results:** Among our 60 patients, 53.3% were male. The average age was  $6.0 \pm 3.3$  years and the mean sedation time was  $23.5 \pm 9.2$  min. The overall success rate was 85.0%. Only minor side effects were reported (6.7%) and they were more frequent for females (Fisher exact test  $p = 0.041$ ). In multivariate modelling, the association between gender and success disappeared and side effects were shown to negatively affect the success rate ( $p = 0.042$ ). **Conclusion:** Provided this technique is used by dentists skills in sedation, with the appropriate staff and equipment at their disposal, moderate sedation can be successfully used in the clinical management of paediatric dental patients and represents a reliable alternative to general anesthesia.

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### Caries Incidence and Progression in Young Adults: Baseline Clinical Data

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This is a prospective cohort study on oral health of young adults aged 18–30 years. The aims were: (1) to analyze caries incidence and progression at clinical as well as radiographic levels; (2) to identify predictors for caries incidence and progression in early adulthood. The subjects were 612 new patients seeking treatment at the University Hospital, Brussels, Belgium in 2010–2011. The participants answered a validated questionnaire about their socio-demographics, oral health related behavior and attitudes, and patient-reported outcomes. Caries experience was summarized in DMFT/S scores ( $D_{\geq 1}$  = decayed active and inactive at non-cavitated and cavitated levels;  $D_{\geq 3}$  = decayed active and inactive at cavitated dentine level; M = missing due to caries, F = filled, T = tooth, S = surface). Inter-examiner reliability of caries scores showed kappa of 0.86. Belgian citizens represented 80% and non-west European 10% of the sample (55.7% female and 44.3% male). Patients were residents in the three regions of Belgium: Brussels (57.4%), Wallonia (22.3%) and Flanders (20.3%). Poor oral health was perceived by 13.0% of patients. About half of the sample had experienced pain or discomfort due to caries whereas periodontal conditions were mentioned in 15.5% of cases only. A limited number of patients were diagnosed as caries-free at  $D_{\geq 3}$ MFS level (13%). Difference between means  $D_{\geq 1}$ MFT 7.05 ( $\pm 2.94$ ) and  $D_{\geq 3}$ MFT 5.51 ( $\pm 4.75$ ) was significant (t test,  $p < 0.0001$ ). The same trend was observed at surface level, 13.58 ( $\pm 14.14$ ) and 11.43 ( $\pm 12.85$ ), respectively ( $p < 0.0001$ ). Occlusal surfaces had significant higher  $D_{\geq 1}$ MFS mean scores of 5.19 ( $\pm 3.97$ ) compared to approximal surfaces of posterior teeth with a mean of 4.00 ( $\pm 5.25$ ) ( $p < 0.0001$ ). In conclusion, our baseline data indicate that the oral health of individuals deteriorates considerably in early adulthood, i.e. between the age of 18 and 30 years.

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### Caries Incidence and Progression in Young Adults: Baseline Radiographic Data

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The oral health status of populations changes from adolescence to early adulthood with a considerable reduction of the percentage of caries-free individuals. Studies examining caries incidence and

progression in young adults might contribute to a better understanding of this phenomenon and support treatment decisions in daily practice. For this purpose, we recruited 612 18–30-year-old new patients seeking treatment at the University Hospital in 2010–2011 in Brussels, Belgium. The patients answered a validated questionnaire about their socio-demographics, oral health-related behavior and attitudes, as well as patient-reported outcomes. Periodontal status of selected teeth and caries experience were assessed at the clinical examination. Standardized bitewing radiographs were taken from all new patients. The approximal surfaces were classified according to the scoring system: 0 = no visible radiolucency, 1 = radiolucency in the outer half of the enamel, 2 = radiolucency in the inner half of the enamel up to the enamel-dentin border, 3 = radiolucency with a broken enamel-dentin border but no obvious progression in the dentin, 4 = radiolucency in the outer half of dentin, 5 = radiolucency in the inner half of the dentin [Mèjare et al.: *Caries Res* 2004;38:130–141]. For occlusal surfaces scores 0, 3, 4 and 5 were applied. Restored surfaces were also recorded. Intra-examiner reliability of caries scores showed kappa of 0.91. Half of the patients were university students or students in other higher education. Brushing more than once a day and interdental cleaning were reported by 60 and 23% of the patients, respectively. The number of patients diagnosed as caries-free at score 3 or higher was limited to 19%. The total mean  $D_{\geq 1}MFS$  was 9.34 ( $\pm 9.15$ ) with decayed component of 3.46 ( $\pm 4.88$ ). The contribution of approximal and occlusal surfaces were significantly different, 4.99 ( $\pm 5.91$ ) and 4.34 ( $\pm 3.87$ ), respectively (t test,  $p < 0.0001$ ). The fact that caries lesions represented one third of mean caries experience of the studied population supports the view that dental disease is still a problem in early adulthood.

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### Approximal ART Restorations Using a Flowable Glass-Ionomer Cement as Liner

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**Aim:** Good survival rates for single-surface ART restorations have been reported while multi-surface ART restorations have not shown similar results. The high-viscosity consistency of the glass ionomer cement (GIC) may lead to its incorrect adaptation into the cavity and thus to restoration failure. The aim of this study was to evaluate the survival rate of approximal ART restorations using a flowable layer of GIC as liner [Bonifácio et al.: *J Dent Child (Chic)* 2010;7:12–16], comparing to conventional ART. **Methods:** A total of 207 primary molars with approximal caries lesions were selected in 6–7-year-old children in Barueri city, Brazil. The patients were randomly allocated to two groups, both restored with Fuji IX (GC Corp): G1 – conventional restoration and G2 – two layers technique. After caries removal with hand instruments, G1 was restored with conventional consistency and G2 was restored using two layers GIC technique (first layer with flowable consistency –

powder/liquid ratio 1:2 and second layer mixed conventionally). All restorations were evaluated after 1, 6, 12, 18 and 30 months according to Roeleveld et al. [*Eur Arch Paediatr Dent* 2006;7:85–91] criteria. Restoration survival was evaluated using Kaplan-Meier survival and logistic regression test was used for testing association with clinical factors ( $\alpha = 5$ ). **Results:** There was difference in success rate between techniques after 30 months, with better performance of two layers technique (OR = 2.29, IC = 1.14–4.60,  $p = 0.020$ ). The overall survival rate of restorations was 60% and the survival rate per group was G1 = 67% and G2 = 59%. No studied factors had an influence in the survival of restorations. The application of a flowable GIC layer in approximal cavities before the insertion of a regular GIC layer improved survival of approximal ART restoration.

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### Clinical Trial of an Antimicrobial Pit and Fissure Sealant

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**Aim:** The aim of this study was to evaluate pits/fissure sealant containing an organo-selenium for clinical retention, plaque and caries formation. **Methods:** 120 subjects, age 7–20 years, at either moderate or high caries risk status, received SeLECT Defense sealant™ (SD, a selenium-containing sealant) on either left or right side of the dentition and UltraSeal XT Plus™ (UXT) on the corresponding tooth on the opposite side. Sealants were evaluated every 3 months for 12 months for clinical retention, plaque and caries formation around sealant. Lost sealants were re-applied but sealant was considered as failure in future analysis. Differences in evaluated factors between UXT and SD at each assessment period were analyzed statistically by McNemar's test. Logistic regression was used to test any association between evaluated factors and other variables. Retention was significantly ( $p = 0.0004$ ) higher in SD (96%) than UXT (81%) after 12 months. No significant difference in retention between the two sealants at 3, 6 or 9 months. SD exhibited 100% prevention of plaque growth around sealant, while plaque growth was observed around 7 and 12% of UXT at 9th and 12th months respectively. No caries formation was observed around either sealant. **Conclusion:** SeLECT Defense™ sealant completely prevented plaque growth around sealant with better clinical retention than UltraSeal XT Plus, which offered only limited protection against plaque accumulation.

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## Practice-Based Randomized Clinical Trial on the Efficacy of Proximal Caries Infiltration in High-Risk Individuals

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Proximal caries infiltration arrests 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 39 young adults, 148 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) after 8–12 months for those individuals having six or more E2/D1 proximal lesions. After approximately 10 months [mean (SD): 309 (56) days], 96 lesion pairs (65%) in 27 patients [age and DMFT at baseline: 23.7 (5.5) and 8.5 (5.6), respectively] of three dentists could be re-evaluated radiographically using standardized bitewing holders. No unwanted effects could be observed. Radiographic progression was recorded in 3/96 test lesions (3%) and 23/96 control lesions (22%) ( $p < 0.001$ ; McNemar test); 0/96 test and 9/96 control lesions progressed from one stage to a deeper one. Thus resin infiltration performed in private practice was more efficacious in reducing lesion progression compared with self-applied non-invasive measures alone over a period of 10 months.

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

## Caries Management: Report from a Japanese Private Dental Clinic

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The aim of this retrospective study is to assess whether the effect of preventive treatments for youth (7–12 years old) is related to frequency of visits to a Japanese private dental clinic. The subjects were selected from the patient database of Sugiyama Dental

Clinic (SDC) on September 30, 2012 ( $n = 13,251$ ), based on two criteria; the preventive treatment of 11–13-year-olds recorded between October 2005 and September 2012 and a 5–6-year treatment period. SDC is a private general dental office in Yachiyo City, a suburban area about an hour from central Tokyo. Water is not fluoridated, and the average 12-year-old DMFT is 0.8 (Yachiyo 2012). 97 subjects were selected, 60 boys and 37 girls, average age 6.8 (SD 0.70) years with DMFT 0.1 (SD 0.33) at the first visit. The average treatment period was 5.1 (SD 0.22) years and the average number of visits 9.5 (SD 4.79). The subjects were grouped into two groups; subjects in group A visited our clinic at least 10 times for preventive treatment, and those in group B fewer than 10 times. In group A ( $n = 50$ ), average increase of fillings were 0.3 surfaces, the proportion of people with at least one new filling was 24%, while in group B ( $n = 47$ ) 1.2 surfaces and 51% ( $p = 0.007$ ). In group A, 56% of fillings resulted from molar-incisor hypomineralisation and other anatomical malformations which obstruct plaque control, while in group B 8%. These results show that consistent caries management (more than 10 maintenance visits in 5-year period from 7–12 years old) leads to reduction of additional fillings on tooth surfaces, which in turn results in a higher rate of fillings due to other factors than caries.

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

## The Results of Caries Infiltration in Pediatric Patients: A Case Series

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**Background:** Treatment of incipient carious lesions has traditionally needed several dental appointments for remineralization but new micro-invasive caries infiltration technology offers a one-appointment plan. **Aim:** To study the results of infiltration technology for incipient caries treatment in pediatric patients. **Methods:** The study, approved by the regional ethics committee, was conducted in Volgograd Pediatric Dental Clinic No. 2 in 2010–2012. 46 children aged 8–17 years were involved in the investigation, the informed consents from parents of 8–14-year-old children and from 15–17-year-olds were obtained. Caries infiltration technology was performed with ICON (DMG, Germany). Treatment was applied in 326 permanent teeth (149 incisors, 83 canines, 59 premolars, and 35 molars). Caries infiltration, according to the manufacturer's instructions, was conducted in 354 incipient carious lesions (ICDAS score 1, 2; radiographic score E1, E2, D1). 286 lesions were localized on vestibular surfaces, 68 lesions were on proximal surfaces. 263 (80.7%) teeth with 287 (81.1%) lesions in 37 (80.4%) children were re-examined after the treatment. The mean follow-up period was 18.3 months (range 9–30 months). The results of caries infiltration were assessed according to the criterion 'presence or absence of caries progression' (mean, 95% confidence interval, CI, were calculated) and by the patients' satisfaction with

the treatment (5-point visual analogue scale, VAS). **Results:** Caries progression was revealed in 4 (1.4%, CI 0.04–2.76) lesions, 283 (98.6%, CI 97.24–99.96) lesions were without the signs of progression. The patients' satisfaction with the treatment results, according to VAS, was 4.7 (CI 4.5–4.9) points for one-appointment treatment, 4.6 (CI 4.4–4.8) points for esthetic improvement, 4.4 (CI 4.2–4.6) points for painless and convenient treatment. **Conclusion:** Caries infiltration technology had high clinical effectiveness and was positively estimated by pediatric patients and their parents.

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### Risk Factors of Early Childhood Caries

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**Aim:** Early childhood caries (ECC) is still a major dental health problem. It is known that ECC is caused by both social-behavioral and microbiological factors. With this cross-sectional cohort study, it can be shown that the known causal factors for ECC cannot fully explain its prevalence. The aim is to show that siblings of children diagnosed with severe ECC have another caries status while having comparable social and behavioral factors. **Materials and Methods:** In three paediatric dental practices in the Netherlands parents (n = 104) of child patients with severe ECC were asked to fill in a questionnaire on risk factors for ECC in their family. Parent filled in details on caries level, bottle use, breastfeeding at will, problems brushing, eating, and medication use of all of their children. To check the level of caries, 20% of the siblings were seen by a trained dental professional. The caries level of the patients was assessed based on the data of the dental care clinics. **Results:** Of all the families (n = 104), it was found that in 93% of the cases (n = 97), the siblings, both older and younger, had less caries than the patient. The additional check at 20 siblings showed that parents are well able to determine the caries level of their children (Cohen's kappa = 0.84). Of the 104 families in this study, comparable risk factors in sibling and patient were found in 45 cases. In all these cases, a significantly different (lower) degree of caries was found (p < 0.05). **Conclusion:** The caries paradigm needs a different approach when studying ECC. Known risk factors for ECC do not fully explain the difference in caries prevalence in one family.

Funding was provided by the paediatric postgraduate training program.

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### Treatment of Deep Carious Second Deciduous Molars: A Prospective Randomized Clinical Trial

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**Aim:** To compare the clinical and radiographic success of indirect pulp treatment and MTA pulpotomy of second primary molars with deep carious lesions. **Materials and Methods:** The study concerns 230 teeth in 119 children (3–7 years old, mean = 4) referred for treatment in general anaesthesia. The study was approved by the Ethics Committee of the VU Medical Centre. Parents signed an informed consent form. Radiographically, the lesion depth was between 1/2 and 3/4 of the dentin thickness and there were no clinical signs of pulpal inflammation. The teeth were randomly assigned to two groups. In the first group, teeth were restored with stainless steel crowns after minimal occlusal, mesial and distal preparation without any carious excavation (IPT). In the second, complete carious excavation resulting in pulp exposure was followed by MTA pulpotomy and placement of a stainless steel crown (MTA). The recall period was on average 25 months (0–39 months). Two independent experienced paediatric dentists scored the radiographs in mutual agreement. Success was defined as the absence of radiographic abnormalities or clinical symptoms indicating pulp inflammation. **Results:** Eventually, a clinical and radiographic follow-up was possible for 80 patients, resulting in 39 teeth treated with IPT and 41 with pulpotomy being available for statistical analysis. There was no statistical difference in the success rate between the IPT (74%) and the MTA (83%) group ( $\chi^2$ : p = 0.3). Preoperative pain reported by parents was found to have no influence in the success rate of the treatments ( $\chi^2$ : p = 0.7). Furthermore, the presence of hypomineralized deciduous molars did not affect the success rate of the teeth in either group ( $\chi^2$ : p = 0.3). **Conclusion:** No differences between treatment pathways were found. Results support the principle of non-invasive restorative dentistry.

Research was founded by the ACTA paediatric postgraduate training programme.



## Session 6

### Erosion

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#### Prevention of Dentin Erosion by Brushing with Anti-Erosive Toothpastes

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This *in vitro* study aimed to investigate the preventive effect of brushing with so-called anti-erosive toothpastes compared to a conventional fluoride toothpaste on dentin erosion. Bovine dentin specimens were eroded in an artificial mouth (6 days, 6 × 30 s/day, flow rate: 2 ml/min) using either citric acid (pH: 2.5) or hydrochloric acid/pepsin solution (pH: 1.6), simulating extrinsic or intrinsic erosive conditions, respectively. Between the erosive attacks, the specimens were rinsed with artificial saliva at 0.5 ml/min flow. Each day, 1 h before the first and 1 h after the last erosive attack, the specimens were brushed for 15 s in an automatic brushing machine at 2.5 N with a conventional fluoride toothpaste slurry (elmex [AmF]) or toothpaste slurries with anti-erosive formulations: Apacare (NaF/1% nHAP), Biorepair (ZnCO<sub>3</sub>-HAP), Chitodent (chitosan), elmex Erosionprotection (NaF/AmF/SnCl<sub>2</sub>/chitosan), mirasensitive hap (NaF/30% HAP), Sensodyne Proenamel (NaF/KNO<sub>3</sub>). Unbrushed specimens served as control. All subgroups contained 12 specimens. Dentin loss was measured profilometrically and statistically analysed using two-way and one-way ANOVA followed by Scheffé's post-hoc tests ( $p < 0.05$ ). Dentin erosion of unbrushed specimens amounted to  $5.1 \pm 1.0 \mu\text{m}$  (extrinsic conditions) or  $12.9 \pm 1.4 \mu\text{m}$  (intrinsic conditions). Under simulated extrinsic erosive conditions, all toothpastes significantly reduced dentin erosion by 24–67%. Biorepair was least effective, while all other toothpastes were not significantly different. Dentin erosion under simulated intrinsic conditions was significantly reduced by 21–40% by all toothpastes except Biorepair. Toothpastes with anti-erosive formulations reduced dentin erosion, especially under simulated extrinsic erosive conditions, but were not superior to a conventional fluoride toothpaste.

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#### Quantitative Evaluation of Abrasion Thickness of Eroded Dentine by Sonic Toothbrush with or without Toothpaste

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This study was carried out to evaluate the eroded dentine-abrading action of a sonic toothbrush by measuring abrasion thickness using an Electron Probe Micro-Analyzer (EPMA). Polished dentine slabs each with an exposed window (4 mm<sup>2</sup>) made using nail varnish were immersed into (A) grapefruit juice (pH 3.3), (B) wine vinegar (pH 2.7) or (C) 0.83 M acetic acid for 60, 120 or 180 min, respectively. The slabs were fixed and the tips of the bristles were placed on the eroded window perpendicular to the window floor, applying a pressure of 1 g per mm<sup>2</sup>. The slabs were then brushed for 1 min using a sonic toothbrush (480 Hz) with either slurry containing non-fluoridated toothpaste and water in a ratio of 1:4 or water. The slabs were cleaned ultrasonically, dipped into acetone to remove the nail varnish and air-dried. Subsequently they were sputter-coated with platinum to examine using secondary-electron imaging. Then they were embedded in methacrylate and cross-sectioned in the middle of the windows. Their surfaces were polished, coated with carbon and examined using back-scattered electron imaging (COMPO). The area of dentine wear within the window was measured in the COMPO image by image analyzing which calculated abrasion thickness. Analysis using ANOVA indicated that abrasion thicknesses were affected by acid solution and working time with a significant interaction between these factors. However, there was an insignificant affect by slurry. The depth of wear ( $\mu\text{m}$ , mean  $\pm$  SD) after 60, 120 and 180 min treatment were  $21.1 \pm 5.1$ ,  $38.2 \pm 6.8$  and  $47.5 \pm 8.1$  in group A,  $43.8 \pm 10.7$ ,  $64.2 \pm 12.8$  and  $73.9 \pm 16.6$  in group B, and  $42.1 \pm 11.7$ ,  $60.0 \pm 12.7$  and  $93.9 \pm 21.0$  in group C, respectively. The results suggested that the influence of toothpaste on eroded dentine wear was limited.

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### Effect of Er:YAG Laser Irradiation in the Erosion Control on Enamel: Analysis by Profilometry

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This study aimed to evaluate in situ the effect of Er:YAG laser irradiation to control the progression of enamel erosive lesions. Bovine incisors were sectioned (5 × 3 × 2.5 mm), flattened and polished. A total of 56 enamel slabs (KHN = 330 ± 10%) had 1/4 of the surface covered with resin composite (control area), and were submitted to initial erosive lesion formation with 1% citric acid (pH = 2.3), 5 min, 2×/day, for 2 days. The slabs were divided in two groups according to surface treatment: irradiated with Er:YAG laser and non-irradiated. After a 2-day lead-in period, 14 volunteers used an intraoral palatal appliance containing two slabs, in 2 phases of 5 days each per group (irradiated and non-irradiated). During the intraoral phase, in a crossed-over design, half of the volunteers immersed the appliance in citric acid (erosive challenge) while other half of the volunteers used deionized water (non-erosive challenge), both treatments for 5 min, 3×/day. Enamel wear was determined by an optical 3D profilometer after initial erosive lesion formation, after surface treatment and after intraoral phase. The wear assessment was verified by means of three readings of each area and also the controlled area (untreated). ANOVA revealed a significant difference between groups ( $p < 0.05$ ). Groups that used deionized water (non-eroded) during intraoral phase presented lower values of wear when compared with the groups that were eroded in intraoral phase, independent of being irradiated (14.14 ± 4.13) or non-irradiated with Er:YAG laser (14.67 ± 4.35). However, the group irradiated with Er:YAG laser followed by intraoral erosion (33.77 ± 14.84) did not differ from group non-irradiated followed by intraoral erosion (36.5 ± 11.43). It can be concluded that Er:YAG laser irradiation did not reduce the progression of erosive lesions on enamel submitted to in situ erosion by citric acid.

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### A Modified in vivo Experimental Model for Evaluating Fluoride Treatment against Initial Erosion Lesions

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The aim of this study was to evaluate a modified version of an experimental in vivo model for initial dental erosion, by testing the protective effect of treating enamel with acidic fluoride solutions. The study was approved by the national committee for research ethics. Maxillary impressions of 8 test persons were used to make hard plastic guides with holes punched out on the labial surfaces of anteriors. Test areas on teeth were isolated with the guide and impression material, holes being repunched through the impression material (mucosal punch, Ø = 4 mm). Test areas with intact pellicle were exposed to 0.18% citric acid (5 ml, 6 ml/min). Acid was collected in coded test tubes (etch I) and the guide was removed. Newly isolated, 2 h later the test areas were exposed to native fluoride solutions for 1 min: 4% SnF<sub>2</sub>, 8% SnF<sub>2</sub>, 0.2% HF, 0.5% NaF, 0.5% APF and H<sub>2</sub>O (negative control). Acid challenges were repeated using the same method under isolation after another 2 h (etch II) and 24 h (etch III). Test areas were rinsed for 5 s with water after all etches/treatments. [Ca] in etches I–III was measured by atom absorption spectroscopy. %Ca reductions after 2 and 24 h were calculated for each tooth and results compared with control (Friedman/Tukey). Median %Ca reductions (2 h/24 h): 4% SnF<sub>2</sub> 71/24, 8% SnF<sub>2</sub> 38/22, HF 85/78, NaF 58/49, APF 58/30 and control 23/24. All test solutions exhibited a positive effect in reducing enamel dissolution, but compared to the control this effect was only significant for HF and 4% SnF<sub>2</sub> after 2 h and for HF after 24 h. The experimental model simulated in vivo conditions, and in this pilot study was able to discriminate between the short-term erosion-inhibiting effects of different acidic solutions.

### Influence of Frequency, Time Interval between Acid Exposures and Saliva on Enamel Erosion

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The aim of this in vitro study was to assess the influence of frequency and the time intervals between acidic exposure (orange juice) on enamel erosion in vitro and to investigate if human saliva protects against enamel erosion. For 15 days, 209 enamel samples were exposed to different exposure regimes to orange juice. During an acid exposure the enamel samples were put in a container with 200 ml orange juice for 4 min. Half of the enamel samples were stored in human stimulated saliva. The other half in water. Both were divided in groups with time intervals of 30, 60 and 120 min

and frequencies of exposure to orange juice varying between 2 and 10 times during one day. During the night and weekends the enamel samples were stored in their own medium, saliva or water. Twice a day saliva was changed. The amount of enamel tissue loss was assessed with a non-contact profilometer. The interexaminer correlation was 0.988. Kruskal-Wallis test was used to analyze the data. Increasing frequency of exposures to orange juice led to increasing enamel loss ( $p < 0.001$ ) for both saliva and water. For frequency 2 was 1.5  $\mu\text{m}$  enamel loss found, this increased to 39.5  $\mu\text{m}$  for frequency 10 (saliva group, time interval 30 min). The storage medium, water or saliva, showed no significant difference in enamel loss. There was no difference found for saliva pretreated enamel samples in tissue loss for different time intervals. Unexpectedly, in water stored enamel samples showed an increase in enamel tissue loss at time intervals 60 and 120 (for example, frequency 2, 8.0  $\mu\text{m}$  and 6.9  $\mu\text{m}$ , respectively) compared to 30 min (3.9  $\mu\text{m}$ ) ( $p = 0.003$ ). Further research into this phenomenon is recommended.

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### Buffering, Undissociated Acid Concentration and Dissolution Rate of Enamel

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To obtain a quantitative description of the effects of buffering properties on erosive potential, the dissolution rate ( $V$ :  $\mu\text{mol}$  hydroxyapatite/ $\text{s}/\text{m}^2$ ) of enamel in solutions of citric, malic and lactic acids, over a pH range (2.4–3.6) typical of soft drinks, was measured using a pH-stat system. Acid concentrations were adjusted to give buffer capacities ( $\beta$ ) of 2–40 mM/pH for each pH. The corresponding undissociated acid concentrations ( $[\text{HA}]$ ) and titratable acidities to pH 5.5 ( $\text{TA}_{5.5}$ ) were calculated.  $V$  increased with  $\beta$ ,  $\text{TA}_{5.5}$  and  $[\text{HA}]$ . The response of  $V$  to  $\beta$  varied with acid type (lactic > malic  $\geq$  citric) and decreased with increasing pH. For example, at pH 2.4  $V$  (lactic) increased from  $73 \pm 54$  (SD) at  $\beta = 10$  to  $366 \pm 72$  at  $\beta = 40$ , while at pH 3.6 the increase was from  $29 \pm 8$  to  $87 \pm 29$ : corresponding data for  $V$  (malic) were from  $86 \pm 6$  to  $216 \pm 15$  (pH 2.4) and from  $27 \pm 4$  to  $36 \pm 3$  (pH 3.6). The response to  $\text{TA}_{5.5}$  varied similarly with acid type but pH > 2.8 had less effect than with  $\beta$  on the response in citric and malic acids, and no effect in lactic acid. The response of  $V$  to  $[\text{HA}]$  showed no systematic dependence on acid type but a small dependence on pH. It appears that  $[\text{HA}]$  is a major rate-controlling factor, probably because undissociated acids are a diffusible source of  $\text{H}^+$  ions in maintaining near-surface dissolution within enamel. However, pH remains a factor independent of  $[\text{HA}]$ .  $\text{TA}_{5.5}$  has a more direct relationship to  $[\text{HA}]$  than  $\beta$  and seems preferable as a practical measure of buffering. The relationship between  $[\text{HA}]$  and  $\text{TA}_{5.5}$  differs between mono- and poly-basic acids, so separate analysis of products according to predominant acid type could improve multivariate models of erosive potential.

This study was funded by the University of Bern.

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### Evaluation of Enamel Erosion and Abrasion in situ

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Eroded enamel is highly susceptible to removal by intra-oral mechanical forces. This study investigated whether the tongue can abrade eroded enamel. Twelve upper removable appliances were constructed with four human enamel specimens, two placed anteriorly and two posteriorly. Both sides of each specimen were exposed to the oral environment whilst retained in the appliance, with an oral cavity facing side and a palatal mucosa facing side. However, one surface facing the palate was subjected to erosion only while the other was eroded and allowed contact from the tongue. Each specimen thus acted as its own control. Appliances were worn from 9:30 to 17:00 h, Monday to Friday for 22 days. Erosion was created by 5 min exposure to 50 ml orange juice 3 $\times$ /daily. Enamel loss was measured using quantitative light-induced fluorescence (QLF) and non-contact light profilometry (NCLP). QLF showed greater reduction in mean fluorescence loss ( $\%\Delta F$ ) from the two anterior specimens than from those placed posteriorly with mean values of 31.0% ( $\pm 6.1$ ) and 30.2% ( $\pm 6.4$ ), 35.0% ( $\pm 5.7$ ) and 37.9% ( $\pm 5.6$ ) respectively. Similarly, NCLP results showed that anterior specimens had greater mean step height (MSH) on their tongue-facing than palatal-facing (control) surfaces. The MSH values ranged from 323.0  $\mu\text{m}$  ( $\pm 144.5$ ) and 305.8  $\mu\text{m}$  ( $\pm 94.3$ ), 266.6  $\mu\text{m}$  ( $\pm 148.1$ ) and 223.3  $\mu\text{m}$  ( $\pm 100.1$ ), respectively. All differences between anterior and posterior specimens and paired surfaces were statistically significant at (t test;  $p < 0.05$ ). Tooth wear is a multi-factorial phenomenon and this study confirms the synergistic effect between erosion and abrasion. The greater enamel surface loss indicated that eroded enamel surfaces were readily abraded by the tongue.

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### Kinetics of Salivary pH Immediately after and following Chewing of Sour Gummy Candies

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Sour candies have been gaining increased market share among children and teenagers. The erosive potential of such confectioneries on teeth have been demonstrated in two laboratory studies [Davies et al.: Br Dent J 2008;204:E3; Wagoner et al.: J Am Dent Assoc 2009;140:906–913]. However, to date, no investigations have evaluated the erosive potential of sour candies in vivo. This clinical trial was undertaken to monitor the kinetics of salivary pH imme-

diately after and following chewing of sour gummy candies. After approval by the local ethics committee, 20 participants were enrolled and had their unstimulated and stimulated whole saliva assessed for flow, pH and buffer capacity. Following a two-arm crossover layout, participants chewed two pieces of a sour and two pieces of an ordinary (as control) gummy candy for 20 s. Participants spat saliva-candy mixture at 18 time points: immediately after chewing ( $t_0$ ) and then after every 15 s intervals up to 1 min, 30 s up to 4 min, 60 s up to 10 min and at 15 min. pH of such collected samples was measured with a calomel pH micro-electrode in conjunction with a pH meter. Kinetics of salivary pH over time as a result of the sour candy consumption fitted an exponential association function ( $r^2 = 0.990$ ). Three-way ANOVA for repeated measures demonstrated significant interaction between the candy version and time points ( $p < 0.001$ ). Tukey's test revealed that up to 120 s after the sour candy had been chewed, salivary pH remained lower ( $t_0$ : 2.96;  $t_{15}$ : 4.93;  $t_{30}$ : 5.69;  $t_{45}$ : 6.36;  $t_{60}$ : 6.61;  $t_{90}$ : 6.86;  $t_{120}$ : 6.84) than that observed when the ordinary version had been chewed. Transitory low salivary pH immediately after and following chewing of sour gummy candies may represent a risk factor for dental erosion.

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### Comparison of Erosion and Erosion/Abrasion Modified by High Fluoride Varnishes

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**Aim:** The aim of the study was to compare the effect of fluoride varnishes on erosion and erosion/abrasion cycles in vitro. **Methods:** A flat area of enamel was taped to expose a window (1 × 3 mm) on 80 polished human enamel samples. Different fluoride varnishes were applied to the surface (Bifluorid10<sup>®</sup> 45,200 ppm F; Duraphat<sup>®</sup> 22,600 ppm F; Fluor Protector 1,000 ppm F) and compared to a control without fluoride (Copal Ether Varnish) and deionized water. Each sample was exposed to 9 cycles of erosion [1 cycle; erosion (0.3% citric acid, pH 3.2, 5 min) + artificial saliva (1 h, pH 7.0)] and erosion-abrasion [1 cycle; erosion + artificial saliva (1 h, pH 7.0) + abrasion (120 linear strokes with 300 g loading)]. The enamel surface was evaluated using mean (SEM) microhardness change ( $\Delta$ KHN) with indents initially recorded adjacent to the taped area and then following cycling within the body of the lesion. Data were analysed using ANOVA and Tukey Kramer multiple comparisons. **Results:** For erosion, all products statistically significantly reduced microhardness change Duraphat<sup>®</sup> 150 (6.1)  $\Delta$ KHN  $p < 0.001$ , Bifluorid10<sup>®</sup> 122 (8.5)  $\Delta$ KHN  $p < 0.001$ , Fluor Protector 138 (5.5)  $\Delta$ KHN  $p < 0.001$  and Copal varnish 158 (7.6)  $\Delta$ KHN  $p < 0.01$  in comparison to the deionized water control 196 (9.4)  $\Delta$ KHN. For erosion-abrasion, the Fluor protector 58 (10.5)  $\Delta$ KHN  $p < 0.001$  and Bifluorid10<sup>®</sup> 81 (8.6)  $\Delta$ KHN  $p < 0.5$  showed significantly reduced microhardness change in comparison to de-

ionized water 125 (8.5)  $\Delta$ KHN. However, Duraphat<sup>®</sup> 109 (10)  $\Delta$ KHN  $p > 0.05$  and Copal Ether 115 (13.7)  $\Delta$ KHN  $p < 0.001$  showed no differences. **Conclusion:** High-concentrated fluoride varnishes reduced surface microhardness change for purely chemical and also chemical/mechanical wear. This suggests that highly concentrated single-application fluoride varnishes penetrate surface enamel and have a persistent effect which is resistant to mechanical processes.

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### Erosion-Inhibiting Interaction of Stannous (Sn<sup>2+</sup>) Ions with Human Enamel Tissue: A Pilot in vitro Study

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Intrinsic and extrinsic acids demineralize human enamel increasing its vulnerability to mechanical alteration which results in gradual tooth erosion. Reduction of teeth susceptibility towards acidic dissolution is one of the preventive strategies. Particularly, application of stannous compounds in dental products (elmex<sup>®</sup> erosion protection) was proven to inhibit erosion progression. This research aimed at the investigation of stannous (Sn<sup>2+</sup>) interaction with the eroded enamel at different incubation times and erosion degrees. Initial in vitro erosion of human enamel was induced by citric acid (pH = 3.6) applied either for 2 (total n = 30) or 4 (total n = 30) min. Afterwards, enamel specimens were treated with elmex<sup>®</sup> erosion protection mouthrinse either for 30 s (n = 20) or 2 min (n = 20) followed by a second erosive challenge. Incubation in tap water instead of the mouthrinse was applied in the control groups (n = 20). The changes of parameters such as surface reflectivity and morphology, calcium release were monitored and compared between treatment groups using Mann-Whitney tests. Energy-dispersive X-ray spectroscopy (EDX) was applied to quantify the uptake of stannous by the eroded enamel. Reduced loss of surface reflectivity (23.6 ± 6.4% vs. 11.0 ± 4.1%) and significantly decreased Ca<sup>2+</sup> dissolution (12.1 ± 3.3 nmol/mm<sup>2</sup> vs. 16.76 ± 3.1,  $p < 0.05$ ) were measured in enamel incubated with elmex<sup>®</sup> erosion protection mouthrinse compared to the control groups, proving inhibition of the erosion by dental rinse. The inhibiting effect of the mouthrinse was independent from the incubation times ( $p > 0.05$ ) and correlated with the stannous uptake. EDX analysis confirmed equal amounts of incorporated stannous in the enamel incubated for 30 s and 2 min in the mouthrinse (1.39 ± 0.54 wt% vs. 0.33 ± 0.17 wt% in control). Therefore, fast stannous uptake was detected after only 30 s of incubation providing significant inhibition of enamel erosion.

### Effects of Acidic Beverages on Human Teeth: An *in vitro* Study

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Families in the South Texas region of United States are often eager to know which of the commercially available beverages are safe to the teeth, since acidic beverages have the potential to cause erosion of tooth enamel. The aim of this study was to evaluate the pH, buffering capacity and erosive potential of a wide variety of beverages available in South Texas, USA. Forty-five beverages composed of fruit juices, soda, sparkling water, flavored water, tea, beer and spring water, were investigated. The initial pH, titratable acidity to raise pH to 7.0, and buffering capacity at the original pH value was measured for each drink. Five enamel blocks with pre-measured surface microhardness were subjected to erosive challenge in each beverage, and the erosion was quantified as the percentage change in surface microhardness (%SMHC) and depth of substance loss (DSL). Data was analyzed statistically using ANOVA followed by Tukey post hoc test, unpaired t test and Pearson correlation (*r*). There was a statistically significant reduction (*p* < 0.01) in SMH with all beverages except spring water. All drinks showed lower pH (pH range = 2.46–4.23) and higher titratable acidity (acidity range = 0.76–7.08 mmol/l NaOH) than spring water (pH = 6.22; acidity = 0.004), and caused significantly higher DSL and higher %SMHC than spring water. There was a significant correlation between initial pH and DSL (*r* = -0.591, *p* < 0.01) and initial pH and %SMHC (*r* = -0.721, *p* < 0.01). Neither titratable acidity nor buffering capacity correlated significantly with %SMHC and DSL. In conclusion, the result of the present study showed that a wide variety of drinks considered as healthy to teeth have a low pH with a high erosive potential.

### Assessment of a Tin-, Fluoride- and Chitosan-Containing Toothpaste on Early Enamel Erosion and Abrasion

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The aim of this study was to assess the effect of a tin-, fluoride- and chitosan-containing toothpaste (Sn-F-Chit-TP) on early enamel erosion and abrasion. A total of 90 lingual surfaces of human premolars were ground and polished, then divided into 3 toothpaste groups (*n* = 30): placebo, sodium fluoride (NaF), Sn-F-Chit-TP. Once a day, the samples were submitted to abrasion (incubation in the toothpaste slurry for 2 min and brushed with 20

toothbrush strokes) and later immersed in tap water (2 min in 10 ml, 25°C, 70 U/min). Then, the samples were submitted to erosion (2 min in 30 ml 1% citric acid, pH = 3.6, 25°C, 70 U/min). Surface microhardness (SMH) was measured initially and after every abrasion and erosion treatment (10 g for 10 s). Also, enamel substance loss was measured with 200 g force indentations made before each abrasion and re-measured after. Brunner-Langer F1\_LD\_F1 and Wilcoxon Rank tests were used for analysis. Statistically significant loss of SMH was found between all groups throughout the study (*p* < 0.001). When the total SMH loss was analyzed, the placebo group had a greater SMH loss (276.0 ± 37.7 KHN) than the NaF (223.9 ± 34.2 KHN) and Sn-F-Chit-TP (219.1 ± 32.2 KHN) (*p* < 0.001), but the latter two did not differ (*p* = 0.610). In relation to substance loss, there was a difference between the groups (*p* < 0.001). The Sn-F-Chit-TP group had a significantly less total substance loss (0.31 ± 0.11 µm) than placebo (1.03 ± 0.28 µm) and NaF (0.98 ± 0.22 µm) (*p* < 0.001), while the latter two lost similar amounts (*p* = 0.390). Both NaF and the tin-, fluoride- and chitosan-containing toothpastes were able to significantly decrease enamel surface softening, but the application of the Sn-F-Chit-TP resulted in significantly less enamel loss compared to the NaF and placebo toothpastes. The tin-, fluoride- and chitosan-containing toothpaste decreased enamel softening and abrasive effect on enamel.

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### Risk Indicators for Dentine Erosion among 18-Year-Olds in Oslo, Norway

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**Aim:** To investigate risk indicators associated with dentine erosion among a group of 18-year-olds in Oslo, Norway. **Methods:** Adolescents (*n* = 552), registered with at least one erosive lesion during dental examination in all Public Dental Health Service clinics in 2008, were offered supplemental examination for dental erosion by a calibrated clinician (A.M.). The distribution and severity grade of the lesions were registered on occlusal surfaces of the first and second molars in both jaws and the labial and palatal surfaces of the upper incisors and canines using a previously tested pictorial manual, Visual Erosion Dental Examination (VEDE). Concurrently with the clinical examination, each participant completed a self-administered questionnaire designed to assess potential risk indicators. Apart from background variables (gender, occupation, national background), the questionnaire included information on selected behavioural (dental hygiene habits, physical exercise) and dietary variables (consumption frequency of acidic drinks and foods), as well as medical history of the participants (medications, gastro-oesophageal reflux and vomiting). Association between dentine erosion and the possible risk indicators were assessed by logistic regression analysis. **Results:** Of 231 adolescents re-examined with dental erosion (response rate 42%), enamel lesions only

were registered in 63%, whereas 37% had at least one lesion extending into dentine. Of the 37 variables considered as potential risk indicators, 13 were significantly associated with dentine erosion in the bivariate analyses. However, regression analysis showed significant association only between dentine exposure and being male (OR 1.8; CI 1.0–3.0), and of western origin (OR 2.4; CI 1.2–4.7). No significant associations between dentine erosion and behavioural, dietary or medical history related variables were found. **Conclusions:** Background variables such as gender and national origin were identified as important risk indicators for dentine exposure among 18-year-olds.

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### In situ Analysis of the Effect of CO<sub>2</sub> Laser to Control Erosive Lesions on Enamel

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This in situ study evaluated the effect of CO<sub>2</sub> laser irradiation to control the progression of erosive lesions on enamel. Fifty-six bovine incisors enamel slabs (KHN = 300 ± 10%) (5 × 3 × 2.5 mm) had 1/4 of the surface covered with resin composite (control area), and were cycled through an alternating erosive and remineralizing regimen. For that, specimens were immersed in citric acid (pH = 2.3), 5 min, 2×/day, for 2 days. Specimens with pre-formed lesions were divided into two groups: CO<sub>2</sub> laser irradiated (λ = 10.6 μm; 0.5 W, ultrapulse) and non-irradiated. Fourteen volunteers (n = 14) were randomly given either irradiated or non-irradiated specimens in the first period (5 days) of this two-period crossover study and then crossed over to the other treatment. During the intraoral phases, volunteers wore palatal devices containing the abovementioned slabs and immersed this appliance extraorally in citric acid (erosive challenge) or in deionized water (control), for 5 min, 3×/day. Enamel wear was determined by an optical 3D profilometer after initial erosive challenge, after surface treatment and after intraoral phase. The wear assessment was verified by means of three readings of each area and also the controlled area. Three-way ANOVA for repeated measures revealed that there was no significant interaction between erosive challenge and CO<sub>2</sub> laser irradiation (p = 0.419). Erosive challenge showed a significant effect (p = 0.001) with an increased tooth wear of enamel [36.01 (16.48)] when compared to non-eroded [18.25 (9.05)], regardless of CO<sub>2</sub> laser irradiation. The factors CO<sub>2</sub> laser irradiation and volunteer presented no significant effect (p = 0.513 and p = 0.544, respectively). We concluded that CO<sub>2</sub> laser irradiation did not reduce in situ the progression of erosive lesions on enamel caused by citric acid.

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### Evolution of the Enamel Erosion Process with Gaseous and Liquid Hydrochloric Acid

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It is known that the disease gastroesophageal reflux may be associated with dental erosion, however, is unknown the effect of gaseous hydrochloric acid in erosive process of the dental enamel. We aimed to evaluate and quantify day by day the volume loss (VL) of enamel in erosive process caused by gaseous and liquid hydrochloric acid. Enamel slabs (4 × 4 × 2 mm) were cut from bovine incisors, ground flat and polished. The samples were isolated with composite resin except for half their outer surface (8 mm<sup>2</sup>), and were randomly divided in 2 groups (n = 10) according to erosive challenge with hydrochloric acid (at pH 2.0): Liquid (L) – 0.03% HCl, 20 s, 6×/day for 5 days; Gaseous (G) – 15.84% HCl, 3 min, 8×/day for 12 days. Between the erosive challenges the samples were stored in artificial saliva at 37°C. The volume loss was analyzed using laser scanning confocal microscope having as reference the surface protected with resin. Data were analyzed by one-way ANOVA (α = 5%). Group L showed significant and gradual increase of VL until the third day (1.25 ± 0.20, 1.59 ± 0.25, 2.09 ± 0.27 μm, respectively) and then stabilized. For group G, it was observed significant increase of VL on the 2nd day, leveling off until the 6th day (2.3 ± 0.52, 3.97 ± 1.74, 6.12 ± 2.19 μm, respectively). On the 8th day (8.30 ± 2.17 μm) there was a new VL increase followed by a new stabilization until the 12th day (10.48 ± 2.74 μm). The VL of the group L (2.15 ± 0.76 μm) was lower compared to the G (6.34 ± 2.39 μm); however the rate of loss in group G was 10 times less than in group L. We can conclude that the erosion by the liquid promoted initially high volume loss, following stabilization of it. Nevertheless, the gas presented oscillatory pattern with periods of major and minor enamel volume loss and low rate of loss.

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### Prevalence and Severity of Dental Erosive Wear among a Group of Norwegian 16–18-Year-Olds

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The aim was to investigate prevalence, distribution and severity of erosive wear in a group of 16–18-year-olds in Western Norway. The study was conducted in 4 Public Dental Health Service (PDHS) clinics. All 16–18-year-olds, scheduled for recall examinations in 2012 (n = 711), were invited to participate and 676 (95%) accepted. The participants were examined for dental erosive wear by six calibrated clinicians as part of their regular examination. Dental erosive wear was registered on a surface level using the Vi-

sual Erosion Dental Examination scoring system (VEDE). Occlusal surfaces of the first and second molars in both jaws and the labial and palatal surfaces of the upper incisors and canines were selected as index surfaces. The kappa values for intra-examiner agreement were (0.32–0.71) and for inter-examiner agreement (0.30–0.79) respectively, indicating fair to substantial agreement. Of the 676 individuals examined, 39% showed no signs of dental erosive wear, 46% had erosive wear in enamel only, whereas 15% had at least one lesion extending into dentine. The palatal surfaces of upper central incisors (35%) and occlusal surfaces of first lower molars (49%) were the most often affected. Cuppings on molars were registered in 38% of the individuals. Erosive wear extending into dentine was significantly more prevalent among males (19%) than females (11%) ( $p = 0.004$ ). The prevalence of dental erosive wear among 16–18-year-olds in Western Norway was 61%. Gender differences in dentine erosion prevalence were found.

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### Quantifying Effectiveness of Fluoride to Reduce Erosion of Natural Surface and Polished Enamel in situ

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Partly for the purpose of measuring erosion, most in situ investigations use flat, polished enamel however this does not truly reflect the enamel surface in vivo. The aim of this study was to determine the protective effect of a high concentration of fluoride against initial erosion on both natural surface and polished enamel. Following ethical approval, 20 healthy individuals participated in this study. Each individual wore lower buccal appliances containing a curved, natural surface specimen and a polished specimen. Lower human incisors were used due to the constraints of the measuring instrument necessitating a curved surface to be within 300  $\mu\text{m}$  height variation over a  $2 \times 1$  mm scanning area. Individuals wore the appliances from 9.00 to 16.00 and were treated with slurry of Colgate Duraphat5000 dentifrice (5,000 ppm F) for 2 min twice a day for 5 days. Specimens were also exposed to an acid challenge by swishing a total of 250 ml orange juice around their mouth over a 10 min period at separate intervals, three times a day. Following each day, the surface of each specimen was scanned using a non-contact profilometer (Proscan2000, Scantron, UK) and the mean  $R_a$  determined. Paired t test between baseline and post treatment was used to analyse the data where  $\alpha = 0.05$ . Polished enamel specimens that were treated with fluoride showed a significant change in surface roughness following 1 day of acid treatment (30 min), which increased following each day of treatment ( $R_a$ : 0.12–0.20,  $p < 0.04$ ). By contrast, natural surface specimens that were treated with fluoride did not show a significant change in surface roughness from baseline until after the fifth day of treatment (150 min acid;  $R_a$ : 0.325,  $p < 0.01$ ). Natural surface specimens treated with a high concentration of fluoride showed prolonged

resistance to acid treatment compared to polished specimens treated with fluoride.

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### Influence of Different Enamel Depths on Initial Erosion Measurements

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This study aimed at assessing the effect of initial erosion on enamel ground to different depths. 20 molars and 20 premolars were divided into two groups: control and erosion. The teeth were cut into lingual and buccal sides and both surfaces were ground to 100  $\mu\text{m}$  depth, and then polished. Initial surface microhardness (SMH) was measured on all teeth. Three consecutive erosive challenges (30 ml of 1% citric acid, pH = 3.6, 25°C, 1 min) were made on the erosion group, then SMH and amount of calcium released to the citric acid were measured. We ground the same teeth again to 200, 400 and 600  $\mu\text{m}$  depths. At each depth, the samples on the erosion group were submitted to 3 erosive challenges and analyzed. There was no difference on initial SMH between both groups. Multivariate linear regression showed no significant difference between lingual and buccal surfaces for SMH loss ( $p = 0.801$ ) or calcium release ( $p = 0.370$ ). Considering the tooth as measuring unit, Brunner-Langer ANOVA models showed that premolars had greater SMH loss than molars, after each erosive challenge ( $p < 0.05$ ), but not significantly different with respect to enamel depth. Total SMH loss for premolars were  $180.4 \pm 23.6$ ,  $185.3 \pm 21.0$ ,  $182.5 \pm 24.0$  and  $178.2 \pm 19.2$  KHN and molars:  $177.4 \pm 31.9$ ,  $171.9 \pm 31.0$ ,  $157.3 \pm 35.5$  KHN and  $166.1 \pm 26.5$  for 100, 200, 400 and 600  $\mu\text{m}$  depths, respectively. Enamel depth significantly influenced calcium release during all three erosive challenges, where greater depths had a tendency for smaller calcium values. We conclude that premolars and molars have different SMH loss and calcium release, while deeper layers of enamel tend to release lower amounts of calcium. Therefore, experiments should use the same type of teeth, and, depending on the methods used, they should be ground to the same depth.

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### Erosion/Abrasion-Preventing Potential of Toothpastes in Dentine and Impact of the Demineralised Organic Matrix

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Little is known about effects of toothpastes on dentine erosion, particularly considering the demineralised organic matrix potentially influencing the effects of active ingredients as well as of abra-

sion. The study sought to investigate whether experimental toothpastes can reduce the erosive/abrasive tissue loss in presence or absence of demineralised collagen. Samples were exposed to erosion/abrasion for 10 days. Between interventions samples were stored in mineral salt solution either without, or with collagenase (from *Clostridium histolyticum* type VII; 100 U/ml) for continuous digestion of the organic matrix. To produce a comparable order of tissue loss, erosion was performed  $6 \times 30$  s/day with 0.5% citric acid in the latter, and  $6 \times 90$  s/day with 1% citric acid in the former case. A NaF (1,400 ppm F<sup>-</sup>) and an amine fluoride (AmF)/NaF/SnCl<sub>2</sub>/chitosan (1,400 ppm F<sup>-</sup>; 3,500 ppm Sn<sup>2+</sup>) formulation were investigated; controls were a SnF<sub>2</sub> gel (970 ppm F<sup>-</sup>; 3,000 ppm Sn<sup>2+</sup>) and placebo. Samples were immersed in slurries ( $2 \times 2$  min/day), or additionally brushed for 15 s within slurry immersion (automated brushing machine, load 200 g). Tissue loss was determined profilometrically ( $\mu\text{m} \pm \text{SD}$ ), ANOVA was used for statistical analysis. Tissue loss (without/with brushing) for placebo, NaF, AmF/NaF/SnCl<sub>2</sub>/chitosan and SnF<sub>2</sub> gel resp. was  $11.6 \pm 3.1/12.2 \pm 2.5$ ,  $12.7 \pm 3.1/10.7 \pm 4.5$ ,  $8.7 \pm 2.1^*/9.7 \pm 2.1^*$  and  $8.8 \pm 1.8^*/10.9 \pm 1.8$  without, and  $10.7 \pm 3.2/11.9 \pm 2.1$ ,  $8.2 \pm 4.0/10.1 \pm 4.1$ ,  $8.7 \pm 2.9/9.1 \pm 1.8^*$ , and  $8.4 \pm 1.9/7.5 \pm 1.5^*$  with continuous digestion of the organic matrix (\* indicates significance compared to the respective placebo groups). NaF revealed no significant effect; AmF/NaF/SnCl<sub>2</sub>/chitosan reduced the tissue loss in the order of 20–25% except when applied without brushing in the absence of the organic matrix. Brushing simulating clinically relevant habits had no impact on tissue loss. Sn<sup>2+</sup>-containing formulations have potential to reduce progression of erosion/abrasion even in the absence of demineralised collagen; seeking for more effective formulations is desirable.

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### Daily Action of Gaseous and Liquid Hydrochloric Acid on Enamel Erosion

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Gastroesophageal reflux disease causes dental erosion, however, is unknown the effect of gaseous hydrochloric acid in erosive process of the dental enamel. The objective was to evaluate and quantify day by day the enamel roughness in erosion process caused by hydrochloric acid gaseous and liquid. Bovine enamel slabs ( $4 \times 4 \times 2$  mm) were cut, ground flat and polished. The specimens were isolated with composite resin except for half their outer surface ( $8 \text{ mm}^2$ ), and were randomly divided into 2 groups ( $n = 10$ ) according to erosive challenge with hydrochloric acid (at pH 2.0): liquid (L), 0.03% HCl, 20 s,  $6 \times$ /day for 5 days; gaseous (G), 15.84% HCl, 3 min,  $8 \times$ /day for 12 days. Between the erosion challenges the specimens were stored in artificial saliva at 37°C. The roughness was analyzed using laser scanning confocal microscope having as reference the surface protected with resin. Data were analyzed by one-way ANOVA ( $\alpha = 5\%$ ). For group L, it was observed significant increase of roughness on 1st day of exposure

( $0.22 \pm 1.11 \mu\text{m}$ ) compared to the control area ( $0.046 \pm 1.11 \mu\text{m}$ ), which decreased in 2nd day ( $0.176 \pm 1.11 \mu\text{m}$ ) and stabilized, observing statistical similarity among days 3, 4 and 5 ( $0.161 \pm 1.11$ ,  $0.176 \pm 1.11$  and  $0.171 \pm 1.11 \mu\text{m}$ ). For G it was observed significant roughness increase on the 2nd day ( $0.26 \pm 1.77 \mu\text{m}$ ), leveling off until the 6th day ( $0.25 \pm 1.77 \mu\text{m}$ ), on the 8th day ( $0.31 \pm 1.77 \mu\text{m}$ ) there is a new roughness increase followed by a new stabilization until the 12th day ( $0.32 \pm 1.77 \mu\text{m}$ ). Group L roughness ( $0.18 \pm 7.07 \mu\text{m}$ ) was lower compared to group G roughness ( $0.29 \pm 7.4 \mu\text{m}$ ). The erosion by the liquid promoted initially high roughness, following stabilization it, the gas presented oscillatory pattern with periods of major and minor enamel roughness and promoted higher roughness surface.

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### Influence of Dentifrice Abrasivity and Remineralization Time on Enamel Toothbrushing Abrasion

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Remineralization of eroded dental surfaces has shown to decrease their susceptibility to toothbrushing abrasion. The influence of dentifrice abrasivity on this protection is not well known and was therefore studied. The following experimental factors were considered: dentifrice slurry abrasivity, at 3 levels (L – low: RDA 69, M – medium: RDA 147, and H – high: RDA 208); remineralization time, at 4 levels (0, 30, 60 and 120 min); and dental substrate, at 2 levels (enamel and root dentin), generating 24 testing groups. Experimental units consisted of slabs of bovine enamel and root dentin cut, embedded in acrylic resin and polished ( $n = 8$ ). They were submitted to a cycling protocol including demineralization with 0.3% citric acid (pH 2.6) for 5 min, remineralization at the test times (0, 30, 60 or 120 min) followed by brushing with the tested slurries (L, M and H) in automated brushing machine for 15 s (45 strokes),  $2 \times$ /day, for 5 days. Surface loss (SL, in micrometers) was determined by optical profilometry. Data was analyzed using mixed-model ANOVA and Fisher's PLSD tests ( $\alpha = 0.05$ ). Remineralization protection was shown for some but not all groups and varied by time. On enamel, it was observed after 30 min for M [SL: 6.5 (standard deviation: 0.7) vs. 8.3 (1.0) at 0 min] and H [8.8 (2.8) vs. 10.7 (1.3) at 0 min], and after 120 min for L [5.1 (0.8) vs. 7.2 (1.4) at 0 min]. For dentin, it was shown only for L after 30 min [6.3 (2.6) vs. 8.8 (1.3) at 0 min]. Root dentin had significantly higher SL than enamel for most of the study groups. Less abrasive dentifrice slurries were able to reduce the toothbrushing abrasive wear on both enamel and root dentin. This protection was enhanced by remineralization for all abrasive levels on enamel, but only for L on root dentin.

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### Comparative Study regarding the Impact of Saliva on Dental Erosion Induced by Various Acidic Beverages

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The aims of this study were to investigate the surface topography and to compare the calcium and phosphorus ions concentration of enamel, dentine and cement following the contact with five acidic drinks in the presence or absence of saliva. 25 caries-free extracted teeth were used in this study. All the teeth were longitudinally sectioned in three slices. One slice had been stored in distilled water (control group). The second slice had been continuously immersed for 12 h in one of the tested beverages: Red Bull, Lipton Green Tea, a commercial apple juice, a natural carbonated mineral water and lemon juice. The third slice had been subjected to 3 cycles of immersion in one of the tested beverages for 1 min, followed by storage in artificial saliva (AFNOR NF S90-701) for 4 h. The samples were analyzed using a scanning electron microscope and an EDX detector. The specimens continuously stored in acidic beverages showed severe erosion of enamel and enlarged dentinal tubules. The specimens successively stored in acidic beverages and saliva showed a slightly eroded surface of enamel and occasionally a film-like layer covering the apparently intact surfaces. The calcium and phosphorus ions concentration in enamel, dentine and cement significantly decreased following continuous storage in all the tested beverages (mean calcium ion concentration (wt%) in enamel (E)/dentine (D)/cement (C): 32.65/28.17/23.93 control group, 30.13/16.45/11.07 apple juice, 30.39/22.84/11.48 Lipton Green Tea, 29.58/24.01/21.46 natural carbonated mineral water, 18.67/15.06/9.22 lemon juice, 23.13/18.09/10.52 Red Bull; mean phosphorus ion concentration (wt%) in E/D/C: 12.87/10.80/9.92 control group, 12.20/7.84/6.55 apple juice, 12.24/8.70/6.98 Lipton Green Tea, 12.82/9.39/9.21 natural carbonated mineral water, 9.90/7.45/6.28 lemon juice, 10.25/7.85/6.79 Red Bull). The decrease of mineral ions concentrations in enamel, dentine and cement was significantly lower when saliva had been used as a storage medium between immersions in acidic beverages ( $p < 0.05$ , ANOVA and Bonferroni test). The calcium and phosphorus ions concentration in dentine and cement were significantly lower when compared to enamel ( $p < 0.05$ , Mann-Whitney test) regardless the presence or absence of saliva.

### Role of High Fluoride Varnishes Investigated by 3D Profilometry

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The aim of the study was to investigate the potential of highly concentrated fluoride varnishes to reduce enamel loss after repeated cycles of citric acid erosion and erosion/abrasion in vitro. Eighty human enamel samples ( $5 \times 3 \times 2$  mm) were used (n per group = 16). A flat area of enamel was taped to expose a window ( $2 \times 2$  mm) to fluoride varnishes; Bifluorid10<sup>®</sup> (45,200 ppm F), Duraphat<sup>®</sup> (22,600 ppm F), Fluor Protector (1,000 ppm F), Copal Ether Varnish (0 ppm F) and deionized water. Following nine cycles of erosion [1 cycle; erosion (0.3% citric acid, pH 3.2, 5 min) + artificial saliva (1 h, pH 7.0)] and erosion/abrasion [1 cycle; erosion + artificial saliva (1 h, pH 7.0) + abrasion (120 linear strokes with 300 g loading)]. The change in enamel surface was evaluated using 3D optical profilometry for mean (SD) normalised volume and images were analysed using surface analysis software (Mountains-Map<sup>®</sup> Universal v6.2). Data were analysed using one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons post-test  $p < 0.05$ . Higher concentrations of fluoride significantly reduced erosion and erosion/abrasion measured by profilometry. Duraphat<sup>®</sup> and Bifluorid10<sup>®</sup> showed statistically reduced normalised volume ( $p < 0.001$ ) ( $5.76 \pm 1.0 \mu\text{m}^3/\mu\text{m}^2$  and  $5.52 \pm 1.2 \mu\text{m}^3/\mu\text{m}^2$  respectively) during erosion versus deionized water ( $8.36 \pm 1.3 \mu\text{m}^3/\mu\text{m}^2$ ) and in comparison to the other varnishes ( $p < 0.001$ ) (FluorProtector<sup>®</sup>  $7.62 \pm 1.2 \mu\text{m}^3/\mu\text{m}^2$  and Copal Ether Varnish  $8.35 \pm 0.9 \mu\text{m}^3/\mu\text{m}^2$ ). For the erosion/abrasion model, Duraphat<sup>®</sup> ( $5.35 \pm 1.0 \mu\text{m}^3/\mu\text{m}^2$ ) and Bifluorid10<sup>®</sup> ( $6.02 \pm 1.0 \mu\text{m}^3/\mu\text{m}^2$ ) showed statistically significantly less volume enamel loss ( $p < 0.001$ ) in comparison to deionized water ( $10.18 \pm 1.4 \mu\text{m}^3/\mu\text{m}^2$ ) and the other products ( $p < 0.001$ ) (FluorProtector<sup>®</sup>  $8.64 \pm 1.2 \mu\text{m}^3/\mu\text{m}^2$  and Copal Ether Varnish  $9.2 \pm 1.4 \mu\text{m}^3/\mu\text{m}^2$ ). Fluor Protector<sup>®</sup> varnish also showed reduced enamel loss versus deionized water after erosion/abrasion ( $p < 0.01$ ). High-concentrated fluoride varnishes effectively reduced enamel wear from erosion and erosion and abrasion. The overall trend of protection from acid and acid/abrasion was similar while different products act in different ways to affect the mineralised tissue.

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## A New Optical Method for the Quantification of the Erosion Inhibition by the Salivary Pellicle Layer

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Possibilities for the quantitative early erosion detection in vivo are attractive long-term goals in dentistry. As a step forwards, a new optical method based on monitoring the specular reflection intensity was recently developed [Rakhmatullina et al.: *J Biomed Opt* 2011;16:107002] and applied in the present study. To prove its applicability, the optical method was used to detect the erosion inhibiting properties of the salivary pellicle in an in vitro erosion model. SEM imaging, microhardness and calcium dissolution analyses were also used. Enamel samples covered with a 15 h in vitro formed salivary pellicle layer (group P, n = 90) and bare enamel surfaces (control group C, n = 90) were compared regarding erosion progression. The erosive challenge consisted of incu-

bating the samples in 1% citric acid (pH = 3.6) for either 2, 4, 8, 10 or 15 min. The optical method proved erosion inhibition by the in vitro formed salivary pellicle in short-term acidic treatments ( $\leq 4$  min) showing significantly higher specular reflection intensities in group P ( $46 \pm 7\%$  after 2 min,  $39 \pm 7\%$  after 4 min). However, only  $42 \pm 6\%$  and  $30 \pm 5\%$  of the reflection intensity was detected in group C after 2 and 4 min erosion. Significantly less ( $p < 0.05$ ) enamel softening was also detected in group P at 2 min ( $34.5 \pm 13.7$  KHN) and 4 min ( $61.0 \pm 12.6$  KHN) erosion compared to group C ( $60.1 \pm 11.9$  KHN and  $84.3 \pm 17.0$  KHN, respectively). SEM images showed rougher, more eroded tissues in group C than in group P. Good agreement of the results obtained from two independent methods showed high applicability of the new optical method for the assessment of early erosive changes. This method can be a potential technique for the fast and non-contact erosion quantification in future in vivo studies.

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## Session 7

# Fluoride and Clinical Studies

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### Synthesis and Characterization of Hydroxyapatite Treated with $\text{TiF}_4$ in vitro

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This in vitro study evaluated the effect of  $\text{TiF}_4$  compared to NaF on hydroxyapatite (HA) structure. HA was obtained by precipitation method and, thereafter, it was suspended in fluoridated solutions (0.5 g/10 ml) according to the groups: A – 1.55%  $\text{TiF}_4$ ; B – 3.10%  $\text{TiF}_4$ ; C – 4.00%  $\text{TiF}_4$ ; D – 2.10% NaF; E – 4.20% NaF; F – 5.42% NaF; and G – control – deionized water (n = 2 samples of HA/group). The  $\text{TiF}_4$  solutions presented their native pH 1.2, and NaF solutions had their pH adjusted to 5.0 (using phosphoric acid) similarly to some commercial NaF-containing products. After 1 min of treatment, the pH of all solutions was adjusted to 7.0 by using 1 M NaOH. HA was suspended in the solutions for further 30 min at 37°C. The HA samples were analysed by infrared spectroscopy and X-ray diffraction. The infrared spectrum showed a reduction in bands corresponding to phosphates deformation and increase in the hydroxyls band in HA treated with  $\text{TiF}_4$ , especially at high concentration. For 2.10% NaF, the infrared spectrum was similar to control. However, the other NaF concentrations presented inconsistent changes, i.e., 5.42% increased the proportion of hydroxyls band, while 4.2% presented a reduction of phosphate and hydroxyl bands. Furthermore, the carbonate bands appeared in HA treated with NaF. The diffractogram identified peaks of HA, calcium fluoride hydride,  $\text{TiO}_2$  and titanium hydrogen phosphate hydrate in  $\text{TiF}_4$  groups, while calcium sodium phosphate, calcium fluoride hydride and HA was seen in NaF groups. Therefore, the results show that  $\text{TiF}_4$  causes changes in HA, which might be related to the effect of this metal fluoride in increasing the acid resistance of enamel compared to NaF.

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### Concentration of Free Fluoride in Toothpastes Marketed in Uzbekistan

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Toothpastes with a high amount of free fluoride ions have proven to be very efficacious in caries prevention. The aim of this study was to determine the concentration of free fluoride ions in most of the toothpastes marketed in Uzbekistan. For this purpose 23 different brands of toothpastes (3 tubes each) which are manufactured by 11 different producers were bought in 2012 in supermarkets from Uzbekistan. Two brands of toothpastes from different producers containing NaF and marketed in Germany served as control. From each toothpaste samples weighing 0.1 g were taken and diluted with 24.9 ml of distilled water and 2.5 ml TISAB III was added. No hydrolysing agents were used. Fluoride concentration was measured with the aid of a F-specific electrode. Eight toothpastes were labelled to contain NaF and the mean F concentration indicated by the manufacturers was 1,281.3 ppm (SD 232.9, range 1,000–1,450). On average 1,223.1 ppm F (SD 213.6, range 913–1,429) were measured. Eleven toothpastes were labelled to contain NaMFP. The mean soluble F concentration indicated by these manufacturers was 972.0 (SD 546; range 39–1,450). In these toothpastes, a mean concentration of free fluoride ions of 183.0 ppm (SD 108.49, range 4–363) was measured. Four brands were labelled to contain NaMFP without indication of F concentration and in them the concentration of free fluoride ions on average was 79.2 ppm (SD 69.69, range 4–183). The German toothpastes which were labelled to contain 1,450 ppm F were found to contain on average 1,405 ppm F. It is concluded that the majority of toothpastes marketed in Uzbekistan contain NaMFP, a fluoride type that in toothpastes offered in Germany nearly has disappeared in recent years. Furthermore, although not expected from the labelled information, in NaMFP toothpastes free fluoride ions were measured but their concentration was very distinctly lower than in NaF toothpastes.

## Sound and Demineralised Enamel Treated with TiF<sub>4</sub> in vitro: KOH-Soluble Fluoride Deposition and SEM-EDXS Analysis

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This in vitro study aimed to evaluate the surface morphology and the presence of elements as well as the deposition of KOH-soluble fluoride on bovine enamel treated with TiF<sub>4</sub> varnishes. Sound and demineralised bovine enamel samples were treated with TiF<sub>4</sub> (concentrations: 1.55%, 3.10% and 4.00%) and NaF (2.10%, 4.20% and 5.42%) varnishes for 12 h. The control group remained untreated. Thereafter, 10 samples (5 sound and 5 demin) per treatment were analysed using scanning electron microscopy and energy-dispersive X-ray spectroscopy (SEM-EDXS). Furthermore, 20 samples (10 sound and 10 demin) per treatment were subjected to KOH-soluble F biopsy. The data were statistically analysed using ANOVA and Tukey tests or Kruskal-Wallis and Dunn tests (for EDXS data), and two-way ANOVA and Bonferroni tests (for KOH-F soluble) ( $p < 0.05$ ). In respect to SEM-EDXS analysis, enamel samples treated with TiF<sub>4</sub> varnishes showed a coating layer with microcracks rich in Ti and F. The amount of Ti and F increased along to the TiF<sub>4</sub> concentration. CaF<sub>2</sub>-like globules were only seen on samples treated with the highest concentrated NaF varnish. Enamel samples treated with 3.10% and 4.00% TiF<sub>4</sub> varnishes showed the greatest alkali-soluble F deposition, which was higher on demineralized (3.10% and 4.00% TiF<sub>4</sub>:  $2.57 \pm 1.05$  and  $3.62 \pm 1.68 \mu\text{g F/mm}^2$ , respectively) compared to sound enamel (3.10% and 4.00% TiF<sub>4</sub>:  $1.10 \pm 0.73$  and  $1.57 \pm 0.96 \mu\text{g F/mm}^2$ , respectively). For NaF, there was no significant difference in F deposition between the demineralised (F min-max means:  $0.07\text{--}0.39 \mu\text{g F/mm}^2$ ) and sound enamel (F min-max means:  $0.05\text{--}0.20 \mu\text{g F/mm}^2$ ). In conclusion, the treatments with TiF<sub>4</sub> varnishes were more effective in increasing fluoride deposition as well as on the formation of a coating on enamel, which might be responsible for the effect of this metal fluoride in increasing the acid resistance of enamel compared to NaF varnishes.

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### A Randomized Controlled Trial of Caries Prevention Effect of a Glass Ionomer Sealant versus a Resin Sealant

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This randomized clinical trial evaluated caries prevention of first permanent molars sealed with a glass-ionomer sealant (GIS) or a conventional resin sealant (RS) over a one-year period in a

high caries children group. Additionally the concentration of fluoride in saliva was evaluated. This study was carried out in the Paediatric Dentistry Department, Dental Faculty, Marmara University, Istanbul, Turkey. Sites with a score of less than 20 with a laser fluorescent device (DIAGNOdent pen) were included in the study. Eighty GIS (Fuji Triage) and 80 RS (Ultraseal XT) restorations were placed on sound first permanent molars of 40 children aged between 7 and 10 years in a randomized order. Mean dft scores were  $6.5 \pm 4.26$  and  $5.15 \pm 2.25$  for GIS and FS groups respectively. All sealants were re-examined clinically one week, 6 months and one year later. Saliva samples were collected from each participant and fluoride concentrations were measured before and after sealant application, one week, 6 months and one year later using a fluoride electrode. Chi-square and one-way ANOVA tests were used between groups. All carious primary teeth were restored. For GIS and FS groups, the total retention rates were 80% and 95% in 6 months and 57% and 82% in one year respectively and the difference was statistically significant ( $p = 0.0096$ ). No caries were detected visually in both groups. Although not significant, fluoride concentrations in saliva remained higher in GIS group in one year (GIS =  $0.060 \pm 0.032$  ppm, RS =  $0.042 \pm 0.035$  ppm;  $p = 0.053$ ). The total retention rate was higher in RS than GIS of pits and fissures of first permanent molars and no caries progression was seen in teeth that had lost GIS partially after one year.

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### Calcium and Fluoride Binding to *Streptococcus mutans* at Below-KSP<sub>CaF<sub>2</sub></sub> Concentrations

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It has been suggested that divalent cations, especially calcium, enhance fluoride binding to dental biofilm bacteria and also that the amount of bacterial-bound calcium doubles in the presence of fluoride. However, these previous studies used calcium and fluoride concentrations that favor the precipitation of calcium fluoride (CaF<sub>2</sub>)-like minerals. Thus, we evaluated the binding capacity of calcium and fluoride to *Streptococcus mutans* using solutions with concentrations which do not exceed the solubility product (KSP) of CaF<sub>2</sub>. Pellets obtained from cultures of *S. mutans* IB1600 were exposed to PIPES buffer, pH 7.0, containing 0 mM Ca + 1 ppm F (G1), 1 mM Ca + 1 ppm F (G2), 10 mM Ca + 1 ppm F (G3), or 1 mM Ca + 10 ppm F (G4), for 60 min ( $n = 2$  experiments). Bacteria were separated from the treatment solution by centrifugation, bound fluoride and calcium were acid-extracted and their concentrations were determined using an inverted fluoride electrode and Arsenazo III reagent, respectively. The concentration of calcium bound to the pellet ( $\mu\text{mol Ca/g}$ ) increased according to calcium concentration in the treatment solution (G1 =  $3.0 \pm 0.6$ ; G2 =  $8.5 \pm 0.9$ ; G3 =  $21.6 \pm 3.3$ ; G4 =  $7.9 \pm 0.4$ ). Similarly, bound fluoride increased in the higher fluoride concentration treatment (G1 =

0.30 ± 0.08; G2 = 0.26 ± 0.17; G3 = 0.23 ± 0.14; G4 = 0.41 ± 0.05), but the presence of more calcium bound to bacteria did not affect its binding. Moreover, although G4 had 1.5× more bound fluoride than G2, the calcium binding capacity to bacteria was not increased. The data suggest that under conditions that prevent CaF<sub>2</sub> precipitation, neither calcium enhances fluoride binding to dental biofilm bacteria nor the presence of fluoride increases the bacterial-bound calcium concentration.

The study was funded by CNPq (569487/2008-0 and PIBIC) and FAPESP (2009/12907-5).

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### The Effect of Amount of Toothpaste Used and Its [F] on Salivary Fluoride Levels

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This study investigated the effect of amount of toothpaste used (g) and its fluoride concentration (ppm) on saliva fluoride concentrations 1 h and 3 h after brushing. The study involved 13 dental students who reported to the clinic at 7 a.m., having eaten breakfast but without brushing their teeth. Initially an 8 ml unstimulated saliva sample was collected (baseline). Participants then brushed their teeth using one of the following 7 treatment regimes for 45 s in each jaw, after which they spat out waste slurry but did not rinse. Further saliva samples were collected in the same way as baseline 1 h and 3 h after the toothbrushing. Between the 1 h and the 3 h sample the student ate an apple and drank 250 ml of water (<0.01 ppm F). Variation in time (from 1 to 20 min) was seen between participants concerning time required to collect the saliva samples. Students brushed at 7 visits using either 0.25 g, 0.5 g or 1 g of 1,000 ppm 'Colgate Smiles' sodium fluoride toothpaste; 0.5 g, 0.75 g of 1,450 ppm Colgate 'Karies kontroll' (sodium monofluorophosphate = 1,000 ppm, sodium fluoride = 450 ppm); and 0.25 g or 0.5 g of 5,000 ppm Colgate sodium fluoride Duraphate toothpaste. Treatment regimes were compared using ANOVA followed by Tickers test: baseline versus 0.25 g 1,000 ppm versus 0.5 g 1,000 ppm versus 1 g 1,000 ppm F toothpaste after 1 h (a; p = 0.009) and 3 h (b; p = 0.325); baseline versus 0.5 g 1,000 ppm versus 0.5 g 1,450 ppm versus 0.5 g 5,000 ppm F toothpaste after 1 h (1c; means 0.079, 0.091, 0.12 and 0.35; p < 0.0001) and 3 h (d; means 0.085, 0.085, 0.089 and 0.116; p = 0.004); baseline versus 1 g 1,000 ppm versus 0.75 g 1,450 ppm versus 0.25 g 5,000 ppm F toothpaste after 1 h (e; p < 0.0001) and 3 h (f; p = 0.005). Concerning a, saliva baseline [F] values were significantly less than saliva [F] values when using 0.5 g and 1.0 g 1,000 ppm F toothpaste. Concerning c, d, e, f baseline, 1,000 and 1,450 ppm saliva [F] values were significantly less than 5,000 ppm saliva [F] values. The data indicate that [F] in saliva is amount-dependent 1 h after toothbrushing, and concentration-dependent both 1 and 3 h after use of F toothpaste, in particular 5,000 ppm toothpaste increases the [F] values significantly in saliva.

The study was supported by Colgate Palmolive.

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### Fluoride Retention in Saliva following Tooth Brushing Using Fluoridated Dentifrices in Three Different Forms

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This crossover study was carried out to evaluate the influence of differences in the form of fluoridated dentifrices on salivary fluoride levels after tooth brushing, using frothy (foam), jellied (gel) and paste types of dentifrices. Eleven consenting subjects (average 23.6 years) brushed their teeth with the same technique using dentifrices (0.5 g) containing 950 ppm fluoride with frothy, jellied or paste forms, respectively. After tooth brushing for 2 min, they spat out once and rinsed their mouths with 15 ml of distilled water for 5 s. Resting saliva samples were collected for 3 min each at different time intervals; 5, 10, 15, 30, 60, 120, 180 and 240 min after the mouth rinsing. Saliva samples taken before brushing served as control. Salivary fluoride concentrations were determined in 10 µl of the filtrates that were obtained by centrifuging (4°C, 5,000 g) the saliva samples for 30 min, using high-performance liquid chromatography (HPLC). Two-way ANOVA followed by Bonferroni test was used to compare the three different forms of dentifrices, regarding salivary fluoride concentrations in each subject. Statistic differences (p < 0.05) were shown in the fluoride concentrations (ppm, mean ± SD) between foam (2.88 ± 2.23) and gel (1.39 ± 1.08) at 5 min after the rinsing. The area under the curve (AUC) of fluoride was calculated on the graph on which the horizontal base line was the sampling time and the vertical scale was the fluoride concentration. Non-parametric tests were used to conduct multiple comparisons of the AUC among the three different forms. The AUC (µm/min, mean ± SD) for the first 60 min showed statistical differences (p < 0.05) between foam (25.5 ± 19.9) and gel (10.9 ± 8.9). The use of the foam type of dentifrice resulted in the retention of more fluoride in saliva.

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### Novel Synthesized CaF<sub>2</sub> Particles as Enamel-Associated Fluoride Storage and Release Forms

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The aim of the present work was to characterize different newly developed CaF<sub>2</sub> particles for their suitability as enamel-associated fluoride storage and release formulations. Recently we have re-

ported the preparation of CaF<sub>2</sub> particles of various sizes and shapes [Köser and Pielas: Eur Cell Mater 2012;23(suppl 2):19]. Solutions with CaF<sub>2</sub> particles in the range between 50 nm and 5 µm and with fluoride concentrations between 450 ppm and 700 ppm were incubated with enamel surfaces for 30–80 s, which correspond to typical application times of mouthrinses and the duration of tooth brushing. Higher coating densities were achieved with smaller particles, up to 40%, and the interaction occurred with both native and polished enamel surfaces. The release of fluoride from CaF<sub>2</sub> particles was highly dependent on the presence of phosphate in the solution, reaching  $5.33 \pm 0.22$  ppm of soluble fluoride within 90 min when 10 mg of particles are dissolved in 100 ml of physiological buffer solution without phosphate, versus  $0.32 \pm 0.02$  ppm in the presence of 3.5 mM phosphate. Furthermore, as a first step towards the elucidation of the effect of calcium fluoride particle association with enamel surfaces we investigated the pellicle formed on either hydroxyapatite or CaF<sub>2</sub> particles. Different protein association patterns were observed and future research will focus on the identification of these proteins and the application of caries and dental erosion models to examine the possible benefit of such synthesized CaF<sub>2</sub> particles for dental care.

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### Kinetics of Fluoride Binding and Release from *Streptococcus mutans*

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It has been suggested that dental biofilm bacteria could bind fluoride acting as a reservoir of this ion. However, the kinetics of binding and release of fluoride from biofilm bacteria has not been investigated, and was assessed in the present study. Pellets obtained from cultures of *Streptococcus mutans* IB1600 were exposed to PIPES buffer, pH 7.0 containing 1, 10, 100 or 225 ppm F at 37°C and the bacteria were separated from the treatment solution by centrifugation. To evaluate fluoride binding the pellets (n = 2 experiments) were treated with the fluoride solutions for 5, 10, 30 or 60 min, centrifuged and fluoride bound to the pellets was acid-extracted. To evaluate fluoride release (n = 3 experiments) the pellets were pretreated with the fluoride solutions for 10 min, centrifuged and the pellets were further treated with a low fluoride (0.019 ppm F) PIPES buffer, pH 7.0, at 37°C, for 10, 30 or 60 min. Fluoride bound and remaining in the pellet after release were determined using an inverted fluoride electrode. The amount of bound fluoride (nmol F/g) increased linearly with the fluoride concentration in the treatment solution, but did not change as a function of time (at 60 min, 1 ppm F =  $303.6 \pm 77.5$ ; 10 ppm F =  $435.1 \pm 43.7$ ; 100 ppm F =  $2,286.0 \pm 1,091.4$ ; 225 ppm F =  $7,139.3 \pm 377.4$ ). The kinetics of fluoride release was also fast and after 10 min, all fluoride was released from the bacteria to the buffered low fluoride treatment. The results suggest that biofilm bacteria can function only as short-term

fluoride reservoirs because although the bound amount is a function of the surrounding fluoride concentration, the release is fast.

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### Development of Techniques for the Analysis of Total Fluoride in Varnishes

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There is no universally accepted method to determine fluoride in varnishes. The toxicity of chloroform, the ISO-proposed solvent for varnishes prior to water extraction, prompted an investigation into the use of hexane. This study aimed at developing a method to test total fluoride in 5% NaF varnishes using hexane either with direct measurement or a modified hexamethyldisiloxane diffusion method. Different combinations of reagents and procedures were tested. Once a method was defined, 0.025 g of each varnish sample (n = 27) were weighed onto paper, placed into polypropylene tubes and 2.25 ml hexane were added. Tubes were capped, vortexed for 1 min and periodically swirled for 20–25 min to dissolve the varnish. Subsequently, 42.25 ml deionized water were added, solutions were vigorously mixed for 15 s, and allowed to separate. Then, the aqueous layer was sampled. Results showed fluoride concentrations ranging from  $25,478 \pm 137$  µg F/g (direct) and  $22,532 \pm 408$  µg F/g (diffusion) to  $4,506 \pm 39$  µg F/g (direct) and  $4,257 \pm 333$  µg F/g (diffusion). 21 products showed >90% recovery of labeled fluoride while six showed <90%. No significant differences were found among the methods ( $20,525 \pm 4,997$  µg F/g for direct vs.  $20,173 \pm 4,698$  µg F/g for diffusion) with an ICC = 0.97 for repeated measures. Results previously obtained using chloroform were not statistically significantly different when compared to those obtained using hexane (p = 0.53). The six products exhibiting <90% recovery of labeled fluoride were more viscous and resistant to dissolution. Hexane fluoride extraction for varnish analysis achieved repeatable values falling within 90% of labeled values when samples were completely dissolved. It is therefore concluded that the direct measurement of fluoride with a choice of hexane or chloroform as solvent can be used to analyze varnishes for total fluoride content.

The study was funded by the Oral Health Research Institute.

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### The Necessity of Making Bitewing Radiographs in the Diagnosis of Caries in Young Children

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Making radiographs in young children can be difficult. That is why bitewing radiographs are not made very often in children un-

der the age of 6. Nevertheless for adequate caries diagnosis they are mandatory. The guidelines of the European Academy of Paediatric Dentistry (EAPD) for the use of bitewing radiographs in children recommend taking bitewings for each 5-year-old child and before that age on indication. Research on early assessment to support this claim is however needed. The aim was to determine the diagnostic value of bitewing radiographs in making a treatment plan at young children. A total of 184 young children (age between 2 and 7 years, mean = 4.3, SD = 1.2) were selected. They were scheduled for treatment in general anesthesia (GA). During anesthesia three different pediatric dentists made treatment plans by clinical judgment only, using mirror, probe to remove dental plaque, light and air. After taking bitewing radiographs they made a second treatment plan. The difference between treatment plans were calculated and analysed. In 184 patients, 1,380 molars were diagnosed for possible treatment. In 24% of the children the treatment plan changed after examining the bitewing radiographs. Proximal caries was missed in 21% of molar teeth. When the child is 2 years old the chance of missing proximal caries was 3% and this percentage increased with age, with the highest percentage in 5-year-old children (37%). Furthermore, in the second treatment plan 30% more extractions were scheduled than were planned in the first treatment plan. This percentage was at its peak when children were 3 years old (36%). Bitewing radiographs are a necessary diagnostic tool in making treatment plans in children. Specific reasons vary with age.

The study was funded by ACTA paediatric postgraduate programme.

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### Survival of Cavitated Primary Teeth Treated by Three Treatment Protocols after 3.5 Years

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Cavitated primary posterior teeth were treated according to conventional treatment using amalgam (CRT), atraumatic restorative treatment using high-viscosity glass-ionomer (ART) and an ultra-conservative treatment protocol, in which small cavities were restored with ART and medium/large cavities regularly cleaned with toothpaste/toothbrush under supervision (UCT). The hypothesis tested was that the survival rates of teeth treated according to CRT and ART were significantly higher than that of UCT after 3.5 years. Teeth extracted because of toothache, sepsis or pulp exposure were failures. The PHREG procedure was used to estimate the survival curves. A total of 302 6–7-year-olds were treated. The numbers of treated teeth were 341 (CRT), 244 (ART) and 281 (109 small ART, 166 open cavities and 6 combinations) for the UCT group. The number of teeth extracted during the 3.5-year study period was 22 (CRT), 16 (ART) and 26 (UCT). There was an age

effect ( $p < 0.0001$ ) but no gender ( $p = 0.71$ ) and no d3mft effect ( $p = 0.75$ ) observed among the three treatment protocol groups at baseline. The 3.5-year cumulative survival rate of teeth treated with CRT was 90.9%, those with ART 90.4% and those with UCT 88.6%, which differences were not statistically significant (Wald test:  $p = 0.13$ ). In terms of tooth survival, the UCT protocol consisting of restoring small cavities using ART and cleaning medium/large cavities with toothpaste/toothbrush under supervision was no different than restoring primary posterior teeth according to either the CRT or ART protocol.

The study was funded by CNPq, FAP-DF, University of Brasília and Radboud University Nijmegen institutional funds.

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### Five-Year Follow-Up of a Randomized Clinical Trial on the Efficacy of Proximal Caries Infiltration

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The aim of this randomized split-mouth placebo-controlled clinical trial was to assess the efficacy of resin infiltration of proximal lesions in combination with self-applied non-operative measures compared with non-operative measures alone to inhibit lesion progression. In 22 young adults, 29 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, pre-product; DMG). A placebo treatment was performed in control lesions. All subjects received instructions for non-cariogenic diet, flossing and fluoridation. At baseline 32% of participants had a low, 36% a moderate, 23% an increased, and 9% a high caries risk (Cariogram 2.01). After 5 years 18 lesion pairs could be re-evaluated radiographically using standardized bitewing holders; another pair received a restoration earlier (response 66%). No unwanted effects were observed. Two lesion pairs dropped out due to a restoration of the study lesions by another dentist. Radiographic progression was recorded in 2/19 test lesions (11%) and 10/19 control lesions (53%) ( $p < 0.021$ ; McNemar test). Over a period of 5 years resin infiltration in combination with self-applied non-operative measures seems to be more efficacious in reducing lesion progression compared with self-applied non-operative measures alone.

The study was supported by DMG, Hamburg and conducted at Charité – Universitätsmedizin Berlin, which is hereby acknowledged.

### Reliability of Diagnosing Molar-Incisor Hypomineralization and Assessing Its Severity under Field Conditions

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The prevalence of molar-incisor hypomineralization (MIH) is relatively high worldwide and it has a significant impact on the restorative treatment needs in children. Therefore, not only caries but also the occurrence and severity of MIH should be assessed in oral health surveys of young people. This study aimed to assess the reliability of MIH diagnosis and its severity amongst forty-one children, 7 to 12 years old. Examiners were trained and calibrated by an expert using the detailed EAPD criteria and clinical pictures. After tooth brushing, examinations were performed by four examiners in the field under natural daylight using a dental mirror. Gauzes were used to control moisture. MIH was assessed at patient and tooth levels (permanent first molars and incisors). The scores were dichotomized into 'no MIH' and 'MIH', and into three categories ('no MIH', 'mild/moderate MIH' or 'severe MIH'). A 'gold standard' was determined by consensus among three experienced examiners. Results were expressed by proportion of agreement (%) and kappa coefficient value (k). Agreement with the 'gold standard' at patient level was: 100% and k = 1 for examiners A, B and C; 75% and k = 0.53 to 0.62 for examiner D. At tooth level, 94.5 to 98.2%; k from 0.80 to 0.93 for all examiners. Inter-examiner agreement at patient level ranged from 91.4 to 97.5% and k from 0.86 to 0.94 within A, B and C; from 74.3 to 80% and k from 0.56 to 0.76 including examiner D. At tooth level, from 93.5 to 97.3% and k from 0.70 to 0.86 for all examiners. MIH diagnosis was reliable under field conditions.

### Genome-Wide Association Study (GWAS) for Molar-Incisor Hypomineralization

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This genome-wide association study (GWAS) investigated the relationship between molar-incisor hypomineralization (MIH) and possible genetic loci. Clinical and genetic data from the 10-year follow-up of 668 children from the Munich GINI-plus and LISA-plus birth cohort studies were analyzed. The dental examinations included the diagnosis of MIH according to the criteria of the European Academy of Paediatric Dentistry (EAPD). Children with MIH were categorized as those with a minimum of one hypomineralized first permanent molar. DNA was extracted from blood samples and analyzed using the Affymetrix Human SNP Array 5.0 or 6.0 (Affymetrix, Santa Clara, USA) for each individual. A total of 2,013,491 single nucleotide polymorphisms (SNPs) were available for analysis. Due to the lack of replication samples, strict quality control criteria were used after data imputation to avoid false-positive results (proper information >0.50, minor allele frequency >7%, HWE p value >0.01). The loci that included a SNP with a p value <10 × 10<sup>-7</sup> for the association with MIH were plotted using LocusZoom to display the linkage disequilibrium. A genome-wide significance threshold of 5 × 10<sup>-8</sup> was assigned. All study protocols were approved by the local ethics committee at the Bavarian General Medical Council. 13.2% (n = 88) of the study population was identified as having MIH. In result of this GWAS Rs13058467, which is located near the SCUBE1 gene on chromosome 22 (p < 3.72 × 10<sup>-7</sup>), was identified as a possible locus linked to MIH when using a threshold of p < 10 × 10<sup>-7</sup>. After considering the limitations of the present study (e.g., limited sample size and lack of an independent replication sample), it can be concluded: (1) replication analyses in an independent cohort study are strongly recommended and (2) large-scale and well-powered studies are needed to investigate a possible genetic link to MIH.

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). GABA GmbH, Lörrach, Germany supported the dental examination and provided oral health care packages for all of the participating children.



## Session 8

# Erosion and Clinical Studies

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### Tin-Containing Fluoride Solution Combined with CO<sub>2</sub> Laser Irradiation for Erosion Prevention

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The aim of the study was to evaluate the effect of combined CO<sub>2</sub> laser and tin-containing fluoride treatment on formation and progression of enamel erosive lesions (10 days) *in vitro*. Ninety human enamel samples were obtained and stored in thymol solution. After surface polishing they were randomly divided into six groups (n = 15) according to different surface treatments: no treatment, as control (C); one CO<sub>2</sub> laser irradiation (L1); two CO<sub>2</sub> laser irradiations (L2); daily application of fluoride solution (F); combined daily fluoride solution + one CO<sub>2</sub> laser irradiation (L1F), combined daily fluoride solution + two CO<sub>2</sub> laser irradiations (L2F). Laser irradiation was performed at 0.3 J/cm<sup>2</sup> (5 μs, 226 Hz, 10.6 μm) on day 1 (L1) and 5 (L2). The fluoride solution contained AmF/NaF (500 ppm F<sup>-</sup>), and SnCl<sub>2</sub> (800 ppm F<sup>-</sup>) at pH 4.5. After surface treatment samples were submitted to an erosive cycling over 10 days, including immersion in citric acid (0.05 M, pH = 2.3, 2 min) 6 times daily and storage in remineralisation solution for at least 1 h between erosive attacks. At the end of each cycling day, enamel surface loss (μm) was measured using a 3D laser profilometer. Data were statistically analysed by means of ANOVA and Tukey tests (α = 0.05). After ten days F (-3.31 ± 2.0 μm) showed significantly lower enamel surface loss than C (-27.22 ± 4.1 μm), L1 (-18.3 ± 4.4 μm) and L2 (-15.4 ± 5.7 μm), but higher than L1F (-1.56 ± 4.0 μm) and L2F (1.2 ± 2.9 μm) (p < 0.05). Tin-containing fluoride solution caused 88% reduction of enamel surface loss while its combination with CO<sub>2</sub> laser irradiation at 0.3 J/cm<sup>2</sup> inhibited erosive loss almost completely. The combination of two CO<sub>2</sub> laser irradiations and treatment with a tin-containing fluoride solution avoided erosion lesion formation and progression after ten days of cycling *in vitro*.

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### Dental Erosion Prevention by Ca-Containing Dentifrices under Low Salivary Flow Rate Simulation

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Ca-containing fluoridated dentifrices were tested in an erosion model simulating low salivary conditions. Enamel and dentin specimens were prepared and randomly assigned into 5 experimental groups (n = 8), according to the test dentifrices: PD: 0 ppm F (placebo), RD: 1,100 ppm F/NaF (reference), HF: 5,000 ppm F/NaF (highly fluoridated), HF+CSP: 5,000 ppm F/NaF+calcium sodium phosphosilicate, and HF+TCP: 5,000 ppm F/NaF+tri-calcium phosphate. Specimens were individually placed in custom-made devices, creating a sealed chamber on the dental surface, connected to a peristaltic pump. Citric acid was injected into the chamber for 2 min (0.6 ml/min) followed by artificial saliva (0.05 ml/min), for 60 min, 4x/day for 3 days. 30 min into the 1st and 4th artificial saliva exposure, specimens were treated with dentifrice slurries for 2 min, followed by the remaining 30 min in saliva. Dental surface loss (SL, in μm), KOH-soluble fluoride and fluoride uptake (in μg/cm<sup>2</sup>) were determined after the cycling phase. Data were analyzed by one-way ANOVA and Tukey tests (α = 0.05). PD presented significantly higher SL, for both substrates (enamel: 5.79 ± 1.72; dentin: 10.50 ± 0.98). For enamel, none of the test dentifrices differed from RD (3.85 ± 0.94) or among each other (HF: 3.85 ± 0.68; HF+CSP: 3.27 ± 0.90; HF+TCP: 3.36 ± 1.07). For dentin, none of the dentifrices differed from RD (7.75 ± 0.77), but HF+TCP (6.97 ± 0.75) showed greater protection than HF (8.49 ± 0.61). HF+TCP presented the highest levels of KOH-soluble fluoride (enamel: 5.31 ± 2.70; dentin: 13.15 ± 2.58) and structurally bond fluoride (enamel: 0.97 ± 0.31; dentin: 1.43 ± 0.38) for both substrates, which was matched only by HF+CSP in the enamel fluoride uptake analysis (0.66 ± 0.14). Under the experimental conditions adopted, all fluoridated dentifrices were able to reduce enamel and dentin SL, with no additional benefit from higher fluoride concentration. Some formulations, especially HF+TCP, promoted

better fluoride delivery to the dental surface/structure; however, it did not significantly translated into better erosion protection.

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### Optical Coherence Tomography for Quantitative 3D Surface Topography Measurement of Erosive Enamel Wear in vitro

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The aim was to validate optical coherence tomography (OCT) for quantitative 3D assessment of enamel erosion in vitro. A clinically approved swept-source Fourier-Domain OCT (VivoSight OCT, Michelson Diagnostics Ltd, UK) underwent a validation process prior to an in vitro investigation comparing the effect of concentrated fluorides on erosive wear of human enamel by erosion (9 cycles of pH 3.2 citric acid erosion) and erosion-tooth brush abrasion (9 cycles of citric acid erosion followed by 2 min of tooth brush abrasion at 300 g loading), modified by fluoride containing surface treatments [Bifluorid10<sup>®</sup> varnish (45,200 ppm F), Duraphat<sup>®</sup> varnish (22,600 ppm F), Fluor Protector varnish (1,000 ppm F), Copal Ether Varnish (0 ppm F) and deionized water (0 ppm F)]. Data were analysed with ANOVA and a Bonferroni multiple comparisons test with  $p < 0.05$  considered significant. The OCT measurement process identified flatness and z axis non-linearity errors which were compensated for in the subsequent comparison with optical profilometry for measurement of simulated enamel wear in vitro. Following the simulated in vitro enamel wear the samples subjected to erosion alone showed no significant differences for the outcomes of the two optical measurement techniques (OCT  $7.9 \pm 1.5 \mu\text{m}$  vs. optical profilometry  $7.3 \pm 0.4 \mu\text{m}$ ,  $p > 0.05$ ); however after erosion-abrasion the OCT measured significantly greater 3D step height enamel loss than the profilometry (respectively  $12.3 \pm 3.0 \mu\text{m}$  vs.  $9.6 \pm 1.9 \mu\text{m}$ ,  $p < 0.001$ ). When the two optical techniques were applied to the effect of fluoride surface treatments in erosion and erosion-abrasion models there was an overall consistent 15–30% increase in the OCT measurement of step height enamel loss ( $p < 0.05$ ), however the between treatment group statistical comparisons were similar to those found with optical profilometry. OCT can be used for quantitative surface topography assessment for dental erosion in vitro, however the influence of measurement uncertainties, such as those demonstrated in this study, highlight the need for further calibration of OCT prior to assessment of worn enamel surfaces.

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### Infiltration of Proximal Caries: Safety and Clinical Effect

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Even after the caries decline, proximal caries is still a problem in clinical dentistry. The slow progression of proximal lesions allows preventive and minimally invasive treatment approaches. Thus, the purpose of this investigation was to test the safety and clinical effect of a new material for the treatment of proximal caries. In 50 patients (27 m, 23 f; mean age  $17.5 \pm 6.2$  years), the infiltration material ICON<sup>®</sup> (DMG, D) was applied on proximal initial lesions (D1–D3 without cavitation, on standardized X-ray) in primary and permanent teeth and subsequently monitored clinically and radiographically by visual-paired examination. In the clinical follow-up after one week, 6 and 12 months and also an X-ray evaluation after one year, all infiltrated surfaces showed smooth transition to enamel, no discoloration, and no inflammatory or allergic signs in the adjacent gingiva. No significant differences ( $p > 0.05$ , Friedman test) were found in plaque accumulation or gingival bleeding at the same tooth surface before infiltration and in the recall. The vitality of all infiltrated teeth stayed positive. On the radiographs, almost all infiltrated lesions ( $n = 42/45$ ) showed no progressions after one year indicating an arrest of the lesions. Three lesions showed minor progression on the standardized X-rays. The reasons for dropout were not associated with the study. Caries infiltration for initial proximal lesions showed no clinical problems and mostly resulted in complete arrest of the initial, non-cavitated caries when examined visually and with standardized radiographs.

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### Partial Caries Removal in Permanent Teeth: 5-Year Follow-Up

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This randomized, multicenter clinical trial evaluated the effectiveness of 2 treatments for deep caries lesions – partial caries removal (PCR) and stepwise excavation (SW) – with respect to the primary outcome of pulp vitality for a 5-year follow-up period. Inclusion criteria were as follows: patients with permanent molars presenting deep caries lesions (lesion affecting  $\geq 1/2$  of the dentin on radiographic examination), positive response to a cold test, ab-

sence of spontaneous pain, negative sensitivity to percussion, and absence of periapical lesions (radiographic examination). Teeth randomly assigned to PCR (test) received incomplete caries removal and filling in a single session. Outcome success was evaluated by assessment of pulp vitality, determined by pulp sensitivity to a cold test and the absence of periapical lesions. Data were analyzed by a Weibull regression model with shared frailty term (survival analysis). At baseline, 299 treatments were executed: PCR, 152 and SW, 147. By the end of the 5-year follow-up period, 229 teeth had been evaluated. Adjusted survival rates were 79% for PCR and 56% for SW ( $p = 0.001$ ). The outcome was favorable when treatment was PCR, compared to SW; when one surface was restored, compared to 2 or more; and when the Midwest region was compared to the South. Association was found between outcome failure and number of absences to follow-up appointment. Patients who failed to show to 3–4 follow-up appointments had significantly more treatment failures than those who missed 0–2 appointments. Patients from the South region were also significantly more absent than those in the Midwest ( $p = 0.00$ ). These results suggest that there is no need to re-open a cavity and perform a second excavation for pulp vitality to be preserved (NCT00887952).

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### Using ICDAS and QLF-I Combined to Predict Caries Lesion Progression

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As a mean to increase sensitivity of visual examination adjunctive aids have been suggested. This study's purpose was to combine a standardized visually-based system, the International Caries Detection and Assessment System (ICDAS) with the sensitive fluorescence-based system, quantitative light-induced fluorescence (QLF) to determine if the predictive ability of the visual method increases. 565 children (5–13 years old) were consented, assented, enrolled and examined at baseline and regular intervals over 48 months with the ICDAS, yearly radiographs and QLF. 338 children completed all the exams. The Spearman correlation coefficients between ICDAS and QLF in combination with ICDAS (QLF-I) and between ICDAS and ICDAS/QLF-I Combo were both 0.89. The addition of QLF exam increased the number of surfaces scored as 1, 2 and 4, with the highest difference among scores 2 (2.54% more surfaces) followed by scores 1 (2.09%) and 4 (0.41%). The odds of progression by age, surface type and ICDAS score at baseline, analyzed by logistic regression, were higher for the ICDAS/QLF-I Combo exam, but when only the first two years were considered, the odds of progression were higher for the ICDAS exam alone. The differences were more pronounced for comparisons with ICDAS score 4 versus unerupted: ICDAS – OR

50.93 (CI 28.67, 90.46)/ICDAS/QLFI – OR 82.35 (39.42, 172.03); followed by sound: 18.15 (10.26, 32.1)/26.73 (12.84, 55.64); inactive score 2: 4.36 (2.29, 8.29)/9.72 (4.05, 23.31); active score 1: 5.59 (3.15, 9.91)/8.79 (4.2, 18.41); inactive score 1: 7.07 (3.87, 12.9)/9.32 (4.19, 20.69); active score 2: 2.8 (1.6, 4.89)/4.38 (2.15, 8.95); and score 3: 0.97 (0.51, 1.83)/1.65 (0.75, 3.6) respectively. In conclusion, the addition of the QLF exam to ICDAS provides a modest increase in predictive ability.

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### Exfoliation Pattern of Primary Molars Treated according to Three Protocols

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The aim of the study was to evaluate the exfoliation pattern of primary molars treated according to three protocols. The hypothesis tested was that there is no difference in the exfoliation pattern of primary molars treated according to conventional restorative treatment using amalgam (CRT), atraumatic restorative treatment using high-viscosity glass-ionomer (ART) and ultra-conservative treatment (UCT), which consisted of restoring small cavities with ART and cleaning medium/large non-restored cavities daily with toothpaste/toothbrush under supervision, protocols. A sample of 302 children aged 6–7 years old from a suburban deprived area of Brazil were followed up for 3.5 years. The numbers of treated molars were 341 (CRT), 244 (ART), and 281 (UCT). Exfoliation percentages were obtained using the PHREG procedure. Differences between the three treatment protocol groups were tested using the Wald test. After 3.5 years, the percentages of all primary molars exfoliated were 51.0% (CRT), 48.7% (ART), and 48.1% (UCT) ( $p = 0.62$ ). Despite the absence of a statistically significant difference between the groups, the pattern of exfoliation during the 3.5 years differed depending on type of molar ( $p < 0.0001$ ). For first primary molars, a higher percentage of molars from the UCT group had exfoliated than that from CRT at the 1-year evaluation ( $p = 0.035$ ). For second primary molars, a higher percentage of molars from the UCT group had exfoliated than those from ART at both 2-year ( $p = 0.009$ ) and 3-year ( $p = 0.036$ ) evaluations, and than that from the CRT group at 3-year evaluation ( $p = 0.036$ ). In conclusion, the three treatment protocols led to similar exfoliation percentages of all primary molars after 3.5 years, but the molars treated according to UCT exfoliated earlier than those of the CRT and ART groups, especially second primary molars.

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### Dental Erosion of Permanent Incisors and Primary Molars in Mixed Dentition Children in Pusan

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The purpose of this study was to survey the prevalence and severity of dental erosion in mixed dentition children, and to assess some relevant risk factors, especially focusing on the newly erupting permanent incisors and nearly exfoliating primary molars with two separate methods and populations. This twofold study was performed by two independent surveys: first was performed in 2011 on 5,116 tooth surfaces of maxillary permanent incisors from 664 children of 8–9 years age using Visual Erosion Dental Examination (VEDE) system modified from Lussi's Index, second, on 4,832 primary molars from 788 children with age of 9–10 years living in Pusan city in 2012 using Modified Linkosalo and Markkanen system by Ganss. Relevant risk factors were assessed by questionnaires asking some consumption modes of erosive beverages and foods. The data were processed statistically with Clopper-Pearson method, chi-squared test, independent t test and logistic regression analysis. The prevalence in young permanent incisors was 36.5% (242/664), showing severity distribution of 58.3%, 26.0%, 15.7% in score 0, 1, 2 separately without score 3, 4, 5, and positive relationship with preference and consumption frequency of carbonated beverages and fruit juices ( $p < 0.05$ ). The total prevalence in primary molars nearing exfoliation was 27% (213/788). By the prevalence of primary teeth, 9.04% (437/4,832) of total primary molars showed erosion, with higher value in mandibular primary molars (287/2,380, 12.06%) than those of maxilla (150/2,452, 6.12%) ( $p < 0.05$ ), and mandibular left first primary molars (17.9%) at top ranking. According to the analysis of risk factors, frequent intake of carbonated drinks and fruit juices, and using straw at drinking showed significant relationship with development of erosion ( $p < 0.05$ ). In conclusion, relatively high prevalence of tooth erosion was found in mixed dentition children both in young permanent incisors and shedding primary molars.

### Saliva Buffering Capacity in Relation to Consumption of Energy Drinks

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**Objective:** To determine the effect produced by the buffering capacity of saliva on the pH of energy drinks. **Methods:** Thirteen energy drinks on the market of Chile were examined. Saliva samples were obtained from three systemically healthy young patients

(aged 18–25 years), who did not present with any pathology that affects the salivary glands and/or any defects in the quality and quantity of saliva. The saliva samples were grouped in a 'pool' of saliva. 5 ml of energy drink was added to each of four tubes. To one tube 5 ml of water was added and the other three were replicates with 5 ml of mix energy drink added. Before adding water or saliva a measurement was performed, first with 5 ml of energy drink, to calculate the pH of the beverage by itself. Then 1 ml of water/saliva were added until 13 ml of solution was completed. The measurements were performed every 3 min to allow the pH meter register the pH correctly. This procedure was performed with each of the energy drinks. **Results:** pH ranges of energy drinks range from  $2.42 \pm 0.008$  (Battery-Gingered<sup>®</sup>) to  $3.44 \pm 0.005$  (Battery Sugar-Free<sup>®</sup>). While the pool of saliva on average had a pH value of 7.99 and 7.05 water. The beverage Speed<sup>®</sup> increases in higher measure the pH after adding the saliva, reaching a pH value of 4.38, while the less pH increase was achieved by the beverage Quick Energy<sup>®</sup> reaching a pH value of 3.37. **Conclusion:** The buffering capacity of saliva is not capable of increasing the pH of energy drinks above the critical pH 5.5 in a solution of 38% (v/v) of energy drink with saliva.

### Protective Effect of Calcium and Fluoride Rinses on Dentin Erosion under Low Salivary Flow Conditions

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Calcium pre-treatment has been suggested to increase the cariostatic effect of sodium fluoride, but its effect on erosion is unclear and was investigated. Dentin specimens were assigned into 6 groups ( $n = 8$ ): deionized water (DIW), NaF (12 mM) or calcium lactate (150 mM) followed by NaF (CaL+NaF); and salivary flow rates: 0.5 or 0.05 ml/min, simulating normal (NF) and low (LF) salivary flow rates, respectively. The specimens were placed in custom-made devices, creating a sealed chamber on the enamel surface connected to a peristaltic pump. Citric acid (0.3%, pH 3.8) was injected into the chamber for 2 min, followed by artificial saliva at 0.5 or 0.05 ml/min for 60 min. This cycle was repeated 4x/day. 30 min after the 1st and 4th erosive challenges, specimens were treated with the rinse solutions. The erosion-remineralization cycles and rinse treatments were performed for 3 days. Surface loss (SL,  $\mu\text{m}$ ) was determined by optical profilometry. KOH-soluble and enamel fluoride uptake ( $\mu\text{g}/\text{cm}^2$ ) were determined at the end of the experiment. Data were analyzed by two-way ANOVA and Tukey test ( $\alpha = 0.05$ ). In NF conditions, DIW showed significantly higher SL ( $8.71 \pm 1.72$ ) than NaF ( $6.41 \pm 1.63$ ) and CaL+NaF ( $5.66 \pm 1.57$ ), which did not differ from each other. In LF conditions, significant differences were observed among all groups (DIW:  $14.55 \pm 2.95$ ; NaF:  $10.08 \pm 1.51$ ; CaL+NaF:  $6.65 \pm 1.19$ ). The within rinse treatments comparison showed that higher SL was found when associated to the low flow, except for CaL+NaF ( $p =$

0.293). Higher KOH-soluble fluoride was observed for CaL+NaF (LF:  $4.25 \pm 2.80$ ; NF:  $2.46 \pm 2.34$ ), followed by NaF (LF:  $2.04 \pm 6.45$ ; NF:  $0.79 \pm 1.52$ ) and DIW (LF:  $0.20 \pm 0.38$ ; NF:  $0.23 \pm 0.24$ ). Fluoride uptake was not different between salivary flows ( $p = 0.636$ ) and DIW (LF:  $0.17 \pm 0.06$ ; NF:  $0.17 \pm 0.07$ ) < NaF (LF:  $0.87 \pm 0.29$ ; NF:  $0.91 \pm 0.28$ ) = CaL+NaF (LF:  $1.31 \pm 0.36$ ; NF:  $1.14 \pm 0.55$ ). It was concluded that the calcium lactate pre-rinse increased KOH-soluble fluoride availability, enhancing the sodium fluoride protection against erosion in low salivary flow conditions.

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### Anti-Erosion Effects of a High Fluoride Dentifrice on Natural and Polished Enamel Surface

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Most studies of the anti-erosion efficacy of fluoride compounds had been carried out with polished samples. However there may be a difference in the response between natural and polished enamel surface. The aim of this study was to evaluate whether there is a difference in the efficacy of a high fluoride dentifrice in reducing the rate of early erosion between polished and natural surface human enamel. A double-blind, split mouth, in situ randomised clinical trial was conducted. 20 volunteers wore two mandibular appliances, each embedded with one human enamel with natural surface (Ns) and one with polished surface (Ps). The participants wore the appliances for 5 days from 09.00 to 16.00. Each day they swished a total of 250 ml orange juice around their mouth for 10 min at three separate intervals. After the 1st and 3rd acid challenge, the appliance carrying samples of the treatment group was subjected to in vitro treatment with Colgate Duraphat5000 for 2 min whilst the other appliance were immersed in deionised water. Surface microhardness measurements were made at baseline and at the end of each day. Percentage of surface microhardness change ( $\Delta$ SMC) was the outcome measure. At the end of the study, the mean difference  $\pm$  SE of  $\Delta$ SMC between the treated and control groups of the Ns and Ps group were  $19.1 \pm 7.2\%$  and  $4.45 \pm 3.89\%$ , respectively. Multiple regression analysis of  $\Delta$ SMC with erosion interval showed that the treatment and control group of the Ns specimens demonstrated significant differences ( $p = 0.036$ ) while the Ps group did not ( $p = 0.248$ ). It can be concluded that treating natural surface enamel with Duraphat 5,000 ppm increased its resistance against concurrent early in vivo erosive challenge with an orange juice challenge regime.

The study was funded by Colgate Palmolive (UK).

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### Effects of Varying Concentrations and Immersion Times of Acid on Human Enamel during Erosion/Abrasion

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The aim was to study the effects of citric acid and abrasion on enamel in vitro. Extracted caries-free teeth were sectioned to produce a total of 360 highly polished enamel samples. Sections were immersed in citric acid (pH 3.2) at 0.3, 0.6 and 1% and for 5, 10, 15, 20 min together with 120 and 240 liner strokes. Each section was embedded in composite, with a window of exposed enamel ( $\sim 1 \times 3$  mm) and separated into 36 groups. Each group of 10 samples, underwent 5 cycles of erosion (1 cycle = immersion in acid  $\rightarrow$  rinse with distilled water) at room temperature, in 80 ml of solution, with agitation (62.5 rpm) at each concentration and immersion time; and in addition for erosion/abrasion (1 cycle = erosion  $\rightarrow$  abrasion on toothbrushing machine, at 120 and 240 with Oral B P40 toothbrush, 290–295 g of force, in non-fluoridated toothpaste slurry). Samples were analysed with non-contact laser profilometry and single/mean step height was calculated using 3 different surface analysis programs (Boddies, Image J and Mountains). Repeatability of the 3 surface analysis programs was calculated using intra-class correlations. 3-way ANOVA and multiple linear regressions was used to analyse the effect of concentration, time and abrasion on step height. There was excellent agreement between the surface analysis techniques (96.7%). The mean step height  $\pm$  standard deviations ( $\mu$ m) for: erosion 0.3% CA/5 min was  $3.66 \pm 0.61$ ; 1% CA/20 min  $40.89 \pm 7.10$ ; erosion/abrasion with 120 LS at 0.3% CA/5 min was  $7.56 \pm 1.26$  and 1% CA/20 min was  $35.10 \pm 5.01$ ; erosion/abrasion with 240 LS at 0.3% CA/5 min was  $5.68 \pm 0.64$  and 1% CA/20 min was  $41.94 \pm 4.44$  ( $p > 0.01$ ). In conclusion, within this laboratory model an increase in time and concentration produced higher step heights for erosion and erosion/abrasion. All experiments showed a limit in step height.

This study was supported by Proctor and Gamble.

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### Efficacy of Silver Diamine Fluoride in Inhibiting Dental Erosion in Different in vitro Models

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This study aimed to assess the efficacy of silver diamine fluoride (SDF) in inhibiting mineral loss during in vitro erosive challenge in primary teeth and to compare it to other usual forms of fluoride.

The sample comprised eighty primary canines. An area on buccal surfaces was delimited by an acid-resistant varnish. Teeth were randomly distributed in eight groups according to pre-treatment solutions: 2% NaF solution (n = 20), 10% SDF solution (n = 20) and distilled water (n = 40). Specimens were immersed in each respective solution for 24 h. Subsequently specimens underwent 10-minute erosive acid challenges using 1% citric acid (n = 10 per group) and 10% citric acid (n = 10 per group). 20 specimens were not exposed to erosive challenge nor to any pre-treatment and remained in distilled water (negative control group). At the end, 3 ml of the citric acid or distilled water solutions were collected for calcium and phosphate concentration analysis by the atomic emission spectrometer and loss of minerals was calculated per area exposed to the challenge ( $\mu\text{g}/\text{mm}^2$ ). Variance analysis and Tukey's test were used to compare groups and different erosive challenges. The specimens treated using SDF presented lower phosphate loss compared to non-pre-treated specimens ( $2.95 \pm 3.10$ ) and to the NaF specimens ( $2.23 \pm 1.69$ ) when exposure to 10% citric acid ( $p < 0.05$ ). When exposure to 1% citric acid lower losses were found for NaF ( $0.15 \pm 0.05$ ) and SDF groups ( $0.13 \pm 0.02$ ) compared to non-pre-treatment ( $1.34 \pm 1.05$ ) ( $p < 0.05$ ). Calcium loss was similar for all groups' exposure to any erosive challenge, independent of pre-treatment received ( $p > 0.05$ ). In conclusion, 24-hour treatment with 10% SDF enables, in vitro, lower phosphate loss on primary teeth in high acid concentration challenge compared to other fluoride products.

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## No Correlation between Caries and Erosion in Adolescents

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There is a distinct caries decline in children and adolescents in many countries. In recent years, other dental hard tissue alterations like erosions have attracted scientific attention, and from some countries an increase in erosion prevalence is reported. As the development both of caries and erosions is influenced by diet, the hypothesis was posed that there is a negative correlation between carious and erosive tooth findings. In a field survey in Hamburg, Germany, 759 schoolchildren aged 12–15 years were examined for caries and dental erosions by two calibrated investigators. Each tooth surface was recorded for caries and erosions separately. Statistical evaluation included determination of prevalence rates and mean number of affected teeth and surfaces as well as correlation analyses. Caries prevalence was 38.3%, and the prevalence of erosions was 25.4%. 10.4% of the children exhibited both caries and erosions, while 46.6% showed none of these findings. The mean DMFT score was  $1.0 \pm 1.8$  (girls  $1.2 \pm 2.0$ , boys  $0.9 \pm 1.7$ ;  $p = 0.015$ ), and on average  $1.2 \pm 3.0$  teeth showed erosions. Neither the prevalence rates of caries and erosions ( $\chi^2$  test:  $p = 0.391$ ) nor the mean number of affected teeth (Pearson correlation:  $p = 0.257$ ) were significantly related. The same result was obtained when the calculation was performed for girls and boys separately. In girls, who exhibited significantly higher DMFT scores than boys, there was a tendency towards lower numbers of erosion-affected teeth in caries-free subjects ( $0.9 \pm 2.6$  teeth), compared with girls with caries ( $1.6 \pm 3.9$ ). It can be concluded that the prevalence of caries and dental erosions and the mean number of involved teeth are independent in schoolchildren in Hamburg.

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