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Abstracts

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Session 1 Microbiology

1

Interproximal Biofilm Removal by Intervallic Use of a Sonic Toothbrush Compared to an Oral Irrigation System

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The purpose of the study was to investigate biofilm removal in interproximal tooth regions after intervallic cleaning with an oral irrigator or a sonic toothbrush.

Three-species biofilms (*S. mutans* UAB 159 (OMZ 918), *S. oralis* SK 248 (OMZ 60), *A. naeslundii* OMZ 745) were grown on hydroxyapatite discs for 3 days in FUM culture media. Every 24 h, metabolic activity at baseline was measured in relative fluorescence units (rfu) using the alamarBlue assay and a fluorescence spectrophotometer. Then, specimens were fixed in interproximal holding devices and underwent treatment with an oral irrigator (WF; Waterpik® Sensonic WP-100E), an active (WPa), or an inactive sonic toothbrush (Wpi; Waterpik® Sensonic SR-3000E) for 10 s (n = 12/group). Untreated biofilms served as controls (CO). After treatment, bacterial activity was remeasured, and specimens were regrown in fresh medium for 24 h until next cleaning procedure. Cleaning was repeated in intervals of three treatment days (d1, d2, d3). Metabolic activity was analyzed for each disc separately, and ratios of baseline and post-treatment rfu values were compared. Results were analyzed using ANOVA with the post-hoc Scheffe test, or Kruskal-Wallis with post-hoc Mann-Whitney test. Averaged baseline values of d1 resulted in 8,117.7±1,615.5 rfu. Highest reduction in metabolic activity occurred with the oral irrigator (residual activity d1: WF 29.0%, WPa 60.6%, WPi 83.5%, CO 84.7%; d2: WF 32.0%, WPa 86.6%, WPi 80.4%, CO 88.1%; d3: WF 17.8%, WPa 61.0%, WPi 83.8%, CO 85.8%).

In conclusion cleaning of interproximal regions was achieved greatest success using an oral irrigator.

2

Molecular Biological Analysis of Bacterial Metabolic Activity of Sound and Cariogenic Biofilm

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The aim of the present study was to establish molecular biological methods to identify the metabolic activity profiles of some caries associated bacteria in the biofilm from sound occlusal surfaces and cavitated occlusal caries lesions. Plaque samples (one each) were obtained from sound occlusal surfaces of subjects without any caries lesions (caries-free subjects, CFS, n = 10) as well as with active cavitated caries lesions (caries active subjects, CAS, n = 13). From subjects with active caries lesions one plaque sample was also taken from a cavitated lesion (Nyvad 3) (CAC, n = 13). Species-specific and genus specific real-time quantitative PCR assays were developed, focusing on *Lactobacilli*, *Bifidobacterium dentium*, *Scardovia wiggsiae* and *Streptococcus mutans* and targeting the 16S-gene or the 16S-rRNA, respectively. As the 16S-rRNA level represents the number of ribosomes, while the 16S-gene level represents the number of cells, the quotient (ribosomes/cell) gives a measure for the metabolic activity of the respective species. A clear difference of the total bacterial metabolic activity between the three plaque/subject groups could be observed. The biofilm of sound surfaces had significantly higher numbers of ribosomes per cell for CFS than CAS (Mann-Whitney test, $\alpha = 5\%$, $p = 0.008$), for CAS activity being slightly lower than for CAC, though not significant. However, CAS and CAC biofilm showed a tendency towards higher metabolic activity of *Lactobacilli*, *S. mutans* and *S.*

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wiggisiae than CFS. In conclusion, we established a 16S targeted real-time quantitative PCR on DNA and RNA level to analyze the differences in metabolic activity of four bacterial species in biofilms from caries lesions or sound surfaces in caries active but also caries-free patients. Nonetheless, high metabolic activity of biofilm of sound surfaces in caries-free subjects needs further exploration.

3

Photodynamic Antimicrobial Chemotherapy on Mature *Streptococcus mutans* Biofilms

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The effect of photodynamic antimicrobial chemotherapy (PACT), by the association of toluidine blue-ortho (TBO-100 µg/ml) and a non-coherent red light (630 nm), energy densities 211.37 and 422.74 J/cm² (1 and 2 min irradiation-time, respectively), on the microbial viability, polysaccharides and topography of mature *Streptococcus mutans* biofilm, was established. Intracellular reactive oxygen species (ROS), and potential cytotoxic effects on oral tissues were determined. *S. mutans* UA159 biofilms were formed on saliva-coated hydroxyapatite discs. PACT was applied in a single dose on the mature biofilm. Chlorhexidine digluconate (CHX) 0.12% was used as positive control. Biofilms were analyzed immediately after treatment and the analysis were performed in triplicates. Variable pressure scanning electron microscopy was used to check biofilm topography. Production of ROS in *S. mutans* biofilm cells was determined by an oxidative-stress-sensitive probe. The cytotoxic effects were evaluated determining LDH (lactate dehydrogenase) by the EpiOralTM tissue model. PACT-2-minutes irradiation achieved a 5-log reduction of colony-forming units/mg of biofilm (CFU/mg), statistically different from all other groups ($p < 0.05$). PACT-1-minute irradiation presented a 3-log reduction when compared to the control. No differences were observed in the amount of soluble and insoluble extracellular polysaccharides among all experimental groups ($p > 0.05$). PACT-2-minutes presented the highest levels of ROS production ($p < 0.0001$) followed by PACT-1-minute ($p < 0.05$). The percent cytotoxicity of PACT-2-minutes irradiation was 1.29%, and CHX, 3.33%. The results show that PACT performed with red light and TBO may be a promising and safe therapy against mature biofilms. Further studies are needed to assess the PACT effects on in situ and in vivo biofilms.

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4

Molecular Detection of Bacteria Associated to Caries Activity in Dental Lesions

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Early childhood caries (ECC) is a microbial infection that severely compromises the dentition of young children. Few studies have focused on oral bacterial community changes within carious lesion activity. The aim of this study was to identify the presence of 7 bacterial species/groups related to dental caries/health: *Actinomyces naeslundii*, *Bifidobacterium* spp., Mitis group, *Lactobacillus acidophilus*, *Lactobacillus casei* group, *Streptococcus gordonii* and *Streptococcus mutans* in active and inactive carious dentine lesions in ECC-children by using quantitative polymerase chain reaction (qPCR). Fifty-six dentine lesions samples, classified as active ($n = 39$) or inactive ($n = 17$), were collected from children aged 2 to 5 years. Relative quantification revealed that *Bifidobacterium* spp. and *L. casei* group were significantly more abundant in active dentine lesions ($p < 0.05$). Concentrations of *A. naeslundii*, Mitis group and *S. gordonii* were not significantly different when comparing dentin lesion activity. The relative proportion of *S. mutans* was significantly greater in inactive than in active lesions ($p < 0.05$). *Bifidobacterium* spp. and *L. casei* group demonstrated a positive correlation ($p = 0.001$) in active lesions. The positive detection of *L. acidophilus* (OR = 15.1) and *S. gordonii* (OR = 7.7) was significantly associated to the active lesions. The data indicates that higher detection levels of *Bifidobacterium* spp. and *L. casei* group may be indicators for dentin lesion activity. Also, the presence of *L. acidophilus* and *S. gordonii* was associated to the lesion activity. Future research should be performed to establish how consistent the levels of certain bacteria might influence the caries activity status of dental lesions.

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Identification and Functional Analysis of Genome Mutations in a Fluoride-Resistant *Streptococcus mutans* Strain

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It is known that fluoride-resistant microorganisms are different from the fluoride-sensitive ones in growth, adherence and metabolic activity. It was hypothesized that these phenotypic differences were due to the stable genotypic changes in the genome of the fluoride-resistant strains. However, until now no study has identified them. The aim of this study is to identify such changes in a fluoride-resistant *Streptococcus mutans* strain (C180-2FR) using whole genome shotgun (WGS) sequencing and to examine the potential function of the identified mutations by comparing the gene expression between the fluoride-sensitive (C180-2) and C180-2FR strains. We performed 50 bp paired-end Illumina shotgun sequencing for both strains. Through extensive bioinformatic analysis, we were able to identify 8 single nucleotide polymorphisms (SNPs) in the genome of C180-2FR, which were further confirmed by Sanger sequencing. The expression of the genes containing or related to the SNPs in C180-2 and C180-2FR was then quantified by real-time PCR. A gene cluster containing genes coding for fluoride antiporters was 10-fold up-regulated in C180-2FR when compared to those in C180-2 ($p < 0.05$). Two SNPs are located in this gene cluster, one in its promoter region and the other in its protein-coding region. In addition, two genes, involved in bacterial growth and membrane transport, were found 2-fold down-regulated in C180-2FR. One SNP was located in the region containing the promoters of these genes. No difference in expression was found for the other SNP-containing genes. In summary, using WGS sequencing, we were able to uncover genetic changes in the genome of a fluoride-resistant strain. These findings can provide new insights into the mechanism of microbial fluoride resistance.

This study was funded by Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam, University of Amsterdam and VU University Amsterdam.

Effects of Chlorhexidine Containing Varnish on Oral and Dental Health in High Risk Patients

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The purpose of this study was to determine the effects of chlorhexidine containing varnish on level of oral acidic bacteria [*Streptococcus mutans* (SM) and *Lactobacilli* (LB)] and on development of White Spot Lesions (WSL) in patients with fixed orthodontic appliances. Altogether 29 patients were examined (mean age 16.5 ± 2.75 ys). At baseline levels of acidic bacteria were determined in saliva and plaque using chairside tests (CRT Bacteria, Ivoclar-Vivadent, Schaan, Liechtenstein) and number of WSL was registered. After placing the fixed orthodontic appliance Cervitec Plus and Placebo varnishes (Ivoclar-Vivadent, Schaan, Liechtenstein) were applied around brackets and tubes randomly in the right and the left quadrants of the same dental arch (Test and Control sides). Varnishes were used according to the instructions of manufacturer's guide, applied monthly during a six months period. Every occasion before application of varnishes the level of SM and LB were determined in saliva and level of SM in plaque on 1st, 3rd, 5th, 6th teeth in the same way. At the sixth month the number of new WSL was determined. Wilcoxon test, descriptive statistics were used. The ratio of saliva samples belonging to low-risk category was significantly more frequent ($p \leq 0.05$) compared to baseline both in SM (76 vs. 52%) and LB (69 vs. 52%) groups from 2nd month. During the six month period level of SM in plaque decreased continuously in both sides. By the end of the period reduction was significantly greater in test, than in Control sides (6.69 ± 1.71 , 4.45 ± 1.60 , respectively) ($p \leq 0.05$). Mean number of new WSL was significantly lower in test (0.06 ± 1.60), than in Control quadrants (1.13 ± 1.50) ($p \leq 0.05$). **Conclusion:** Chlorhexidine varnish can reduce level of acidic bacteria in saliva and plaque, and development of caries lesions.

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Microscopic Monitoring of Extracellular pH in Dental Biofilms

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pH in dental biofilm is a key virulence factor for the development of caries lesions. The complex three-dimensional architecture of dental biofilms leads to steep gradients of nutrients and metabolites, including organic acids, across the biofilm. For decades, measuring pH in dental biofilm has been limited to monitoring bulk pH with electrodes. Although pH microelectrodes with a better spatial resolution have been developed, they do not permit to monitor horizontal pH gradients in real-time. Quantitative fluorescent microscopic techniques, such as fluorescence lifetime imaging or pH ratiometry, can be employed to map the pH landscape in dental biofilm with more detail. However, when pH sensitive fluorescent probes are used to visualize pH in biofilms, it is crucial to differentiate between extracellular and intracellular pH. Intracellular microbial pH and pH in the extracellular matrix differ considerably, and only extracellular pH in dental biofilms affects the underlying tooth. We here developed a method to reliably monitor extracellular pH in dental biofilm microscopically with the ratiometric pH-sensitive dye C-SNARF-4. Fluorescent emissions of C-SNARF-4 can be used to calculate extracellular pH irrespective of the dye concentration. We showed that at pH <6, C-SNARF-4 stained 15 bacterial species frequently isolated from dental biofilm and visualized the entire bacterial biomass in dental biofilms grown intraorally on glass slabs mounted on individually designed lower jaw splints. We then employed digital image analysis to remove the bacterial biomass from the microscopic images and adequately calculate extracellular pH values. As a proof of concept, we monitored the extracellular pH drop in six replicate dental biofilms fermenting glucose. The observed pH drops differed between biofilms and also between different microscopic fields of view within the same biofilm, with pH values ranging from 6.6 to 5.3, five min after exposure to glucose. The combination of pH ratiometry with C-SNARF-4 and digital image analysis allows accurate monitoring of extracellular pH in dental biofilms in three dimensions in real-time.

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Transcriptomic Analysis of 20 Lactobacillus Species in Root Caries: An RNA-Seq Approach

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The aim of the study is to evaluate the gene expression profiles of lactobacilli present in sound root surface biofilms (SRS) and in root caries (RC). Root surface biofilms were collected from 10 volunteers for the SRS group. Carious dentin was collected from 30 volunteers with active RC. Total bacterial RNA was extracted from each collected sample. Some carious dentin samples were pooled (disease group; n = 9). cDNA libraries were prepared and sequenced on the Illumina Hi-Seq2500 system. Sequence reads were mapped to 20 lactobacilli genomes related to RC. Count data were obtained and normalized using the R package DESeq to obtain gene expression (NGE) values. Median (25th–75th) of the gene expression, number of transcripts and metabolic pathways were analysed. Lactobacilli presented lower gene expression in SRS (NGE median = 32.1; 25th–75th = 16.8–80.1) than in RC (NGE median = 10,116.4; 25th–75th = 984.8–26,305.7). The total proportion of transcripts in the SRS group comprised only 9% of expressed genes while in the RC it was 45%. The highest gene expression median in SRS was from *L. acidophilus* (NGE median = 972.38). The species with highest gene expression in RC were *L. delbrukii*, *L. gasseri* and *L. rhamnosus*. In the SRS, *L. gasseri* produced the highest proportion of transcripts (57.8%), whereas *L. rhamnosus* presented the highest proportion of transcripts (95.9%) in RC. Almost all species presented high gene expression for *atp* (ATP synthase, Energy metabolism), *eno* (enolase, Glycolysis/Gluconeogenesis), *rpoA/rpoB* (DNA-directed RNA polymerase, Nucleotide metabolism) and *slpX* (surfacylayer protein) genes and some ribosomal proteins, meaning that the glycolysis pathway is active and the cells are protecting themselves against stress condition in the carious environment. Lactobacilli in root caries are processing sugar, growing and expressing virulence factors.

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Dental Biofilm Structure Under Xylitol or Sucrose Exposure

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Carbohydrates and saccharides are the most important energy sources for microorganisms in dental biofilms. By degradation of carbohydrates biofilm bacteria produce acids and contribute causally to caries development and progression. Extracellular polysaccharides (EPS) synthesised by many bacteria are an essential mechanism of pathogenesis in the biofilm matrix. They influence diffusion behaviour in biofilms and promote accumulation of nutrients and metabolic products.

Using sugar substitutes is a complementary way for caries prevention. The sugar alcohol xylitol cannot be metabolised efficiently by oral microorganisms.

The aim of this study was to investigate the effect of xylitol or sucrose on biofilm morphology and vitality during in vivo dental biofilm formation. Intraoral splints supplied with standardized human enamel slivers were used for in vivo generation of biofilms. The biofilms were exposed ex vivo either to 50 ml of a xylitol (25%) solution, sucrose (10%) solution or saline (control) six times during a period of 24 h. Biofilms were analyzed by CLSM after staining with Syto9/propidium iodide or fluorescently labelled Concanavalin A and wheat germ agglutinin. Biofilm thickness (BT), surface area coverage (C), vitality (V), and EPS production were measured. Statistical analysis was performed using ANOVA and the Tukey-Kramer test.

BT was significantly reduced after xylitol exposure (25 µm) compared to sucrose exposure (42 µm) or the control group (41 µm). C (xylitol 36%, sucrose 44%, control 42%) and V (xylitol 82%, sucrose 78%, control 82%) were similar. Xylitol led to a statistically significant reduction of the mean EPS activity (55%) compared to the control (62%). Sucrose exposure did not influence mean EPS activity (58%) significantly.

In conclusion, xylitol and sucrose exposure during initial dental biofilm formation had minor influence on biofilm morphology, but metabolic activity like EPS production was changed moderately.

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Effect of Maltitol on Growth and Adhesion of Two Caries-Associated Streptococcal Species

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According to previous clinical studies, maltitol exhibits interesting effects on dental plaque reduction. Therefore, we aimed at investigating maltitol effects on in vitro bacterial growth and adhe-

sion of various streptococci. Materials & Methods. Two streptococci strains (*S. mutans* ATCC25175 – SM, and *S. sobrius* ATCC33478 – SS) were grown in Brain Heart Infusion medium (BHI) at 37°C under aerobic conditions for 48 h in 96-well plates. SweetPearl® maltitol effects on growth and adhesion were investigated at 2 different concentrations (5 and 10%, w/w) in BHI. Bacterial growth was assessed using both optical density measurements and bacterial counting. Adhesion was evaluated through exopolysaccharides titration using the crystal violet methodology. Anhydrous dextrose and BHI were used as positive and negative controls, respectively. Experiments were performed in triplicates. Maltitol supplementation slowed down both SM and SS growth in a dose dependent manner after 24 h of incubation, but this effect was no longer observed after 48 h. The larger effects were observed in the BHI medium supplemented with 10% maltitol, consequently, adhesion experiments focused on the 10% dose. After 48 h of incubation, no effect of maltitol was observed on SM adhesion but SS adhesion exhibited an 84% decrease compared to BHI. In addition, anhydrous dextrose induced a 92% increase in SS adhesion compared to BHI. Maltitol slowed down SS and SM in vitro growth until 24 h of incubation, and displayed a sharp decrease in SS adhesion after 48 h. According to these results, in vivo maltitol may potentially affect adhesion and numbers of MS and SS biofilm development.

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Serum Component Promotes *Streptococcus mutans* Aggregation and Internalization by Vein Endothelial Cells

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Streptococcus mutans, a major pathogen of dental caries, may also cause infective endocarditis (IE) and an important step in the pathogenesis of IE is heart valve bacterial infection. Recently, two 120-kDa cell surface collagen-binding proteins (CBPs) of *S. mutans* were characterized (Cnm and Cbm). CBP-positive strains have been shown to possess high properties of invasion of human umbilical vein endothelial cells (HUVEC). *S. mutans* invasion of host cells is considered to be an important virulence factor for development of IE, though the mechanism by which the bacteria are internalized by non-phagocytic cells remains to be elucidated. In the present study, we analyzed the ability of CBP-positive *S. mutans* to invade HUVEC by focusing on bacterial interaction with serum and serum components. We used the *S. mutans* blood isolate TW295 (Cnm+) and its Cnm-defective mutant strain TW295CND, as well as oral isolate MT8148 (CBP-). Bacterial aggregation of TW295 was observed following addition of serum obtained from healthy human volunteers, whereas that was not observed with CBP-negative strains. When TW295 organisms were cultured with HUVEC, prominent masses of aggregated bacteria invaded the cells in the presence of serum, while significantly reduced invasion was seen in cultures without serum ($P < 0.001$). On the other hand, no such invasion abilities were observed in cultures of CBP-negative strains with or without serum. In addition,

TW295 showed a higher level of invasion in the presence of type IV collagen, a component of the extracellular matrix in serum. These results suggest that aggregation of CBP-positive *S. mutans* induced by a serum component may be involved in bacterial internalization in HUVEC with cytoskeletal rearrangement, which may contribute to the virulence of IE.

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Host Immune Response Mediates Infective Endocarditis Caused by *Streptococcus mutans*

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Streptococcus mutans, a pathogen responsible for dental caries, is occasionally isolated from the blood of patients with infective endocarditis (IE). We previously demonstrated that bacterial DNA of *S. mutans* with 120-kDa collagen-binding proteins (CBPs: Cnm, Cbm) was frequently detected in heart valve specimens extirpated from IE patients. However, host response to *S. mutans* infection in cardiovascular diseases remains unclear. In the present study, alterations of gene expression profiles in human vein endothelial cells (HUVEC) infected by *S. mutans* were evaluated using DNA microarray analysis. In addition, we performed histopathological evaluations using IE model rats to examine the involvement of host molecules. *S. mutans* TW295 (CBP+) was isolated from the blood of a patient with bacteremia, from which CBP-defective mutant strain, TW295CND, was developed. HUVEC were separately exposed to both bacterial strains for 90 minutes, then expressions of altered genes were examined using DNA microarray analysis. Genes encoding ARHGAP9, a member of the RhoGAP domain, known to disrupt the actin cytoskeleton, were prominently up-regulated in cells infected with TW295 as compared to those with TW295CND. In addition, E-selectin which mediates inflammatory cells such as leukocytes, was also upregulated in the TW295-infected cells. Furthermore analysis of heart valves extirpated from IE model rats with catheter-induced artificial impairment of the aortic valve showed that the TW295-infected group displayed prominent vegetation masses as compared to TW295CND-infected group. Also, hematoxylin-eosin-stained sections of aortic valve lesions showed destruction of the endothelial cell layer and inflammatory cells around aortic valves in rats infected with TW295. These results suggest that elevated ARHGAP9 and E-selectin expression in endothelial cells induced by CBP-positive *S. mutans* is an important risk factor for IE aggravation.

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Monitoring of the Efficacy of Caries Preventive Infiltration by ICON *in vitro*

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The infiltration of enamel lesions by ICON could be an alternative strategy of caries treatment. The aim of the study was to compare the penetration of *S. sobrinus* into human enamel *in vitro* after protection by ICON. 16 radiographically caries-free extracted teeth (18, 28, 38, 48) from 11- to 16-year-olds were cleaned ultrasonically with de-ionized water and, after air-drying, embedded in epoxy resin (Epofix, Struers, Germany). After 8-hours of setting at room temperature, the specimens were ground on both sides (a, b) with SiC-paper 1200 (particle size 13–16 µm) (Exakt-Mikroschleif-System 400CS, Exakt Apparatebau Norderstedt, Germany). Enamel was removed in circular areas sized 3 mm in diameter; the mean depth of removed enamel was (a) 176±50 µm or (b) 198±51 µm; the amounts did not differ significantly (Wilcoxon test $p = 0.103$). The specimens were autoclaved. Under aseptic conditions (Heraeus Lamin Air, HBB 2448, Hanau, Germany) one area (a) of the enamel surface of each specimen was covered with ICON (DMG, Hamburg, Germany) whereas one area (b) remained as the untreated control. The specimens were incubated for 8 weeks anaerobically at 37±2°C in 20 ml Balmelli broth inoculated with 1 ml of 24 h-cultures of *S. sobrinus* OMZ 176. After 8 days each, the teeth were transferred into 20 ml of fresh medium. After incubation, the teeth were prepared into fragments and viewed in a scanning electron microscope [Kneist et al: Caries Res 2013;47:439]. A bacterial penetration into the covered enamel areas (a) could not be detected. In the untreated enamel areas (b: n = 11 of 16) *S. sobrinus* reached depths of 1151±342 µm (min 520, max 1800). Within the limitation of an *in vitro* study, it can be concluded that an infiltration of enamel lesions with ICON inhibits bacterial invasion.

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Modification of Methylene Blue Used for Antimicrobial Photodynamic Therapy Over *Streptococcus mutans* Biofilm

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Photodynamic therapy involves the activation of photosensitizers by light in the presence of oxygen, resulting in the production of reactive radicals capable of inducing cell death. Reduction of bacteria levels can provide additional means of preventing dental caries. In this way, modifications on the photosensitizers have been made to improve its effect mainly over biofilms. Then, this study evaluated the susceptibility of *S. mutans* (ATCC 25175) biofilm to photodynamic therapy after sensitization with unmodified (U) and modified (M) methylene blue – MB (5, 10 and 20 μM) by potassium iodide (20 μM) and exposure to red light at 665 nm (Lumacare[®]) under 60 J and 5 min pre-irradiation time. In addition, the $^1\text{O}_2$ production was determined using SOG (Singlet Oxygen Generation) and HPF (Hydroxyphenyl Fluorescein) assays (Life Technologies). Biofilm was induced in BHI broth supplemented with 1% sucrose for 7 days in a 48 well-plate. Serial dilutions were seeded onto brain heart infusion agar to determine viability in colony-forming units per milliliter (CFU/ml). Additionally, confocal laser scanning microscopy (CLSM) was performed using BacLight LIVE/DEAD system. Eight different groups were analyzed: L–D– (control), L–D+ (drug group, U and M methylene blue), L+D– (light group), and L+D+ (photodynamic therapy group, U and M methylene blue). Results were analyzed by two-way ANOVA and Tukey's test ($p < 0.05$). PDT using modified and unmodified MB was effective in reducing *S. mutans* counts mainly under 10 μM by 10^3 and 10^4 CFU/ml, respectively ($p < 0.05$) compared to their untreated controls (10^7 CFU/ml) log 10. Unmodified MB generated 1.6 times more $^1\text{O}_2$ than modified. Since in vitro $^1\text{O}_2$ production does not indicate that $^1\text{O}_2$ was generated in the bacterial activity site, the bactericidal action against *S. mutans* cannot be related to in vitro singlet $^1\text{O}_2$ generation rate. In vitro experiments demonstrated that modified MB at 10 μM effectively reduced this microorganism.

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Virulence Gene Expression of *Streptococcus mutans* in Active Dentine Lesions from ECC-Children

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Streptococcus mutans (SM) are organisms strongly associated with the development of dental caries, thus their genetic and phenotypic responses were investigated in active dentinal lesions. This study aimed to verify the prevalence and quantity of viable SM as well as to analyze their expression of virulence genes in active and arrested dentine lesions of children with early childhood caries (ECC). Dentine samples from 29 active and 16 arrested lesions were harvested from pre-school children (age 2–5 years). Total RNA was extracted and the RT-qPCR reactions were performed for SM identification, quantification and analysis of virulence gene expression (atpD, fabM, nox, pdhA and aguD). Although expressed in low quantity in relation to total bacteria, no statistically significant differences were found for SM prevalence and abundance in active and arrested caries lesions ($p > 0.05$). SM expressed all studied virulence genes in both groups, but pdhA and aguD were more expressed in arrested lesions ($p \leq 0.05$). Although present in low quantity, SM is part of the viable microbial community in dentine lesions and their pdhA and aguD genes had an increased expression in arrested caries, probably due to the unfavorable environmental conditions for microbial growth, inherent to this type of lesions. In this way, regardless of the SM prevalence in dentinal lesions of ECC, the SM role in caries activity in dentine remains unclear.

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Deciphering the Microbial Composition of Initial Oral Biofilms by High-Throughput-Sequencing

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Limited data are available for the microbiological composition of initial oral biofilms using high-throughput sequencing. The aim of this in-situ-study was to investigate 2- and 4-hour biofilms using a combination of 16S rRNA gene amplification and high-throughput sequencing using reversible terminators. Polished bovine enamel slabs ($n = 96$) mounted on acrylic splints were worn by 24

volunteers in their upper jaws for 2 and for 4 h. DNA was prepared using lysozyme, proteinase K and a standard DNA preparation kit (Qiagen). V1 and V2 hypervariable regions of the 16S rRNA genes were amplified by polymerase chain reaction using primers with universal adaptor sequences at their 5'-ends. Sequencing on the MiSeq, Illumina, yielded up to 30,000 reads per sample. Sequences were mapped using TUIT, a BLAST-based tool for taxonomic classification of nucleotide sequences. A total of 5.8×10^6 sequences were assigned to overall 221 genera. While after 2 h 163 genera were detected, after 4 h 191 genera were present. Most frequently detected after 2 h were members of the genera *Rothia*, *Streptococcus*, *Gemella*, *Veillonella*, *Actinomyces*, *Prevotella*, *Neisseria*, *Fusobacterium* (in 100% each), *Vibrio* and *Haemophilus* (in >90%). From 4 h biofilms *Rothia*, *Streptococcus* and *Corynebacterium* were detected in all samples and the genera *Gemella*, *Veillonella*, *Actinomyces*, *Prevotella*, *Neisseria*, *Vibrio*, *Haemophilus*, but also *Flavobacterium*, *Fusobacterium* and *Porphyromonas* were present in >90%. Most abundant were *Streptococcus* (2 h: 45%/4 h: 46%), *Rothia* (10%/6%), *Gemella* (4.5%/5.1%), *Veillonella* (2.6%/3.3%), *Actinomyces* (2.6%/2.7%). Nine further genera were detected at >1% in both biofilms. While the analysis did not demonstrate significant quantitative differences of microbioms of initial biofilms after 2- and 4 h (number genera, Wilcoxon-test, $p > 0.05$), the analyzed biofilms at an early stage showed an unexpected high overall heterogeneity.

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Effect of the Combination of Starch Hydrolysates with Sucrose Evaluated by Three-Species Biofilm Model

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It has been considered that starch hydrolysates could increase the cariogenic potential of sucrose due to the changes provoked on biofilm matrix. Thus, this in vitro study aimed to evaluate if starch hydrolysates of different dextrose equivalents (DE) would enhance the cariogenicity of sucrose using a validated three-species biofilm model. *Actinomyces naeslundii* (ATCC 12104), *Streptococcus gordonii* (ATCC 35105) and *Streptococcus mutans* (UA159) biofilms were formed on saliva-coated bovine enamel and root dentine slabs in batch culture and were grown in ultra-filtered tryptone-yeast extract broth at 37°C, 10% CO₂ during 4 days. The biofilms (n = 12) were exposed 8x/day to one of the following solutions: 0.9% NaCl (negative control); 10% sucrose (positive control); 1% DE5 + 9% sucrose or 1% DE40 + 9% sucrose. The acidogenicity of the biofilm was estimated evaluating the culture medium pH every day, after the last sugar challenge and after overnight starvation. After 96 h of growth, the biofilms were collected and viable microorganisms, intracellular polysaccharide

(IP), and soluble (SEPS) and insoluble extracellular polysaccharides (IEPS) were quantified. Dentine and enamel demineralization was assessed by percentage of surface hardness loss (%SHL). Biofilms exposed to 10% sucrose, or the combination 1% DE5 + 9% sucrose or 1% DE40 + 9% sucrose were more acidogenic ($p < 0.05$), presented higher bacterial counts ($p < 0.05$), formed more IP, SEPS and IEPS ($p < 0.0001$), and caused higher demineralization ($p < 0.0001$) on either enamel or dentine than those exposed to 0.9% NaCl group. However, for all variables, there were no differences ($p > 0.05$) among the exposure to 10% sucrose and the combinations 1% DE5 + 9% sucrose or 1% DE40 + 9% sucrose. The findings suggest that starch hydrolysates do not increase the cariogenic potential of sucrose.

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Prevention of Biofilm Formation by Exposure of Bacteria to Silver Nanoparticles

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Aim: To establish the potential of a novel silver nanoparticle formulation for preventing biofilm formation. **Method:** In vitro cultures of multiple *Streptococcus* spp. and *Enterococcus faecalis* were grown in 300 µl 96-well microtitre plates containing BHI media with 20% sucrose added, incubated at 37°C for 48 hr anaerobically, forming monoculture biofilms. A suspension of monodisperse 6.7–9.2 nm sized silver nanoparticles was added (30 µl, effective silver concentration 62.7 µg/ml) to separate cultures of the same bacteria prior to growth. The crystal violet assay was used to determine the mass of biofilm produced by measuring the amount of crystal violet binding to adherent bacterial cells, resolubilising and measuring the optical density of supernatant collected into further microplates with a microplate spectrophotometer. Positive controls without silver present and negative controls where bacteria were exposed to 70% isopropyl alcohol were used. All cultures were replicated in triplicate. **Results:** Monoculture biofilms were successfully generated for all organisms tested. In the presence of silver nanoparticles, biofilm formation was prevented as noted by absence of crystal violet staining, for *S. gordonii* DL1, C219, G102 and ATCC10558 strains, *S. mutans* UA159, *S. mitis* I18 and *E. faecalis* JH22 (all $p < 0.01$). There was a high degree of consistency between triplicate replicate results for each organism tested (CV 0.75% to 2.5%). For *S. oralis* ATCC6249 and ATCC9811, and *S. sanguinis* D1 and ATCC10556, biofilms formed were not adherent so the crystal violet assay could not be used, however, qualitative observation of the wells indicated non-adherent biofilm formation was prevented for these organisms when silver was present. **Conclusions:** The crystal violet assay provided a rapid, reproducible and quantitative assay for evaluating biofilm formation. In the

presence of monodisperse silver nanoparticles, biofilm formation for several *Streptococcus* spp. and *E. faecalis* was prevented.

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Comparison of Gene Expression between Fluoride-Sensitive and Fluoride-Resistant *Streptococcus mutans* Strains

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Fluoride inhibits acidogenicity of *Streptococcus mutans* and is most effective at acidic pH values. This inhibitory effect is reduced in the fluoride-resistant (FR) *S. mutans* strain. Recently, we compared the genome sequences of the *S. mutans* C180-2FR strain with the fluoride-sensitive C180-2 strain and confirmed several single-nucleotide-polymorphisms (SNPs), located in or related to 9 different genes in the genome of the FR strain. Our aim is to compare the expression of these genes as well as 7 genes involved in the glycolytic pathway between the FR and FS strains. To this end, the log-phase cells of C180-2 and C180-2FR were incubated with or without 0.4% glucose at pH 7 or pH 5.5 in the presence or absence of NaF (15 mM for pH7; 1.5 mM for pH 5.5) for 1 h. The cells were collected for RNA isolation. The expression of 16 genes was quantified using quantitative PCR. The differences in expression levels between C180-2FR and C180-2 were calculated as the fold changes. Multiple analysis of variance (MANOVA) was used to analyze the significance of the fold change of each gene and the effects of the environmental factors (glucose, fluoride and pH) on the fold changes. At all conditions, a gene cluster containing 2 SNPs was highly expressed in C180-2FR, as compared to C180-2 ($p < 0.001$). This cluster contains genes encoding fluoride antiporters. The application of the environmental factors caused differential gene expression. For 9 genes, the changes in expression were significantly different between C180FR and C180-2 ($p < 0.05$). Out of 9 genes, 5 are involved in glucose uptake, while 4 contain SNPs. This study may provide novel insights into the mechanisms related to fluoride antimicrobial effect and bacterial fluoride resistance.

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Caries Risk Assessment in Adults Using Survival Analysis with Classification and Regression Trees

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Objective: The purpose of this study was to identify the factors which affected the onset of primary and secondary caries in adults, and to identify those patients with high or low risk of caries by using survival analysis aided by Classification and Regression Trees (survival CART). **Experimental Approach:** A clinical data set of 732 patients aged between 20 to 64 years in nine Japanese general practices was analysed. The following parameters of 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. Those factors, which were identified as statistically significant by the Cox proportional hazards regression analysis, were subjected to analysis using survival CART. **Main Results:** The incidence of primary caries was associated with SM ($p = 0.0016$), LB ($p = 0.0033$) and compliance to a preventive programme ($p = 0.0006$), while that of secondary caries was associated with DMFT ($p < 0.0001$), SM ($p < 0.0001$) and LB ($p < 0.0001$). Survival CART was able to identify high risk patients against primary caries according to their poor compliance to a preventive programme and levels of SM ($>=10^6$ CFU/ml) with odds ratio of 3.66 (95% CI: 1.96–6.39, $p = 0.0002$). In the case of secondary caries identification of those at high risk by LB ($>=10^4$ CFU/ml) and DMFT (>19) showed odds ratio of 3.04 (95% CI: 2.00–4.61, $p < 0.0001$). **Conclusions:** Cariogenic bacteria were the most influential factors associated with the incidence of caries. Preventive programmes were effective in preventing the incidence of primary caries. Survival CART is an effective way of predicting the caries risk of an individual patient.

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Caries Risk Assessment in Patients Infected with *Helicobacter Pylori*

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The purpose of present study was to investigate the effect of *Helicobacter pylori* infection on caries risk. A total of 29 adult subjects were examined. The experimental study group represented 10 patients with *H. pylori* infection (mean age 34.8 ± 12.1 yr), diagnosed by dBest One Step H. Pylori Saliva Test™. Ethical approval and patients' written consent were obtained. The experimental group was compared with the age and gender match group of 19

non-infected control subjects (mean age 37.8±14.9). Patients of both groups had on average 28 teeth. At the time of experiment they all had similar periodontal status, oral hygiene and dietary habits. 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 *H. pylori* infection on growth of salivary *Streptococcus mutans*, *Lactobacillus* and *Candida albicans* was investigated. Dental status was recorded and caries was assessed clinically according to ICDAS criteria. We observed no significant differences in salivary pH value, buffering capacity and stimulated salivary flow rate between the two groups. Patients with *H. pylori* infection showed no significant differences in CFU/ml count of *Streptococcus mutans*, *Lactobacillus* and *C. albicans* compared to non-infected controls. Patients with *H. pylori* infection showed statistically significantly less ICDAS 5 and 6 scored carious teeth (t-test, 1.1±0.3 vs. 2.2±2.2, p = 0.042) compared to non-infected controls. We concluded that *H. pylori* infection does not increase caries risk.

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Anticariogenic Activity of Egg Ovalbumin after Sucrose Exposure to the Oral Biofilm on Enamel, in situ

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Objectives: Previous studies of our group showed that egg ovalbumin reduces cariogenicity when presented to a single-species biofilm of *Streptococcus mutans* after a cariogenic challenge induced by sucrose in an experimental caries model. The aim of the present study was to test whether the effect of ovalbumin was replicated when the experimental exposure occurred in a clinical setting of an in-situ caries model. **Methods:** Twelve healthy volunteers aged between 21 and 35 years-old participated in this split-mouth, crossing-over and double-blind randomized controlled trial. During two phases of 14 days each with a 7-day washout phase, volunteers wore palatal appliances containing triplicate bovine enamel slabs at each side. Whole biofilms formed on the enamel slabs were extraorally challenged with a drop 20% sucrose 8 times per day for 5 min and relocated. Only after 4 of the sucrose exposures, slabs were immediately exposed to 2 different treatments per volunteer in each phase, for 5 additional min with a drop of either: A) 200 µg/ml ovalbumin, B) 100 µg/ml ovalbumin, C) 50 µg/ml ovalbumin or D) 0.9% NaCl (caries-positive control). After each phase, biofilms were recovered to analyze biomass, *S. mutans*, *Lactobacillus*, *S. sanguinis*, total streptococci and polysaccharide formation. Enamel demineralization was estimated by the percentage of surface Knoop-microhardness loss (%SHL). **Results:** Biofilms exposed to 20% sucrose followed by 200 µg/ml and 100 µg/ml ovalbumin showed a dose-dependent reduction (p < 0.05) in the %SHL with (38.4±3.1) and (40.0±3.3), respectively,

when compared with the caries-positive control (44.2±9.2). Only exposure to 200 µg/ml ovalbumin decreased biomass and insoluble polysaccharide formation (p < 0.05). No difference was observed in the number of either bacterial species among treatments (p > 0.05). **Conclusion:** Egg ovalbumin exposed after a highly cariogenic challenge with sucrose appears to reduce the cariogenicity of the oral biofilm in enamel, without a bactericidal effect.

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Effect of Over-the-Counter Fluoridated Products Regimens on Root Caries Inhibition

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This study aimed to evaluate the effect of fluoridated dentifrice (FD) and fluoridated mouthwash (FM) under different regimens on root caries (RC) inhibition. Biofilms formed by *Streptococcus mutans* and *Lactobacillus casei* were grown on the surface of bovine root dentine slabs which were exposed to one of the following treatments: T1 – distilled and deionized water 3x/day; T2 – FD (1,450 ppm F) 2x/day; T3 – FD 2x/day + FM (226 ppm F) 1x/day; T4 – FD 3x/day. Percentage of surface microhardness change (%SMC) was determined on slabs after 3 days of treatment. Lesion depth (LD; µm), integrated mineral loss (IML; vol%µm) and the percentage of change (Δ%) in the ratio of fluorapatite (Fap) and hydroxyapatite (HAp) to amide were also calculated via transversal microradiography and micro-Raman spectroscopy. Experiments were performed in triplicate. %SMC in T4 was statistically lower compared with T2, but not different compared with T3 (T1: -61.7±9.3a; T2: -38.9±13.1b; T3: -29.9±14.0bc; T4: -25.8±10.1c). LD of slabs treated with T4 was statistically lower compared with T2 and T3, which were not statistically different between them (T1: 96.2±21.0a; T2: 55.7±36.1b; T3: 58.1±39.2b; T4: 38.0±23.5c) (p < 0.05). No statistical differences were found for IML and Δ% among the fluoridated treatments (IML-T1: 2427.8±789.1a; T2: 720.6±322.3b; T3: 873.9±551.9b; T4: 656.9±371.3b; Fap-T1: -34.1±27.6a; T2: -5.5±11.4b; T3: -3.5±13.1b; T4: 3.0±21.6b; HAp-T1: -25.2±20.6a; T2: -11.8±21.7ab; T3: -14.4±15.9ab; T4: 4.2±15.5b). %SMC, LD and IML were negatively correlated to FD frequency of use (r = -0.81; -0.68 and -0.78, respectively; p < 0.001) while Δ% (for Fap and HAp) was positively correlated (r = 0.60 and 0.54, respectively; p < 0.05). The use of FD 3x/day may be more effective than the use of FD 2x/day or the tested association between FD and FM on RC inhibition.

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Session 2 Clinical Studies

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The Impact of Dental Caries on the Quality of Life of Disadvantaged Adolescents

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This study assessed the extent to which dental caries, adjusted for socio-demographic and oral health behavior determinants, impact on oral-health-related quality of life of disadvantaged adolescents. All students attending the 6th to 8th grades of the same public school in the region of Itapoã, Federal District of Brazil (n = 1,122) where water is fluoridated were screened. A total of 618 were selected and examined. The adolescents were 10–15 years old (54.2% female), had at least one permanent second molar without filling or sealant and were enrolled in a prospective controlled clinical trial on occlusal caries. Parents answered a questionnaire about family's socio-economic status. Adolescents answered a questionnaire about demographic and oral health behavior determinants in addition to the Child Perception Questionnaire (CPQ11-14) used for assessment of oral health related quality of life. The questions of the CPQ11-14 were answered on a five-point scale as never (score 0), once/twice (score 1), sometimes (score 2), often (score 3) and every day/almost every day (score 4). The test-retest of the questionnaire and the CPQ11-14 in 5% of the sample showed kappa 0.76 and Intraclass correlation coefficient of 0.63, respectively. Clinical and radiographic examinations for dental caries were carried out. The kappa value for inter-examiner reliability for caries in 5% of the sample was 0.81. The D₃MFT = 0 (D₃ = cavitated dentine lesions, M = missing, F = filled, T = tooth) was observed for 47.4% of the adolescents. The outcome was a high score on oral health related quality of life (median split >9). The prevalence of adolescents with at least one domain being impacted

‘often’ or ‘every day/almost every day’ was 34.8%. Logistic hierarchical regression analysis showed that adolescents with absolute D₃T scores ≥5 were significantly more likely to report a high impact on their oral health related quality of life than adolescents with lower scores (p = 0.03). In conclusion, this study indicated that a high number of cavitated caries lesions with dentine penetration had a significant adverse effect on the oral health related quality of life of disadvantaged adolescents.

The study was supported by the Brazilian National Council for Scientific and Technological Development (CNPq).

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Onset, Development and Arrest of Occlusal Caries in Young Permanent Teeth

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This study describes baseline data of a controlled clinical trial on the onset, development and arrest of occlusal caries in young permanent teeth of adolescents. In the disadvantaged region of Itapoã, Federal District of Brazil, where water is fluoridated, all adolescents attending 6th to 8th grades (n = 1,122) of one public school were screened, 618 were selected and examined. The adolescents, 54.2% female, were 10–15 years old (median = 12 yr), had at least one permanent second molar without filling or sealant. The examination recorded occlusal plaque, status of tooth surfaces and stage of eruption of posterior teeth. Two bitewing radiographs were taken and used for caries detection and treatment planning. Caries status was summarized in DMFT/S scores (D₁ = decayed active, inactive at non-cavitated and cavitated dentine levels; D₃ = decayed active and inactive at cavitated dentine level; M = missing

due to caries, F = filled, T = tooth, S = surface). The adolescents' teeth were professionally cleaned and dried before the examination for caries. Inter-examiner reliability of caries scores showed non-weighted Kappa of 0.81. D₁MFS = 0 was observed for 22.1% of the adolescents and D₃MFT = 0 for 47.4%. The difference between mean D₁MFT 4.59 (±3.56) and D₃MFT 1.43 (±2.05) was significant (p < 0.0001, t-test). The same trend was observed at surface level, 6.36 (±6.05) and 2.43 (±4.11) respectively (p < 0.0001). On occlusal surfaces, active lesions were observed in less than 5% of premolars in contrast to 30% of second molars. Further, the number of active non-cavitated lesions on partially erupted second molars was significantly higher than on those fully erupted (p < 0.001, Chi-square test). Finally, inactive lesions were mainly identified on permanent first molars. In conclusion, the findings underline the need to implement non-operative strategies to control caries progression on occlusal surfaces of young permanent teeth, particularly on erupting molars.

The study was supported by the Brazilian National Council for Scientific and Technological Development (CNPq).

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Occlusal ART Restorations Using Three Different Filling Materials: Preliminary Results

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The most used filling material for Atraumatic Restorative Treatment (ART) is the high viscosity glass ionomer cement (GIC). However, some other restorative materials can also be used, as compomer (COM) and the newly developed material called glass carbomer cement (CAR). **Objective:** To evaluate if survival rate (SR) is affected by different fillings materials on ART occlusal restorations in primary molars. **Material and Methods:** A total of 281 children (4–8 years old) with an occlusal carious lesion were selected and randomly allocated into the three restoration material groups: GIC (Equia Fil – GC Corp.), COM (Dyract – Dentsply) and CAR restorations using (Glass Carbomer – GCP Dental). The children were treated on school grounds, following ART principles. Restorations were evaluated after 6 months, using ART criteria, by a calibrated examiner. Restorations SR were evaluated using Kaplan – Meier survival analysis and Log-rank test. Cox regression analysis was used for testing association with clinical parameters $\alpha = 0.05$. **Results:** The overall SR was 83.9% and the SR per group was GIC = 90%; COM = 81.7% and CAR = 81.2%. There was no significant difference among the three materials tested (RR = 1.05, CI = 0.72–1.53, p = 0.79). Cox regression analysis showed no influence of any investigated clinical features in the survival rate of restorations. **Conclusion:** There was no difference in the survival rate of the three materials tested in in primary molars occlusal cavities.

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Relationship between Food Preference, Prop Sensitivity and Dental Caries Experience of Dental Students

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Aim: The aim of this study was to evaluate bitter, spicy and creamy food preferences of a group of young individuals, to determine their taster status with 6-Propyl-2-thiouracil (PROP) and to investigate the correlation between dental caries experience, bitter food selection and PROP sensation. **The Experimental Approach:** Participants were 210 3rd and 4th year dental students of Marmara University, Dental Faculty, Istanbul, Turkey (77 males, 116 females; age 20 to 29). Participants filled out an anonymous questionnaire for age, height, weight, smoking, drinking habits, hunger, thirst, mood, DMFT and a preference questionnaire comprised of 38 bitter, spicy or creamy food items with 10-point scale (0 = 'dislike' to 9 = 'love'). Then they were asked to taste twice filter papers soaked to a 3.2 mmol/l PROP solution (Sigma-Aldrich) and to rate the bitter sensation experience as 1 = 'none' to 9 = 'highest'. Based on PROP scores, participants categorized as non-tasters, medium-tasters and supertasters. The food preferences reported by participants were categorized into flavor dimensions using a principal component factor analysis with varimax rotation [Catanzaro et al.: *Appetite* 2013;68:124–131] and all flavor dimensions, PROP categories and caries experience were compared using SPSS 17.0. **Results:** The study group was found 104 (56%), 63 (34%), 18 (10%) for non-taster, medium and supertasters respectively. For the five flavor dimensions found, only spicy foods significantly correlated with PROP sensitivity (p = 0.044). Two food-items showed significant correlation with PROP sensitivity (chilli-pepper and red-pepper p = 0.022 and p = 0.037 respectively). Twenty-five (13.5%) participants were caries free, the group was divided as 48.6% and 51.4% as low and high caries respectively. A significant difference was found between PROP sensitivity and caries experience (p = 0.040). **Conclusions:** PROP sensitivity and bitter sensation seem to influence food selection and may have effect on caries experience.

Evaluation of Failures of Non-Restorative Cavity Treatment with Long-Term Cases

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Failures of Non-Restorative Cavity Treatment (NRCT) have been reported to occur between 10–25%. In order to improve the prognosis of the NRCT (no caries removal/opening-up cavity/oral health instructions/fluoride varnish), reasons for failures should be evaluated. The aim of this report of cases was to analyze the factors causing failure of NRCT in three long-term clinically well indicated and documented cases. **Case 1:** Food impaction: boy, 7 yrs.; tooth # 54/55; follow-up 24 months; reasons for failure: Insufficient self-care, slicing too vertical, traditional oral health instructions. After 16 months restorations (54/55) were performed to prevent pulpitis. After motivational interviewing with the mother in absence of her child (mother felt safe to show self-reflection about the situation at home), the compliance improved substantially. **Case 2:** Periodontal (bone) destruction by food impaction: girl, 6 yrs; tooth # 74/75; follow-up 36 months; reasons for failure (see case 1); extraction of tooth 74 after 22 months because of deep angular bone defect. After reviewing similarly as in case 1 the compliance improved. Meanwhile the treatment of the deep carious lesion in 75 was successfully continued by NRCT. **Case 3:** Pulp involvement: boy, 6 yrs.; # 75 occlusal lesion; follow-up 60 months; reasons for failure: insufficient self-care, insufficient lesion opening, traditional oral health instructions; extraction after 32 months because of irreversible pulpitis. Traditional oral care instructions were continued. There was no improvement of self-care. Conclusion: Insufficient self-care, insufficient lesion opening/slicing and ineffective oral health instructions are reasons for failure.

Schoolchildren Multicentre RCT Sample Groups Baseline Data Comparison: ICCMS™ vs. Colombian Health-System Caries Managements

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The International Caries Classification & Management System (ICCMS™), a recently developed health-outcomes system focuses in maintaining health/preserving tooth structure through caries-process staging and activity assessment followed by risk-adjusted preventive care, control of initial non-cavitated caries lesions (d/D: ICCMS™In), and conservative restorative treatment of deep dentinal and cavitated moderate/extensive caries lesions (d/D: ICCMS™Mod, Ext equivalent to d/D-3). The Colombian-Health System classifies the whole population as high-caries risk and only detects cavitated/deep dentinal caries lesions (d/D-3). It focuses on systematic-risk-preventive care and caries-lesion restorative treatment. **Aim:** This study aimed to compare in a schoolchildren caries managements' multicentre RCT Group A (ICCMS™) vs. Group B (Colombian-Health-System) gender and caries related baseline data. **Materials and Methods:** After IRB (UEB008-2014) and informed consent from parents of 360 7-yr. olds in 9 Colombian dental schools' clinics, comparison of the two groups (n = 180 each) was conducted in terms of: gender, caries risk likelihood (low/moderate/high), prevalence of caries experience (dmf/DMF-S, d/D: ICCMS™Mod, Ext) and-mean number of surfaces with caries experience (both dmf/DMF-S, d/D:ICCMS™Mod, Ext; and dmf/DMF-S, d/D:ICCMS™In, Mod, Ext). **Results:** The sample corresponded to 194 girls and 166 boys (Group-A: 105 and 74; Group-B: 89 and 92, respectively) (X^2 ; $p = 0.07$). The prevalence of caries experience (dmf/DMF-S, d/D:ICCMS™Mod, Ext) was 74.7% (Group-A: 76.5%; Group-B: 72.9%) X^2 ; $p = 0.27$). The mean dmf/DMF-S (d/D: ICCMS™Mod, Ext) was 6.8 ± 8.2 (Group-A: 7.4 ± 8.5 ; Group-B: 6.3 ± 7.8) (Mann-Whitney test; $p = 0.21$), increasing to 15.3 ± 12.9 (Group-A: 15.9 ± 13.1 ; Group-B: 14.8 ± 12.6) (Mann-Whitney test; $p = 0.47$), when including d/D: ICCMS™In. The percentage distribution of caries-risk likelihood was: high: 54.2%; moderate: 35.8%; low: 10.0% (Group-A: 57.5%, 33.5%, and 9.0%, and Group-B: 50.8%, 38.1%, and 11.1%, respectively) X^2 ; $p = 0.43$). **Conclusion:** The baseline data of this schoolchildren caries managements' multicentre RCT shows that Group A (ICCMS™) and Group B (Colombian-Health-System) individuals are similar in terms of gender and caries related characteristics.

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

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Objectives: Occlusal surfaces of erupting permanent molars are highly prone to caries. Self-assembling peptide (P11-4) has been proven to enhance biomimetic mineralization of early carious lesions. Aim of this study was to evaluate safety, clinical applicability and effect of using P11-4 (Curodont™Repair) in non-invasive treatment of early occlusal lesions on erupting permanent molars. **Methods:** 70 patients (28 females, mean age 10.03 ± 2.7 years, dft 2.8 ± 3.1 , DMFT 1.3 ± 2.5) with active early occlusal lesions (ICDAS-II: 1-3) on first or second permanent molars at eruption were allocated in this randomized, controlled, single blinded post-marketing study to test (Curodont™Repair+Duraphat®) or control (Duraphat®) groups. Safety and applicability were evaluated using dentist's/patient's questionnaires on adverse events, difficulties of application and satisfaction. Lesions were assessed at baseline and recalls after 3 and 6 months regarding caries activity according to Nyvad criteria, clinical status (ICDAS-II) and with Diagnodent®. At every recall, oral health instructions were given and fluoride varnish was applied on all lesions. **Results:** Data showed good patient acceptance for Curodont™Repair. Investigators considered the application as much easier than a composite filling or even a fissure sealant. No adverse events or allergic reactions were observed after application. After 6 months ($n = 62$; 31 test lesions), two test and three control lesions had progressed and had to be sealed, while nine test and two control lesions showed regression. All other test and control lesions were stable. 24 test lesions were inactive in contrast to only 11 control lesions (OR 7.6, $p = 0.0006$). Regressions in Diagnodent® readings were obvious in the test group in comparison to the controls (means = -18.57 ± 19.77 , -1.06 ± 25.84 resp., $p = 0.005$). **Conclusion:** Results suggest that Curodont™ Repair may present a simple, safe and effective non-invasive treatment for early occlusal carious lesions on erupting teeth in conjunction with topical fluoride.

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

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Using an Imperfect Placebo in a Trial for Arresting Initial Approximal Caries Lesions in Children

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This study aimed to investigate the effects of an imperfect placebo used for controlling a randomized clinical trial comparing treatments for arresting initial approximal caries in primary molars. One-hundred forty one children presenting at least one approximal surface of primary molars with initial caries lesion were included. Children were allocated in three groups of active interventions: silver diamine fluoride (SDF) ($n = 47$), resin infiltration ($n = 47$) and control group (active treatment: only flossing) ($n = 47$). Depending on the allocation, patients were orientated to flossing and received the active treatment and respective placebo therapy(ies). We used the placebo to not underestimate daily flossing by those who received other active treatments. However, an imperfect placebo was used to 'simulate' resin infiltration, since just the rubber dam was positioned and no local anesthesia and clamps were used. To assess the effect of using the imperfect placebo, we compared the groups regarding discomfort reported by patients, flossing compliance and occurrence of plaque reduction. We also recorded additional time and costs, as well the cost-efficacy of using the placebo in this study. Children who received the 'active' resin infiltration reported higher levels of discomfort ($p < 0.01$). However, similar compliance (SDF: 47%; infiltrant: 37%; control: 45%, $p = 0.47$) were achieved among groups.

SDF and infiltrant groups reduced similarly the plaque compared to control ($RR_{SDF} = 1.3$; 95% CI: 0.7–2.3; $RR_{infiltrant} = 1.5$; 95% CI: 0.8–2.7). On average, 1.8 dollars per child (SD: 0.8) were spent with the placebo. Considering the potential for similar plaque reduction, the cost-efficacy ratio of including the placebo was 0.53, 1.75, 1.77, respectively, for the groups infiltrant, SDF and control. In conclusion, using the imperfect placebo may be useful and cost-effective to guarantee similar children's compliance and plaque reduction when treatments with different complexities are offered.

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Increased Caries Incidence in Tooth Surfaces Adjacent to Recently Placed Class II Restorations

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The aim of the present study was to compare caries incidence in approximal surfaces adjacent to newly placed composite restorations with the caries incidence of the contralateral teeth. Data from a practice based study, where 4030 posterior tooth-colored restorations placed in permanent teeth by clinicians working in a Public Dental Health Service in Norway and followed for more than 4 years were used. The study was approved by the Regional Committee for Medical Research Ethics. For the present retrospective study with split-mouth design, a subsample of patients with a sound surface adjacent to a Class II composite restoration with an intact contralateral pair of teeth in the opposite quadrant in the same jaw was selected (n = 193).

At the end of the follow-up period, the study restorations and their adjacent surfaces were evaluated both clinically and radiographically. Status of the contra-lateral tooth pair at baseline and end point was based on recordings from routine dental examinations, retrospectively extracted from the electronic dental records.

One hundred and ninety three patients (mean age 9.3 years, SD = 3.4) fulfilled the inclusion criteria. The surfaces were followed on average for 4.8 years (maximum 7 years). Follow-up observations revealed that 41% of adjacent surfaces remained sound compared with 67% of the control surfaces (p = 0.000, McNemar's test). In individuals with DMFT ≥ 5 (n = 104) a lower proportion (36%) of adjacent surfaces remained sound compared with 47% in individuals with less caries experience (n = 89), (p = 0.068, Chi squared test). In the DMFT ≥ 5 group 24% of the approximal surfaces were restored compared to 9% in low caries experience group (p = 0.004, Chi squared test). Caries incidence in approximal surfaces adjacent to newly placed composite Class II restorations was significantly higher compared with the contralateral control surface without a restoration.

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Stain Reduction Efficacy of Two Experimental Dentifrices Containing Alumina and Sodium Tripolyphosphate

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This study compared dental stain reduction from two experimental dentifrices and a comparator dentifrice after 3 and 8 weeks as indicated by the Modified Lobene Stain Index (MLSI). This was

a single centre, examiner-blind, randomised, stratified (baseline total MLSI, smoking), three treatment, parallel group study in healthy volunteers with a baseline MLSI Intensity x Area (IxA) score ≥ 15 on facial surfaces of six maxillary and six mandibular incisors. Experimental dentifrice 1 contained 5% w/w sodium tripolyphosphate (STP), 1% w/w alumina abrasive, plus 0.3% w/w zinc chloride. Experimental dentifrice 2 contained 5% w/w STP and 2% w/w alumina abrasive. A non STP Comparator dentifrice contained 14% w/w silica abrasive. All three dentifrices contained 927 ppm sodium fluoride. Subjects brushed twice daily for 8 weeks ± 2 days. Of 287 screened subjects, 283 were randomised, 273 completed the study and 276 were analysed as the intent-to-treat population. The change from baseline for each variable was analysed using ANCOVA. All treatments produced significant reductions in overall MLSI at Week 8 compared to baseline (p < 0.0001). Similar significance at Week 3 was shown for Experimental dentifrice 1 and the Comparator dentifrices (p < 0.0001). Experimental dentifrice 1 was significantly better, but Experimental dentifrice 2 was significantly less effective in reducing stain than the Comparator dentifrice at both Weeks 3 and 8 (both p < 0.0001). The rank order of effectiveness of the three treatments (Experimental 1 > Comparator > Experimental 2) was unexpected and is unexplained in terms of biological mechanism. This is contrary to the findings from numerous previous clinical and in-vitro whitening studies showing superiority or at minimum no difference of STP products (in a silica base) versus non-STP products.

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Changes in Dentine Tubule Occlusion Following 4 Weeks Use of an Occluding Toothpaste

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This clinical study examined whether dental impressions could detect changes in dentine patency in sensitive dentine following on brushing application and 4 weeks twice daily brushing with an occluding toothpaste (8% w/w strontium acetate), compared to a non-occluding toothpaste (standard fluoride toothpaste), and whether changes observed were reflected in clinical assessment of dentinal hypersensitivity.

This was a single site, examiner blind, parallel, two treatment arm, randomised clinical trial. Participants were aged 18 or over, in good general health with at least one sensitive tooth (evaporative air sensitivity, Schiff score ≥ 2), and patent dentine tubules as determined by visual grading of electron micrographs of replica impressions (Aquasil[®]) of the dentine. Following a washout period, subjects returned for baseline sensitivity and dentine patency assessments (occlusion score; clinical sensitivity assessment (Schiff and visual analogue scale (VAS) following an evaporative stimuli (air-blast), and an ice probe to the selected tooth)). Eligible subjects (Schiff ≥ 2 (air and ice), and patent dentine score ≥ 3) were randomised to treatment. After a single brushing treatment at

baseline, and after 4 weeks of twice-daily home brushing, occlusion and sensitivity were re-evaluated. The per protocol population was the primary population for evaluation of efficacy. All statistical tests were 2 sided and were performed at the 5% significance level. There were no adjustments for multiplicity in this exploratory study. A Wilcoxon rank sum test was performed to test for differences between treatments for the dentine patency, evaporative air Schiff and thermal Schiff scores. ANCOVA was used to test treatment differences for evaporative air VAS and thermal VAS scores. Wilcoxon signed rank test was performed to look at changes from baseline within treatments for dentine patency, evaporative air Schiff and thermal Schiff scores. ANCOVA was used to test changes from baseline within treatments for evaporative air VAS and thermal VAS scores. After 4 weeks, evaporative air Schiff and VAS and thermal Schiff scores were significantly lower than baseline for both treatments ($p < 0.05$), while thermal VAS score was only significantly lower for the non-occluding toothpaste ($p < 0.0001$). Dentine patency scores for both treatments decreased markedly from baseline to 4 weeks, but did not achieve statistical significance. The only statistically significant difference between treatments was change from baseline thermal VAS (25.9 mm) after 4 weeks ($p = 0.0114$) in favour of the non-occluding toothpaste.

In this method development study, both occluding and non-occluding toothpastes resulted in reduced clinical sensitivity and tubule occlusion. It is hypothesised that the impression material may have sheared off within the dentine tubules, and provided some relief from sensitivity.

This study was funded by GSK Consumer Healthcare.

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Dentine Tubule Occlusion Conferred by a Stannous Fluoride Dentifrice, Applied Using Dry or Wet Brushing

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This study compared the dentine tubule occlusion conferred by a 0.454% w/w stannous fluoride (SnF₂) test dentifrice, applied using either a dry or wetted toothbrush, with a non-occluding control dentifrice (0.76% w/w sodium monofluorophosphate) and a reference control (water) after 4 days twice daily treatment in an in situ acid challenge model (days 3–4). The study was a randomized, controlled, single centre, analyst-blind, split mouth, four treatment, crossover, in situ study in healthy subjects. Subjects wore lower right and left buccal intra-oral appliances (~5 hours/day), each fitted with four dentine samples. Samples were brushed twice daily with the test dentifrice ex vivo, using either a dry or pre-wetted brush. The non-occluding control was applied from a 'dry' brush; the appliance was brushed in water as reference control. One treatment was applied to each appliance. Two 10 minute in vivo acid challenges occurred on Days 3 and 4. One sample was removed after each treatment day. Tubule occlusion was measured

from scanning electron micrographs using a visual scoring index. The primary efficacy variable was the mean occlusion score. The per protocol was the primary analysis population. This variable was analysed using Analysis of Variance (ANOVA) based on a mixed model with subject included as a random factor, and treatment, period and side of mouth included as fixed factors. All testing was two-sided at the 5% significance level with no adjustment for multiplicity. Twenty eight subjects were screened/randomised, 26 subjects completed the study. There were no significant differences between the test dentifrice application methods after Day 2, and after acid challenges (Days 3 and 4) ($p = 0.3170$, $p = 0.5795$, $p = 0.2106$, respectively). The 'dry' applied test dentifrice demonstrated significantly greater occlusion than negative control on Day 2 ($p = 0.0391$). Both 'dry' and 'wet' application demonstrated significantly greater occlusion than the negative control after the first ($p < 0.0001$, $p = 0.0002$, respectively) and second ($p = 0.0237$, $p = 0.0006$, respectively) acid challenges.

In conclusion, there was no difference between 'dry' or 'wet' brushing methods with regards to the short-term dentine tubule occlusion of a 0.454% SnF₂ dentifrice.

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3-Year Retention Survival Rates of Composite Resin and ART Sealants Using Two Retention Assessment Criteria

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Objectives: To test the null-hypothesis that there is no difference in the cumulative rate of retained composite resin (CR) and a newly formulated high-viscosity glass-ionomer ART sealants in first permanent molars calculated according to the traditional and a modified retention assessment criteria over 3 years. **Methods:** this cluster-randomized controlled clinical trial covered 123 schoolchildren of 6–7 years old. At baseline, high-caries risk pits and fissures of fully erupted first molars were treated with CR and ART sealants. Evaluations were performed after 0.5, 1, 2 and 3 years by two calibrated evaluators. Retention was scored for free-smooth surface and for each of the three sections in which the occlusal surface was divided equally. The modified criterion differed from the traditional one by determining an occlusal sealant a failure when at least one section had no visible sealant material. Data were analyzed using a Proportional Hazard Rate Regression Model, Wald-test, ANOVA and t-test using the Jackknife procedure. **Results:** cumulative survival rates for retained CR and ART sealants in pits and fissures of free-smooth and occlusal surfaces for both traditional and modified criteria were not statistically significantly different over the 3 year period (occlusal surfaces, for both criteria, $p = 0.05$; free-smooth surfaces, $p = 0.34$). Higher sur-

vival rates of retained CR sealants in occlusal surfaces were observed at longer evaluation intervals (at 3 y, traditional criterion: CR, 66.3 (4.9)% and ART, 50.8 (8.0)%; at 3 y, modified criterion: CR, 29.8 (5.4)% and ART, 15.5 (5.0)%). Retention survival rates were statistically significantly lower for the modified criteria. Despite low sealant retention rates, survival rates of cavitated dentine carious lesion-free occlusal surfaces were high after 3 years. **Conclusions:** No statistically significant difference in the cumulative survival rate of retained CR and ART sealants was obtained. The modified retention assessment criterion should be used in future sealant retention studies.

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Toothbrushing Systematics Index (TSI) for the Analysis of Timed-Event Sequential Data

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Toothbrushing systematics is an important part of oral hygiene instruction aiming to achieve complete tooth cleaning. Observational studies presented first approaches to describe brushing systematics by simple classifications (e.g. according to Rateitschak), but were linked to predefined sequences, unsuitable to take clinically meaningful variations into account. The aim of the present study was to develop an index to objectively quantify the degree of toothbrushing systematics, regardless of which type, from timed-event sequential data. Parameters of interest were: completeness of brushing (reaching all areas of the dentition), isochronal brushing (distribution of brushing duration regarding the areas), alternation frequency (number of changes between areas) and brushing duration. Three algorithms were developed to analyse these parameters by timed-event sequential data and were applied to brushing parameters of 2560 simulated subjects generated by the observation-software INTERACT (Mangold International). Their values were composed to an index value ranging from 0 (non-systematics = brushing incompletely, unequal distribution of brushing duration, frequent alternations, brushing duration too short) to 3 (perfect systematics). The TSI was validated by the analysis of observational data of 26 real participants who firstly brushed habitually (T1), and afterwards according to the brushing systematics by Rateitschak (T2) [Schlueter et al.: Clin Oral Invest 2010;14:99–106]. The TSI values of the simulated matrix showed an approximately linear course and ranged between 0.06 (least systematics simulation) and 2.94 (perfect systematics simulation) (MW±SD: 1.41±0.64). Real participants mean TSI values were (T1) 1.86±0.33 and (T2) 2.57±0.12 (p < 0.001; paired-samples t-test).

The TSI was able to reflect simulated non-systematics to perfect systematics and was able to discriminate between uninstructed tooth brushing and adopted toothbrushing systematics after in-

struction in real subjects. It might be a suitable tool for toothbrushing instruction studies.

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Conventional and Atraumatic Restorative Treatment for Managing Dental Caries in Young Children: A Behaviour Perspective

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Objectives: To compare pre-school children's level of anxiety, pain, and clinical behaviour regarding the treatment of Early Childhood Caries according to two restorative protocols. **Methods:** 32 children aged 4–5-years-old, presenting at least one cavitated dentine lesion and no tooth with pulp exposure and/or pain, were randomly allocated to two treatment groups: Atraumatic Restorative Treatment (ART) and Conventional Composite Treatment (CCT). Anxiety, pain, and clinical behavioural were assessed by the Facial Image Scale, the Wong-Baker Scale and the Frankl Scale, respectively. Whether the child cried, required local anaesthesia and the time spent to perform both procedures were recorded. Treatment was provided in two sessions for children with two cavitated lesions of the same location and severity. Data were analysed using Crosstabs and the Chi-square test. **Results:** 24 ART and 25 CCT restorations were performed. Mean time spent to perform ART and CCT restorations (1st session) were 11.43 and 25.05 minutes, respectively (p < 0.001). The same pattern was observed during the 2nd session. Local anaesthesia was required by one child from the ART group, and all, except one, from the CCT group for both sessions. No statistically significant difference was observed between groups regarding the level of anxiety for both 1st (p = 0.35) and 2nd sessions (p = 0.47). Neither for pain (p = 1.0). Clinical behaviour, at the 1st session, was more positive for children treated according to ART in comparison to those children from the CCT group (p = 0.02). Only children from the CCT group cried (p = 0.043). **Conclusions:** Pre-school children were more collaborative when treated according to ART; local anaesthesia was administered more frequently to children from the CCT group. The conventional treatment took longer to be performed in comparison to ART.

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Association between Dental Fluorosis and Caries in School Children in Villavicencio, Colombia, 2013

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Aim: The aim of this study was to determine the association between occurrence of dental fluorosis and dental caries on a sample of 8–12 yr-old children from the area of Villavicencio, Colombia.

Materials and Methods: The work was approved by the Bioethics Committee of the University and informed consent was obtained from the parents. The study was descriptive and conducted by calibrated dentists employed at the Universidad Cooperativa de Colombia in a total 8 public schools. A total of 459 students from a population of >30,000 schoolchildren aged between 8–12 years were selected using probability sampling. The diagnosis of fluorosis was performed by the Thylstrup/Fejerskov (TFI) index and caries was scored by the ICDAS two digit scoring system, which for the purpose of this study was also transformed to DMFT values. Outcome variables: Sex, age, ICDAS, DMF-T where the D component was defined as ICDAS scores 3 or above. **Results:** Of the 459 participants 223 (49%) were girls and 236 (51%) were boys. Fluorosis prevalence (TFI >0) was 65.8% (n = 302). TFI was not associated to gender (p > 0.5). The prevalence of dental caries with DMF-T = 27% and = 80% by the full ICDAS. Increasing age among the participants was associated with increasing number of lesions scored by the ICDAS (p < 0.001). There was no association between the occurrence of dental fluorosis and caries at the DMFT level using Spearman's rho (rs) (rs = -0.05 p = 0.27) neither at the full ICDAS level (rs = 0.21, p = 0.66). **Conclusion:** In this sample of 8–12 yr-old children from Villavicencio, Colombia there was no association between the occurrence of dental fluorosis and dental caries.

Acknowledgement to Universidad Cooperativa de Colombia sede Villavicencio.

The Role of Unstimulated Saliva as a Risk Factor for the Development of Root Caries

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Aim: The aim of the study was to evaluate whether the levels of unstimulated saliva and its pH could be regarded as risk indicators for the development of root caries lesions. **Material and Methods:** The subjects who took part in the study were 603 patients referred for treatment in the Faculty of Dental Medicine, Sofia. 212 of them were males (35.2%) and 391 (64.8%) – females. Their age varied

from 25 to 85. Two hundred fifty six of them had gingival recessions. Dental examination was carried out with a dental mirror and a probe. No radiographs were taken. Root caries lesions, restorations of those lesions and secondary caries lesions were recorded separately. The ratio between the sum of the number of root caries and restorations on root surfaces and teeth with recessions was estimated. The flow rate of unstimulated saliva was measured in all patients with recessions. The quantity of collected saliva was measured using automatic pipette. Intraoral salivary pH measurement was done in the region of upper first molar vestibulary (gl. parotis), lower first molar lingually and on the back of the tongue. Extraoral measurement of the pH of the collected in the containers saliva was done. **Results:** The average pH at the upper region was 6.87 in patients with root caries and 7.32 in those without, for the lower jaw – 7.03 and 7.65, on the back of the tongue – 7.06 and 7.56 respectively, extraorally – 7.00 and 7.34 respectively. Flow rate for 5 min. of unstimulated saliva was 1.37 in patients with root caries, compared with 3.07 in those without. The binary logistic regression analysis revealed that both quantity of unstimulated saliva (p = 0.000) and its pH (p = 0.000) are risk factors. Both parameters are metric. The decrease of unstimulated saliva with one leads to rise of the risk of development of root caries with 0.103, the 95% confidence interval being between .048 and .220. The reduce of pH with one leads to reduce of risk with .088, the 95% confidence interval being between 0.028 and 0.274. **Conclusion:** Based on the presented study we may conclude that both quantity and pH of unstimulated saliva may be regarded as risk factors.

Influence of Bitewing Radiographs on Treatment Decision of Caries Lesions in Primary Teeth

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Aim: This study aimed to assess the influence of bitewing radiographs on treatment decision of caries lesions in the primary dentition by experienced dentists. **Materials and Methods:** 40 dentists, students of a graduate program, agreed to participate in this study; 57.5% of the participants had specialist or master's degree title. A questionnaire comprising 10 clinical cases with photographs of primary teeth with carious lesions in different stages was developed and projected on a screen to the dentists. Firstly, each case was presented with the description of the lesion (ICDAS code and activity status), and patient's age. At this point the participants only had access to the clinical photograph of the lesion, which represented the visual examination. Right after the presentation of the 10 clinical images, the same cases were presented again, but associated with bitewing radiographs settled side by side to the clinical image (visual and radiograph examination). In each case the dentists were inquired about their treatment decision and they had to choose among eight different options. The changes between the treatment options considering the visual examination or visual and radiographic examination were assessed by Sign test

and presented in percentages. **Results:** In three cases, the association of bitewing radiographs changed the dentists' decision for a more invasive treatment (19.4%, 17%, 20%). In one ICDAS-5 case the association with bitewing radiograph changed the decision to a less invasive treatment (7.5%). **Conclusion:** The majority of the participants decided for an invasive treatment, particularly when bitewing radiographs were associated.

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Calibration of PBRN Dentists in CAMBRA Research

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Objective: To standardize and calibrate dentists participating in a Caries Management by Risk Assessment – Practice-Based Research Network (CAMBRA-PBRN) study. **Methods:** To minimize inter-examiner variability in data collection, including classification of carious lesions and recording of existing restorations, participating dentists were trained and calibrated in accurate DMFS (decayed, missing, filled surfaces) charting. To detect and diagnose non-cavitated caries lesions, the dentists were also trained in ICDAS (International Caries Detection and Assessment) scores 0, 1, and 2 for posterior occlusal surfaces. (IRB #10-04804). 30 dentists were calibrated to a single gold standard examiner (BJ) during 6 calibration sessions, between 2011 and 2014. Kappa statistics were used to determine inter-examiner reliability on 13 or more patients, aged 12–65 (average age 38±15), per examiner during each session, resulting in 94 patients being seen over the course of all 6 sessions. To participate in the main study, examiners needed to achieve a minimum required kappa of 0.75. During the calibration process, examiners scored between 1,036 and 2,220 tooth surfaces. **Results:** The kappa values (unweighted kappa) of the participating dentists compared to the gold standard examiner ranged from 0.75 to 0.90, with an average kappa of 0.84±0.03. 90% of the examiners achieved overall kappa values above 0.8. However, separate reliability for assessment of non-cavitated lesions, as in other studies, was lower (0.55±0.15). Multiple subcategories were evaluated. All dentists reached sufficient reliability values to proceed into the study; nevertheless, one dentist discontinued with the study due to scheduling conflicts. **Conclusion:** The high inter-examiner reliability results have shown thus far that dentists who work in primarily non-research based practices can be effectively standardized and calibrated in data collection, based on specific guidelines created to anticipate potential research study scenarios.

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Caries Management of 6- to 20-Year-Olds; Report from a Japanese Private General Practitioner

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Objective: Maintaining sound teeth in the paediatric to young adult period is essential to lifetime oral health. In Japan, however, mainstream caries treatment has not shifted to long-term preventive care from childhood. The aim of this retrospective study is to investigate the effect of preventive treatment for 6- to 20-year-old. **Methods:** 56 subjects (25 boys and 31 girls) were selected from the patient database of Sugiyama Dental Clinic as at December 20, 2014, based on the following criteria: received preventive treatment between 5 and 7-years-old and then between 18 and 21-years-old (average treatment period 13.4±1.11 years). The subjects were then divided into three groups A, B, and C; subjects in Group-A visited SDC 1 to 12 times for preventive treatment (n = 15, average treatment period = 13.1±1.06 years), Group-B 13 to 24 times (n = 20, 13.2±1.27 years), and Group-C 25 times and more (n = 21, 13.9±0.91 years). **Results:** In Group-A DMFT increase was 4.9±4.50, Group-B DMFT increase was 2.3±2.98 and Group-C DMFT increase was 2.0±2.28. Comparison of DMFT increase between A and B (p-value = 0.048) and A and C (p-value = 0.026) suggests frequency of preventive care visit matters. Interruption of preventive care was also linked with DMFT increase (p-value = 0.033); with interruption longer than one year (n = 40, 13.5±1.20 years) DMFT increase was 3.3±3.49 while without interruption (n = 16, 13.3±0.93 years) DMFT increase 1.9±3.15. **Conclusion:** In the groups of 'frequent visits for preventive care' and 'without interruption', the DMFT increase was comparatively lower. This study was based on clinical data of single dental office and the number of subjects was limited; further investigations, if they reinforce this result, may lead to shift in caries treatment in Japan.

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Approximal ART Restorations Using Three Different Filling Materials: Preliminary Results

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Good survival rates for single-surface Atraumatic Restorative Treatment (ART) restorations have been reported, while multi-surface ART restorations have not shown similar results. The physical-mechanical characteristics of the glass ionomer cement (GIC) may contribute to these failures. **Objective:** The aim of this study was to evaluate the survival rate of approximal ART restorations using three different filling materials. **Methods:** A total of 290 primary molars with approximal caries lesions were selected in 4–7 years old children in Barueri city, Brazil. Only one tooth was selected per child. The patients were randomly allocated in three groups: G1 – high-viscosity GIC (EQUIA Fil – GC Corp); G2 – compomer (Dyract Extra – Dentsply) and G3 – glass carbomer cement (GCC – Glass Carbomer – GCP Dental). All treatments were performed following the ART premises and all restorations were evaluated after 2 and 6 months according to Roeleveld et al. [Eur Arch Paediatr Dent 2006;7:85–91] criteria. Restoration survival was evaluated using Kaplan-Meier survival analysis and log-rank test, while Cox regression analysis was used for testing association with clinical factors ($\alpha = 5$). **Results:** There was difference in survival rate between the materials tested, with a poorer performance for GCC (RR = 1.33, CI = 1.05–1.69, $p = 0.017$). The overall survival rate of restorations was 58.3% and the survival rate per group was G1 = 62%; G2 = 58% and G3 = 48% (no difference between G1 and G2). Cox regression test revealed that presence of cavitated lesion in adjacent tooth surface and high dmft had an negative influence on restoration survival. **Conclusions:** This study suggests that GCC is less suitable for the use in approximal ART restorations in primary molars compared to high-viscosity GIC and compomer in short time evaluation.

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Evaluation of Resin Infiltration of Post-Orthodontic Caries Lesions

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The aim of this study was to assess clinically the masking effect of resin infiltration of post-orthodontic caries lesions. In 29 adolescents 240 non-cavitated caries lesions (ICDAS 2) located from first premolar to first premolar were treated by resin infiltration. Prior to treatment, a digital picture of the anterior teeth was taken. Following etching (maximum repetition 3x) with 15% HCl (Icon etch, DMG) the teeth were dried with alcohol (Icon dry). Finally, the infiltrant (Icon infiltrant) was applied and light-cured followed by polishing. Digital pictures of the final result were taken one week after treatment. Using a numerical scale from zero (no lesions visible) to ten (all teeth involved, more than half of surface, high contrast), four experienced dentists evaluated the pre- and post-treatment severity of lesions on the teeth portraits. The success of treatment was assessed by five categories: deteriorated (1), unchanged (2), improved, but not satisfying (3), improved and no further treatment required (4), completely masked (5). The severity of aesthetic impairment due to white spot lesions was rated with an average of 4.7 points (SD 2) and 1.7 points (SD 1.4) before (interobserver reliability; Fleiss kappa: 0.35 = fair) and after (0.31 = fair) the treatment, respectively. In one patient the result was classified as unchanged, in five patients as improved but not satisfying, in 21 patients as improved and no further treatment required, and in two patients as completely masked (Fleiss kappa: 0.57 = moderate). For 8/240 teeth further treatment with composite filling was recommended. Our results show that resin infiltration of post-orthodontic caries lesions provides satisfying to excellent results in the majority of patients.

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Dental Caries Experience in 12-Year-Old Children with Chronic General Illnesses

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Aim: To determine dental caries experience and treatment needs in 12-year-old children with chronic general diseases. **Methods:** The cross-sectional study was carried out in Pediatric Hospital № 8 in Volgograd, Russia. The parents' informed consents were obtained before children hospitalization. The children (N = 287), aged 12 years, who were hospitalized in 2014 for treatment of chronic general diseases, were examined by a calibrated dentist in the dental room of the hospital. The children had cardiovascular disease (N = 95), digestive system disease (N = 96), or renal disease (N = 96). Caries prevalence (DMFT >0), DMFT-index according to WHO criteria, standard deviation (SD), care index (% FT/DMFT), and 95% confidence interval (CI) were calculated. **Results:** In 12-year-old children with general diseases caries prevalence was 79.1% (CI 74.4–83.8), mean DMFT-index was 3.19 (CI 2.77–3.61). The number of untreated teeth was more than the number of filled teeth; the care index was 46.4% (CI 43.2–49.6). There were no statistically significant differences (t-test, $p > 0.05$) between mean DMFT-values in the children with different diseases: DMFT±SD was 3.62±2.69 in the children with cardiovascular disease, 3.01±2.26 in the children with digestive system disease and 2.92±2.44 in the children with renal disease. However, the care index in the children with cardiovascular disease was substantially higher (Chi-square-test, $p < 0.001$) than in the children with digestive system disease or renal disease: 85.2% (CI 84.2–86.2) versus 21.5% (CI 17.4–25.6) and 23.3% (CI 19.0–27.6) respectively. **Conclusions:** High prevalence of dental caries was revealed in 12-year-olds with chronic general illnesses. Caries treatment needs in the children with digestive system disease or renal disease were substantially higher than in the children with cardiovascular disease.

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Difference in Remineralization Below and Adjacent to Fluoride Varnish in vitro

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The remineralization efficacy of caries lesion may be different in where fluoride varnish (FV) was applied on the lesion (direct) or around the lesion (indirect). The aim of this study was to compare direct and indirect remineralization potential of FV on the artificial caries lesion in vitro. One hundred twenty polished round bovine enamel specimens (3.0 mm diameter) were exposed to demineralization gel (pH 5.0, Lactic acid, Carbopol ETD2050, saturated hydroxyapatite) for 24 h to induce artificial caries lesion formation. The demineralized specimens were allocated to four varnish groups (n = 30) based on the Vickers microhardness number (VHN). The VHN mean value of all demineralized specimens was 71.5±16.5. Four fluoride varnishes used in this study contained 5% sodium fluoride as follows: Duraphat, Enamelpro, MI and ClinproWhite varnish. Half the specimens from each group (n = 15) had the FV applied (10 mg per specimen) and the other specimens (n = 15) were not treated. The both specimens treated with and without FV were immersed together in 20 ml of artificial saliva at 37°C. The FV was removed from the specimen after 24 h, and the immersing process was continued for 48 h. The VHN was measured to evaluate the difference between de-remineralization procedures. The indirect remineralization potential ($\Delta 134.1 \pm 30.4$) was significantly higher than the direct one ($\Delta 65.4 \pm 29.7$) ($p < 0.01$). All varnish products showed a significant difference between direct and indirect potential ($p < 0.01$). The differences of VHN between indirect and direct increase were 111.8 (Enamelpro), 66.9 (MI), 66.7 (Clinpro White), 27.4 (Duraphat), respectively. Based on this in vitro study, it could be concluded that the remineralization effect of fluoride varnishes would be higher in the vicinity than the underneath of the varnish treated surface.

Toothpaste Concentration Effect and Its Combination with APF on Enamel and Dentine De- and Remineralization

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High-fluoride toothpaste (FT) (5,000-ppm) or combination of ways of F use such APF plus standard-FT (1,100-ppm) have been advocated to control caries in high risk patients, mainly those with exposed root dentine. Nevertheless, the comparative ability of these interventions, to reduce demineralization or enhance remineralization, either on enamel or dentine, have not been assessed. In a double-blind, crossover, in situ study 18 subjects wore palatal appliance containing two sound enamel and two dentine slabs in one side, and two carious enamel and two carious dentine ones in the other side. Biofilms was allowed to accumulate on slabs, and the sound slabs were exposed to 20% sucrose 8x/day, the carious were 3x/day. Treatments groups were: placebo toothpaste (PT), 1,100 ppm FT (1100-FT), 5,000 ppm FT (5000-FT) or combination of previous application on slabs of acidulated phosphate fluoride (APF-gel; 12,300-ppm-F) with daily use of 1100-FT (APF+1100-FT). Toothpastes were used twice/day. After 14 days, demineralization of the original sound slabs and remineralization of the carious were assessed by cross-sectional hardness and the lesion area (ΔS ; $\text{kg}/\text{mm}^2 \times \mu\text{m}$) was calculated. The dose-response effect for FD-concentration (0,1100,5000) was significant for all variables ($p < 0.05$), except for dentine remineralization. ANOVA showed that, for dentine, the combination of APF+1100-FT was significantly more effective than 5000-FT on reduction of demineralization (362 ± 340 vs. 677 ± 337) and enhancement of remineralization (1033 ± 472 vs. 360 ± 272) ($p < 0.05$). For enamel, the difference between APF+1100-FT and 5000-FT, either in demineralization (4429 ± 2074 vs. 4460 ± 3286) or remineralization (6255 ± 1764 vs. 6547 ± 1980), was not significant ($p > 0.05$). The findings suggest that either the inhibition of demineralization or enhancement of remineralization of either enamel or root-dentine are FT-concentration dependent but the combination of APF with daily exposure to conventional-FT would be more effective for root dentine.

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Evaluation of Caries Risk Assessment System 'Apple-Examination' for Motivating Children to Improve Their Lifestyle Habits

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Background: We developed an original oral health examination system designed to motivate children to improve their lifestyle habits. The system was examined based on intraoral examination, X-ray examination, bacterial tests of the tooth surface, and risk factors in the patient's lifestyle habits. We focus on the conversation with the patients during the examination and adopt their opinion of self-evaluation in the final assessment to help the patients change their habits by themselves. Four apple colors are assigned corresponding to the four phases of caries risk so that the patients and their parents can easily understand the risk. We named the system 'Apple-examination'. **Objective:** This study aims to evaluate caries risk assessment system we developed for motivating children to improve their lifestyle habit using a questionnaire. **Methods:** Patients received 'Apple-examination' from April 1st to September 30th, 2014. We asked to fill in the questionnaire 5 patients who could answer by themselves and 39 parents. We investigated whether their lifestyle habits regarded as caries risk factors showed improvement after 'Apple-examination'. **Results:** Of 42 answered questionnaires, we analyzed 40 where patient's consent was obtained. As a result, lifestyle habits have 'improved clearly' in 9 patients, 'became slightly better' in 31 patients. Duration of the improvement in their lifestyle habits lasted was 'more than 3 months' in 21 patients, '1 to 3 months' in 15 patients, and 'less than 1 month' in 4 patients. **Conclusion:** It was found that the lifestyle habits of all patients have been improved after 'Apple-examination'. Lifestyle habits of half the patients were improved for more than three months. It was shown that 'Apple-examination' was useful.

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Investigation of Taste Perception as a Factor for Caries Risk Assessment of School Children

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Aim: The aim of this study was to evaluate whether or not salty, sweet, bitter and sour taste perceptions could add any valuable information in determining caries risk assessment of the school children. **The Experimental Approach:** Two hundred children (99 boys, 101 girls age 6 to 13) who attended to Paediatric Dentistry Department of Marmara University were included in the study.

Caries risk assessments of children were performed using Cariogram protocols. Each child then tasted twice 50 ml of sodium chloride (2 mg/l and 4 mg/l), sucrose (12 g/l and 24 g/l), caffeine (0.27 g/l and 0.54 g/l) and citric acid (0.6 g/l and 1.20 g/l) solutions at random order and asked for their taste perception. The children rinsed their mouth with tap water between solutions and the same tasting protocol was followed a week later. Chi-square and Pearson correlations were done using SPSS Statistics version 20. **Results:** Low, medium and high caries risk groups were 63 (31.5%), 67 (33.5%) and 70 (35%) respectively. Taste perception of the whole study group decreased from sucrose solutions (86% and 92%), to citric acid (80% and 87.5%), sodium chloride (61% and 75.5%) and caffeine solutions (9.5% and 38.5%). No significant difference was found between any taste perception and caries risk groups. Sweet, salty and bitter tastes were positively correlated with age ($r = 0.14$; $p = 0.046$; $r = 0.214$; $p = 0.002$; $r = 0.157$; $p = 0.026$, respectively). When occlusal and approximal caries scores of the children were separated and age of the children was controlled, a weak positive correlation was found between occlusal caries and sweet (24 g/l sucrose) taste perception ($r = 0.232$; $p = 0.021$) and a weak negative correlation between approximal caries and salty (4 mg/l NaCl) perception ($r = -0.225$; $p = 0.025$) if children were between 6 and 9. Our study showed that school children's sweet, salty, bitter and sour taste perceptions failed to correlate to Cariogram caries prediction results and age of the child could affect the results.

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Dentin-Pulp Complex Response after Partial Caries Removal in Primary Teeth: A Randomized Clinical Trial

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Objective: This randomized clinical trial analyzed the dentin-pulp complex response in primary teeth after the partial caries removal (PCR) compared to the total caries removal (TCR) during the 6-month period. **Methodology:** Forty four deciduous molars with active caries lesions in the inner half of dentin and vital pulp were randomly divided into two groups: 1) TCR group ($n = 22$), in which the decayed tissue was completely removed with the aid of dye, and 2) PCR group ($n = 22$), in which carious dentin was completely removed from the dentino-enamel junction and lateral walls, keeping the affected dentin in pulpar wall. The main outcome was percentage of the level of oxygen saturation (%SaO₂) of pulpal blood flow assessed by a pulse oximeter adapted for human dental anatomy (before and immediately after procedures restoration of the cavity, 7 days, at 1–6 month posttreatment). Secondary outcomes were intraoperative pulp exposure and pulp necrosis. **Results:** There was no statistical difference in %SaO₂ values in the comparison into the group or between groups, before and 6

months posttreatment: PCR (87.48 ± 3.24 ; 88.42 ± 2.61) and TCR (89.44 ± 4.10 ; 89.20 ± 4.18) ($p > 0.05$). Pulp exposure occurred in 5 teeth (TCR – 4/PCR – 1) and two necrosis cases were recorded in TCR group. **Conclusion:** The dentin-pulp complex response assessed by %SaO₂ did not differ between PCR and TCR after 6 months follow-up. The lower percentage of intra-operative pulp exposure and post-operative pulp complications were observed in PCR group suggests that PCR seems to be a minimal invasive option for caries removal for primary teeth.

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Effect of Self-Set Goal Approaching Tooth Brushing Instruction (TBI) According to Caries Risk in Elementary School Children

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Aim: The aim of this study was to evaluate the effect of the tooth brushing instruction by self-set goal approaching in elementary school children classified into groups according to caries risk level. **Method:** The subjects ($n = 170$) were students of G elementary school in Gwangju, Korea. Students and their parents filled out questionnaires on behavior and attitude to self oral care. We conducted an oral examination of children including tooth and plaque status and calculated DMFT index and plaque control rate (PCR). We used PCR in this study which is the value obtained by subtracting O'Leary plaque index from 100 (%). Then we classified children into moderate or high risk group by caries risk level according to ADA caries risk assessment (CAMBRA). We showed students their PCR and plaque disclosing image and they set up their own goals of PCR individually in TBI procedure. Six months later, we conducted an oral examination again and compared the first outcome with the second. **Result:** The mean PCR of high risk group ($n = 126$) was increased from 28.01% to 40.05% and that of moderate risk group ($n = 44$) was increased from 31.43% to 37.54%. PCR of high risk group and moderate group were both significantly increased from baseline ($p < 0.001$). And the mean PCR of high risk group were significantly more increased than that of moderate risk group ($p = 0.046$). But average PCR of both groups was not significantly different ($p = 0.813$). **Conclusion:** A self-set goal approaching TBI was effective for most students and this approach was particularly more effective in high caries risk group.

This study was supported from own funds and the authors declare to have no conflicts of interest.

In vivo Comparison of Radiographic and Directly Assessed Clinical Caries Status of Posterior Approximal Surfaces

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The aim of this study was to compare conventional radiographic assessment of lesion depth with clinical assessment by means of direct visual inspection of approximal surfaces following elective temporary separation of the teeth. Forty-two patients were examined. These patients were 18 years old and over and attended the dental clinics at the University of Chile and the Austral University for general dental treatment.

A comparison of the status of 333 permanent posterior approximal surfaces was made on the basis of their appearance on posterior bitewing radiographs and the findings of the presence or absence of cavitation by direct visual examination in vivo after temporary tooth separation was achieved by means of elastomeric separation over a one-week period. Radiolucencies were scored according to the category system proposed by Pitts (1984). Upon clinical examination, cavitation was found in 1.5 percent of surfaces, which in turn did not display radiolucencies upon radiographic examination. Cavitation was also detected in 6.67 percent of surfaces, which upon radiographic examination displayed radiolucencies within the outer half of enamel and in 6.81 percent of surfaces that displayed radiolucencies within the inner half of enamel. Of the surfaces with radiolucencies within the outer half of dentine, 38.46 percent were cavitated whereas 80 percent of the surfaces that presented with radiolucencies within the inner half of dentine were also cavitated. These results may contribute to a re-evaluation of the optimal threshold for restorative intervention at approximal sites.

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Criteria to Evaluate the Survival Rate of Atraumatic Restorative Treatment: Restoration versus Tooth Survival

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Objective: Lately, the need of repairing defective restorations in the primary dentition or even the need of restoring carious primary molars is being questioned. The aim of this study was to compare the difference in outcome using two evaluation criteria for the survival rate (SR) caries lesions treated using the Atrau-

matic Restorative Treatment (ART) technique in primary molars. The SR was evaluated on a restoration – and on a tooth level. **Methods:** A total of 208 primary molars with proximal carious lesions were selected in 208 6–7 years old children of Barueri city, Brazil. All teeth were treated according to the ART protocol and were filled with Glass Ionomer Cement (Fuji IX – GC Corp.). The restorations were evaluated after 1, 6, 12, 18, 24 and 36 months according to criteria for proximal ART restorations [Roeleveld et al.: Eur Arch Paediatr Dent 2006;7:85–91]. The tooth survival was evaluated by clinical failure rates, scored as minor failure (reversible failures, possible to be repaired) and major failure (irreversible failures such as pulpitis, abscess, etc.). Kaplan-Meier survival analysis and Log-rank test were used to compare the SR of restorations and teeth, while Cox regression analysis was used for testing the association with clinical factors ($\alpha = 5$). **Results:** There was a higher SR for teeth compared to restorations ($p < 0.001$). After 36 months, the SR for restorations was 53.1% and for teeth this was 92.7%. A Cox regression test revealed that there was no influence of clinical factors in the SR for teeth or restorations. **Conclusions:** The SR for teeth is higher than that for ART restorations in proximal cavities of primary molars.

Fluoride Concentration of the Six Most Sold Toothpaste Brands in Colombia

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Several studies have found inconsistencies between the labeled concentration of total fluoride in the toothpaste tube and the total soluble fluoride concentration (TSF), which corresponds to the potentially active one to control caries and that should be ≥ 1000 ppm F. **Objective:** To evaluate the concentration of total soluble fluoride in toothpastes sold in five Colombian cities. **Methods:** The six most frequently sold toothpastes in Colombia, five familiar (A: Colgate-Triple-Acción[®] 1450 ppm F, B: Fortident-Cuatriacción[®] 1450 ppm F, C: Fluocardent-Triple-Acción[®] 1500 ppm F, D: Kolynos-Super-Blanco[®] 1450 ppm F, E: Oral-B-Prosulud-Limpieza Profunda[®] 1450 ppm F) and one children's toothpaste (F: Colgate-Smiles[®] 1100 ppm F) were obtained each from three different stores in the five representative Colombian cities: Bogotá, Medellín, Cartagena, Cali, and Villavicencio, for a total of 15 toothpaste tubes from each brand ($n = 3$ for each city). All toothpaste were within their expire date of use (fresh samples). For each toothpaste tube, TSF was assessed in the slurry's supernatant (1%) after acid treatment to hydrolysis MFP. Fluoride was determined using an ion-specific electrode coupled to an ion analyzer, previously calibrated with F standards. A one-way ANOVA test was used to search differences in TSF concentrations of the same brand among cities ($p < 0.05$). **Results:** All toothpastes had TSF mean values lower than the total fluoride declared in the label but higher than 1000 ppm (A: 1399.4 ± 28.9 ppm; B: 1427.3 ± 39.6 ppm; C:

1410.5±47.9 ppm; D: 1054.4±21.1 ppm; E: 1262.0±18.2 ppm; F: 1018.4±55.0 ppm). The toothpastes sold in the different cities did not differ statistically in TSF concentrations. **Conclusion:** The fresh samples of the top-selling fluoride toothpastes sold in Colombia's main cities have fluoride concentration able to control dental caries but the stability of TSF found is unknown and should be further evaluated.

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Physical Properties of Dental Fluoride Varnishes

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The aim of this study was to compare the ability of dental fluoride varnishes to deliver fluoride, calcium and phosphate ions. Five varnishes were analyzed: 1) MI Varnish (MIV, GC Corp.); 2) Clinpro White Varnish (CWV, 3M ESPE); 3) Duraphat (DRP, Colgate); 4) Profluorid varnish (PFR, VOCO); 5) Flor-Opal varnish (FLO, Ultradent). Two varnishes contained a calcium phosphate compound in addition to fluoride: MIV with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and CWV with functionalized tricalcium phosphate (fTCP). To determine the fluoride, calcium and phosphate ion release, the varnishes were applied onto an acrylic plate (20 mm x 40 mm) and immersed in 25 ml of deionized distilled water (n = 5 per group). After one day of incubation with gentle agitation, the amount of fluoride, calcium and phosphate ion release was measured using an ion-specific electrode, atomic absorption spectrophotometry and colorimetry respectively; and expressed as mg/g of varnish. Data were statistically analyzed (ANOVA, Tukey's test). At Day 1 fluoride ion release of MIV, CWV, DRP, PFR and FLO was 31.64±1.53, 11.32±1.40, 1.50±0.10, 7.76±0.46 and 20.02±1.40 (Mean ± SD, mg/g) respectively. At Day 1 calcium ion release was 2.16±0.05, 0.23±0.04, 0.08±0.02, 0.08±0.02 and 0.19±0.04 (Mean ± SD, mg/g) respectively. Moreover, at Day 1 phosphate ion release was 6.04±0.36, 0.43±0.11, 0.19±0.05, 0.17±0.09 and 3.40±0.39 (Mean ± SD, mg/g) respectively. This study determined that MI Varnish containing CPP-ACP had significantly higher fluoride, calcium and phosphate ion release than the other tested fluoride varnishes (p < 0.05).

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Oral Biofilm Uptake of Mineral Ions Released from Experimental Toothpaste Containing S-PRG Filler

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Oral biofilm uptake of mineral ions released from experimental toothpaste containing surface pre-reacted glass-ionomer (S-PRG) filler was estimated by depth-specific analysis. Eighteen subjects (19–39 years) wore in-situ plaque-generating devices comprising a pair of enamel slabs (4 mm²) on their upper molars and plaque was allowed to form. A slurry of toothpaste containing S-PRG filler (φ 3 μm, 5 wt%) was filtered; the filtrate contained Al (369.4 ppm), B (506.4 ppm), F (193.0 ppm) and Sr (1216.0 ppm). Each device was removed after three days and immersed in the filtrate for one minute, then reinserted at the same location. After thirty minutes, the device was removed and samples were separated into outer, middle and inner layered fractions (each 300 μm thick) and each fraction was serially sectioned, with 2x2 μm sections taken first followed by 4x4 μm sections. This was repeated until the required thickness was obtained. Samples treated by filtrate without S-PRG filler extract served as control. Fluoride and the other three mineral ions extracted from the thicker sections were quantified using a fluoride-selective electrode and ICP-atomic emission spectroscopy, respectively. The results were corrected for biomass volume estimated by area measurement of stained 2 μm sections. The average uptake ratios (S-PRG/control) of Al, B, F and Sr were 0.97, 0.84, 47.7 and 9.13 in the outer, 0.89, 0.96, 16.8 and 7.03 in the middle, and 1.08, 1.12, 10.2 and 6.45 in the inner layer, respectively. Although ANOVA analysis indicated that depth-specific concentrations of minerals were affected by short exposure to S-PRG filtrate, the biofilm showed significant uptake of only F and Sr. The results suggested that toothpaste containing S-PRG filler promotes the diffusion of F and Sr into oral biofilms.

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Effect of Fluoride on Cell Membrane Protein Expression in Mesenchymal Cells of Mice with Different Susceptibilities to Fluorosis

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A/J and 129P3/J mouse strains have different susceptibilities to dental fluorosis and differ with respect to several features of fluoride (F) metabolism due to their genetic backgrounds. This study evaluated the effect of F on cell membrane protein expression in mesenchymal cells from A/J and 129P3/J mice. The mesenchymal cells were exposed to 0, 10⁻⁵ M (F1) or 5x10⁻⁶ M (F2) of F for 6 h.

Membrane proteins were extracted by ultracentrifugation and solubilized in Urea. Fifty µg of protein were digested with trypsin Gold. The peptides were analyzed by LC-MS/MS. Difference in expression among the groups was obtained using PLGS software and expressed as $p < 0.05$ for down-regulated and $p > 0.95$ for up-regulated proteins. Uniprot ID was used to generate a list of Gene Ontology and in silico interaction analysis was performed by Cytoscape. Comparative analysis between A/J and 129P3/J controls showed 5 and 6 proteins down and up regulated, respectively, in A/J cell line. Sixty one and 39 proteins were exclusive to A/J and 129P3/J, respectively. When A/J_F1 and 129P3/J_F1 were compared, 2 proteins were down and up regulated, respectively, in A/J cell line. Additionally, 33 were found exclusively in 129P3/J. In group F2, 6 and 9 proteins were found down and up regulated, respectively, in A/J cell line. In addition, 10 and 17 proteins were exclusive to A/J and 129P3/J, respectively. In silico analysis showed that around 100 proteins were related with membrane term. After pathway analysis several interactions related with mitochondrial processes were found. The ADP/ATP translocase 2, that performs the exchange of cytoplasmic ADP with mitochondrial ATP across the mitochondrial inner membrane was up regulated in A/J_C, but after exposure to the lowest F concentration, it was found down regulated, in respect to 129P3/J. This may lead to a disturbance in ATP synthesis, since mitochondrial transmembrane complex might have been compromised, which should be evaluated in further studies. A/J and 129P3/J mesenchymal cells have different membrane protein profiles that are affected by F exposure.

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Novel Varnish Formulation Provides Tooth Strengthening Benefits in Reduced Time

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Fluoride-varnish is often applied to teeth to remineralize lesions. Although tooth-varnishes contain 22,624-ppm (2.26%) fluoride-ion, differences in fluoride-release kinetics and coating-composition influence time for varnish to remineralize. **Aim:** compare kinetics of fluoride-release of three formulations: [Experimental-ToothCoating (A; Coating A is not a rosin-ester, but a unique permeable-polymer formulation), 3MTMESPETMClinproTMWhiteVarnish(B); Dentsply[®]Nupro[®]WhiteVarnish(C); and artificial-saliva-control(D)] to surface-microhardness and fluoride-uptake in an in-vitro-remineralization-study. **Approach:** Sample prep: Mount and polish bovine enamel (5x5 mm blocks) in acrylic. Prepare caries-like lesions by immersing blocks in pH5.0-Carboxpol-907/lactic-acid demineralization-solution (96 hrs, 37°C). Measure baseline-lesion Vickers-microhardness (200 g load, 15 sec) and stratify samples into equivalent treatment-groups (n = 10). Remineralization: coat samples, immerse in artificial-saliva (4 hr, 37°C, pH = 7), remove coating-residue, immerse in artificial-saliva (24 hr), rinse with deionized (DI)-water, soak in acid-demineralization-solution (24 hr), rinse, measure microhardness and fluoride-uptake. Calculate percent sur-

face-microhardness change from baseline-lesion for each treatment group using one-way-ANOVA and Tukey's-t-test ($p < 0.05$). Fluoride release: coat varnish (0.05 g) on slide, soak in DI-water (50-ml)/TISAB (5-ml), measure using fluoride-ion-selective-electrode (ISE) (24-hrs). Fluoride-uptake: dissolve 1-mm-diameter x100 µm deep-enamel-biopsy in 0.5 M-perchloric acid (150 µl), measure using fluoride-ISE in 1N-NaOH (100 µl) and TISAB-II (150 µl). **Results:** Mean surface-microhardness percent-change from baseline-lesion to treated-acid-challenged samples (SD); A: 14.5% (28.9); B: 10.7% (18.0); C: -14.3% (8.5); D: -25.9% (11.7); A&B are statistically different from C&D. Baseline-lesion microhardness values are not significantly different, demonstrating balanced groups at study initiation. After acid-challenge, A&B increased tooth microhardness from lesion whereas C&D became soft. Fluoride-uptake after-challenge (µgF/cm², SD): A: 17.3 (5.1); B: 14.1 (7.1); C: 7.2 (3.3); D: 4.0 (0.9); A&B are statistically different from C&D. Percent of theoretical fluoride-ion released in DI-water at 0.5, 4.0 and 24.0 hours: A: 70.6, 76.5, 93.0; B: 10.4, 36.8, 71.5; C: 23.6, 26.3, 36.2. The fluoride released from A&C is immediate and slower after 30-minutes, whereas fluoride released from B is more-constant over time. Formulation-C requires longer exposure to remineralize. **Conclusion:** In 4-hours, coating-A enables highest increase in tooth microhardness. The unique fluoride-release kinetics, coating-composition, and fluoride-ion permeation through coating-A enables remineralization in a shorter time.

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Salivary Fluoride Retention Following Toothbrushing with Experimental Toothpastes Containing Three Different Concentrations of S-PRG Fillers

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This crossover study was carried out to evaluate salivary fluoride retention after toothbrushing with experimental toothpaste containing three different concentrations (1, 5 or 20 wt%) of surface pre-reacted glass-ionomer (S-PRG) filler from which fluoride ions are released gradually, respectively. Seven consenting subjects (Ave. 36.4 years) brushed their teeth in the same manner using 0.5 g of toothpaste containing S-PRG filler (ϕ 1 µm) for 2 minutes. After toothbrushing, they spat out once and rinsed their mouths with 15 ml of distilled water for 5 seconds. Resting saliva samples were collected for 3 minutes each at different time intervals; 5, 10, 15, 30, 60, 120 and 180 minutes following mouth rinsing. Saliva samples collected after brushing using toothpaste without S-PRG filler (0 wt%) served as control and pre-brushing saliva samples

served as baseline. One-tenth volumes of low-level TISAB were added into the saliva samples and then fluoride concentrations were determined using a fluoride-selective electrode. ANOVA analysis indicated that salivary fluoride concentrations were affected by concentrations of S-PRG filler in toothpaste and time intervals between mouth rinsing and saliva collection with a significant interaction between these factors. Salivary fluoride concentrations (ppm, mean±SEM) after toothbrushing with toothpaste containing 1, 5 and 20 wt% S-PRG filler were peak 5 minutes after mouth rinsing, indicating, 1.24±0.27, 3.83±0.65 and 3.10±0.42, respectively, although the concentration of saliva exposed to toothpaste without S-PRG filler was 0.23±0.003. There were no significant differences in salivary fluoride concentrations between toothpaste containing 0 wt% and 1 wt% and between toothpaste containing 5 wt% and 20 wt% S-PRG filler at any time intervals. The results suggested that tooth brushing with toothpaste containing more than 5 wt% of S-PRG filler promotes fluoride retention in saliva.

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Fluoride Excretion Estimation in a Spot Urine Sample of 12-Years Old Adolescents

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The aim of this study was to determine how representative is a spot urine sample for the estimation of excretion of fluoride. A 24-h urine sample and a morning spot urine sample were collected from 13 Chilean adolescents. All the individuals had been residing in the same fluoridated area. All the urine samples were analysed, at most 24 h after collection, in duplicate. The fluoride content was determined directly using a fluoride ion selective electrode. Urinary creatinine was measured by the Jaffe method. Using the ratio Fluoride/Creatinine, we estimated the fluoride content of 24 hrs urine sample. A poor correlation between fluoride excretion estimated using the F/Cr ratio in a morning spot urine sample and fluoride excretion in a 24-h urine sample was found. The media of fluoride content estimation from F/Cr ratio was 1.22 mg/day and the media of fluoride content from the 24 hrs urine sample was 0.23 mg/day.

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Alkali-Soluble Fluoride Formation on Enamel and Dentine by APF and Its Retention in situ

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Alkali-soluble fluoride products formed on sound enamel by professional fluoride application are considered responsible for the anticaries effect of this way of fluoride use but there are scarce data about formation on dentine. Also, there is very few data about the in vivo retention of these chemical products on dental substrates. Therefore, we evaluated in situ the formation and retention of alkali-soluble products formed on enamel and dentine slabs by acidulated fluoride application (APF-gel). APF-gel (1.23% F, pH 3.5) was applied in 72 slabs of enamel and 72 of root dentine placed in palatal appliances of 18 volunteers (n = 18). Half of slabs of enamel and dentine were removed after application for determination of fluoride formed and the rest after 14 days. During these 14 days, biofilm was allowed to accumulate on slabs that were exposed 8x/day to one drop of a 20% sucrose, simulating high cariogenic challenge. Twice/day, the volunteers used 1,100 µg/g fluoride toothpaste (0.9 g). Alkali-soluble fluoride formed and retained on the slabs was extracted with 1 M KOH and analyzed with ion specific electrode. Baseline alkali-soluble fluoride was also determined in 18 slabs of enamel and dentine not subjected to any treatment. The results (Avg ± SD; n = 18) found for enamel and dentine were (µg F/cm²) respectively: Baseline = 0.11±0.03 and 0.13±0.05; Formed = 22.4±25.8 and 103.4±48.0; Retained = 4.3±3.7 and 19.4±12.1. The concentration of alkali-soluble fluoride formed on dentine was 5 times higher than that formed in enamel. For both dental substrates, these concentrations decreased around 80% after 14 days of high cariogenic challenge under fluoride toothpaste use. In conclusion, higher concentration of alkali-soluble fluoride is retained in dentine than in enamel after APF-gel application because the concentration formed is higher and the percentage leached out is similar.

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Development and Evaluation of Online Oral Hygiene, Prevention of Infection Module in E-Bug: European Project

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e-Bug is a web-based PHE-funded project to develop and disseminate, across Europe, a junior and senior school educational resource for teachers covering microbes, hygiene, antibiotics and prevention of infection. e-Bug has demonstrated knowledge acquisition in 9–11 year olds with hand and respiratory hygiene modules. To-date there has been no oral health module. Working in partnership with the e-Bug team at Public Health England, our research group has developed a new module 'Oral Hygiene, Prevention of Infection'. The module is evidence-based, follows cognitive and social theories of learning, aiming to impart oral health related knowledge and support development of healthy behaviours, specifically: reducing amount and frequency of free sugars intake; increasing and improving tooth brushing with fluoride toothpaste. **Aim:** To work with teachers, local education and health authorities to develop the new Oral Health module; evaluate teachers' views of content and structure to inform completion of materials. **Methods:** Evaluation conducted with school teachers in London. Three, one hour focus groups were set up commencing with presentation of the lesson plan and supplementary materials followed by semi-structured interviews to establish teachers' views. **Results:** Teachers across Years 3, 4 and 5 contributed covering child ages 7–10 years. Teachers were very positive to using the online lesson plan and engaged well with the materials. Several themes emerged regarding lesson duration, nutrition content and the feasibility of some of the activities. **Conclusions:** Following modifications to the module, it will be incorporated into e-Bug online and made available for evaluation with schoolchildren in selected primary schools. This research team is developing a European research consortium to examine the effectiveness of the Oral Hygiene e-Bug module in the development of healthy sugar and brushing behaviours in schoolchildren.

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Applicability of an in situ Model to Evaluate Fluoride Mouthrinse Effect on Enamel De-Remineralization

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In situ caries models, such as the 'Piracicaba in situ model', have been successfully used to assess cariogenicity of carbohydrates and the anticaries effect of fluoride from toothpastes and professional use. Here we studied the applicability of the Piracicaba model to test the effect of fluoride mouthrinses on enamel de- and remineralization. In a double-blind, crossover, in situ study, 10 volunteers wore a palatal appliance containing 8 bovine enamel slabs, covered by a plastic mesh and exposed to 20% sucrose. Four slabs were sound and were treated with sucrose 8x/day to assess enamel demineralization; 4 slabs had incipient caries lesions and were treated with sucrose 3x/day to assess enamel remineralization. The tested mouthrinses (0, 250 or 1000 ppm F, from NaF) were used 2 times/day. After 14 days, biofilm and the dental slabs were collected for analysis. Fluoride concentration was determined in biofilm fluid and solids. On enamel, percentage of surface hardness loss (%SHL) (initially sound blocks) and recovery (%SHR) (initially carious blocks) were determined. Model dose response was assessed by linear regression. The %SHL and %SHR were 80.1±23.1; 59.5±24.1; 42.0±18.3 and 3.7±14.9; 23.7±19.8; 29.4±9.3 for groups 0, 250 and 1000 ppm F, respectively, which were linearly correlated to fluoride concentration ($p = 0.001$ and 0.005). F concentration in the biofilm fluid and solids also increased linearly ($p < 0.01$) with F concentration in the rinse. The results show that the Piracicaba in situ model presents dose-response to assess the effect of fluoride from mouthrinses on enamel de- and remineralization.

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Quantitative Study of the Pore Volume of Human Fluorotic Enamel Filled with Resin Infiltrant

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Capillarity theory predicts that the pore volume infiltrated by a liquid in a body with tubular capillaries is directly proportional to the capillary radius. The expected volume available for infiltration is the loosely bound water volume (water volume replaced by air), which can be related to the radius available for infiltration.

Our aim was to test the hypothesis that the proportion of the pore volume infiltrated by resin infiltrant (Icon, DMG) ($V_{\text{Infiltrant}}$) is correlated and agrees with the proportion of the pore volume with loosely bound water ($V_{\text{loosewater}}$). Seven human fluorotic third molars (4 unerupted and 3 erupted; TF scores ranging from 4 to 7; fluoride content of inner coronal dentin ranged from 143 to 934 μg Fluoride/g) were prepared and resin infiltration was performed during 10 minutes in ground sections of fluorotic enamel whose cut surfaces were protected with nail varnish. Mineral volume was quantified by microradiography, and non-mineral volumes and penetration depths were quantified from interpretation of birefringence. Volumes were measured at histological points ($n = 98$) along transversal lines traced from the enamel surface to the enamel-dentin junction. No well-mineralized surface layer was found. Infiltration depths ranged from 380 to 1600 μm . $V_{\text{Infiltrant}}$ ranged from 1.8 to 17.7% (mean of $9.8\% \pm 4.2\%$), was lower than $V_{\text{loosewater}}$ (t-test: $p < 0.00001$; 95% CI: $-5.6\%/-4.4\%$), and correlated positively with $V_{\text{loosewater}}$ ($R = 0.71$; power $>99\%$) and negatively with the air volume remained after infiltration ($R = -0.74$, power $>99\%$). From an agreement plot, $V_{\text{loosewater}}$ exceeded $V_{\text{Infiltrant}}$ in 5% (1/3 of $V_{\text{loosewater}}$) on average. In conclusion, $V_{\text{loosewater}}$ and $V_{\text{Infiltrant}}$ correlated well but lacked good agreement, indicating that organic matter, firmly bound water and unpredicted amounts of air remained in enamel pores after resin infiltration.

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Effects of Polychlorinated Biphenyl 169 and Polychlorinated Biphenyl 155 on Tooth Development in Rat

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Polyhalogenated aromatic hydrocarbons (PAH), which include polychlorinated-biphenyls (PCB) and -dioxins (PCDD), are persistent and widespread lipophilic environmental pollutants. Studies have shown that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a model compound for PAH, can impair tooth development. The aim of our study was to investigate the adverse effects of two hexachlorobiphenyls, dioxin like planar PCB-169 and non-dioxin like non-planar PCB-155, individually and in combination, on different stages of tooth development in lactationally exposed rat offspring. Lactating Wistar dams ($n = 15$) were intraperitoneally administered a total of 3 mg/kg b.w. PCB-169 (group 1, $n = 4$), 12 mg/kg b.w. PCB-155 (group 2, $n = 4$), PCB-169 and PCB-155 together (group 3, $n = 3$), or olive oil (group 4, $n = 4$) only. Offspring were exposed to PCB-155 and/or to PCB-169 via mothers milk and sacrificed on postnatal days 9 and 22. PCB blood concentrations were analysed. Mandible halves with teeth in situ were prepared for histological examination. On longitudinal sections through lower molars presence and developmental stage of third

molars and shape of developing tooth crowns and roots of all molars were recorded. Tooth crown and root dimensions and thickness of dental hard tissues were measured. Additionally, changes in oral epithelium, enamel organ, dental pulp, periodontal ligament and alveolar bone were recorded. Statistically significant differences between groups were explored using Chi square-test and analysis of variance test (ANOVA). PCB blood concentrations confirmed offspring PCB exposure via mothers milk. PCB-169 and combination PCB-155 and PCB-169 exposed offspring had statistically significantly smaller teeth with shorter and in some cases fused roots, thinner dental hard tissues and frequently unmineralized enamel matrix remnants. PCB-169, individually and in combination with PCB-155, negatively affected tooth crown and root morphogenesis and hard tissue formation in lactationally exposed rat offspring.

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Tooth Loss Due to Dental Caries: A Case for Personalized Dentistry

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Certain risk factors such as tobacco use, diabetes, genetic variations on the IL1 gene, and other inflammatory conditions are hypothesized to predict tooth loss in patients treated in a large medical center. Tooth loss trends are hypothesized to be greater in patients with more risk factors. DNA samples for 881 individuals were taken from the Dental Registry and DNA Repository at University of Pittsburgh School of Dental Medicine. Clinical data for all 4,137 subjects in the registry were also available. SNP genotyping was performed on the samples for IL1 α (rs1800587) and IL1 β (rs1143634). IL1 positive status was determined as having one or more of the recessive alleles for either SNP. Tooth loss status was determined based on dental records and data gathered for age, sex, ethnicity, and self-reported medical history. Various statistical analyses were performed to determine if differences were statistically significant. Tooth loss averages increased with age by all risk factors (smoking, diabetes, hypertension, and interleukin genotypes; $p = 4.07E-13$) and by number of risk factors ($p = 0.006$). Since all those variables are associated with aging, we analyzed the data again by dividing the sample in adults younger than 50 years of age and older than 50 years of age. The same trend can be seen in both groups however the trend of increased tooth loss as one accumulates risk factors is much clearer in people younger than 50 years of age. Tooth loss in younger individuals is more likely due to dental caries than periodontal diseases. These trends suggest that older patients and those with more risk factors should seek further preventive care to reduce future tooth loss.

Session 4

Erosion: Lab-Based Studies and Hard Tissues

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The Protecting Effect of Adults and Children Salivary Pellicles against Initial Enamel Erosion

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This experiment aimed at assessing the protecting effect of salivary pellicles made from adults or children saliva against enamel erosion. Stimulated saliva was collected, centrifuged and stored as separate pools of adults (20–30 year-olds) and children (7–13 year-olds) saliva. Forty-five premolar enamel specimens were divided into three groups (n = 15): no pellicle control (NP), adults pellicle (AP) and children pellicle (CP). NP specimens received no saliva, while salivary pellicles were formed on AP and CP specimens using the respective saliva (humid chamber; 37°C; 2 h). Excess saliva was removed and the specimens were individually eroded (10 ml 1% citric acid; pH 3.6; 25°C, 1 min). Calcium released, enamel surface reflection intensity loss (SRI-loss) and microhardness loss (SMH-loss) were measured. The cycle consisting of pellicle formation, erosion and measurements was repeated 4 times. Differences between groups were tested with nonparametric ANOVA, and post-hoc Wilcoxon Rank Sum tests. Mean calcium release increased with erosion time to 4.9±0.7, 4.7±0.5, and 4.9±0.7 nmol Ca²⁺/mm² enamel, for NP, AP and CP, respectively, after 4 min erosion (p = 0.150). SMH-loss significantly increased with erosion time. After 4 min, NP presented greater SMH-loss (92.2±8.1 KHN; p < 0.05) than AP (80.0±12.3 KHN) and CP (86.5±10.9 KHN), with no difference between AP and CP (p = 0.250). SRI-loss also increased with erosion time. AP presented early protection, with significantly lower SRI-loss than NP and CP after 1 and 2 min erosion (p < 0.01), but after 4 min, AP (65.5±7.7%) and CP (64.9±7.1%) presented lower SRI-loss than NP (81.6±8.1%; p < 0.01). Both adults and children pellicles protected enamel against initial erosion, reducing SMH-loss and SRI-loss after 4 min, but SRI results show that adults pellicle presented an earlier protection.

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Evaluation of an Optical Pen-Size-Reflectometer in Detecting Erosive Tooth Wear in Permanent and Primary Teeth

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Objective: To evaluate the effectiveness of an optical pen-size-reflectometer in detecting erosive tooth wear in permanent and primary teeth. **Materials and Methods:** 75 permanent upper incisors and 80 primary upper canines were selected from a pool of extracted teeth. A trained examiner assessed erosion on their buccal surfaces according to the BEWE index (weighted kappa coefficients were 0.82 for permanent and 0.46 for primary teeth). Two different examiners (A and B) used the optical reflectometer to evaluate the enamel reflection factor (ERF) of the buccal surfaces of the teeth. Both examiners evaluated each tooth twice, and the intra- and inter-examiner reliability was calculated by means of intraclass correlation coefficients (ICC). Later, the teeth were dichotomized into either eroded teeth (ET) or sound teeth (ST), and the ERF averages of both groups were compared with Mann-Whitney U test (p < 0.05). **Results:** Strong ICC values were observed for intra- and inter-examiner reproducibility in permanent and primary teeth (ICC 0.83–0.92). In both examiners' evaluation eroded teeth (ET) presented higher ERF values than sound teeth (ST) for permanent and primary teeth. ERF values for permanent teeth were: examiner A ET 25.61±8.12 and ST 19.17±6.72 (p = 0.001); and examiner B, ET 35.26±10.25 and ST 24.82±7.76 (p < 0.001). For primary teeth ERF values were: examiner A, ET 18.07±5.80 and ST

13.56±4.05 ($p < 0.001$); and examiner B, ET 26.09±7.71 and ST 19.63±6.52 ($p = 0.001$). **Conclusions:** The reproducibility of the ERF values was excellent according to ICC values. The optical reflectometer maybe a useful tool to detect erosive tooth wear lesions on the buccal surfaces of both permanent and primary teeth, specially when used by the same examiner.

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In-vitro Effect of Different Vinegar Varieties on Enamel Surfaces of Molars

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The aim of this in-vitro study was to analyze dental enamel specimens after exposure to different vinegars for erosive changes. 24 surgically removed wisdom teeth from various young patients were used. After removal of roots and residual tissue, the crowns of the teeth were cut in half, fixed and embedded in 12-well plates with Venus (Heraeus Kulzer, Hanau, Germany) so that an average enamel area of 34 mm² was exposed. One half per tooth ($n = 6$ per vinegar) was incubated with 4 ml of a vinegar (Condimento Rosato, pH = 2.7; Condimento Balsamico Bianco, pH = 2.7, Vinagre de Jerez sherry vinegar, pH = 2.7; Bio-Ess apple cider vinegar, pH = 3.0) for 8 h at 37°C, the other half, incubated with saline, served as control ($n = 24$). Afterward, the amounts of calcium in the supernatants were measured colorimetrically using kits (Randox, Laboratories, Krefeld, Germany), corrected for calcium contents in the vinegars and expressed in mg/34 mm². Surface roughness (Ra) was determined ($n = 48$) profilometrically (Perthometer, Mahr, Göttingen, Germany), data were evaluated with the MarSurfX20 software. A visual examination in a digital microscope (VHX, Keyence, Neu-Isenburg, Germany) followed at magnifications of x300 and x500. The following amounts of calcium were released (mean±SD, mg/34 mm²): Condimento Rosato 0.14±0.06; Condimento Balsamico Bianco 1.43±0.17; Vinagre de Jerez sherry vinegar 2.33±0.35; Bio-Ess apple cider vinegar 2.29±0.61. Except for the last two, the differences were statistically significant ($p < 0.05$; one-way ANOVA, Tukey post-hoc test). No significant differences for Ra were found between controls and specimens exposed to vinegar (paired t-test), and also not among the controls or among the exposed specimens. Visual analyses revealed irregular enamel surface structures in controls and in specimens incubated with vinegar. In conclusion, erosive changes from in-vitro incubation of specimens from wisdom teeth with vinegars could only be expressed as calcium loss, but not as change in surface roughness.

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Erosive Potential of Various Vinegars in-vitro with Specimens from Extracted Front Teeth

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The objective of this study was to assess in vitro the erosive potential of various vinegars using dental enamel specimens.

Caries-free front teeth, removed for orthodontic reasons or from patients with periodontitis, were included in this study. The crowns were separated from the roots, polished with SiC and aluminium oxide discs of decreasing particle size, cut in half, fixed and embedded in 12-well plates with Venus (Heraeus Kulzer, Hanau, Germany) with an average exposed enamel area of 16 mm². One half per tooth ($n = 6$ per vinegar) was then incubated with 4 ml of a vinegar (Ortalli Bianco pH = 2.4, Apple cider vinegar, pH = 3.2; Aceto di Vino Rosso, pH = 2.8, raspberry vinegar, pH = 2.75;) for 8 h at 37°C; the second half, incubated with saline, served as control ($n = 24$). To determine calcium released into the supernatants, kits (Randox, Laboratories, Krefeld, Germany) were used, the amounts were corrected for calcium in the vinegars and expressed in mg/16 mm². Surface roughness (Ra, Rq, Rz, Rmax, Rt) was determined ($n = 48$) profilometrically (Perthometer, Mahr, Göttingen, Germany), using the MarSurfX20 software. The enamel surfaces were also visually examined in a digital microscope (VHX, Keyence, Neu-Isenburg, Germany) at magnifications of x300/x500. The following amounts of calcium were released (mean±SD, mg/16 mm²): Ortalli Bianco 0.13±0.08, Apple cider vinegar, 2.29±0.57; Aceto di Vino Rosso 2.60±0.94; raspberry vinegar 2.60±0.66. The amount of calcium released by Ortalli Bianco was statistically significantly lower ($p < 0.001$; one-way ANOVA, Tukey post-hoc test). Significant differences in the values for surface roughness between controls and specimens exposed to vinegar were only found for raspberry vinegar (paired t-test) where an increase was observed (e.g. 1.77±0.7 µm for Ra). Visual analyses of the enamel surfaces confirmed the results from the determination of the surface roughness. It can be concluded that in-vitro-exposure to vinegars caused loss of dental enamel and alterations in surface topography.

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Effect of Extrinsic and Intrinsic Acids in the Development of Enamel Erosion and Erosive Wear

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This in vitro study quantified the progression of 'Erosion' to 'Erosive wear' phase, when enamel was exposed to the most important extrinsic and intrinsic acids for 30 s to 120 minutes, by using surface microhardness and contact profilometry, respectively. Forty enamel samples were randomly divided into 2 groups: 1) 0.05 M citric acid (pH 2.5) simulating extrinsic erosion; 2) 0.01 M hydrochloric acid (pH 2.2) simulating intrinsic erosion. The samples were submitted to acid challenges (25°C, under agitation) for 30 seconds to 120 minutes. After each period of acid challenge, the samples were evaluated by hardness (until hardness increasing and/or baseline indentation was no longer visible), which is indicated to measure the stage of softening 'Erosion'. The samples were also analysed by profilometry, indicated to quantify 'Erosive wear'. The "'Erosion' phase" was measurable up to 1 minute and 2 minutes for hydrochloric and citric acids, respectively. In this stage, there was no significant difference between the acids ($p > 0.05$). Erosive wear was significantly increased over time for both types of acids, which was clearer from 10 to 120 minutes compared to the earlier times. There was no significant difference between the acids from 2 to 8 minutes. However, after 8 minutes citric acid was more aggressive than hydrochloric acid ($p < 0.001$). The progression of enamel erosion from softening to wear is highly dependent on the type of acid, being citric acid more aggressive in later stages.

The Erosion-Protective Effect of Enamel Proteins

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This study analyzed the protective effect of enamel proteins against initial erosion in mature enamel. From 40 human molars, we ground away 100 µm of enamel on the buccal or lingual sides. The resulting surfaces were polished, while the opposite sides were ground to obtain specimens with coplanar surfaces. We deproteinized half of these specimens by individually immersing them in hydrazine hydrate for two weeks at 70°C. This procedure completely removes all proteins at and close to the surface. The other half of specimens served as controls and was incubated for two weeks at 70°C in mineral solution. We then determined the exposed surface area and measured the initial surface microhardness (SMH) and reflection intensity (SRI) of each sample before individually immersing them in 10 ml of citric acid (1%, pH 3.6) for a total of 10 min (25°C, 70 rpm dynamic conditions). After 1, 2, 4, 6,

8 and 10 min, the samples were removed from the acidic solutions and their SMH and SRI measured, while the acidic solutions were analyzed by atomic absorption spectroscopy to determine the calcium release. The deproteinized samples released significantly more calcium ($p < 0.0001$), and their SMH as well as their SRI values decreased faster than those of the control group. Statistical analysis confirmed that the progression of the loss of SMH ($p < 0.0001$) and SRI ($p = 0.002$) and the progression of calcium release ($p < 0.0001$) differed significantly between the two groups. Therefore, we conclude that enamel proteins play a role in the natural resistance of enamel against initial erosion, slowing down calcium release and surface softening.

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Gels Containing MMP Inhibitors to Preserve the Organic Matrix Increase Recovery of Eroded Dentin Specimens

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Eighty human root dentin blocks (4x4x2 mm) were demineralized by immersion in 1% citric acid solution (pH2.3) for 60 min (20 ml/specimen). After demineralization, tissue loss was evaluated by profilometry. In 40 specimens, the demineralized organic matrix (DOM) was removed by immersion in 0.5 ml of mineral solution containing type VII collagenase from *Clostridium histolyticum* (100 U/ml) for 96 h and profilometry was performed again. Groups with DOM preserved or not were allocated to subgroups that differed with respect to the type of gel that was applied (4 min) before remineralization and after 7 days of remineralization, as follows: Placebo gel (no active, control), gel containing 0.2 mM Galardin (MMPinhibitor), gel containing 500 µM E-64 (Cysteine Cathepsin-CC inhibitor) and gel containing 10 mM chlorhexidine-CHX (MMP and CC inhibitor). Specimens were immersed in remineralizing solution (1.45 mM CaCl₂, 5.4 mM K₂HPO₄, 0.05 mM ZnCl₂, 100 mM TRIS, pH 7.0) for 14 days (20 ml/specimen). Profilometry was performed again after days 7 and 14. Data (with DOM and without DOM) were analyzed by 2-way repeated-measures ANOVA and Tukey's test ($p < 0.05$). For the specimens that had the DOM removed, dentin loss showed a significant decrease with time, regardless the treatment with the gels that had no influence on dentin loss. When the DOM was preserved, significant differences were detected among the times and treatments, with significant interaction between these criteria. In the beginning of the experiment, dentin losses were similar for all the treatments. At days 7 and 14, only the groups treated with MMP inhibitors (galardin and CHX) had dentin losses significantly lower than those found for placebo gel. The preservation of the DOM by the application of MMP inhibitors significantly increased the recovery of eroded dentin specimens.

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Structural Changes in Erosion/Abrasion Lesions and Their Importance to Clinical Treatment

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The aim of this study was to analyze the changes that occur in the hard tissues of the teeth affected by abrasion and the related morphological changes in the dental pulp of these teeth, to find out how much of the tooth health is preserved and the eventual clinical implications of these changes. For the study we used 46 extracted teeth diagnosed with abrasion in different stages. These teeth were used for histological and immunohistochemical research. The hard tissues were decalcified, stained with methylene blue and sectioned. The dental pulp was prepared for histology and stained with Hematoxylin and Eosin and Goldner-Szeckelly stain. For the immunohistochemical study the tissue sections were embedded in paraffin and we investigated the epitopes: CD31, CD34, CD8, S100. On the hard tissue sectioned we observed alterations in the dentinal tubules within a layer of reactionary dentin, and due to the loss of enamel this layer was found close to or at the surface of the teeth. The histology study showed the presence of calcified deposits, of various sizes, in the dental pulp. Also, the smooth muscular and collagenous fibers were disorganized to various extents. The immunohistochemistry demonstrated alterations of different stages for nerves and blood vessels within the dental pulp, many new-formed vessels situated mainly under the odontoblastic layer in variable number depending on the abrasion level and destruction on the smooth muscles and nervous fibers was confirmed. In conclusion we have shown that surface enamel abrasion results in anatomical changes in the underlying pulp.

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Influence of Erosive Challenge in Deciduous Teeth Undergoing Restorative Procedures with Different Adhesive Protocols – in vitro Study

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Aim: To evaluate the influence of erosive challenge in the tooth-restoration interface of deciduous teeth submitted to different adhesive protocols (etch-and-rinse and self-etching adhesives, with and without fluoride). **Methods:** In this in vitro study, 40 deciduous molars were cut mesio-distally, embedded in polystyrene resin, grinded and polished. The samples were randomly divided ac-

ording to the adhesive system: G1 (Adper Single Bond 2[®]), G2 (Universal Single Bond[®]), G3 (Optibond FL[®]) and G4 (Bond Force[®]). After conducting standardized cavity preparation (2x2 mm), adhesive systems were applied and the samples were restored with composite resin. Half of the samples were submitted to erosive and abrasive cycles, and the other half (control group) remained immersed in artificial saliva during the experimental phase (n = 10). In the erosive challenge, the samples were immersed in 50 ml Coca-Cola[®] (pH 3.6) for 1 minute at 25°C, under constant shaking, three times per day for five days. The samples were brushed once a day using fluoridated toothpaste and artificial saliva. For microleakage analysis, samples were submersed in methylene blue 1%, cross-sectioned, and analyzed at 40x magnification. Cross-sectional microhardness (CSMH) was performed with 50 g/5 s indentations in enamel at 25 µm, 50 µm and 100 µm from the eroded surface and at 25 µm, 75 µm and 125 µm from the adhesive interface. **Results:** Considering the microleakage, in 7.5% of the samples, no dye infiltration were observed, in 30% dye was observed only at enamel and in 62.5% the dye infiltrated through amelo-dentin junction. No significant difference was observed on CSMH at different depths in all groups (Two-Way-ANOVA, p ≥ 0.05). **Conclusions:** No significant changes were observed in both test and control groups on microleakage or CSMH in deciduous teeth submitted to different adhesive protocols.

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The Effect of Therapeutic Products in Combination on Prevention of Tooth Surface Loss in vitro

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Aims: To investigate therapeutic products for daily use, in combination, for prevention of enamel surface loss in vitro. **Methods:** 15 bovine enamel specimens were used in each of four groups; 1. Elmex[®] sensitive plus toothpaste (AmF, 1400 ppm F) plus Elmex[®] erosion mouth rinse (MR) (500 ppm F as AmF/NaF and 800 ppm Sn as SnCl₂), used twice/day (Elmex TP plus MR), 2. Pronamel toothpaste (NaF, 1450 ppm F+ 5%KNO₃) plus Pronamel (NaF) mouth rinse, twice/day (Pronamel TP plus MR), 3. Pronamel toothpaste 2/day plus GC tooth mousse (TM) once/day for 5 mins (Pronamel TP plus TM), 4. 0 ppm F toothpaste control. pH cycling was performed using 0.3% citric acid (pH 3.6), 2 min/five times/day. Abrasive challenge was provided with automated toothbrushing (15 strokes, 300 g load) for 2x2 min/day during the total experimental period of 28 days. The enamel slabs were left in day artificial saliva between each erosive challenge for 1 hour and incubated overnight in night saliva at 37 C. Surface loss was determined by profilometry after 7, 14, 21 and 28 days. **Results:** after 28 days significantly less enamel surface loss (µm) was observed with Elmex TP plus MR (0.40±0.23) and Pronamel TP plus MR (0.60±0.28) compared with Pronamel TP plus TM (1.84±1.85) and 0 ppm F control (2.65±1.79). Although Elmex TP plus MR gave consistently better protection at all-time points compared with

Pronamel TP plus MR, this did not reach statistical significance. **Conclusion:** The topical applications of combinations of toothpaste and mouthrinse significantly reduced surface loss of enamel in our model, whereas the use of a NaF toothpaste in combination with a widely used remineralising mousse containing casein phosphopeptide and amorphous calcium phosphate did not offer any significant protection.

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Influence of Polymers on the Protective Effect of Fluoride Over Enamel Erosion Development

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The aim of this study was to evaluate if the incorporation of different polymers (Carbopol 980, Carboxymethylcellulose-CMC and Aristoflex AVC) in a fluoridated solution (NaF 900 ppm) would increase the protective effect of fluoride on initial enamel erosion. The concentration of the polymers (1% w/v) was pre-determined to achieve solutions with similar viscosity, which were intended to simulate a mouth rinse. Ultrapure water was used as control. Bovine enamel specimens (n = 13 per group) were prepared and initial Knoop microhardness (KHN1) was measured. The specimens were submitted to 2 min immersion in 0.3% citric acid; 1 min treatment with polymer solutions, with or without sodium fluoride (900 ppm); 60 min storage in artificial saliva; and a new 2 min immersion in citric acid. Microhardness was assessed after the first acid challenge (KHN2), after the first immersion in the treatment solutions (KHN3), and after the second acid challenge (KHN4). Percentage of remineralization (%rem=[KHN3-KHN2/KHN2]*100) and of protection (%prot=[KHN4-KHN2/KHN2]*100) were determined. Data were analyzed with 2-way ANOVA and Tukey's tests (p < 0.05). Regarding rehardening properties, no solution containing the polymers differed significantly from control. When associated with NaF, Carbopol (11.42±6.41) presented similar %rem compared to NaF solution without polymers (14.47±6.75), significantly higher than Aristoflex (7.33±5.30) and CMC (5.80±6.25). For initial erosion protection, the solutions with the polymers isolated did not significantly reduce enamel demineralization compared to the control. When associated to NaF, Carbopol (-3,00±7.34) presented significantly higher protection than the NaF solution without polymers (17.61±8.10), Aristoflex (17.80±5.84) and CMC (18.22±8.72). It was concluded that Carbopol did not influence NaF remineralization potential, but it enhanced NaF protection against initial enamel erosion. Carbopol is therefore a promising active agent to be added to NaF solutions intended to prevent or control enamel erosion.

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Effects of Conventional and Anti-Erosion Fluoride Products on Enamel Erosion

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Objectives: In this in vitro study the protective effect of different agents on enamel erosion was compared. **Methods:** In total 60 bovine incisor enamel discs were ground, polished and divided into 5 treatment groups and a control group (n = 10): Stannous fluoride 970 ppm F and ACP (SnF-ACP), fluoride only as 1450 ppm F (NaF), Stannous fluoride (1100 ppm F) with 350 ppm NaF (SnF-NaF), NaF 900 ppm F paste with Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), Stannous fluoride (1400 ppm F) chitosan (SnF-Ch), Negative Control (NoF). The treatment-erosion-cycle (10 days) consisted of treating the samples with the assigned slurry (dilution 7 g: 23.3 ml, 2x2 min/day) and rinsing for 15 sec with deionized water. Subsequently the samples were submitted to an erosive challenge (0.65% citric acid, pH = 3.6, 2 x 10 min/day). The remainder of the day the specimens were kept in remineralization solution (1.5 mmol/l Ca, 0.9 mmol/l PO₄, 130 mmol/l KCl, 20 mmol/l HEPES, pH: 7.0. 0.5 Mm sodium azide is added to prevent bacterial growth.) At the end of day 5, half of the specimens were taken out for profilometric measurements and the remaining specimens were measured at day 10. Statistical analysis was done with repeated measures ANOVA and one-way ANOVA tests. **Results:** After 5 days of cycling enamel loss of the specimens with profilometry was 9 µm (SnF-ACP), 6 µm (NaF), 6 µm (SnF-NaF), 16 µm (CPP-ACP), 1 µm (SnF-Ch), 17 µm (NoF). Specimens in all groups showed significantly lower substance loss than the specimens in group CPP-ACP and NoF (p < 0.05). After 10 days of cycling, enamel loss of the specimens with profilometry was 13 µm (SnF-ACP), 23 µm (NaF), 9 µm (SnF-NaF), 31 µm (CPP-ACP), 10 µm (SnF-Ch), 45 µm (NoF). The group containing SnF-NaF showed significantly higher prevention as compared to SnF-ACP and NaF (p < 0.05). Stannous fluoride with NaF and Stannous fluoride with chitosan (SnF-Ch) prevented erosion indicated by a reduced substance loss measured with profilometry. **Conclusion:** It is concluded that SnF₂-containing dental products are able to reduce erosive tooth wear in vitro.

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Interaction between Toothpaste Abrasivity and Toothbrush Filament Stiffness on the Development of Erosive-Abrasive Lesions

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This in vitro study investigated the interaction between toothpaste abrasivity and toothbrush filament stiffness on the development of erosive-abrasive lesions in enamel and dentin, independently. The study followed a 2 (high/low-level abrasive; silica) × 3 (bristle stiffness; soft/medium/hard) × 2 (cycling time; 3/5 d) factorial design. Bovine enamel and dentin specimens were cut, embedded, and polished. Protective tapes were placed on the surface of the specimens, leaving an area of 1×4 mm exposed in the center. Specimens (n = 8) were subjected to 5 d of erosion/abrasion cycling: erosion (5 min, 4×/d, 0.3% citric acid, pH 3.75), abrasion (15 s, 2×/d, 45 strokes each, 150 g load, automated brushing machine), fluoride treatment (15 s with abrasion and 45 s without abrasion; 275 ppm F as NaF in abrasive slurry) with exposure to artificial saliva between erosion and abrasion/Fluoride exposure (1 h) and all other times (overnight). Non-contact profilometry was used to determine surface loss (SL) after 3 and 5 d of cycling. Data were analyzed using three-way ANOVA (factors: abrasive/bristle stiffness/time) with separate analyses conducted for enamel and dentin. For enamel, only 'cycling time' was found to affect surface loss with 5>3 d. Overall, there was little SL (mean range: 0.76–1.85 µm). For dentin (mean SL range: 1.87–5.91 µm), significantly higher SL was found for 5 vs. 3 d, with particularly large differences for hard stiffness/high-level abrasive, and medium stiffness/medium abrasive. Hard stiffness resulted in significantly higher SL than medium stiffness for high abrasive after 5 d, with no other significant stiffness differences. High abrasive had significantly higher SL than medium abrasive overall with strong effects for all combinations, except medium stiffness after 5 d. In conclusion, the interplay between abrasivity and filament stiffness appears to be more relevant for dentin than enamel.

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Erosion Potential of Mineral Effervescent Tablets Available in Switzerland

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Objectives: To investigate the erosive potential of seven commercially available mineral effervescent-tablets (ET). **Methods:** Ninety-six bovine enamel samples were prepared and randomly assigned to eight groups (G1-G8). Percycle, samples (n = 12/

group) were immersed (2 min) in solution (10 ml/sample) freshly prepared from water supplemented with: none (control-group, G1), Actilife Calcium ET (G2), Actilife Calcium+Magnesium ET (G3), Actilife Magnesium ET (G4), Optisana Magnesium ET (G5), Sunlife Calcium ET (G6), Sunlife Magnesium ET (G7) and Well&Active Magnesium ET (G8) according manufactures' instructions. Enamel loss was measured using profilometry after 10 and 20 erosive cycles and compared by Kruskal-Wallis and Mann-Whitney tests. Additionally, saturation of the solutions with respect to hydroxyapatite (HA), octacalciumphosphate (OCP) and calcium-fluoride (CF) was determined [Shellis: Comput Appl Biosci 1988;4:373–379]. **Results:** After 10 and 20 cycles, no tooth loss was observed for G1 (control), G2, G3 and G6. Significantly highest enamel loss (mean ± SD) after 10 and 20 erosive cycles was observed for G5 (3.66±0.41 µm and 8.32±1.00 µm, respectively) and G8 (3.22±0.54 µm and 8.55±1.21 µm, respectively). The lowest erosive wear after 10 cycles was observed for group G7 (0.45±0.28 µm) and after 20 cycles for groups G4 and G7 (3.11±0.78 µm and 2.59±0.89 µm, respectively). In all groups, except G1-G3 and G6, the tooth wear after 20 cycles was significantly higher compared to that after 10 cycles (p < 0.05, respectively). All solutions, except G1 (control), presented an acidic pH between 4.21 and 4.71 and were undersaturated with respect to HA, OCP and CF. **Conclusion:** All ET with magnesium alone showed an erosive potential and patients should be informed accordingly.

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Erosive Potential of Juices from Amazonian Brazilian Fruits

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Considering that the erosive potential of Amazonian Brazilian fruits is unknown, the purpose of this study was to determine the erosive effects of the juices of araçá-boi (AR), camu-camu (CA), cupuaçu (CU), taperebá (TA) and umbu (UM) on enamel. The pH of the juices was determined and their erosive effect was studied in vitro and in vivo. For the in vitro erosive cycling, bovine enamel slabs, with pre-determined surface hardness (SH), were randomized (n = 5/group) for treatments with the juices, 1% citric acid solution pH 3.75 (positive control; C+) and purified water (negative control; C-). The cycling lasted for 5 days. The slabs were treated 4x/day for 2 min and were immersed in remineralizing solution between the treatments for 2 h. Percentage of SH loss (%SHL) was determined at the end of each day of cycling and surface loss (µm) was analyzed by profilometry at the end of the study. For the in vivo study, volunteers (n = 12) sipped the treatment solution, held for 10 s in the mouth and expectorated solution and saliva. After 15 up to 120 s, saliva was collected and pH was determined. The pH data of each saliva collection were used to calculate the area under the curve of pH versus time (AUC). pH values (mean±SD, n = 6) were: AR: 2.9±0.1^b; CA: 2.8±0.1^{bc}; CU: 3.5±0.1^a; TA: 2.7±0.1^c; UM: 2.5±0.1^d. %SHL after 3 days of cycling was: C-:

3.3±6.0^a; C+: 50.7±6.7^b; AR: 77.9±4.2^d; CA: 65.3±3.9^c; CU: 53.8±6.9^b; TA: 74.8±4.8^{cd}; UM: 77.3±3.4^d. Surface loss was: C-: 0.2±0.2^a; C+: 0.5±0.4^{ab}; AR: 2.3±1.0^{bc}; CA: 1.5±0.9^{bc}; CU: 0.5±0.3^{ab}; TA: 2.1±1.2^{bc}; UM: 3.2±1.1^c. AUC was C-: 588±30^a; C+: 506±47^b; AR: 509±40^b; CA: 522±40^b; CU: 509±55^b; TA: 510±47^b; UM: 490±60^b. Distinct letters indicate statistically significant difference by Tukey test. The juices of Amazonian fruits may be erosive for enamel, with araçá-boi, taperebá and umbu juices potentially presenting the most erosive effect.

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Adhesive System as an Alternative Material for Color Masking of White Spot Lesions

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Objectives: The aim of this study was to evaluate the color masking effect of artificial white spots lesions (WSL) infiltrated by a commercial infiltrant resin in comparison to different adhesive systems. **Methods:** 144 enamel/dentin specimens were obtained from bovine incisors. The baseline color was evaluated using a reflectance spectrophotometer, according to CIELab system. Artificial WSL were produced and the color was evaluated again. The samples were divided into eight groups: Control-artificial saliva for 7 days; IC-infiltrating resin Icon (DMG); EC-EquiaCoat (GC); FU-Futurabond U (Voco); SBU-Single Bond U (3M/ESPE); SBMP-Scotchbond MP (3M/ESPE); OB-OptibondFL (Kerr); BF-Bioforty (Biodinamica). The adhesive systems were applied using the same method recommended to Icon. After treatments, the final color evaluation was performed. The values of ΔL , Δa , Δb and ΔE were calculated in relation to the baseline measurement. Data were analyzed by one-way ANOVA and Tukey tests. **Results:** After artificial WSL, the L^* values increased and the b^* values reduced. After treatments, the L^* values were reduced compared to the color after WSL. For ΔL values, only SBMP, OB and BF did not show difference in relation to the control. The other resinous materials presented significant differences to the Control ($p = 0.000$), although the IC showed a higher effect in L^* reduction, resulting in negative ΔL values. For b^* coordinate, the values increased after the treatments. For Δb values, all groups showed differences in relation to the Control and no differences among them ($p = 0.001$). In relation to ΔE , all groups showed differences in relation to the control group and no significant differences among them ($p = 0.001$). **Conclusions:** The commercial infiltrant produced a higher lightness change, although for general color difference, all the adhesive system tested were able to perform the color masking of artificial WSL.

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Radiopaque Tagging of Caries Lesions: A Microradiographic and Microbiological Study

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Objectives: Caries lesions remaining after selective excavation are radiographically detectable, which causes diagnostic uncertainty. Radiopaque tagging has been suggested for resolving this uncertainty. The aim of this study was to evaluate if tagging (masking) effects depend on the depth of the lesion and to assess antibacterial effects of radiopaque tagging. **Methods:** Human dentinal samples were demineralized using acetic acid (pH 5.0) for 2, 4, 6, 8 and 10 weeks ($n = 34$ /group). The resulting lesions (mean [SD] depths: 282 [52] to 554 [66] μm , mineral loss [ΔZ]: 3560 [1050] to 5005 [1470]) were evaluated using wavelength-independent microradiography, treated with one of two radiopaquetagging materials (SnCl_2 or SnF_2) in aqueous solution (70 and 30 wt%), and re-evaluated microradiographically. Relative tagging effects ($\Delta\Delta Z$ in % compared with baseline) were determined and submitted to repeated measures ANOVA for statistical analysis. To evaluate antimicrobial effects, 40 dentin specimens were pre-demineralized (0.5 MEDTA for 24 h, followed by acetic acid for 14 days) and submitted to a constant-culture bacterial invasion model employing *Lactobacillus rhamnosus* (ATCC 12116) Samples were divided into 4 groups: No treatment (negative control), 0.2% chlorhexidine (positive control), SnCl_2 or SnF_2 ($n = 10$ /group). Dentin was sampled using rose-head burs, weighed, and colony-forming units/mg determined. **Results:** The tagging effect was significantly influenced by the radiopaque material ($p < 0.001$ /ANOVA), with higher relative tagging by SnCl_2 than SnF_2 . Tagging effect decreased linearly with increasing lesion depths ($R^2=0.489$, $p < 0.001$). The median (25th/75th percentiles) number of viable bacteria was 7.3 (0.5/10.4), 3.7 (0.3/7.3), 0 (0/0) and 0 (0/0) $\times 10^5$ CFU/mg in control, chlorhexidine-, SnCl_2 - and SnF_2 -treated dentin, respectively. **Conclusions:** Radiopaque tagging effects are less pronounced in deeper compared with shallower lesions. Application of tagging materials reduces bacterial numbers.

FS, HML and SP hold a patent regarding radiopaque tagging of residual lesions (AZ: DE/10/2014/003/037.7). FS receives a grant from the German Research Foundation (DFG: SCHW 1766/2-1).

Effect of Surface Pre-Treatment on Resin Infiltration of Active and Inactive Natural Enamel Caries Lesions

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To allow resin infiltration of the lesion body the surface of non-cavitated caries lesions needs to be pre-treated. Therefore, it was the aim of the present in vitro study to evaluate the effect of different pre-treatment protocols on the resin infiltration of active and inactive natural enamel caries lesions. Proximal white spot lesions (active n = 60 and inactive n = 60) in extracted molars and premolars were etched with either 15% hydrochloric or 37% phosphoric acid gel for 60 s as well as 120 s (n = 15/group). Etching gels were passively applied on top of the lesions using small plastic spatula. After rinsing and drying, all lesions were resin infiltrated (Icon; DMG) for 3 min and the material was light cured for 60 s. Subsequently, lesions were cut into 200 µm sections for examination using dual fluorescence staining and confocal microscopy. Lesion depth (LD) and penetration depth (PD) were measured at three defined points along each lesion. Percentage of penetration (PP) was calculated for each lesion ($PP = \text{mean PD} / \text{mean LD} \times 100$) and statistically analyzed. Mean (SD) LDs of active and inactive lesions were 820 (352) µm and 682 (247) µm, respectively. Active lesions (N = 54) were significantly deeper infiltrated than inactive lesions (N = 51) ($P < 0.05$; ANOVA). Pre-treatment with 15% hydrochloric acid resulted in significantly deeper penetration [mean PP (SD)] after 120 s etching [active lesions: 76 (23)%, inactive lesions: 46 (13)%], compared with 37% phosphoric acid [active lesions: 24 (16)%, inactive lesions: 18 (14)%] ($P < 0.05$; ANOVA). Although shorter application of etchant resulted in reduced PP, influence of application time was not significant ($P > 0.05$; ANOVA). It can be concluded that surface pre-treatment of caries lesions with 15% hydrochloric acid is more effective to obtain deeper resin infiltration compared with using 37% phosphoric acid.

The study was supported by DMG, the manufacturer of Icon, which is hereby acknowledged. HML & SP receive royalties from Icon sales.

The Relationship between Enamel Porosity and Depth of Erosive Lesions Investigated by SEM and CLSM

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The inter-prismatic regions of enamel can act as diffusion pathways for acids and dissolved ions, and can influence the demineralisation process. In this study, the effects of acid erosion on the depth and distribution of enamel pores were considered. Scanning electron microscopy (SEM) was used for imaging fractured cross-sections through erosive lesions to measure the depth of the demineralised enamel. Confocal laser-scanning microscopy (CLSM) was also utilised to obtain tomographic images of enamel pores. 21 polished bovine enamel discs were randomised into seven groups which were immersed in 1% citric acid, pH = 3.8 for 0, 15, 30, 45, 60, 90 and 120 minutes after which discs were rinsed, then soaked in fluorophore solution (Rhodamine B, 0.1 mM) and imaged by CLSM to determine porosity depth (PD). The discs were subsequently fractured and cross-sections were imaged by backscattered SEM to measure lesion depth (LD). Comparisons between LD and PD were made using linear least squares regression and ANOVA. The mean LDs (SD) for 0–120 minute acid exposure were 0.0 ± 0.0 , 5.3 ± 0.5 , 7.9 ± 1.1 , 9.9 ± 0.8 , 10.0 ± 0.3 , 11.2 ± 0.8 and 14.2 ± 0.2 µm. CLSM images demonstrated mean PDs (SD) that were proportional to the extent of acid exposure (4.1 ± 1.2 , 6.1 ± 1.8 , 6.6 ± 0.4 , 10.0 ± 4.9 , 10.4 ± 0.78 , 11.3 ± 4.1 and 13.1 ± 3.4 µm for 0–120 minute acid exposure). Regression analysis showed that there was a positive linear correlation between PD and LD ($R^2 = 0.910$, $P < 0.001$). In conclusion, CLSM was found to be a powerful technique for studying both quantitative and qualitative aspects of enamel demineralisation, demonstrating that enamel was porous to a depth that was directly correlated with LD as measured by SEM.

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Longitudinal Monitoring of Early Dentine Erosion with Bearing Curve

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Objectives: This study aimed to assess the potential of bearing curve roughness parameters for longitudinal monitoring of early dentine erosion. **Methodology:** 20 polished dentine samples were prepared from external surfaces of 10 human permanent premolar roots. A window was created on each sample and half of it was

covered with adhesive tape creating a control and exposed area. Baseline images were obtained with a non-contact profilometer (Alicona 3D Optical Infinite Focus). Samples were then immersed in 0.003 M citric acid for 10 minutes, three times a day in a cycling model for 3 days. In between erosive challenges, samples were stored in a remineralisation solution for up to 3 hours followed by overnight storage in similar remineralisation solution. Images were taken at the beginning of each cycling day. An area composed of 800 measurement lines was drawn on each image. The Bearing Curve parameters, Core roughness (R_k), Peak Roughness (R_{pk}) and Valley Roughness (R_{vk}) were extracted for both the control and eroded areas. The outcome measure used was the fraction of the roughness parameters of each time point from baseline (fR_k , fR_{pk} or fR_{vk}). **Results:** Repeated measures showed that fR_{pk} , fR_k and fR_{vk} (2.11 ± 1.71 , 1.73 ± 0.61 , 2.09 ± 1.41) for the eroded area detected significant ($P < 0.05$) erosion-interval related increase in roughness from baseline to day 3 while fR_{pk} , fR_k and fR_{vk} (1.01 ± 1.09 , 0.95 ± 0.27 , 0.82 ± 0.49) for the control area did not. Paired t-test revealed that fR_{pk} , fR_k and fR_{vk} for the eroded group showed a detection threshold of 1 day, 2 days, and 3 days from baseline respectively. **Conclusion:** Bearing curve parameters R_{pk} , R_k and R_{vk} can be used for longitudinal monitoring of early dentine erosion with R_{pk} being the most sensitive parameter.

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In-vitro Investigation of Timing of Stannous Fluoride and Sodium Fluoride Application to Prevent Enamel Erosion

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Objectives: The aim of this in-vitro study was to investigate the timing and effectiveness of a stannous fluoride (SnF_2) mouthrinse and a sodium fluoride (NaF) mouthrinse to prevent enamel erosion. **Materials and Methods:** 80 specimens were prepared from extracted human molars (REC ref 12/LO/1836) and randomly assigned to two groups (group 1 = one erosive cycle and group 2 = five erosive cycles). Both groups tested 225 ppm mouthrinses of stannous (PeriomedTM3MTMESPE) and sodium (FluoriguardTM-ColgateTM) fluoride. Fluoride was either applied before erosion (Fluoride 1 min, water-rinse 2 mins, deionised water 30 mins, 0.3% citric acid pH3.2 10 mins, water-rinse 2 mins) or after erosion (0.3% citric acid pH3.2 10 mins, water-rinse 2 mins, fluoride 1 min, water-rinse 2 mins, deionised water 30 mins). Mean step height was measured using non-contacting profilometry and the microhardness change was calculated by subtracting the KHN (Knoop Hardness Number) of the eroded area from the KHN of the reference area. As data were normal, one-way ANOVA and independent t-tests was used. **Results:** Statistically less step height occurred when SnF_2 was applied before erosion for both one cycle (before: $1.3 \mu m$ (SD = 0.63) and after: $2.3 \mu m$ (SD = 0.48); $p < 0.0001$) and five cycles ($4.6 \mu m$ (SD = 0.7) and $6.2 \mu m$ (SD = 0.8) respectively;

$p < 0.0001$). In contrast, significantly less step height occurred when NaF was applied after erosion (before $10.5 \mu m$ (SD = 1.1) and after $9.5 \mu m$ (SD = 0.87); $p = 0.035$). Microhardness change after one cycle was statistically greater for both SnF_2 and NaF when applied before the erosive challenge; SnF_2 before: 152.8 KHN (SD = 22.9) and after: 93.9 KHN (SD = 34.7); $p = 0.011$, NaF before: 133.3 KHN (SD = 27.6) and after: 102.1 KHN (SD = 21.1) $p = 0.035$.

Conclusion: Application of stannous fluoride inhibited erosion to a greater extent than sodium fluoride, particularly when applied before the erosive challenge. Hardness change and step height may be influenced by both the metal ion and timing of application.

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Surface Layer Erosion of Pit and Fissure Caries Lesions in Preparation for Resin Infiltration

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The penetration of a low-viscosity resin (infiltrant) into caries lesions depends on the erosion of the relatively impermeable surface layer (SL) that normally covers the lesion body. Previous studies investigated pre-treatments in order to enhance the infiltration into occlusal lesions but did not measure the SL-reduction directly. Therefore, the present study aimed to evaluate the effect of different pre-treatments on the erosion of the SL and consequently on the penetration of an infiltrant into occlusal lesions. Sixty extracted human molars showing ICDAS-2 lesions were randomly allocated to 5 groups. A small area of each lesion was covered with resin (Tetric EvoFlow; Ivoclar Vivadent) before etching. Twelve lesions each were etched with 15% HCl-gel (H120) or 37% H_3PO_4 -gel (P120) for 120 s. Further, the HCl-gel or two experimental etchants (H_3PO_4 -gel or HCl-gel, each including abrasives) were applied for 120 s or 30 s using a brush (surface pressure 150 g) (H30B, P120BA, H30BA). After rinsing and drying all lesions were infiltrated for 180 s (Icon; DMG). From each tooth sections were prepared that showed unetched and etched SL parts and the infiltration. The thickness, the erosion of the SL, the lesion and penetration depth were measured using dual staining and confocal-laser-scanning-microscopy. The thickness of the unetched SL [median (Q25/Q75)] was 33 (23/51) μm . The H120 [87 (69/100)%] and H30B [86 (73/100)%] showed significantly higher SL-reduction compared with P120BA [48 (26/75)%] or P120 [58 (33/64)%] but only in H30BA [100 (100/100)%] the SL was eroded completely ($p < 0.05$; Mann-Whitney test). While four groups showed only a slight infiltration the lesion areas were almost completely penetrated in H30BA ($p < 0.05$). Thus, HCl-gel including abrasives that was rubbed on the enamel surface with a brush seems to be most effective to erode the SL in preparation for resin infiltration.

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

Session 5

Diagnosics and Erosion: Clinical Studies

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Morphological Analysis of the Proximal/Occlusal area between Primary Molar Teeth Related to Dental Caries

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Aim: To determine the relationship between tooth morphology of the proximal surfaces and presence/absence of caries in primary molar teeth. **Methods:** After ethical approval and signed consent by parents, 36 4-yr-old Colombian children participated. Initially, the first and second upper/lower primary molar teeth, randomly selected from one side of the mouth, were separated with an elastic band. Two days after insertion the bands were removed, distal and mesial surfaces were cleaned and dried, and silicone impressions followed by bitewing radiographs were taken. Resin models of the impressions were made and 1.6X stereomicroscopic images were obtained from the occlusal view. Morphology of the proximal surfaces of the primary molar teeth was scored as: 0-convex mesial and distal surface with minor plaque-retention morphology; 1-concave mesial or distal surface with plaque-retention morphology in one surface, and 2-concave mesial and distal surface with plaque-retention morphology in both surfaces. Caries status of the mesial surfaces of the second primary molar teeth and the distal surfaces of the first primary molar teeth was radiographically assessed according to ICDAS: -score 0 (absence) vs. scores 1 to 6 (presence). Morphology and the caries status for each surface were scored twice. One randomly-selected scored surface from each patient was used in the analysis for a total of 36 pairs of observations (visual and radiographic). This procedure was repeated three times. Cramér coefficient (C) was used to measure the correlation between morphology and presence/absence of caries. **Results:** Prevalence of caries ($d_{ICDAS1-6mf-s}$) was 79% and

mean caries experience 1.97 ± 1.67 . Intra-examiner reproducibility (weighted Kappa) for radiographic-ICDAS and dental-morphology scoring was 0.76 and 0.81, respectively. The C values between pairs of observations was 0.50, 0.52, and 0.58, (p-values <0.05). **Conclusion:** This study shows a substantial and significant correlation between tooth morphology of proximal surfaces in the first and second primary molar teeth and the presence/absence of caries.

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Post-Eruptive Breakdown of MIH Opacities Recorded Over 12 Months

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Objective: To determine whether Molar-incisor Hypomineralization (MIH) enamel opacities can predict enamel/dentine post-eruptive breakdown. **Methods:** A longitudinal 12-month follow-up study was conducted with 179 children, aged 6–12 years, diagnosed as having MIH in a previous survey. MIH defects were recorded by EAPD criterion. Teeth with MIH opacities at baseline were re-assessed after 12 months. Chi-square test was used to compare the proportion of severe MIH at baseline and after 1 year and the proportion of opacities that progressed to breakdown. Odds ratio was calculated to assess whether yellow/brown opacities at baseline would have a higher chance of progressing to breakdown. **Results:** 50.3% of the sample was male and 49.7% female (mean age of 9.04 ± 1.44). At baseline, 126 (70.4%) children presented mild MIH (only opacities), 12 (6.7%) moderate MIH (enamel breakdown not exposing dentin), and 41 (22.9%) severe MIH (enamel breakdown exposing dentin/atypical restoration). After 1 year, the number of children with severe MIH increased to 69 (38.5%) ($p < 0.01$). Progression to enamel breakdown was observed in 20.5% and in 31.5%

($p = 0.04$) of the permanent first molars with white and yellow/brown opacities, respectively. Progression to severe breakdown was observed in 12.0% and in 19.1% ($p = 0.10$) of the permanent first molars with white and yellow/brown opacities, respectively. Presence of yellow-brown opacities increased the risk of enamel breakdown compared to white opacities (OR 1.78 (CI 1.03–3.08; $p = 0.04$), but color of the opacity did not predict breakdown to severe stages of MIH (OR 1.74 (CI 0.90–3.36; $p = 0.10$)). **Conclusion:** MIH enamel opacities run a considerable risk of enamel breakdown in young school children, in particular the yellow-brown opacities.

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In vivo Assessment of Incisal Tooth Wear by Ultrasonic System: A Pilot Study

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Tooth wear is a common problem with a prevalence of 97% of the population. Loss of dental hard tissues due to erosion, abrasion, attrition and abfraction has been an important condition to diagnose and follow longitudinally in clinical situations. Due to the lack of an effective and generally accepted diagnostic method, the early reliable and reproducible diagnosis of pathologic tooth wear is still impossible. Clinicians need a practical and reproducible method for obtaining baseline follow-up data to provide a quantitative longitudinal evaluation of tooth wear. This study aimed in vivo evaluation of the effectiveness of ultrasonic system for quantitative measurement of incisal tooth wear. 4 maxillary incisors from 5 patients were chosen and patients used only toothpaste. 4 maxillary incisors from other 5 other patients were chosen and patients used the same toothpaste and occlusal splints. Baseline and monthly measurements were achieved by ultrasonic system and digital radiography for 3 months. Two measurements were achieved by first researcher and one other was achieved by second researcher for each period. Cast analysis were considered as control method.

Student t and Mann Whitney U tests were used to compare the two groups. There was no statistically significant difference between two groups for both ultrasonic system and digital radiography ($p > 0.05$). Paired sample t test was used to assess the readings within the groups. The obtained data from both groups showed a gradual decline at monthly measurements by ultrasonic system and were found statistically meaningful ($p < 0.05$, $p < 0.01$) correlating with the cast analysis. For the digital radiography 1st and 2nd month-readings ($p > 0.05$) were not significant in the toothpaste group, while 3rd was statistically meaningful ($p < 0.05$). For the toothpaste and occlusal splint group there was no significant difference among all monthly measurements. Both researchers were in moderate agreement for the ultrasonic and digital radiographic readings. Ultrasonic system seems to be a promising method for the evaluation of incisal tooth wear under the conditions of this study. Digital radiography didn't seem to be sensitive enough compared with the ultrasonic system. Funded by Marmara University Faculty of Dentistry Department of Restorative Dentistry.

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Use of the Fluorescence-Based Camera VistaProof for Monitoring Sound Teeth and Incipient Carious Lesions

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Objective: Sound tooth surfaces and incipient lesions require regular monitoring in order to detect a possible incurrence or progression of caries early on. The present study aimed to evaluate the ability of the fluorescence-based camera VistaProof (VP) to monitor sound tooth surfaces and incipient lesions in children. **Subjects and Method:** The study was approved by the ethics committee and informed consent was completed by the parents of the participating children. Visual examination (ICDAS as reference value) and VP measurements were performed on occlusal surfaces of 35 patients (average age 9.1 years) with ICDAS codes 0-2. All examinations were repeated after 6 and 12 months. Correlation of the methods was calculated using Spearman's rank correlation coefficient (r_s). Wilcoxon test was used to examine whether the VistaProof identified changes as well as the ICDAS method ($\alpha = 0.05$). **Results:** 419 posterior teeth were included in the study (205 deciduous molars, 145 permanent molars and 69 premolars). Significant positive correlation was found between ICDAS and VP findings ($r_s: 0.66-0.77$, $p < 0.001$). The Wilcoxon test showed no significant differences between all examination times for the ICDAS findings ($t1/t2: p = 0.46$, $t2/t3: p = 0.17$, $t1/t3: p = 0.14$). Significant differences for the fluorescence measurements were ascertained between the first and the second examination ($p = 0.03$) but neither for $t2/t3$ ($p = 0.11$) nor for $t1/t3$ ($p = 0.42$). **Conclusion:** The VP is able to support visual examinations and monitoring of occlusal surfaces of deciduous and permanent posterior teeth.

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Association of ICDAS, LAA and Radiography with Treatment Decisions on Approximal Caries Lesions in Primary Molars

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This in vivo study evaluated the performance of the International Caries Detection & Assessment System (ICDAS), Lesion Activity Assessment (LAA) and bitewing radiography (BW) to detect caries lesions after tooth separation on approximal surfaces and determined their association with treatment decisions (TD).

The sample consisted of 89 primary molars with 166 approximal surfaces in 56 children aged 8–12 years presenting at least one primary molar in an advanced root resorption stage. The children were clinically evaluated by a single examiner in 2 sessions: (first) before and (second) after temporary tooth separation. After extraction, the teeth were sectioned and the presence/extent of caries lesions was analyzed in a stereomicroscope. Unweighted Kappa values for intraexaminer reproducibility were 0.40 (ICDAS), 0.35 (LAA) and 0.56 (TD). The sensitivities, specificities and accuracies values for ICDAS before/after tooth separation were 0.55/0.88, 0.73/0.26 and 0.60/0.69, respectively. The sensitivity, specificity and accuracy values for BW were 0.49, 0.79 and 0.58. Spearman's correlations between ICDAS and BW for first/second sessions were 0.95/0.96, between ICDAS and TD were 0.95/0.88, between ICDAS and LAA were 0.91/0.63, between BW and TD were 0.81/0.70, between LAA and TD were 0.83/0.72, and between LAA and BW were 0.64/0.63 ($p < 0.05$). In general, the examiner determined the TD in cases of ICDAS 0 and inactive ICDAS 1 and 2 as preventive treatment; active ICDAS 1, 2 and 3 as therapeutic treatment; and active ICDAS 4, 5 and 6 as restorative treatment. It was concluded that the ICDAS assessment after tooth separation presented better clinical performance than BW for the detection of approximal caries lesions in primary molars. In addition, there was a high correlation between treatment decisions based on ICDAS, LAA and BW, highlighting the functional similarity of these tools in the clinical decision-making process.

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Relative System Sensitivity of Clinically Approved Calcvivis Caries Activity Imaging Device with Differing Photoprotein Concentrations

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Aim: To determine relative sensitivity, linearity, limit of detection of formulations with differing photoprotein concentration using calcium chloride solutions of varying concentration and extracted teeth. **Methods:** Previous ORCA posters reported a luminescent assay for assessing caries lesion activity and erosive challenges (2008/9/10/13/14). The device consists of a sensitive intraoral camera capable of imaging very low level, transient luminescence when a photoprotein is applied to tooth surfaces. Using a dark box, images of 100 μ l of varying concentrations of CaCl_2 (0 to 1.0 mM/l) in micro-titre wells were obtained and total light output measured using imagej software. Similar imaging of 28 extracted teeth using varying concentrations of photoprotein was performed. **Results:** Increasing the CaCl_2 concentration increased the relative light output. The increase in light output was close to linear at the range of CaCl_2 assessed (0 to 1.0 mM/l [R^2 values 0.91 to 0.97]). Although increasing the photoprotein concentration increases the light output, particularly at relatively high concentrations of CaCl_2 , it has only a marginal effect on the limit of detection. Teeth categorised as; sound/no visible decay, or mild to mod-

erate decay showed stronger luminescence in fissure patterns as the concentration of photoprotein increased. **Conclusions:** At fixed concentration of protein increasing the CaCl_2 concentration increased the light output in a linear relation at the range of CaCl_2 assessed (0 to 1.0 mM/l [R^2 values 0.91 to 0.97]). There was a clear dose response curve (increasing the protein concentration increases light output) in the ex-vivo models. It is possible that the concentration of the current formulation could be increased to give a stronger signal in clinical use without giving rise to significant, increased background luminescence. This possibility will be investigated in a further study.

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Detection and Assessment of Carious Lesions by Optical Coherence Tomography vs. ICDAS-II

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Objective: Evaluation of in-vivo and in-vitro performance of spectral domain optical coherence tomography (SD-OCT) for detection and assessment of carious lesions compared to ICDAS-II. **Methods:** 49 vestibular carious lesions of 43 incisors and 6 premolars were visually examined in vivo by an experienced dentist and categorized according to ICDAS-II (codes 0-6). Additionally 250 lesions of 51 freshly extracted and cleaned incisors (25), premolars (11) and molars (15) were similarly assessed. One region of interest was defined, each in the direction of the maximum lesion extension, followed by 2D-imaging with SD-OCT (center wavelength 1310 nm; Telesto II, Thorlabs GmbH, Dachau, Germany). OCT signals were categorized according to the lesion depth extent: 0: sound, 1: lesion limited to first quarter of enamel, 2: lesion extended up to whole enamel without cavitation, 3: lesion limited to enamel with cavitation, 4: cavitation in enamel, demineralization of dentin, 5: cavitation with dentin involvement, 6: extensive cavitation into dentin. Scoring of lesions with both methods was statistically compared (weighted Cohen's kappa coefficient (K)). **Results:** In-vivo study: Agreement for ICDAS-II code 0 was 50%. Values for codes 1-4 were 22%, 31%, 60% and 0% ($K = 0.21$). In-vitro study: Assessment of code 0 resulted in 62% agreement, values for codes 1-6 were 18%, 19%, 12%, 16%, 40% and 75% ($K = 0.28$). OCT images obtained information on lesion extension and early findings of demineralization of enamel and dentin resulting in different categorizations. **Conclusion:** Despite only fair agreement between ICDAS-II and OCT, the tomography can complement visual inspection.

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Clinical Evaluation of Fluoride Effects on Early Caries Lesion Using the QLF-D

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The aims of this clinical study were to investigate changes in mineral contents of early caries lesions after professional fluoride treatment, and to identify the value for initial fluorescence loss ($\Delta F_{(0)}$) which can recover one or four weeks after the treatment using QLF-D. For this clinical study, early caries lesions developed on primary teeth of 31 children aged between 2 and 12 years were examined, and 1.23% APF gel was applied for one minute. QLF-D images were taken before the fluoride application, and during the follow-up period (one and four weeks), each after prophylaxis process. Then, fluorescence loss ($\Delta F_{(0)}$, $\Delta F_{(1)}$, $\Delta F_{(4)}$, %) and recovery rate ($R_{\Delta F_{(1)}}$, $R_{\Delta F_{(4)}}$) of the lesions were evaluated. Based on the results of the paired t-test performed on the 101 lesions of the 31 subjects one week after the fluoride application, the recovered group ($R_{\Delta F_{(1)}} \geq 0$, $N = 68$) showed approximately 21.2% recovery $\Delta F_{(1)}$, while the unrecovered group ($R_{\Delta F_{(1)}} < 0$, $N = 33$) showed about 22.8% deterioration $\Delta F_{(1)}$ ($p < 0.01$). When only the 90 lesions of the 27 subjects were analyzed four weeks after the fluoride application due to the drop-out, the recovered group ($R_{\Delta F_{(4)}} \geq 0$, $N = 53$) showed approximately 23.5% recovery $\Delta F_{(4)}$, while the unrecovered group ($R_{\Delta F_{(4)}} < 0$, $N = 37$) showed about 30.8% deterioration $\Delta F_{(4)}$ ($p < 0.01$). From the results of the ROC analysis, the $\Delta F_{(0)}$ of early caries lesion, which could be maintained or recovered after the fluoride application, was found to be less than -11.8% for $R_{\Delta F_{(1)}} \geq 0$, and less than -13.0% for $R_{\Delta F_{(4)}} \geq 0$ ($p < 0.01$). In conclusion, we could detect changes in mineral contents during the remineralization from the fluoride application using QLF-D, and found the $\Delta F_{(0)}$ which had the fluoride effect of remineralization and inhibition of demineralization after one and four weeks.

Current State of Caries Detection and Excavation in Germany – A Survey among Dental Schools

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Recognition and treatment of carious lesions are cornerstones of dental practice. Minimal invasive caries excavation and restoration methods have been introduced in recent years. The aim of this study was to scrutinize the caries excavation methods taught at German universities in order to be able to develop a treatment guideline. A questionnaire concerning caries excavation methods, deep carious lesions lining and instruments employed was sent to all operative dentistry departments of German universities ($n =$

34). The answers obtained from 23 returned questionnaires were investigated by means of the SPSS 22 software. The results showed that 56% of the universities teach that infected dentin should be visually discarded and with a probe. Approximately 30% employ also the caries detector and fluorescence aided caries excavation (ca. 12%) methods. 30% of the academicians stated that dentin should be white and hard during probing. All other academicians agreed that confined caries could be left in case its removal would lead to a pulp opening. Most universities (56%) teach the use of calcium hydroxide in case of deep carious lesions. The employment of adhesive materials (13%) and Biodentine or mineral trioxide aggregate (3%) is noticeably less recommended. During caries excavation most academicians (60%) teach the use of a round bud bur and hand excavators to remove hard dentin. The use of a polymer bur is the most common one when employing selective instruments; however, no agreement concerning this matter was established. Although caries excavation is performed more frequently with a round bud bur and hand excavators the results showed that the employment of fluorescence aided caries excavation and self limiting polymer burs also are employed. The results reinforce the actual tendency of minimal invasive caries removal.

Caries Risk Profile for Dental Students in a Saudi Arabian Dental College Using Cariogram Software

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Aim: The aim of the study was to examine the relationship between caries related factors and caries risk determined by clinical judgment supported by the use of Cariogram software. Also to enforce the concept of caries risk assessment for dental students by using validated risk assessment aids. **The Experimental Approach:** This study was conducted as part of a course in preventive dentistry taught at the pre-clinical level in the College of Dentistry, University of Dammam, Saudi Arabia. Data were collected by clinical examination as caries was scored using WHO criteria, oral hygiene was scored using the Silness-Löe plaque index and a dietary questionnaire was used to gather information needed to develop a risk profile for each participant. In addition, a bacterial count analysis using the Caries Risk Test (CRT) for both mutans streptococci (SM) and Lactobacilli (L) was performed. The caries risk of each individual, expressed as the chance to avoid new caries, was then obtained by using the Cariogram software. **Main Results:** There were 70 (37 males and 33 females) dental students who participated in the study. Variables that showed a significant correlation with the cariogram software this included: Past caries experience, SM counts, fluoridation program and buffer capacity of the saliva. There was no significant gender difference for the risk of developing caries using the software. **Conclusion:** The caries risk profile was determined for the dental students, were those with high DMFT ran a higher risk of developing caries. As they had significantly higher numbers of SM and L and had less chance of

avoiding new cavities according to the Cariogram. It is a useful educational tool for dental students in discussions with patients about their caries risk.

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Relationship between Dental Plaque Amounts and Caries Activity Test Using Saliva

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The acidogenicity of oral biofilm is related with the tooth demineralization and the caries activity. The Snyder test is simple colorimetric test range from green to yellow and representative acidogenic test of oral bacteria which shows a positive correlation with lactobacillus count. The amount of dental plaque is also important factor to predict caries activity. The aim of this study was to evaluate the relationships between modified Snyder test, dental plaque index, and oral bacterial counts. Thirty subjects (male: 15, female: 15, mean age: 30.0) participated in this study. One examiner recorded dental plaque using Quigley-Hein index (QHI). The modified Snyder test were carried out using stimulated saliva and modified medium (distilled water 1 L, beef extract 3 g, dextrose 20 g, Agar 8 g, bromocresol green 0.04 g, pH 5.5). Lactobacillus, *Streptococcus mutans*, and total species in stimulated saliva were cultured in specific media at 37°C, for 48 hours, and the colonies were counted. The relationship between the dental plaque index and the results of each test were analyzed by SPSS statistical analysis program. The subjects were divided to 3 groups (low, medium, high risk) according to results of modified Snyder test. The anterior QHI of low, medium, and high risk group were 1.49±0.25, 2.43±0.99, and 2.93±1.07, respectively. The anterior QHI also was correlated with the colony counts of Lactobacillus, *S. mutans*, and total species ($P < 0.05$). This study confirmed the association between new modified Snyder test and the amount of dental plaque and oral bacterial counts. It could be concluded that the new caries activity test medium suggested in this study could be used to evaluate caries activity related with oral biofilm but this requires further investigation.

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Enhancement of Detecting Dental Caries and Filling Body by Using Portable QLF Device on Epidemiological Tooth Examination

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The aim of this study was to evaluate applicability of portable QLF device in epidemiological tooth examination for enhancing the detecting dental caries and filling body. Two examiners (1 epidemiologic expert and 1 non-expert dentist) carried out visual tooth examination for 52 subjects aged 25 to 34 using only dental operating light, dental mirror and air-drying syringe without dental explorer. And then pictures or movies on every tooth surfaces were taken under visual ray and 405 nm blue ray respectively using portable QLF device Qraycam™ (All in one Bio Co. Seoul, South Korea). Two examiner performed tooth examination from the movies or images taken under visible ray and 405 nm blue ray by Qraycam™ after more than 7 days from the day carried out visual examination. Agreement for two examiner and Receiver Operating Characteristics (ROC) were evaluated with gold standard derived from visual and Qraycam™ examination. The expert examiner decided the gold standard by synthesizing clearly defined tooth surface status from all three (visible, visible ray image and 405 nm blue ray image) results. The results of visual examination, visible ray image examination and 405 nm blue ray image examination showed very good kappa agreement (expert Kappa = 0.969, 0.941, 0.963, non expert's Kappa = 0.946, 0.928, 0.947, $P < 0.001$) with gold standard. Accuracy of detecting dental caries and filling body was enhanced by reading 405 nm blue ray image on ROC analysis in non expert. Tooth examination of Qraycam™ images revealed high agreement with gold standard and showed advanced accuracy for detecting caries and filling body. This results imply that portable QLF would enhance the quality of oral epidemiologic tooth survey.

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Clinical Monitoring of Inactive Non-Cavitated Caries Lesions in Occlusal Surfaces of Permanent Teeth

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The aim of this retrospective observational study was to evaluate the diagnostic changes and progression of inactive non-cavitated lesions on occlusal surfaces of permanent teeth. This evaluation was performed by analyzing the records performed by fifteen examiners of patients treated at the Faculty of Dentistry of the Federal University of Rio Grande do Sul (UFRGS), in treatment for at least five years and presenting at least one inactive non-cavitated caries lesion. 12,802 records were analyzed, and 550 were enrolled in the study. A total of 2171 inactive non-cavitated caries lesions were included in the study, with at least two evaluations (mean time between exams: 14 months). The results indicate that most of the inactive non-cavitated lesions remained unchanged (85.54%, $n = 1857$). Of the examined lesions, 9.12% ($n = 198$) developed to inactive cavitated lesions. As for lesions that changed their diagnosis: 2.12% ($n = 46$) have been restored; 1.20% ($n = 26$) were diagnosed as active cavitated lesions; 0.60% ($n = 13$) changed his diagnosis to active lesions without cavity; in 1.29% ($n = 28$) were extracted teeth (13 teeth were third molars); 0.05% ($n = 1$), the lesions were sealed; and 0.09% ($n = 2$) were crowns. Of the variables analyzed, gender, dental plaque, gingival bleeding and patient's caries activity were not associated with the outcome change in diagnosis. Previous caries experience (DMFT and DMF-S) and change of examiner between evaluations were significantly associated with the change of diagnosis ($p < 0.05$). It was observed that most of the inactive non-cavitated caries lesions remained unchanged, confirming the diagnosis of inactivation of the disease process and a possible lack of need for therapeutic intervention for these lesions.

Molar Incisor Hypomineralization (MIH) in 8- and 9-Year-Old Children in Bosnia-Herzegovina

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Aim: Molar Incisor Hypomineralization (MIH) is one of the major developmental defects of dental enamel affecting permanent first molars and incisors, and is a challenge for dental health personnel. The aim of the study was to determine the prevalence of MIH in a group of 8- and 9 year-old children in the city of Kljuc, a Northern part of Bosnia-Herzegovina, and to describe the distribution of affected teeth. **Materials and Methods:** All 8- and 9-year-olds ($n = 104$) living in Kljuc (born 2004/2005) were invited to participate, and 103 (62 boys and 41 girls) were examined by a calibrated clinician (EC). Written, informed consent was obtained

from all participants' relatives, as well as a questionnaire designed to assess potential risk indicators. Index teeth were permanent first molars and incisors (12 teeth). Opacities (>1 mm), defects, atypical restorations and previous extractions caused by MIH were registered. **Results:** The prevalence of MIH was 11.7% ($n = 12$), significantly higher for girls than for boys (14.6% vs. 9.7%; $p < 0.05$). The upper first molars and the upper incisors were 1.8 times ($p < 0.02$) and 2.3 ($p < 0.05$) more frequently affected than the lower ones, respectively. It was found that 30% of the affected molars had opacities, 34.8% had defects and 35.2% atypical restorations. No molars had been extracted due to MIH. Uses of the penicillin due to tonsillitis in the first 5 years were associated with a higher prevalence of MIH (41.7% vs. 19.6%). **Conclusion:** The prevalence of MIH (11.7%) supports the data previously published from Bosnia-Herzegovina, but is some higher than reported prevalence from other countries in the same age group. Girls had higher prevalence of MIH than boys, and first molars were almost twice as affected in the maxilla as in the mandible.

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Developmental Defects of Enamel in Primary Teeth – Findings of a Regional German Birth Cohort Study

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Aim: This study was to assess the prevalence of developmental defects of enamel in 3- to 4-year-old Thuringian children in 2013 as part of a prospective cohort study. **Material and Methods:** Subjects ($n = 755$) were participants in the intersectoral oral health program for prevention of early childhood caries of the city of Jena, Thuringia. Children of the birth cohort 2009/2010 were invited to dental examination in the Jena University Hospital in first year of life. They were included in a risk related recall system with continuous dental care over 3 to 4 years. Dental caries was scored at d_1 -level using WHO diagnostic criteria without radiography. Enamel defects were assessed according to the modified DDE Index. Data were analysed statistically using multivariate analysis. **Results:** Children aged 3.3 ± 0.5 years and 49.0% were females. Caries prevalence was 26.9% and caries experience 0.6 ± 3.0 $d_{3-4}mfs$. Prevalence of enamel defects was 4.1% ($n = 31$). Demarcated opacities were the most common defects ($n = 22$) and hypoplastic the least common ones ($n = 3$). 5 children had diffuse opacities and 1 child a combination. Second primary molars and incisors were more affected than other teeth. Enamel defects were associated with preterm birth ($p = 0.002$; OR = 5.06). **Conclusions:** Preterm children are at higher risk to suffer from enamel defects.

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Individual Susceptibility to Dental Erosions in situ

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Aim: To test the enamel susceptibility to erosion for different individuals. **Methods:** Three sound premolars extracted due to orthodontic treatment were collected from each of eight young persons (donors). Two enamel specimens were made from each tooth. Individual mandibular acrylic mouth appliances were fabricated for six healthy volunteers (carriers). One specimen from each donor (n = 8) was mounted on the buccal sides of each appliance. The carriers wore the appliances at all times for nine days, except while eating, during oral hygiene procedures and experimental acid exposures. The appliances were immersed and gently stirred in 300 ml of 0.01 M HCl with pH 2.2 at 37° C for three minutes two times per day for nine days. The enamel specimens were analysed at baseline and after the study period by a white light interferometer. The difference between the two images showed the changes in topography, and thereby the amount of enamel loss (µm). Mean enamel loss for the six specimens from each donor was calculated (outcome 1). Mean enamel loss for the eight specimens (one from each donor) within each carrier expresses the variation in oral environment (outcome 2). **Results:** Mean enamel loss varied significantly between the donor teeth; outcome 1 (p = 0.009); mean 45.04 µm (range 32.2–52.7 µm). When comparing the enamel loss of the eight specimens worn by each of the six carriers; outcome 2 (p = 0.004); the mean value was 45.04 µm (range 32.7–52.6 µm). **Conclusion:** The results indicate that the susceptibility to dental erosion is influenced by individual factors both in the enamel and in the oral environment. This may help to explain the variation in prevalence and severity of dental erosions among patients exposed to similar acidic challenges.

The study was funded by the University of Oslo, Norway.

In-vivo Application of Optical Coherence Tomography (OCT) in Detection of Early Enamel Demineralisation

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This cross-over randomised controlled clinical trial aimed to: 1. Evaluate the effect of an in-vivo erosive challenge compared to a non-erosive control challenge on enamel of healthy volunteers and 2. Assess the impact of the pellicle layer on enamel subjected to the erosive challenge compared with the non-erosive control challenge. 30 healthy volunteers (REC 13136) had baseline OCT images taken of the labial enamel of the maxillary incisors. In order to provide clinically relevant erosive/control challenges, subjects underwent 6 cycles of rinsing, each consisting of either 10 orange juice (pH 3.8) or 10 water (pH 6.9) one minute rinses, followed by OCT imaging. Prior to each rinse, pellicle was removed from the enamel on one side using wet cotton wool. After a two week wash out period, subjects were randomly allocated to the alternative rinse. Changes in the surface/subsurface optical properties of the enamel were quantified using a custom-designed algorithm (ImageJ). Shapiro-Wilk's test demonstrated a normal distribution and means (SD) of the optical changes for pellicle-present and pellicle-removed enamel were analysed for erosion versus control challenges. Random effects models assessed overall effects of orange juice versus water, order of treatment, presence/absence of pellicle and rinsing duration. Random effects model showed enamel subjected to orange juice to have overall higher mean (SD) changes in optical properties, 0.64 (0.15) versus water control, 0.61 (0.15) (p < 0.05). Differences were observed between the pellicle-removed versus the pellicle-present enamel, however the pattern was complicated. In conclusion OCT detected optical changes in enamel during an erosive challenge. Further investigations into surface events occurring during early enamel erosion should aim to identify suitable targets for preventing the progression of erosion leading to enamel loss.

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Impact of Fluoride Dentifrices with Different Surfactant Systems on in situ Erosion Prevention of Demineralisation

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This study compared the in situ prevention of demineralisation from an experimental fluoride dentifrice, a marketed fluoride dentifrice and their respective non-fluoride placebo formulations. This was a randomized, placebo-controlled, single centre, analyst-blind, 4-period crossover design. Experimental and Comparator dentifrices contained 1150 ppm (0.2542% w/w) fluoride as sodium fluoride. The Experimental dentifrice contained a modified surfactant system: 0.706% w/w oleoyl sarcosine (OS) + 0.45% w/w tegobetaine; the Comparator contained 1.2% w/w tegobetaine. Fluoride-free placebo dentifrices contained respective matching surfactants. Demineralisation/remineralisation extent was evaluated by surface microhardness (SMH). Subjects wore an intra-oral palatal appliance carrying six bovine enamel specimens. During each treatment, enamel specimens were exposed to three treatment/erosion challenge cycles, 60 minutes apart: 2 minutes product exposure (30 seconds brushing, 90 seconds swishing with slurry); 15 minutes rest; 20 minutes fresh orange juice erosive challenge. Two specimens were removed after each cycle. Thirty subjects were screened; all 28 randomised subjects completed the study. Mean percentage change in enamel SMH was analyzed using ANOVA incorporating factors for treatment group, treatment visit and subject. Demineralisation prevention by the Experimental dentifrice was significantly different compared to Experimental Placebo (% change from baseline -7.7, -19.5, -32.3 for 20, 40, 60 minutes respectively) and Placebo Comparator dentifrices (-7.7, -15.4, -30.3 respectively) ($p = 0.0002$, 20 minutes; $p < 0.0001$, 40 and 60 minutes). Similar significant differences occurred between the Comparator dentifrice and Placebo dentifrices at all timepoints (all $p < 0.0001$). No significant differences were found between the active fluoride treatments or between the placebo treatments. Following treatment/erosion cycles, fluoride-containing treatments demonstrated significantly greater SMH than placebos. Modifying the comparator dentifrice surfactant system did not impact fluoride efficacy with respect to demineralisation prevention following erosive challenges.

This study was funded by GSK Consumer Healthcare.

Evaluation of a Fluoride Dentifrice/Mouthrinse Regimen in a Short-Term in situ Remineralisation Model

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Early stage dental erosion may be prevented by the use of fluoride-containing products to promote remineralisation of eroded enamel and prevent further tissue loss. This randomised, controlled, in situ study investigated the ability of a fluoride mouthrinse administered after brushing with a fluoride dentifrice to render acid-softened enamel more resistant to demineralisation, as determined by % relative erosion resistance (%RER), and to remineralise acid-softened enamel, as determined by % surface microhardness recovery (%SMHR), compared to fluoride dentifrice alone. Enamel fluoride uptake (EFU) was also measured. Five treatment regimens (fluoride paste/rinse immediately or 1 hour post-brushing, fluoride paste/no mouthrinse, placebo paste/fluoride rinse 1 hour post brushing or placebo paste/no mouthrinse) were evaluated using a crossover design in subjects ($n = 24$) wearing a palatal appliance holding bovine enamel specimens in situ for 4 hours post-brushing. Statistical analyses were based on analysis of variance (ANOVA) for all endpoints.

The primary comparison showed fluoride paste/mouthrinse (1 h) regimen conferred 31.9% better %RER than fluoride paste alone ($p = 0.0094$). No significant difference in %SMHR was observed between these two groups ($p = 0.1801$). All fluoride-containing treatments performed significantly better than placebo paste/no mouthrinse for %RER ($p < 0.0001$) and %SMHR ($p < 0.01$). Fluoride paste/mouthrinse (1 h) significantly increased EFU versus fluoride paste/no mouthrinse (33.9%; $p < 0.0001$) and placebo paste/fluoride mouthrinse (24.3%; $p = 0.0003$). There was no significant alteration in EFU with use of fluoride mouthrinse immediately or 1 h after fluoride dentifrice. In conclusion, fluoride mouthrinse administered 1 h after fluoride toothpaste rendered enamel significantly more resistant to second extra-oral erosive challenge and increased EFU compared to fluoride dentifrice alone. These findings highlight the potential importance of using a fluoride mouthrinse in addition to fluoride dentifrice in the management of dental erosion.

This study was funded by GSK Consumer Healthcare.

Effect of Mouthrinsing with Two Fluoride Solutions on Enamel Solubility Using an in vivo Experimental Model

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The aim of this pilot study was to compare the erosion-protective effect of mouthrinsing daily for one month with two fluoride-containing solutions, using a cross-over design experimental in vivo model. Six test subjects were enrolled and individual splints were fabricated from upper jaw models. Subjects used standard NaF-toothpaste prior to and during the study. Using the splints and light-bodied impression material, circular test windows of labial enamel (4 mm diam.) were isolated on healthy anterior teeth (n = 36). Enamel was exposed to citric acid (CA, 50 mmol/l) using a peristaltic pump (5 ml @ 6 ml/min) and CA was collected in coded test tubes (etch I). Test subjects rinsed for 28 days with rinse A (control – NaF: 0.05% F, pH 5) or rinse B (Elmex Erosion Protection: 0.05% F, 0.08% SnCl₂, pH 4.5) before a new CA exposure was performed (etch II). Following 28-day wash-out, the same procedure was repeated for all subjects rinsing with the other F-solution. Teeth were rinsed with water (5 s) after etching. Enamel dissolution was assessed using atom absorption spectroscopy of calcium (Ca) in CA. Mean Ca (ppm) and % reduction in Ca was calculated for rinse A and B at the individual level. There was no significant difference in mean Ca in etch I between rinse A (0.801±0.346) and rinse B (0.778±0.357). Rinse B resulted in lower but non-significant mean Ca concentration in etch II than rinse A (A: 1.106±0.702, B: 0.598±0.226; p > 0.05). The mean Ca reduction for rinse A was 6.7% (2 of 6 individuals showed no effect) and for rinse B 24.1% (all individuals showed effect). These preliminary results suggest that rinsing with the commercial rinse may have a beneficial effect compared to the NaF control.

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Awareness, Attitudes and Diet Related Dental Erosion among a Group of Vietnamese Adults

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This study was to describe the prevalence of dental erosion and its association with awareness, attitudes and dietary habits in a group of Vietnamese adults. First-year students of the Ho Chi Minh University of Medicine and Pharmacy were invited to participate. Students were examined for dental erosion using the Basic erosive Wear Examination (BEWE) and interviewed about their awareness, attitudes and dietary habits related to dental erosion. Of the subjects 69.6% (n = 276) showed signs of dental erosion. Severe erosion (BEWE 3) was found in 16.3% subjects. The total BEWE scores were significantly different between age groups (<30, 30–35, >35 year old) (Kruskal Wallis, p < 0.001). Dental erosion was associated with age (Chi-Square, p < 0.001). There was no significant differences in both prevalence and severity between gender. Half of subjects having BEWE 2 (49,4%) and BEWE 3 (44,4%) had not perceived dental erosion. There was no significant association between BEWE score and both perception and attitude toward dental erosion. 75.8% subjects with BEWE ≥2 selected incorrect methods for erosive status. Compared to subjects with no erosion, those with mild erosion had a significantly higher consumption of soft drinks and snacks (Chi-Square, p < 0.001). Consumption of drinks and foods with an erosive potential were frequent (78.3% consumed acidic fruit juices, 74.6% consumed acidic foods, 55.0% consumed soft drinks). The results suggested that erosive dental wear is of concern for this studied population. There should be increased awareness of erosive dietary foods as the consumption of acidic foods and drinks are very popular.

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Caries Increment and Dietary Habits in 6 and 12 Years Old Children in Riga, Latvia*J. Gudkina^{a,b}, B.T. Amaechi^c, S.H. Abrams^{d,e}, A. Brinkmane^{a,b}**j.gudkina@inbox.lv*^aRiga Stradins University, Latvia, ^bInstitute of Stomatology, Riga Latvia, ^cUniversity of Texas Health Science Center, San Antonio, USA, ^dQuantum Dental Technologies, and ^eFour Cell Consulting, Toronto, Canada

This study (1) compared the dietary habits, and (2) investigated the caries increment, in 6 and 12 years old children in Riga, Latvia in a 3-year period. Methods: Thirty-eight 6 and thirty-nine 12 years old children were examined visually and with bitewing radiographs for dental caries at baseline and after 3 years. Decayed, missing, and filled surfaces (dmfs/DMFS) in all teeth were scored by one calibrated examiner using the Radike's caries scoring criteria. Dietary habits questionnaires, on snacking, beverages consumption and sugar intake, were completed by all participants and/or their parents. The data were analyzed using t-test, chi-square test, Wilcoxon and Fisher's tests ($\alpha = 0.05$). Results: The mean (SD) values of caries experience at baseline/3-year period in 6- vs.12-year olds were as follows. DMFS: 0.72 (1.02)/3.13 (3.13) ($p = 0.0000$) vs. 6.79 (5.14)/14.79 (9.86) ($p = 0.0000$); dmfs: 11.26 (8.71)/7.74 (4.86) ($p = 0.0780$) vs. 3.57 (2.03)/1.5 (0.71). Changes in dietary habits between baseline and 3-year time points were only significant for consumption of soft drinks ($p = 0.032$) and sugared tea ($p = 0.018$) for the 6 years old, and for sugared tea ($p = 0.017$) and daily amount of sugar tea spoons ($p = 0.0095$) for the 12-years old. Further data examination showed positive caries increment in all the 6 and 12 years old children that reported significant increase in consumption of soft drinks/sugared tea and increase intake of sugared tea and daily amount of teaspoons of sugar respectively. Conclusions: The result of the present study thus suggests that the increased prevalence of caries in permanent teeth among the 6 and 12 years old Riga children within 3 years may be related to the concomitant increase in sugar intake within the same period.

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Impact of National Health Insurance Coverage on Pit and Fissure Sealing Experience*J.-S. Choi^{a,b}, S.-M. Bae^c, D.-Y. Park^{a,b}**jguitar@gwnu.ac.kr*^aDepartment of Preventive and Public Health Dentistry, College of Dentistry, Gangneung-Wonju National University, Korea, ^bResearch Institute of Oral Science, Gangneung-Wonju National University, Korea, ^cDepartment of Dental hygiene, College of Dentistry, Gangneung-Wonju National University, Korea

The purpose of this study is to review the change of supplied amount of pit and fissure sealing (PFS) after including PFS into the list of coverage treatments in National Health Insurance (NHI), and to check out whether there is a difference of supplied amount among the area where the dental treatment accessibility is different based on data of Korean National Oral Health Survey (KNOHS). PFS was included in NHI at December 2009. The data year used as 'before covered (BC)' was 2006, and that of 'after covered (AC)' was 2012 when Korean National Oral Health Survey was done. Areas were classified as Urban and Rural. Reduction of Out-of-pocket expense according to NHI coverage almost doubled PFS experience of 12-year-olds from 33% in BC to 63% in AC. The number of urban 12-year-olds who have experienced PFS was estimated as 208,476 in BC, and 343,892 in AC. However, the number of rural 12-year-olds was estimated as 23,845 in BC, and 24,546 in AC, and average number of sealed teeth per person in rural area was decreased from 2.23 in BC to 1.78 in AC. Rate of PFS experience of 12-year-olds will be more increased until 2016 when 6-year-olds at the time of NHI coverage started reach the age of 12. However, increased experience is mainly led by urban area and number of teeth sealed in rural area is even decreased. Although PFS is covered by NHI, geographic accessibility and affordability of out-of-pocket money make inequality of PFS experience.

To reduce inequality, supply of PFS in rural area by community oral health program should be strengthened. And also, waiving out-of-pocket money for PFS in NHI should be considered.

Association between Caries in Young Children and Breastfeeding Patterns

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Aim: To study caries experience in 1- to 3-year-olds in relation to different patterns of breast feeding. **Methods:** A retrospective study was conducted in Volgograd with the permission of the Regional Ethics Committee. Children aged one year (155) or 2 years (119) were examined during their routine visits at 3 pediatric clinics; children aged 3 years (115) were examined in 6 kindergartens located in different districts of Volgograd. Parents' informed consent was obtained. Mothers were interviewed about the following breastfeeding peculiarities: first breast feeding (immediately or a few days after birth); exclusive breastfeeding (lack or ≤ 3 months or within 4–6 months duration); whole period of breast feeding (in months); the number of breast feedings per day in 2- and 3-year-olds (0–2 or 3–4 or 5–6 and more). Caries prevalence, d3-4mft values, relative risk ratios (RR), 95% confidence intervals (CI) and P-values were calculated. **Results:** Caries prevalence in 1-, 2- and 3-year-olds was 8.39% (CI 4.03–12.77), 31.93% (CI 23.55–40.31), and 59.13% (CI 50.15–68.11), mean d3-4mft values were 0.35 ± 0.08 (CI 0.34–0.36), 1.36 ± 0.04 (CI 1.35–1.37), and 2.85 ± 0.20 (CI 2.81–2.89) respectively. Dmft values increased in the children who either did not receive the first breast feeding immediately after birth (RR = 1.81, $P < 0.0001$), or who did not have exclusive breastfeeding (RR = 2.32, $P < 0.001$), who had short (≤ 3 months) (RR = 2.04, $p = 0.0006$) or prolonged (> 12 months) breastfeeding (RR = 2.04, $p = 0.0005$). In 2- and 3-year-olds the dmft values increased with the number of daily breast feedings (RR = 1.76, $p = 0.0299$). **Conclusions:** Caries risk in young children is associated with the following breastfeeding patterns: delay of first breast feeding after birth, lack of exclusive breast feeding, a short or prolonged breast feeding period as well as a high number of daily breast feedings for 2- and 3-year-olds.

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Anemia and Dental Caries in Pregnant Women: A Prospective Cohort Study

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The relationship between anemia and dental caries activity during pregnancy is not clear. The aim of this study was to evaluate the effect of anemia during pregnancy on the risk of developing tooth decay in women. This is a prospective cohort study, developed in São Luís, Maranhão, Brazil. The outcome of interest was new dental caries lesions initiating during pregnancy, according to Nyvad's criteria. Pregnant women up to 16 weeks of gestational age (GA) in prenatal care in the Maternal and Child Health Unit of the University Hospital of the Federal University of Maranhão were included in this research. The main independent variable was the presence of anemia (diagnosed by means of serum Iron – Fe, ferritin, hemoglobin, erythrocyte, hematocrit, mean corpuscular volume – MCV, mean corpuscular hemoglobin – MCH, mean corpuscular hemoglobin concentration – MCHC and Red Cell Distribution Width – RDW). Patients were evaluated in two stages: up to 16 weeks of GA (T1) and in the last trimester of pregnancy (T2). Associations of interest were estimated using incidence ratio risk (IRR) and 95% confidence intervals (95% CI) in Poisson regression analyzes. Fifty-nine pregnant women were evaluated in two stages. The incidence of caries was 52.5% ($n = 31$). The average gestational age at T1 was $11.6 (\pm 3.3)$ weeks and $31.7 (\pm 5.0)$ in T2. Risk associations were observed between caries and hemoglobin parameters (IRR = 2.74; 95% CI: 1.40 to 5.36) and hematocrit (IRR = 1.40 and 95% CI: 1.17 to 1.68). However, the RDW behaved as a protective factor (IRR = 0.18; 95% CI: 0.07–0.47) in T1. In T2, the risk association was observed for the erythrocyte (IRR = 2.51; 95% CI: 1.10 to 5.73) and RDW (IRR = 2.58; 95% CI: 1.29 to 5, 12). We conclude that the hematological parameters of anemia in pregnant women influence the incidence of dental caries in this stage of life.

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Are Markers of Metabolic Syndrome Associated with Caries in Children?

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Sugar consumption has been associated with metabolic syndrome, however it remains unknown if markers of this systemic condition are associated with caries in children. The objective of this study was to investigate the association between sugar consumption, markers of metabolic syndrome and caries in children from 12–16 y. In this study, a subpopulation of children aged 12 to 16 years from the NHANES III data (n = 1582) was selected. A theoretical model was built to adjust for sex, age, ethnicity, poverty, candies consumption and markers of metabolic syndrome (body mass index, HDL cholesterol, triglycerides and glycated hemoglobin). Cavities in permanent teeth were the outcome was considered as a multinomial variable (0; 1 or 2 lesions; 3 or more lesions). A multinomial logistic regression analyses were performed. Age (Prevalence Ratio – PR: 1.4; Confidence Interval – CI: 1.24, 1.59); high candies consumption (PR: 1.82; CI: 1.14, 2.91), and low HDL cholesterol (PR: 1.58; CI: 1.01, 2.47) were associated with high caries (3 or more cavities). Conclusion: More cavities in permanent teeth were associated with high sugar consumption and low HDL levels, suggesting that high caries in children may relate to later risk of metabolic syndrome, which is associated to chronic diseases.

Understanding the Factors Influencing Preschool Child Oral Health

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Aim: This study aimed to explore the knowledge and views of mothers living in Sydney South-West regarding risk and preventive factors affecting the oral health of their preschool-aged child. **Experimental Approach:** Mother-infant dyads (n = 21) were selected from a population-based cohort study that began in 2010 focusing on preschool children living in disadvantaged areas serviced by the Sydney and South West Sydney Local Health Districts.

This nested qualitative study involved semi-structured interviews to explore mother's views on factors influencing early childhood oral health. Interviews were audio-recorded, transcribed verbatim, and subsequently analysed using thematic coding. **Main Results:** There was a general lack of understanding of the importance of oral health in terms of children's overall growth and development. Although caregivers indicated the significance of prevention, they did not feel the need to visit a dental health professional for young children unless the child complained of pain. While most mothers were aware of the role of sugary foods and drinks in dental decay, a significant proportion did not associate bottle-feeding with dental caries. The majority of mothers did not realise the importance of brushing their child's teeth with a fluoride toothpaste until he/she was 2 years old, and were worried about the risk of swallowing the toothpaste by their young child. **Conclusions:** Mothers residing in disadvantaged areas of Sydney and Sydney South West were not health literate about their child's oral health. Results from this cohort study may be useful for oral health promotion to pregnant mothers and/or new mothers to reduce the burden of dental caries in young children in disadvantaged areas.

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Dental Caries Prevalence among Moscow 3- and 6-Years-Old Children in 2012

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Caries prevalence in primary dentition remains high in many Russian regions. Punctually applied preventive strategies related to dental ages as suggested in the literature [Ekstrand & Qvist: Int J Paediatr Dent, 2014 Epub ahead of print] may be one way to control caries in primary dentition among children in Moscow. **Aim:** The aim of the study was to describe baseline data on dental caries prevalence in primary dentition of 3- and 6-year-old children in Moscow. A cross-sectional survey was conducted in all districts in Moscow in 2012. The study population comprised 44580 three-year-olds and 59600 six-year-olds corresponding to about ½ of the total population of 3- and 6-yr-olds in Moscow in 2012. Caries was scored using d2mft index criteria, including separate evaluation of the decayed, missed and filled components. **Results:** A total of 31% of examined 3-yr-olds had d2mft ≥ 0, with mean dmft index value equal to 1.24±0.08. A total of 42% of 6-yr-olds had d2mft ≥ 0, with mean dmft index value on 4.72±0.24. At the age of 3, the mean number of decayed teeth was 0.68±0.05; of filled ones, 0.22±0.03 and of extracted ones due to caries, 0.34±0.03. The corresponding figures for 6-year-olds were 0.91±0.06, 3.35±0.19 and 0.46±0.04, respectively. Mean DMFT Index value among the 6-year-olds was 0.40±0.04. Prevalence of cavities and fillings increased significantly (p < 0.05) with age. The results of the survey revealed that in

3-yr-olds dental caries prevalence is moderately high however, significantly higher in the 6-yr-olds. Therefore the implementation of punctually applied preventive strategies at the earliest age is highly recommended for the child population in Moscow.

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Development of a 3D Caries Process and Lesions Detection Model as a Teaching Tool

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Objective: To develop a digital, interactive, three-dimensional (3D) model of the caries process and the detection of lesions using current caries paradigm concepts as an attempt of complementing the ICDAS e-learning course. **Methods:** Pedagogic planning was performed prior to the construction of this tool. This included objectives; a survey to explore the perceived difficulty degree of caries-diagnosis associated topics answered by dental undergraduate (n = 90) and postgraduate students (n = 23) and caries-lecture dental staff (n = 8); literature review of key concepts based on the cariology teaching European curriculum, and national and international experts' consultation (n = 4). **Results:** The analyses of previous steps resulted in the design of an educational script strategy that included the following topics to be addressed in this order: 1.) Dental anatomy, 2.) Plaque formation areas, 3.) De/re-mineralisation process, 4.) Caries lesion initiation and progression on occlusal surfaces, and 5.) ICDAS scores with histological correlation. The development of the 3D model used the refined 'Virtual Man-Project[®]' graphical digital laboratory with multiple 3D software forms. A computer graphic modelling and pre-visualization phase was conducted. Afterwards, a draft video revision was conducted by co-authors and agreed suggestions were incorporated. The final video was edited. Lastly, explanatory subtitles as well as generated textured and rendered format were done. This process resulted in a 6-minute 3D video for dentists and dental students to support the teaching/learning caries process and the detection of caries lesions using ICDAS. This tool will be further tested in a study in terms of utility, usability, and users' satisfaction in different population contexts. **Conclusion:** The pedagogical development of this video combines modern technology and current cariology paradigm concepts resulting in a more friendly caries teaching/learning tool.

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Caries Experience and Treatment Need among 6- to 17-Year Old Psychiatric Patients

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Aim: To analyze caries prevalence and treatment need of patients hospitalized at the Child and Adolescent Psychiatric Clinic. **Material and Methods:** 81 hospitalized patients, 64.2% male, aged 6 to 17 years (mean 10.6±2.4 years) were included. Dental examinations were provided by one dentist at the patient's bedside and caries scored by WHO-criteria. Mental disorders were diagnosed following ICD-10 and DSM-IV. **Results:** Caries prevalence was 60.5% in the permanent dentition (PermD) and 85.7% in the primary dentition (PrimD). 2.32 (±2.79) DMFT, 1.77 (±2.23) DT, 0.01 (±0.11) MT, 0.54 (±1.08) FT and 3.63 (±2.77) dmft, 1.8 (±1.97) dt, 0.59 (±1.32) mt and 1.24 (±1.74) ft were recorded. Higher caries experience and untreated caries was observed in both dentitions in the study group compared with regional data of children at the same age. In 80% of children with affective disorders, 75% with personality and behavior disorders and 66.7% with neurotic, stress-related and somatoform disorders (NSSD) caries occurred in the PermD accounting 2.80, 4.00 and 3.40 DMFT respectively. In the PrimD, 94.4% of children with NSSD and 82.9% with behavioral and emotional disorders with onset usually occurring in childhood/adolescence had dental caries with 3.67 and 3.56 dmft respectively. Regarding psychosocial and environmental factors, highest caries prevalence in the PermD was recorded in children with acute stressful life events (ASE) 78.3%, abnormal immediate environment (AIE) 57.6% and abnormal educational conditions (AEC) 57.4%, having 3.57, 2.27 and 2.38 DMFT respectively. Caries in the PrimD occurred in all children with ASE (5.08 dmft), 89.7% with AIE (3.83 dmft) and 85.3% with AEC (3.65 dmft). **Conclusions:** High caries prevalence, DMFT/dmft and untreated caries was found in children and adolescents hospitalized with psychiatric disorders and psychosocial and environmental problems.

Food Advertising to Children on UK Television: Implications for Dental Health

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This study examined the prevalence of food advertising on UK television watched by children, with specific focus on foods potentially detrimental to dental health.

Data were collected from 352 hours of television recorded from ITV 1 channel in 2012. One weekday and one weekend day every month (6 am–10 pm) were recorded. Of the targeted 384 hours, 32 hours were missing. Recorded hours were scanned for both food and non-food adverts. All adverts were coded according to a coding scheme and food adverts were further coded based on their effect on dental health.

9151 adverts were coded. Food products were the second most commonly advertised products (16.7%; $n = 1532$). Adverts for foods considered potentially harmful to dental health comprised nearly two-thirds of all food adverts (61%; $n = 934$), and of these, 96.6% were cariogenic foods and 11% acidogenic foods. Adverts for cariogenic foods with high and very high sugar levels (>9 g/100 g or 100 ml) represented 43% of all cariogenic food adverts, with almost 50% of the latter being sticky foods. The proportion of adverts for foods that are potentially harmful to dental health was significantly higher than the proportion of adverts for foods that are potentially non-harmful to dental health (65.9%; $p = 0.011$) during peak children's viewing hours, which were between 17:30 and 22:00 hours on weekdays, and between 19:00 and 21:00 hours on weekend days. Although the proportion of adverts for foods potentially harmful to dental health was less than 1% around children's programmes, they were shown significantly more often during other programmes watched by children in greater numbers ($p < 0.001$). Children are exposed to a considerably high proportion of advertisements for foods that are potentially detrimental to their dental health during children's peak viewing hours and around programmes watched by significant numbers of young people.

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Incidence and Progression of Dental Erosion among Brazilian Adolescents

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Objective: This population-based longitudinal study aimed to estimate the incidence, progression and risk factors for dental erosion among Brazilian adolescents. **Methods:** 801 schoolchildren attending 42 schools (33 public and 9 private) were examined at 12 years of age and after a mean period of 2.5 years (standard deviation [SD] = ± 0.35). Clinical examinations were conducted at the schools by two calibrated examiners (intra- and inter-examiner kappa values ≥ 0.72). After tooth cleaning and drying, permanent incisors and first molars were classified according to the Basic Erosive Wear Examination (BEWE). Questionnaires were used to collect data on socio-demographics, dietary habits, brushing frequency, and general health. Poisson regression analysis was used to assess the association between erosion incidence and explanatory variables. Incidence risk ratios (IRR) and their respective 95% CI were estimated. **Results:** Out of 801 schoolchildren (mean age = 14.8; SD = ± 0.5), 680 were free of erosive wear at the baseline examination (at risk for erosion development) and 121 already presented dental erosion (at risk for erosion progression). Over the study period, 49 schoolchildren developed dental erosion (incidence rate of 7.14%; 95% CI = 5.20–9.07). Among those who already presented with erosion at baseline, 31 showed new or more advanced lesions (25.42%; 95% CI = 17.56–33.27). Boys were more likely to develop dental erosion than girls (IRR = 1.88; 95% CI = 1.06–3.32). **Conclusion:** The incidence of dental erosion observed in this population over 2.5 years, the higher susceptibility among boys and the progression of erosive lesions in a quarter of the affected individuals should be taken in consideration when designing preventive strategies for dental erosion.

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Fixed Orthodontic Treatment and Caries Activity among Adolescents and Young Adults

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Aim: To assess the effect of the duration of fixed orthodontic treatment on caries activity among adolescents and young adults. This cross-section study included individuals with no fixed orthodontic appliances (G0) and patients undergoing orthodontic treatment for one year (G1), 2 years (G2) or 3 years (G3). Participants completed a structured questionnaire on socio-economic characteristics and oral hygiene habits. Clinical examinations were

conducted by a single examiner after tooth cleaning and drying. Non-cavitated and cavitated caries were recorded and classified according to lesion activity based on clinical characteristics (surface texture and brightness). The prevalence and extent of active caries lesions were compared among groups (Wald test). The association between independent variables and the number of active caries lesions was assessed (Poisson regression models). Rate ratios (RR) and 95% confidence intervals were estimated. The group was considered the main independent variable. A total of 254 10–30-year-old individuals were included in the study, equally distributed among groups. The prevalence and extent of active caries lesions increased linearly as the duration of orthodontic treatment increased (G0: 6.1% and 0.06; G1: 27.3% and 0.61; G2: 73.7% and 2.12; G3: 72.3% and 1.95). These differences were statistically significant among G0, G1 and G2 ($p < 0.001$). The prevalence and extent of caries activity were similar between G2 and G3 ($p > 0.05$). Group, age and the use of dental floss were significantly associated with the outcome in this study. Compared to G0, individuals undergoing fixed orthodontic treatment presented increased risks for active caries lesions (G1: RR = 2.53, 95% CI = 1.50–3.56; G2: RR = 3.85, 95% CI = 2.84–4.85; G3: RR = 3.94, 95% CI = 2.93–4.96), adjusted for age and the use of dental floss. **Conclusion:** The duration of fixed orthodontic treatment was significantly associated with caries activity in this population.

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Is the Presence of Initial Caries Lesions a Predictor of Quality of Life Impairment in Preschool Children?

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Since non-cavitated caries lesions are considered initial stages of more severe conditions, our hypothesis is that the presence of initial caries lesions in preschool children could be a predictor of worsening in their oral health-related quality of life (OHRQoL). Therefore, this 2-year cohort study aimed to assess the risk of OHRQoL impairment in preschool children with different stages of dental caries. During an epidemiological survey, we examined 478 children (12–59 months-old) for dental caries using the ICDAS and their parents answered the Early Childhood Oral Health Impact Scale (ECOHIS). Subjects were categorized in children with no caries lesions ($n = 138$), children with only non-cavitated lesions ($n = 120$); children with at least one moderate lesion (ICDAS scores 3 and 4, $n = 30$) and children with dentine cavitated lesions ($n = 89$). After 2 years, 352 children (73.6% follow-up rate) were re-examined and their parents completed a new ECOHIS questionnaire. Influence of presence of caries at the baseline on OHRQoL impairment was evaluated through Poisson regression considering two dichotomized outcomes: worsening (any increase in the ECOHIS scores) and severe worsening (increase higher than

2.3 units – one-half of standard deviation at the baseline). Relative risk (RR) values and 95% confidence intervals (95% CI) were calculated. Compared with caries free children, individuals with moderate lesions (RR; 95% CI = 2.00; 1.30 to 3.07) or with extensive lesions at the baseline presented worsening on the OHRQoL (1.59; 1.09 to 2.30). However, children with only initial caries lesions did not present higher risk of worsening (1.02; 0.69 to 1.54). The same trends were observed considering severe worsening of OHRQoL. In conclusion, preschool children with only initial caries lesions do not present higher risk of OHRQoL impairment; however, moderate and extensive lesions are predictors of worsening of the OHRQoL.

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A 2-Year Cohort Study on Natural History of Dental Caries Lesions in Belarusian Adults

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The progression of caries lesions in adults considering lesion severity and activity stages is not well documented in the literature. The objective of our study was to evaluate the natural history of caries lesions in Belarusian adult population. The convenience sample of 491 adults aged 18–55 years was examined at the baseline. A total of 322 adults were reexamined in two years. Dental examinations were performed by a trained and calibrated examiner with intra-oral mirrors, ball-ended and sharp probes, using dental equipment with standardized drying and light capacity. The teeth of each patient were brushed before the examinations. For caries lesions detection and severity assessment the ICDAS criteria were used. Lesion activity was assessed by the Nyvad criteria. Considering all examined tooth surfaces, 14%, 33% and 48% of active caries lesions with ICDAS scores of (1+2), 3 and 4 respectively became frankly cavitated, restored or missed due to caries in the follow up, while the results for the inactive lesions with the same ICDAS severity scores were 10%, 20% and 23% respectively ($p < 0.01$). In conclusion, in adult population progression lesions into cavitation may vary depending on lesion activity and severity status.

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Exposure to Water Fluoridation and Social Inequalities in Child Caries Experience

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Aim: Water fluoridation (WF) providing passive access to fluoride can deliver larger benefits for high-risk (socially disadvantaged) people. The study aims to measure and compare socioeconomic inequalities in child dental caries between children in fluoridated (F) and non-fluoridated (NF) areas. **Methods:** A population-based study in Queensland, Australia was used. Children in F and NF areas were selected through a two-staged, stratified random sample selection process. Parents completed a questionnaire detailing parental education (low, medium, and high), household income (low, medium and high) and child oral health behaviours. Children were examined by trained and calibrated examination teams to estimate surface-level dental caries experience (dmfs/DMFS). Data were analysed using procedures for complex sample. Multivariable mixed effect models were generated to produce model-adjusted estimates of dmfs for 5–8-year-old and DMFS for 9–14-year-old children separately for F and NF areas. Sophisticated measures of socioeconomic inequality in health (Absolute Concentration Index (ACI), Slope Index of Inequality (SII), Relative Concentration Index (RCI), and Relative Index of Inequality (RII)) were estimated for F and NF areas. **Results:** Data of 2149 5–8-year-old and 3025 9–14-year-old children were used. NF-area children had significantly higher mean dmfs and DMFS than that of F-area children (4.31 (3.78–4.85) vs 2.75 (2.32–3.17) and 1.41 (1.24–1.59) vs 0.82 (0.69–0.95), respectively). Multivariable models for both F and NF areas showed that socioeconomic disadvantaged children had significantly higher rate of caries than the advantageous counterparts controlling for oral health behaviours and dental visiting patterns. Measures of socioeconomic inequality in oral health showed significant higher concentration of dental caries among socially disadvantaged groups in NF areas but not in F areas. For example, SII by parental education for DMFS: –0.002 (–0.486, 0.482) vs. –0.857 (–1.37, –0.344) in F and NF respectively. **Conclusion:** WF reduced socioeconomic inequality in child oral health.

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Educational Status as a Risk Indicator for Coronal and Root Caries among Brazilian Adults

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This cross-sectional study aimed to assess the association between educational status and coronal and root caries in adults from South Brazil. A multistage sampling strategy was used to draw a representative sample of 1,023 individuals aged ≥ 35 years. Questionnaires were used to collect data on socio-demographic characteristics, oral hygiene habits, dental care, and smoking. Oral examination assessed gingival bleeding, gingival recession, and coronal and root caries experience. Survey negative binomial regression models were used for data analysis (incidence rate ratio/95% confidence interval). The mean coronal DMFT and root DFT scores were 18.73 (95% CI 18.29–19.17) and 1.15 (95% CI 0.95–1.34). Individuals aged 45–59 years (IRR 1.29, 95% CI 1.19–1.40) or ≥ 60 years (IRR 1.39, 95% CI 1.29–1.49) and women (IRR 1.09, 95% CI 1.04–1.14) were more likely to have coronal caries. The likelihood of coronal caries decreased with high educational status (IRR 0.88, 95% CI 0.78–0.99) and proximal tooth cleaning frequency (IRR 0.94, 95% CI 0.91–0.97). Individuals with higher coronal DMFT scores (IRR 1.11, 95% CI 1.07–1.14) and a larger number of teeth with recession (IRR 1.18, 95% CI 1.15–1.22) were more likely to have root caries. The likelihood of root caries decreased with a higher tooth brushing frequency (IRR 0.75, 95% CI 0.59–0.96) and a larger number of retained teeth (IRR 0.92, 95% CI 0.89–0.95). A high educational status increased the probability of root caries by 74% (IRR 1.74, 95% CI 1.11–2.72). In conclusion, educational status was a risk indicator for both coronal and root caries in this Brazilian population. However, adults with a high educational status should only be considered at high risk for root caries.

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Systemic Calcium/Phosphorus and Caries in Pregnancy: A Prospective Cohort Study

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The mobilization of calcium (Ca) and phosphorus (P) of bone reserves is regulated by the action of parathyroid hormone, vitamin D and calcitonin. However, there is no consensus about the effect of systemic concentrations of Ca and P on the dental enamel and caries incidence in pregnant women. The aim was to assess whether changes in the concentrations of these ions in the blood and saliva are associated with the development of dental caries in pregnant women. In this prospective cohort study, 59 patients were evaluated at three time points (T1: in the 1st trimester of pregnancy, T2: 3rd trimester; T3: postpartum). At each time a dental examination was conducted for diagnosis of caries, according to the criterion of Nyvad. Blood and saliva were collected for analysis of total and ionized Ca and P. In addition, it was assessed socioeconomic status, dietary/hygiene habits and access to health services. Associations were evaluated by incidence risk ratio (IRR) in hierarchical multiple regression analysis ($\alpha = 5\%$). Twenty-eight women had no new caries lesions (Group 1–G1) and 31 had at least one (Group 2–G2). After adjustment, women with higher total serum Ca rates in T1 (IRR = 754.9; $p < 0.001$), ionized serum Ca in T1 (IRR = 2.4; $p = 0.03$), T2 (IRR = 21.6; $p = 0.04$) and T3 (IRR = 5.5; $p = 0.007$) had higher caries risk. High serum levels of P in T1 (IRR = 10.4; $p = 0.001$) and low levels in T2 (IRR = 0.4; $p = 0.04$) and T3 (IRR = 0.4; $p = 0.03$) were associated with higher incidence of caries. Increased salivary levels of Ca (IRR = 0.2; $p = 0.001$) and P (IRR = 0.8; $p = 0.03$) showed a protective effect. It was concluded that changes in serum and salivary Ca and P during pregnancy are associated with caries incidence. It is possible that the increased serum levels of Ca are indicating mobilization of this ion of mineralized maternal reserves, making it bioavailable to the fetus and thus increasing the risk of caries incidence in pregnant women. Tooth decay in pregnancy may be influenced by the serum and salivary calcium and phosphorus ions, reinforcing the importance of balanced nutrition in oral health.

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Features of Dental Services in Primary Health Centers in the Capital of Brazilian States

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Primary health care centers are places with provision of public health services in primary care and must offer dental services capable of performing diagnosis, prevention and basic treatment of caries. The aim of this study was to analyze sociodemographic characteristics and structure of dental services in primary health centers in the capital of Brazilian states. Descriptive ecological study, the unit of analysis was the state capital of the federation of Brazil. The variables were obtained from different databases: dental services characteristics in 2013 (Survey of the First Census of primary health care centers, Ministry of Health), sociodemographic characteristics in 2013 (Brazilian Institute of Geography and Statistics; United Nations Program for Development-website). The characteristics of services were: oral health services coverage in primary care was 41% (ranging from 7.3% Midwest Region to 85% Northeast), ratio of dentists by primary care centers was 1.4 (0.05 Midwest region to 2.55 Southern). The number of dentists that working in primary care centers was 144 (3 Midwest Region to 552 Southeast). The percentage of primary care centers with dentists in the capital was 74% (5% to 100% Midwest Region). The ratio of dentists that working in primary care centers by residents was 17,308, ranging from 3,970 (Northeast Region) to 187109 (the Midwest). The average population of the capital was 1,712,100 inhabitants (242,070 North to 11,376,685 Southeast), while the population density in the capital was 2,540 (12.57 to 7786.44 Northeast). There is great variation in sociodemographic characteristics of capital and low coverage of public oral health services in primary care to the population of the capital, noting that not all centers have primary dental health team. The presence and distribution of the best dentists in the health centers that offer primary care for dental caries is an essential condition for the control of this disease.

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Caries Experience and Educational Status are the Best Predictors for Long-Term Caries Development in Schoolchildren

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Objective: After the caries decline, caries trends have shown a non-normal distribution. In order to allocate resources for prevention properly, valid and easy predictors for caries incidence are needed. The objective of this 10-year-cohort-study was to assess the socioeconomic influence on the caries development in schoolchildren. **Methods:** The oral status and the parental educational background were documented for 521 children (5.8±0.5 yr) who took part in the compulsory school entry examination in Greifswald/Germany. In 10th grade, 170 (33%) of these pupils (16.5±0.4 yr) could be re-examined. The relationship between the children's and parental educational status as well as the caries prevalence and increment were analysed. For comparisons at baseline and in the follow-up, drop-outs (n = 351; 5.8±0.6 yr) and drop-ins (n = 364; 16.9±0.6 yr) were taken into consideration. **Results:** In this 10 year period, caries levels rose from 0.1 DMFS (±0.6) to 5.1 DMFS (±6.7), while the drop-ins (other 10th-graders) displayed a significantly higher caries experience (6.6±8.8 DMFS; p < 0.001). In children attending higher school education in 10th grade, baseline caries experience (6.1±7.4 dmfs) was only half compared to the children who later on attended lower school education (11.1±11.4 dmfs; p < 0.005). Furthermore, the 10 year caries increment was significantly lower when the father had a university degree (2.7±3.2 ΔDMFS; vs. others: 5.1±6.6; p = 0.02; OR 4.6). Similarly, a low baseline caries experience in the deciduous dentition correlated with a low caries increment in the permanent dentition (OR 3.6). **Conclusions:** This study confirms that caries in the deciduous dentition and the parental educational level were associated with the caries increment.

Dental Caries Profile and Associated Risk Factors among Adolescent School Children in Urban Indian City

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Aim: To study the dental caries experience among adolescent school children in Chennai city using ICDAS-II scoring system. The secondary objective was to identify associated risk indicators to different thresholds of dental caries defined by ICDAS. **Methodology:** Two hundred and thirty-seven children (13–17 years) from 5 schools across Chennai city were included using simple random sampling. After obtaining assent to participate in the study and satisfying the selection criteria, 200 children were screened for dental caries using ICDAS-II. The population was assessed for the following risk indicators: Socio-demographic status, habits, diet, plaque and salivary parameters. Prevalence of dental caries was estimated at the following thresholds: Normal (ICDAS-0/1), incipient caries (ICDAS-2), enamel caries (ICDAS-3/4) and dentin caries (ICDAS-5/6). Inter-observer correlation was 0.91. Backward logistic regression analysis was performed to identify risk indicators at different thresholds and crude Odds ratio (OR) was calculated for significant risk indicators. **Results:** The proportions of children at different caries thresholds were: ICDAS-2 – 55% (95% CI: 48%–62%), ICDAS-3/4 – 51% (95% CI: 44%–58%) and ICDAS-5/6 – 25% (95% CI: 19%–31%). Reduced salivary pH was a significant risk indicator for enamel caries and dentin caries (OR 6.24, 95% CI 1.18–32.78 and 1.73, 95% CI 1.18–1.92 respectively) and saliva flow rate was a significant risk indicator for incipient caries and enamel caries (OR 4.48, 95% CI 2.94–8.23 and 3.97, 95% CI 2.65–7.03 respectively). Low buffering capacity was associated with incipient caries OR 5.71, 95% CI 2.82–18.2). A non-significant value using Hosmer-Lemeshow Goodness of fit test indicated that all three models predict the true estimate of the population. **Conclusion:** The proportions of children with incipient caries and enamel caries were high considering their age group. The risk indicators associated with incipient caries were different from those associated with enamel and dentin caries.

Session 7

De- and Re-Mineralization

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Demineralization Characteristics of Enamel Depending on Baseline Mineral Loss and Lesion Depth in situ

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The aim of this double-blinded, randomized, cross-over, in situ study was to evaluate the response of sound enamel as well as shallow and deep caries-like enamel lesions after the application of three different fluoride compounds. In each of three experimental legs of four weeks 21 participants wore intraoral mandibular appliances with each two bovine enamel specimens inserted in the buccal flanges (1 mm below the acrylic under a plastic mesh). Specimens included sound enamel and either a pre-demineralized shallow (pH 4.95; 7 days) or deep (21 days) lesion. The three randomly allocated treatments (application only) included the following dentifrices: 1,100 ppm F⁻ as NaF (NaF), 1,100 ppm F⁻ as SnF₂ (SnF₂) and a fluoride-free (0 ppm F⁻), negative control (FF). Differences in integrated mineral loss ($\Delta\Delta Z$), lesion depth (ΔLD) and the ratio of mineral loss and lesion depth ($\Delta'R$ -value) were calculated between values before and after the in situ period using TMR. Six participants did not complete the study; two were excluded due to protocol violation. Mean (SD) baseline mineral loss, lesion depth and $\Delta'R$ -value were 2930 (1487) vol% $\times\mu\text{m}$, 100 (31) μm and 29 (3) vol% for shallow and 5208 (1600) vol% $\times\mu\text{m}$, 149 (34) μm and 35 (3) vol% for deep lesions. Irrespectively of the treatment, higher baseline values led to lower increase of $\Delta\Delta Z$, ΔLD and $\Delta'R$. Sound surfaces showed a significantly higher increase of $\Delta\Delta Z$, ΔLD and $\Delta'R$ compared to shallow and deep lesions ($p < 0.05$, t-test). Shallow lesions showed a significantly higher percentage change of $\Delta\Delta Z$, ΔLD compared with deep lesions ($p < 0.05$, t-test). In conclusion, baseline mineral loss and lesion depth directly affects in situ lesions' response to fluoride. Treatment groups should therefore be well-balanced with respect to baseline mineral loss and lesion depth.

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Effect of Bioglass on Root Caries – An in vitro Optical Surface Profilometry Study

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Optical profilometry is a useful method for measuring de- and remineralisation in root caries. The aim of this study was to assess the effect of two dental products containing different concentrations of bioglass (NovaMin) on the changes in surface roughness of root caries using non-contact optical surface profilometry. A total of 18 extracted teeth with root caries were obtained from the Dental Emergency Clinics, Barts and the London Trust, UK. They were randomly assigned to the control and two test groups. Either dentifrice containing 5% NovaMin with 1450 ppm fluoride (n: 6), or prophy paste containing 15% NovaMin (n: 6) were applied to the test groups. A dentifrice containing 1450 ppm fluoride without bioglass (n: 6) was applied to the control group. Surface roughness was measured at baseline and after the application of the dental products at 0.5 h, 1 h, 4 h, 12 h, 24 h, 48 h, and 168 h using non-contact optical profilometry. All teeth were immersed in a remineralising solution containing 1.4 mmol of CaCl₂ at 37°C in between measurements. The results showed that the surface roughness of root carious lesions for prophy paste containing 15% NovaMin group decreased significantly by 28% between baseline and 168 h ($p = 0.002$) whilst there was no significant difference in dentifrice containing 5% NovaMin with 1450 ppm fluoride group ($p = 0.40$) and in the control group ($p = 0.05$) at baseline and after 168 h. Changes in surface roughness were significantly different between two test groups at baseline and after 168 h. However, there was no significant difference in changes in surface roughness for two test groups when compared to the control group. Conclusion derived from this lab based study, the use of high concentration bioglass has a potential to remineralise root carious lesions.

In situ Study to Investigate Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) Toothpaste in Orthodontic Patients

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The study aimed to investigate the remineralisation of enamel subsurface lesions with CPP-ACP toothpaste compared with regular fluoride toothpaste in orthodontic patients using an in situ cross-over randomised control trial design.

12 orthodontic patients had demineralised sub-surface lesions attached onto a fixed orthodontic appliance via a custom made stainless steel enamel carrier. All patients had a 4 week period of using a standard fluoride tooth paste (1450 ppm) alone or in addition to CPP-ACP paste application (Tooth Mousse™ GC Corporation, Europe) with a 4 week wash out period in between. Transverse MicroRadiography (TMR) was used to analyse and compare the mineral content profiles between the control and experimental samples including Mineral loss ΔZ (vol% μ m), Lesion depth Ld (μ m) and Lesion width Lw (μ m) and percentage changes in these parameters calculated. Post-treatment measurements were compared using analysis of covariance (ANCOVA), adjusting for the baseline measurements, and for the order in which the treatments were received. Results showed significant remineralisation occurred in both treatment groups compared with baselines, as demonstrated by reductions in Mineral Loss ΔZ , Lesion depth and Lesion width. Mineral loss ΔZ reduced by 15.4% and 23.6% for Fluoride and CPP-ACP groups respectively, however differences between these groups were not statistically significant ($p = 0.066$). Lesion depth reduced by 2.4% and 10.1% for Fluoride and CPP-ACP groups respectively, with a statistically significant difference between these groups ($p = 0.003$). Lesion width reduced by 4.7% and 16.2% for Fluoride and CPP-ACP groups respectively, with a statistically significant difference between these groups ($p = 0.001$).

In conclusion, CPP-ACP paste combined with fluoride toothpaste showed a significantly greater reduction in both lesion depth and lesion width than fluoride toothpaste only.

Incongruent Dissolution of Fluorapatite in Acids

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Aim: The aim of this study was to establish the sequence of ionic detachment occurring during fluorapatite (FAP) dissolution in acids. **Methods:** Single FAP crystals of 1–2 mm in size were etched in 0.1 M H₃PO₄ solution for 10–15 s, followed by washing

in acetone to stop the dissolution process and remove the traces of unreacted acid. Afterwards, the etched crystals were dried and their surface was studied by infrared (IR) and Auger-electron spectroscopy (AES), scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDX). Then, the entire procedure was repeated. **Results:** The results obtained with AES, IR and EDX indicated that chemical changes occurred with the FAP surface during its interaction with H₃PO₄. Namely, a very thin surface layer was found to have lost almost all fluorine. Similar measurements for calcium and phosphorus indicated that the surface composition of FAP changed toward formation of acidic calcium phosphates. Namely, the atomic ratio Ca/P was found to have reduced from 1.65 \pm 0.05 (FAP) to 1.30 \pm 0.05 for FAP crystals treated in acid. Thus, calcium was partly dissolved from FAP surface, while phosphate remained on the surface. Based on these results and additional data taken from the literature, the following sequence of ionic detachment was found to occur during FAP dissolution in acids. Fluorine was dissolved first. Most likely, this was due to its specific location in the crystallographic channels oriented along the c-axis. Calcium was dissolved afterwards, followed by dissolution of phosphate. **Conclusions:** A nonstoichiometric (incongruent) detachment of major FAP constituents was found during its chemical dissolution in acids.

Effect of Phosphoryl Oligosaccharides Calcium in Presence of Fluoride or Protein on Remineralization of Early Carious Lesions

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Phosphoryl oligosaccharides of calcium (POs-Ca[®]) is a material containing bioavailable calcium. In the presence of low-level fluoride (>100-ppm) enhanced remineralization of enamel lesion with POs-Ca, whereas higher fluoride impaired the effect by forming insoluble precipitation. On the other hand, pellicle precursor proteins (PPPs) such as statherin and proline-rich protein which contains saliva are known to prevent mineral precipitation on tooth surfaces, and facilitate remineralization. Casein is used as an alternative of PPP. The aim of this study was to investigate the effect of remineralization of the enamel and dentin early caries lesions by POs-Ca in the presence of 300/1000-ppm fluoride and casein in artificial saliva, using transversal microradiography (TMR). We prepared early-stage caries lesions of bovine dentin and enamel under acid condition. Then, they were treated with artificial saliva containing 6 mM calcium and 3.6 mM phosphate ions 10 μ g/ml Casein with 1000-ppm/300-ppm fluoride from NaF at 37°C for 24 hrs. POs-Ca or CaCl₂ was added as the source of calcium. Then, thin sections were prepared by cutting perpendicular to the surface, and analyzed by TMR. The results showed that for mineral recovery rates of remineralization ($p < 0.05$, A Mann-Whitney U-test) were 36.1 \pm 15.0% for POs-Ca with fluoride (1000-ppm) and 0.7 \pm 13.2% for CaCl₂ with fluoride (1000-ppm) to mineral loss. While, dentin mineral recovery rates of remineralization were 1.2 \pm 6.8% for POs-Ca with fluoride (1000-ppm), 1.5 \pm 10.5%

for CaCl₂ with fluoride (1000-ppm), 34.3±5.8% for POs-Ca with fluoride (300-ppm), and 15.1±7.3% for CaCl₂ with fluoride (300-ppm) to mineral loss. These results indicate that POs-Ca with high-level fluoride enhances remineralization of both dentin and enamel caries lesions in the presence of casein much better than inorganic calcium.

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Effect of Scandium on Bovine Enamel Under pH-Cycling Conditions

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This study evaluated the impact of a novel antibacterial scandium doped phosphate-based glasses (Sc-PBGs) on enamel mineralisation under pH-cycling conditions.

Scandium doped and scandium free control, C-PBG, rods (5 x 2 mm) were produced using a conventional melt quenching method, at 1100°C for 1 h. The effect of Sc-PBG on bovine enamel was investigated under pH cycling condition. Enamel blocks were assigned to one of 4 conditions (Sc-PBG, C-PBG, NaF and Water) and exposed to 5 days of cyclic exposure to an acidic challenge (2.0 mM CaCl₂, 2.0 mM KH₂PO₄, 0.04 ppm F⁻ form NaF and 0.075 mM acetic acid adjusted to pH 4.7 with 1.0 M NaOH) (6 h Demineralisation/18 h Remineralisation), followed by 2 days in remineralising solution (1.5 mM CaCl₂, 0.9 mM KH₂PO₄, 150 mM KCl, 0.05 ppm F⁻ form NaF and 20 mM HEPES adjusted to pH 7.0 with 1.0 M NaOH). Non-contact surface profilometry, NCSP (Proscan 2000, Scantron Industrial Products LTD) was used to calculate mean surface roughness, Ra. Integrated mineral loss (ΔZ) was analysed using transverse micro-radiography (TMR2006, Inspektor Research Systems, Amsterdam, The Netherlands). Statistical analyses were conducted using the GraphPad software (San Diego, California, USA), Tukey-Kramer multiple comparison tests were used to compare values. The Ra values of enamel sections exposed to Sc-PBG (4.43±2.5 μm), C-PBG (3.42±0.99 μm) and NaF (7.73±4.53 μm) showed a decreasing but statistically non-significant (p > 0.12) trend compared with baseline. TMR analyses showed mineral losses for Sc-PBG (2859.44±168.05 vol%.μm), C-PBG (1464.89±375.49 vol%.μm) and NaF (3240.05±808.73 vol%.μm) compared with baselines (≤ 536.56±114.32 vol%.μm) for all samples (p < 0.05) but patterns of unusual lesion formation with appearance of several layers of alternating hyper- and hypo-mineralisation, occurred in the presence of Sc-PBG.

Sc-PBGs may have the potential to protect the enamel surface under conditions representative of the oral environment. Further work will be required to confirm whether it has an application to prevent or reduce caries.

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Enamel Fluoride Uptake Measurements from Sealants Formulated with Microencapsulated Remineralizing Agents

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Objectives: Caries at the interface of dental materials and mineralized tooth structure continues to be a significant issue in oral healthcare. The objective of this study was to demonstrate that sealants with microencapsulated remineralizing agents with sustained release of fluoride, calcium and phosphate ions promotes enamel fluoride uptake by demineralized tooth structure. **Experimental Approach:** Sealants that contained 5% (w/w) microcapsules with aqueous solutions of 5M Ca(NO₃)₂ or 0.8M NaF or 6.0M K₂HPO₄ or a mixture of all three were prepared. Ion release profiles were measured as a function of time. Enamel fluoride uptake by demineralized tooth structure was determined by a modified version of FDA Method 40. In the Method 40 experiment, the enamel surface is soaked in acid before and after treatment with a fluoride releasing material. The amount of fluoride in the acid is measured using ion specific electrodes. The amount of enamel was derived from the amount of calcium in the acid by AA. The amount of fluoride associated with the enamel before and after treatment is therefore reported as microgram of fluoride per gram of enamel. All sets of data was n = 12. **Main Results:** Sustained release of fluoride, calcium and phosphate ions from a sealant was demonstrated. Fluoride uptake by demineralized enamel was significantly increased compared to a control sealant manufactured without microcapsules. Bovine enamel that contained 2.2±2.1 μg F/g of enamel prior to exposure to a sealant without microcapsules had 2.3±0.5 after 90 days. Enamel exposed to sealant with 5% (w/w) NaF microcapsules went from 3.5±3.5 μg F/g of enamel of enamel prior to exposure to 148±76 after 90 days. Enamel exposed to sealant with 2% (w/w) NaF, 2% (w/w) Ca(NO₃)₂ and 1% (w/w) K₂HPO₄ microcapsules went from 1.7±0.7 μg F/g of enamel prior to exposure to 190±137 after 90 days. **Conclusions:** Sealants with encapsulated remineralizing agents are capable of releasing biologically available fluoride, calcium, and phosphate ions.

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Using the Canary System to Evaluate the Resistance of Resin Infiltration to Demineralization

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In this *in vitro* study, the ability of Resin Infiltration (RI) to resist enamel breakdown by acid demineralization was monitored by The Canary System (CS). 135 visually detected early caries lesions on smooth and proximal surfaces of extracted human teeth were scanned with CS (Baseline reading). 75 lesions in the treatment group (TG) were treated by Resin Infiltration (DMG ICON), while 60 lesions in control group (CG) were not infiltrated. All lesions (treated/untreated) were then subjected to demineralization with acidified gel (pH 4.5). During demineralization, CS scanning was repeated on each lesion at baseline (pre-treatment), and days 0, 7, 14, 30 and 50, and the Canary Number (CN) based on tooth structure (0–20 indicates sound structure; 21–70 indicates early caries; 71–100 indicates advanced caries) was recorded. For the following, asterisk (*) indicates significant differences in CN from baseline (Wilcoxon Signed-Rank Test, $p < 0.05$). For smooth surfaces, CN \pm SE over the course of the study progressed from 24 \pm 2* to 32 \pm 1* (TG) and 42 \pm 2* to 60 \pm 3* (CG). For proximal surfaces, CN \pm SE over the course of the study progressed from 13 \pm 1* to 18 \pm 2* (TG) and 30 \pm 2 to 42 \pm 2* (CG). Polarized Light Microscopy histologically confirmed the presence/absence of caries. Overall, this study demonstrates that lesion infiltration with DMG ICON is able to significantly delay progression of existing caries, and that the status of an infiltrated caries lesion can be monitored using The Canary System.

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Effect of Zinc and Fluoride on Secondary Demineralisation of Enamel Lesions *in vitro*

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Clinical and *in vitro* data suggest that fluoride is more effective at inhibiting initiation, rather than progression, of caries. Zinc, despite being a crystal-growth inhibitor for apatites, does not reduce the anti-caries effectiveness of fluoride toothpastes. Hence the aim was to investigate the effect of zinc and fluoride on secondary demineralisation of enamel lesions at plaque fluid concentrations. Sound bovine enamel blocks were demineralised in three

acidified methyl cellulose gels, partially-saturated with respect to enamel (37°C, 10 d, 14 d, 18 d). Mineral-loss (ΔZ , units vol%. μ m) was measured using transverse microradiography (TMR) and lesions assigned to one of four treatment groups per lesion type (i.e. twelve treatment groups, 8 lesions/group). Lesions were further demineralised (20 h, 37°C) in one of four solutions. The base solution (nonF/nonZn, was 1.00 mmol/l calcium chloride dihydrate, 4.00 mmol/l potassium dihydrogen orthophosphate, 50 mmol/l acetic acid, 130 mmol/l potassium chloride, pH 4.90. Three further solutions were created by the addition of zinc (Zn, 231 μ mol/l, as zinc acetate), fluoride (F, 10.5 μ mol/l, as NaF), or zinc and fluoride (ZnF, 231 μ mol/l, 10.5 μ mol/l). Subsequently, DDZ was measured again and net mineral change (DDZ) calculated. Data were analysed (ANCOVA). Differences were deemed to be significant at $p < 0.05$. Mean DDZ (SD) values for nonF/nonZn, Zn, F, ZnF respectively, were: 10 d: 1160 (366)^a, 443 (343)^b, 855 (280)^a, 374 (238)^b. 14 d: 1070 (404)^a, 579 (212)^b, 993 (454)^a, 399 (382)^b. 18 d: 1500 (519)^a, 923 (636)^b, 1520 (458)^a, 639 (441)^c. (For each lesion type, means with same letter not significantly different.) The data support those from previous mechanistic studies, where relatively high concentrations of fluoride were needed to inhibit lesion progression. In conclusion, zinc reduced enamel dissolution *in vitro* under simulated plaque-fluid conditions, whereas fluoride did not, suggesting a possible complementary anti-demineralisation mechanism for zinc.

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Combined Effect of Stannous and Fluoride Ions on HAP Powder Dissolution Inhibition: An ISE Study

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Stannous ions, individually and in combination with fluoride ions, are sometimes used in oral health care products to prevent demineralization. However, the mechanism by which stannous ions inhibit dental hard tissue mineral dissolution remains obscure. Computer interfaced ion selective electrodes (ISEs) allow continuous measurements enabling monitoring of calcium ion (Ca²⁺) release into demineralizing solutions to obtain kinetic, and thermodynamic (equilibrium), dissolution data. The aim was to use Ca²⁺ ISEs to investigate the cariostatic efficacy of stannous ions, with and without fluoride ions, in reducing Ca²⁺ release from calcium hydroxyapatite (HAP) powders. HAP powder (size 2–6 μ m, specific surface area 6–8 m²/g) was obtained from PlasmaBio-tal (UK). Caries-simulating acetic acid solutions (pH = 4.0) containing either; sodium fluoride (NaF); tin acetate (SnAcetate); or tin fluoride (SnF₂), at concentrations of either; 0.0 ppm (control); 0.01 ppm; 0.1 ppm; or 0.5 ppm, were prepared using analytical grade reagents. Ca²⁺ ISEs were immersed into each solution, and 0.2 g HAP powder added and stirred, and [Ca²⁺] measured every 10 s for 1 h (equilibrium achieved). pH changes were monitored, but remained constant throughout. All experiments were carried out at 25.0 \pm 2.0°C. For NaF (control); 0.01, 0.05 and 0.1 ppm, the

percentage inhibition at equilibrium was 69.1 ± 1.0 , 79.3 ± 1.0 and 89.1 ± 1.0 respectively. For SnAc; 0.01, 0.05 and 0.1 ppm, the percentage inhibition at equilibrium was 89.3 ± 1.0 , 98.3 ± 1.0 and 98.6 ± 1.0 respectively. For SnF; 0.01, 0.05 and 0.1 ppm, the percentage inhibition at equilibrium was 98.6 ± 1.0 , 98.7 ± 1.0 and 99.1 ± 1.0 respectively. Stannous ions are effective inhibitors of hydroxyapatite dissolution. The time-dependent data indicated that inhibition was rapid. However, the hydroxyapatite dissolution inhibition effects of stannous ions and fluoride ions are not additive, suggesting different mechanisms of HAP of dissolution inhibition.

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Comparative Study Regarding the Reduction of Dental Hard Tissues Demineralization Using Different Commercial Remineralizing Products

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The aims of this study were to investigate the surface topography and to compare the calcium and phosphorus ions concentration in enamel, dentine and cement when three different commercial remineralizing products are used after demineralization of the dental hard tissues. 20 caries-free extracted teeth were split in two halves. A free window of enamel (E), dentine (D) and cement (C) in the cervical area of the slices was preserved. The slices were randomly divided in four groups (A-D). In control group (group A) the slices were subjected to a demineralization period of 1 hour, three times a day, for 14 days. Between the demineralization cycles, the samples were stored in artificial saliva (AFNOR NF S90-701). In groups B, C and D the same demineralization cycles were applied, but the first and the last demineralization period were followed by the application of Colgate Total® toothpaste (Colgate Company) for 3 minutes (group B), MI Paste Plus (GC Corporation) for 3 minutes (group C) and Home Care Fluoride gel (Alpha-Dent Company) for 1 minute (group D). The samples were analyzed using a scanning electron microscope coupled with a EDX detector. SEM aspects showed a reduction of demineralization areas after using all the remineralizing solutions. The mean calcium and phosphorus ions concentration in E, D, C increased after using the remineralizing products. The mean calcium ion concentration (wt%±SD) in E/D/C was: $30.57 \pm 0.21/15.46 \pm 0.44/13.12 \pm 0.22$ group A, $30.90 \pm 0.36/15.91 \pm 0.27/13.77 \pm 0.32$ group B, $32.25 \pm 0.44/20.41 \pm 0.11/14.92 \pm 0.25$ group C, $38.63 \pm 0.33/24.89 \pm 0.14/16.55 \pm 0.61$ group D. The increase of calcium and phosphorus ions concentrations were significantly higher in groups C and D when comparing to groups A and B (ANOVA and post hoc Bonferroni tests, $p < 0.05$). MI Paste Plus and Home Care Fluoride gel showed a significantly higher remineralization capacity when comparing to Colgate Total® toothpaste.

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Effect of Differential Fluoride Treatments on Mineralization in Incipient Lesions

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The aim of the present study was to investigate the ability of aqueous NaF solutions of different concentrations applied for varying durations to promote enamel remineralization and prevent further demineralization of caries lesions under dynamic conditions simulating exposure to cariogenic acids. Ninety 4x4 mm ground and polished human enamel specimens were used in this study. Two opposing strips of nail varnish were painted onto the specimens creating an approximate 2 mm wide window in the center. The unprotected area was demineralized for 5 days at 37°C using a lesion forming solution. After demineralization, an experimental 1.5 mm wide window was created on the specimens using nail varnish. Specimens were subsequently stratified into five treatment groups (n = 18/group) balanced by their post-demineralization surface microhardness values. The daily treatment regimen included 4 hours/day acid challenge in the lesion forming solution and four treatment periods with NaF solutions: 1000 ppm F x 1 min (A); 500 ppm F x 2 min (B); 200 ppm F x 5 min (C); 50 ppm F x 20 min (D); 25 ppm F x 40 min (E). The remaining time specimens were stored in a remineralization system consisting of a 50:50 mixture of pooled human and artificial saliva. The regimen was repeated for 20 days at room temperature. After the cycling period, blinded samples were evaluated for change in surface microhardness (Δ SMH, VHN), enamel fluoride uptake (EFU, $\mu\text{gF}/\text{cm}^3$) and change in integrated mineral loss (Δ M, vol% μm) via transverse microradiography. The results (means \pm SD) were as following: A: 22.5 ± 8.9 , 2379 ± 586 , 279 ± 457 ; B: 20.8 ± 7.9 , 2432 ± 751 , 141 ± 501 ; C: 20.1 ± 8.8 , 2180 ± 622 , 236 ± 515 ; D: 19.1 ± 7.1 , 2367 ± 493 , 97 ± 507 ; E: 17.6 ± 6.8 , 2294 ± 411 , 49 ± 497 . There were no statistical differences between all 5 treatment groups ($P > 0.05$). Based on these results it can be concluded that all treatments were equally effective.

Treatment of Fabricated Caries Lesions; Self-Assembling Peptides vs. Fluoride

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Non-invasive interventions for early stage enamel caries include fluoride-containing varnishes. Recently, a biomimetic self-assembling peptide, which mimics the function of the extracellular protein matrix during enamel development, supporting de novo apatite crystallisation and growth, has been shown to have clinical benefit in promoting enamel remineralisation. The aim of this study was to assess the ability of self-assembling peptides (SAPs) and to re-mineralise artificial caries-like lesions in human teeth in vitro. Thin slabs were prepared from human permanent teeth obtained with consent from the Research Tissues Bank at Leeds following surgical extraction. Several coats of acid-resistant nail varnish were applied to each slab to delineate a window on the enamel surface of approx. 3x3 mm. The slabs were divided into groups and demineralised in a solution of 0.05 M acetic acid, 2.2 mM NaH₂PO₄, 2.2 mM CaCl₂, pH 4.4 for 96 hours. Lesions were assessed by micro-CT to ensure the presence of an intact surface zone and lesion depth not greater than 120 µm (range 80–120 µm). Lesions were treated with water (control), fluoride varnish or self assembling peptide P11-4 (CH₃COQRFWEFEQQNH₂). Each slab was then placed in re-mineralising solution (Ca(NO₃)₂ 1.5 mM, KH₂PO₄ 0.9 mM, KCl 130 mM, Tris 60 mM, pH 7.4) for 120 hours at 37°C, replacing the solution every 24 hours. Lesions were then assessed by quantitative micro-CT (resolution 5.58 mm, 0.4 step, average 5), with five samples per group (paired). Re-mineralisation was observed with both the fluoride varnish and the SAPs compared with untreated controls, however, mineral density in the lesions that were treated with the SAPs was approx. 40% greater than the demineralised slabs. The results suggest that the use of self-assembling peptides in the treatment of early caries might offer an alternative treatment strategy for early caries either instead of, or in conjunction with, traditional fluoride treatment.

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Comparison of Mineral Density Profiles of Hypomineralised and Sound Teeth Obtained Using Micro-Computed Tomography

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Objectives: Molar Incisor Hypomineralisation (MIH) is a common condition with average worldwide prevalence of 15% (range 2–40%) of children. It presents significant issues with poor physical characteristics of enamel sometimes leading to rapid post-eruptive breakdown, restoration failure and sometimes extraction. Examination of dentine related to MIH has been limited with increased inter-globular dentine the only structural difference identified and bacterial penetration into the dentine under macroscopically intact hypomineralised enamel reported. Dentine has an important role in supporting the overlying hypomineralised enamel and for retention of restorations; any change in the dentine may play a role in the poor outcomes of MIH-affected teeth. **Methods:** A convenience sample comprising 19 hypomineralised first permanent molars (FPM), two sound FPM and two sound premolars was collected. MIH lesions were classified as white/cream, yellow/brown with or without post-eruptive breakdown. Micro-computed tomography (µCT) was used to estimate mineral density. Measurements were taken at three depths in enamel (outer, middle and inner thirds) and four depths in dentine (DEJ, outer, middle and inner thirds). Linear mixed-modelling approach was used for statistical analyses. **Results:** 101 mineral density profiles were obtained from hypomineralised lesions and sound enamel areas on the 23 teeth. Enamel mineral density was significantly more variable than dentine measurements for both hypomineralised and sound enamel. Mineral density at DEJ was significantly lower in hypomineralised compared with sound enamel. In dentine, the mineral density measurements from the DEJ to inner-third dentine under hypomineralised lesions increased by over 4.3% whereas for sound enamel it decreased by 6.4% ($P < 0.001$). **Conclusions:** The mineral density of dentine from superficial to deep readings under hypomineralised enamel was abnormal with reversed density to normal dentine.

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Session 8 Diagnostics: Lab-Based Studies

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The in vitro Impact of Toothpaste Extracts on Cell Viability

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Aim: Tooth-brushing with a toothpaste is worldwide the most common form of oral hygiene to physically remove dental plaque. Toothpastes contain a large number of ingredients. The most important active ingredients that occur in almost all toothpastes are surfactants, abrasives and fluoride. Surfactants are responsible for the intraoral dispersion and the foaming of the toothpaste, while abrasives physically clean the teeth and fluorides prevent of caries. Besides the undisputed positive effect, surfactants, particularly sodium-lauryl-sulfate, have been proposed as a component that enables toothpastes to produce cytotoxic effects in vitro. However, sodium-lauryl-sulfate is not the single surfactant used in toothpastes and up to now no study investigated the in vitro cytotoxicity of toothpastes regarding their surfactants. **Experimental Approach:** The in vitro cytotoxicity of nine commercially available toothpastes with four different surfactants was evaluated. Toothpastes were diluted in serum-free media, centrifuged and filter sterilized. The half-lethal concentration of the toothpaste-conditioned medium was calculated based on formazan formation by gingival fibroblasts, oral squamous cell carcinoma HSC-2 cells and L929 cells. Furthermore assays for proliferation and live-dead staining were performed. **Results:** Toothpastes containing sodium-lauryl-sulfate but also amine-fluoride resulted in significantly inhibited cell viability with a half-lethal concentration of approximately 1% toothpaste-conditioned medium. Toothpastes containing cocamidopropyl-betaine and Steareth-20 showed higher half-lethal concentration values, of about 10% and 70% tooth-

paste-conditioned medium, respectively. Proliferation and live-dead data were consistent with the cell viability analysis. **Conclusions:** Our results demonstrate that the type of surfactant in toothpastes can be associated with changes in in vitro cell toxicity.

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Detection and Assessment of Carious Lesions by Optical Coherence Tomography vs. X-Ray Microtomography

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Objective: Evaluation of performance of spectral domain optical coherence tomography (SD-OCT) and X-ray microtomography (μ CT) for detection and assessment of cavitated and uncavitated carious lesions. **Methods:** 51 extracted human molars, premolars and incisors with 238 carious lesions on occlusal, approximal and smooth surfaces (ICDAS-II code 0-6) were visually selected. One region of interest was marked by two drill-holes each in the direction of maximum extension of lesion, followed by imaging with SD-OCT (2D, 3D, center wavelength 1310 nm; Telesto II, Thorlabs GmbH, Dachau, Germany). μ CT images were used as validation standard (Skyscan1172-100-50, Bruker MicroCT, Kontich, Belgium). μ CT and OCT signals were categorized according to the lesion extent adapted to the seven-category ICDAS-II scale: 0: sound, 1: lesion limited to first quarter of enamel, 2: lesion extended up to whole enamel without cavitation, 3: lesion limited to enamel with cavitation, 4: cavitation in enamel, demineralization of dentin, 5: cavitation with dentin involvement, 6: extensive cavitation into dentin. Scoring of lesions was statistically compared (weighted Cohen's kappa coefficient (K); McNemar-test, $\alpha = 0.05$). **Results:** Codes 2-6 showed almost a perfect agreement (80–100%, K = 0.87; p: 0.001–1.00). In contrast, the agreement was low with code 1 (33%, p < 0.001). Altogether, code 1 was detected 9.7 times more often with OCT than with μ CT. Agree-

ment for sound enamel (code 0) was 54% ($p < 0.001$). **Conclusion:** OCT seems to be more suitable to assess sound surfaces and to detect early enamel carious lesions than μ CT.

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Utilization of QLF-D for Monitoring the Remineralization Potential of Early Caries Lesions after Fluoride Application

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The aim of this study was to analyze the initial fluorescence loss (ΔF) using quantitative light-induced fluorescence-digital (QLF-D) systems for screening enamel lesions to be effectively remineralized by fluoride in situ. In a double blind, randomized, in situ study over 20 days, 20 adult volunteers wore intraoral appliance containing slabs of bovine enamel. To generate artificial enamel lesions of varying severity, 80 specimens were immersed in demineralizing gel for 3 to 20 days. Specimens were covered with fluoride gel for 1 min. The ΔF and ΔF_{\max} for the enamel specimens were assessed three times using the QLF-D systems: immediately after demineralization and after 1 week and 4 weeks of fluoride application. From the results of the repeated-measures ANOVA analysis, the ΔF significantly increased according to the treatment period ($p < 0.001$). From the ROC curve analysis, when $\Delta F_{(0)}$ of the initial lesions was -11.5% , $\Delta F_{(1)}$ could be recovered up to -6% after 1 week after fluoride application, and cutoff value of the $\Delta F_{(0)}$ for recovery of the $\Delta F_{(4)}$ up to -10% was found to be -25.50% . Our study results indicate that clinicians can predict the recovery rate of early caries lesions using the QLF-D systems, which results in more effective treatment plans.

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Changes in Red Fluorescence and Bacterial Composition of Microcosm Biofilm during Maturation

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The aim of this study was to investigate the bacterial composition of microcosm biofilm at different maturation stages. Dental microcosm biofilms were grown on bovine enamel discs. They were initiated from one donor saliva for 4 h, and then grown in 0.5% sucrose growth media which were replaced daily for 10 days. On days 1, 3, 7, and 10 after the incubation, fluorescence images of the biofilms were captured using the QLF-D (Inspektor Research Systems bv., The Netherlands) and the red fluorescence intensity was quantified by calculating the red/green ratio (R/G value). Total and aciduric bacteria within biofilm were counted. This whole procedure was repeated three times. The respective phylogenetic diversity of 10 species (*Actinomyces israelii*, *Lactobacillus casei*, *Prevotella intermedia*, *Streptococcus anginosus*, *Streptococcus mitis*, *Treponema denticola*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Streptococcus mutans*, *Veillonella* spp.) was analyzed by 16S rRNA real-time quantitative PCR analysis. The results show that total and aciduric CFU were significantly increased until 7 days ($p < 0.05$). R/G ratio was significantly correlated with the total CFU ($r = 0.74$, $p = 0.001$), and aciduric CFU ($r = 0.85$, $p = 0.001$). The most abundant species was *Veillonella* sp. in each stage and *Streptococcus anginosus* was rapidly increased in the late stage. In conclusion different ratio of each oral bacterium exists in microcosm biofilms. Moreover this composition constantly changes during biofilm maturation. This diversity should affect auto-fluorescence and acidogenicity of biofilms.

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In vitro Assessment of Two Fluorescence Techniques in Detecting Initial Caries Lesions

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Objective: To evaluate the in-vitro performance of two fluorescence-based techniques used on incipient caries lesions and to compare the measurements to visual findings. **Methods:** 51 freshly extracted permanent teeth (molars, premolars, incisors) were selected. Only teeth with sound surfaces and uncavitated caries lesions were subjected to examination and coding according to ICDAS-II criteria 0-2 ($n = 114$). Teeth were cleaned and subsequently dried for 5 s. The ICDAS score of each investiga-

tion site was obtained and used as reference standard. Both DIAGNOdent™ Pen (DDpen) readings of each site were recorded (A) and photographs using the QLF-D Biluminator™2 (QLF) were taken to obtain the quantitative parameters ΔF (fluorescence loss) and ΔR (presence of porphyrins) by image analyses (B). Values of both systems were scored by one investigator either according to Lussi (1993, DDpen) or to the manufacturer's recommendations (QLF) and statistically compared with ICDAS-II scores 0-2 (weighted Cohen's kappa coefficient, K). **Results:** A -ICDAS vs. DDpen: Agreement for code 0 was 100% and 9% for summarized values of initial caries lesions (code 1+2). B -ICDAS vs. QLF: Agreement for sound surfaces (code 0) was 29% both for ΔR and ΔF . Regarding ΔR for codes 1 and 2 the agreements were 47% and 20% respectively (K = 0.376) and 56% for code 1+2. For ΔF the agreement was 27% (code 1), 7% (code 2; K = 0.285) and 16%(code 1+2). **Conclusions:** The QLF-system was better than the DDpen for the detection of non-cavitated carious lesions.

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Validation of Quantitative Light-Induced Fluorescence-Digital (QLF-D) for the Detection of Approximal Caries in vitro

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This study aimed to assess the ability of the quantitative light-induced fluorescence-digital (QLF-D) in detecting approximal caries, and to compare the performance with those of the International Caries Detection and Assessment System II (ICDAS II) and digital radiography (DR). Extracted permanent teeth (n = 100) were selected and mounted in pairs. The simulation pairs were assessed by one calibrated dentist using each detection method. After all the examinations, the teeth (n = 95) were sectioned and examined histologically as gold standard. The modalities were compared in terms of sensitivity, specificity, areas under receiver operating characteristic curves (AUROC) for enamel (D₁) and dentin (D₃) levels. The intra-examiner reliability was assessed for all modalities. At D₁ threshold, the ICDAS II presented the highest sensitivity (0.80) while the DR showed the highest specificity (0.89); however, the methods with the greatest AUC values at D₁ threshold were DR and QLF-D (0.80 and 0.80 respectively). At D₃ threshold, the methods with the highest sensitivity were ICDAS II and QLF-D (0.64 and 0.64 respectively) while the method with the lowest sensitivity was DR (0.50). However, with regard to the AUC values at D₃ threshold, the QLF-D presented the highest value (0.76). All modalities showed to have excellent intra-examiner reliability. The newly developed QLF-D was not only able to detect proximal caries, but also showed to have comparable performance to the visual inspection and radiography in detecting proximal caries.

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Effects of Carious Lesion on Water Migration into Dentin-Pulp Complex: A Magnetic Resonance Micro-Imaging Study

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Aim: Monitoring of water migration processes from the lesion to pulp chamber provides objective and quantitative data for an accurate assessment of carious lesion effect on dentin-pulp complex. This work aims to quantify influence of caries lesion size and location on dentin-pulp complex based on its water migration properties by using magnetic resonance (MR) micro-imaging. **Experimental Approach:** Progress of water migration was monitored in various carious teeth (scored ICDAS 5 or 6) by sequential MR imaging using the high-resolution 3D T1 weighted spin-echo sequence after the application of 0.05 ml (0.001 mM) of gadolinium-based MR contrast agent (gadopentetate dimeglumine, Magn- evist). Relative intensity profiles were calculated across four representative pulp cross-sections for each lesion group (occlusal or approximal). The water migration process was quantitatively analysed by digital image analysis of the acquired images of carious teeth. **Results:** Severely decayed teeth with occlusal carious lesions yielded the fastest water migration and had the highest signal intensities within the lesions. Relative signal intensity in the pulp cross-sections distant to the site of lesion increased monotonically in teeth with occlusal lesions (SI occlusal = 6±1.5 a.u., n = 5), however non-monotonically signal increase was observed in teeth with approximal lesions (SI approximal = 5±0.5 a.u., n = 10). Differences in relative signal intensity profiles (as a function of time) between the both types of lesions were found statistically significant (p < 0.005). Our experimental results as well as descriptive model indicate that fluid migration into dental-pulp complex highly depends on the lesion location as well as on its size.

The study was supported by Ministry of Higher Education, Science and Technology, Slovenia.

Surface Roughness and Surface Layer Characteristics of Active and Inactive Caries Lesions

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The aim of the present study was to correlate surface roughness and histological characteristics of non-cavitated lesions in vitro. A detailed characterization of in particular the surface layer might help to improve the detection and assessment of lesion activity. 119 human teeth with natural non-cavitated caries lesions (ICDAS 1-2) were selected. After cleaning with a polishing paste average surface roughness (Ra) was measured for the lesion (Ra.lesion) and for a sound part of the same tooth (Ra.sound) using 3D laser profilometer. For each tooth differences in surface roughness were calculated ($\Delta Ra = Ra.lesion - Ra.sound$). Afterwards samples were cut through the middle of the lesion and one half was prepared for confocal laser microscopy (CLSM) and the other for transversal microradiography (TMR). Both methods were used to measure surface layer thickness (SLT). Data were then analysed using Spearman's rank correlation coefficient ($\alpha = 0.05$). Due to preparation losses 60 samples could be evaluated with all three methods. The ΔRa means ($23.6 \pm 17.0 \mu m$) showed a significant but very weak correlation with SLT ($101.7 \pm 130.1 \mu m$, $p = 0.039$, $r = -0.193$) as measured with CLSM, but not with that of TMR (SLT = $118.4 \pm 78.4 \mu m$, $p > 0.05$). It can be concluded that there seems to be a weak correlation between surface roughness and surface layer thickness of non-cavitated caries lesions.

Use of Plaque-Fluid Representative pH-Cycling Conditions to Model Post-Eruptive Enamel Maturation in vitro

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The present study aimed to determine whether exposure to pH-cycling conditions representative of plaque fluid in addition to zinc and fluoride could be used as a basis to model in vitro the decreased susceptibility to acid challenges observed in post-eruptively matured enamel. 48 polished bovine enamel blocks were subjected to a demineralisation pre-treatment representative of plaque fluid (pH 5.11, 2.25 mM Calcium Chloride Dihydrate, 17.65 mM Potassium Dihydrogen phosphate, 32.9 mM lactic acid, 4.25 μM NaF) for 0 or 3 hrs, before exposure to pH-cycling for 0 or 20 days (30 min Demineralisation at 9 am, 12:30 pm and 4 pm, stored in remineralisation solution (pH 6.58, 20 mM HEPES, 1 mM

calcium chloride dihydrate, 12.7 mM potassium dihydrogen orthophosphate, 130 mM KCl, 5.7 μM NaF). At each solution change, blocks were subjected to one of 4 treatment groups (untreated control, 231 $\mu mol/l$ Zn sulphate, 12 mM NaF and Zn and NaF combined). The model was tested through a standard 72 h demineralisation challenge and results analysed using Quantitative light induced fluorescence (QLF-D), Multispectral imaging (MSI) and Transverse Microradiography (TMR). For all analysis methods significant decreases in mineral/fluorescence loss was observed for all pH-cycled blocks in comparison to un-cycled controls (QLF-D: $p = 0.008$, MSI: $p = 0.028$ and TMR: $p = 0.021$, $n = 24$, Independent samples Mann-Whitney U test). A non-significant decrease in fluorescence/mineral loss was observed between blocks exposed to fluoride and zinc treatment groups in comparison with untreated controls ($n = 12$). Observed effects were more pronounced in enamel not subjected to a pre-treatment in comparison with that exposed to 3 h demineralisation prior to pH-cycling. These initial results indicate that exposure to plaque-fluid relevant pH-cycling conditions in the presence of zinc and fluoride may provide a basis for the development of an in vitro model of post-eruptive maturation.

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Artificial Saliva Supplemented with Mucin Stimulate Interleukin-8 Expression in Oral Fibroblasts

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Objective: Artificial saliva is widely used to overcome reduced natural salivary flow. In vitro, natural saliva provokes a substantial inflammatory response in oral fibroblasts. However, if artificial saliva changes the expression of inflammatory mediators remains unknown. Here, we investigated the ability of commercial preparations of artificial saliva to change the expression of inflammatory chemokines in oral fibroblasts by means of RT-PCR and immunoassays. Western blot analysis and pharmacologic inhibitors were used to determine the signaling pathways involved. **Results:** We report that A.S Saliva Orthana[®] containing porcine gastric mucin, but not Aldiamed[®], Glandosane[®] and Saliva natura[®], provoked a more than 10-fold increased interleukin (IL)-8 expression in gingiva and palate fibroblasts. Supporting their potential role as the pro-inflammatory component, mucins isolated from the bovine salivary gland, but also recombinant human mucin 1, substantially increased IL-8 expression in vitro. IL-8 expression in response to A.S Saliva Orthana[®] required nuclear factor-kappa B and mitogen-activated protein kinase signaling. In contrast to natural saliva, A.S Saliva Orthana[®] did not promote CXCL6, CCL7, and CCL20 chemokine expression. **Conclusions:** A.S Saliva Orthana[®] enhanced IL-8 expression in oral fibroblasts. A similar response was observed with isolated mammalian mucins, suggest-

ing that their containing of mucins can differentiate artificial saliva.

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Red Fluorescence of Dental Plaque in Relation to the Caries Status in Children

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The aim of this cross-sectional study was to assess the correlation between the amount of red plaque fluorescence in children and their caries status. The study protocol was approved by the Temple University Health Sciences IRB and informed consent/assent was obtained from parent and child. Fifty children (28 female, 22 male) at Temple dental pediatric clinic with mean age of 10 years (SD 2.3, range 6–14 yr) were instructed not to brush their teeth on the evening and morning before the appointment. Red fluorescent plaque from the buccal surfaces of all teeth up to the first molars was recorded using a QLF-D camera. After professional cleaning, caries was assessed using ICDAS II, complemented by recent bitewings. The area of red fluorescence related to the total tooth area of the teeth was calculated using dedicated software (QA2v1.25, Inspektor Research Systems) and expressed in a Simple Plaque Score (SPSTM; range: 0 (no fluorescing plaque) to 5 (teeth nearly completely covered with fluorescing plaque)). dmft/DMFT-scores were calculated using ICDAS scores 2 and 3 as cut-off and normalized for the number of teeth present. Inter- and intraobserver agreement for the ICDAS (Cronbach's alpha) was 0.997 and 0.921, respectively. The mode of the SPS was 0 (median 1). The mean dmft/DMFT was 4.6 (SD 3.6; ICDAS-cut-off 3) and 14.3 (SD 5.3; ICDAS-cut-off 2). The correlation (Spearman's rho) between red fluorescent plaque (SPS) and caries was weak with ICDAS-cut-off 3 ($r_s = 0.33$, $p = 0.02$), while the correlation with ICDAS-cut-off 2 was moderate ($r_s = 0.49$, $p < 0.001$). A significant moderate correlation was found between red fluorescent dental plaque and the caries at an ICDAS-cut-off score of 2. A longitudinal study to correlate caries activity with plaque fluorescence should be undertaken.

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Correlation between Nyvad and ICDAS-LAA Caries Activity Assessment Systems and Lesion Surface Roughness

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The present study had two aims; first to evaluate if differences in caries lesion surface roughness can be detected between lesions classified as active and inactive according to visual-tactile methods. Second, to determine sensitivity and specificity of the detection and assessment systems (Nyvad, ICDAS-LAA), if lesion surface roughness is assumed as the main indicator of lesion activity. 119 human teeth with natural non-cavitated caries lesions (ICDAS 1 or 2) were selected and cleaned with a polishing paste, which had no significant effect on surface roughness. Two examiners were calibrated for the two caries activity assessment systems: Nyvad and ICDAS-LAA ($\kappa > 0.8$ for inter- and intraexaminer reliability). All lesions were then assessed with both systems and afterwards analysed using a 3D laser profilometer. Average surface roughness (Ra) was measured for the lesion (Ra.lesion) and for a sound part of the same tooth (Ra.sound). For each tooth differences in surface roughness were calculated ($\Delta Ra = Ra.lesion - Ra.sound$). Additionally, the sensitivity and specificity of both visual-tactile methods were calculated after determining the best ΔRa thresholds for lesion activity using the ROC curve. No significant differences of ΔRa means were observed between lesions classified as active ($n = 55$; $31.9 \pm 18.3 \mu m$ -Nyvad; $n = 85$; $29.6 \pm 17.6 \mu m$ -LAA) and those classified as inactive ($n = 64$; $34.6 \pm 21.1 \mu m$ -Nyvad; $n = 34$; $39.9 \pm 19.5 \mu m$ -LAA) ($p > 0.05$; Mann-Whitney test). The most suitable ΔRa threshold for Nyvad and LAA was $40 \mu m$, resulting in a sensitivity/specificity of 45%/48% and 65%/76%, respectively. It can be concluded that natural non-cavitated caries lesions classified as active or inactive by two visual-tactile methods do not show significant differences in surface roughness under the conditions of this in vitro study.

Influence of Sample Spectrum Used for Training on Student's Performance in Caries Detection Using ICDAS

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We investigated if different sample spectrum used for training could interfere with undergraduate and graduate students' performance and their types of errors when detecting caries lesions. Two samples of primary extracted teeth (sample 1 (S1): n = 13 teeth; sample 2 (S2): n = 12 teeth) were prepared comprising all ICDAS scores and similar levels of difficulty. They were different regarding the number of non-cavitated surfaces. Six undergraduate and six graduate students examined each sample. Benchmark examiners' scores and histology were used as references. Bland Altman and consistency analyses were performed to check type of errors and systematic differences between students and benchmark examiners. Types of errors in different samples were compared using chi-square test. ROC analyses were used to assess student's performance. A comparison of ROC curves were performed between samples. Compared to benchmark examiners, number of false results was similar for S1 (undergraduate: 37%; graduate: 18%) and S2 (undergraduate: 43%; graduate: 19%), ($p > 0.05$). However, among the errors, a higher frequency of classifying lesions score 1 as score 2 were observed for the undergraduate students in S1 (34%) than in S2 (3%), ($p = 0.005$). Considering the severity by benchmark scores, similar areas under ROC curves were also found (graduate – S1: 1.0; S2: 0.99; $p = 0.15$ | undergraduate – S1: 0.95; S2: 0.92; $p = 0.36$). The same trend was observed when histology was the reference. Similar patterns of differences between benchmark and students' scores were found in both samples, with a slight increase in the amplitude of the differences in S2. No systematic errors were identified despite the sample. Concluding, the sample spectrum does not influence the student's performance in detecting caries lesions or the occurrence of systematic errors, but it may expose the examiners to different types of errors during a training activity.

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Identifying Naturally Occurring Infected, Affected and Healthy Dentine Using Optical Coherence Tomography (OCT)

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Aim: To identify and differentiate layers of carious and healthy dentine using backscattered intensity (BSI) of Swept Source Optical Coherence Tomography (SS-OCT). **Methodology:** 26 extracted permanent human carious molars of ICDAS code 4 and 5 were selected. All teeth had soft, yellow or light/dark brown occluso-proximal caries. The lesions were transversely sectioned and area with maximum thickness of diseased and healthy dentine was identified (ROI) for investigation. A 3.05 mm copper grid with 85 mm inter-bar distance was placed next to the ROIs to aid localization. 3-D scans measuring 3×3.5×2.0 mm (x-y-z) were acquired of the ROIs using a Swept-Source-OCT (OCS1300S, Thorlabs Inc.) The OCT volume data was aligned to the surface of the dentine and an Enface view was generated. A 2000X magnification SEM image was captured from each of the inter-bar spaces using a Field-Emission-Scanning-Electron-Microscopy (Quanta 200F, UK). From these SEM images, Infected (ID), Affected (AD) and Healthy (HD) Dentine were identified using Pugacha et al 2009's histological classification. The fractional difference of the backscattered intensity (BSI) between surface and 15 mm sub-surface for ID, AD and HD were obtained from the mean BSI depth profiles. **Results:** The Leven test showed good homogeneity of variance. One-way ANOVA was undertaken using metric formula between 3 groups (ID, AD and HD) and it yielded significant differences amongst the groups, $p = 0.001$. Post hoc comparison using Tukeys HSD test indicated the mean fractional difference of BSI between surface and 15 mm sub-surface of ID (0.62 ± 0.31) was significantly different than AD (0.86 ± 0.36) and from that of HD (0.11 ± 0.22). **Conclusion:** OCT was able to differentiate between infected, affected and healthy dentin by quantifying the backscattered intensity gradient.

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