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Abstracts

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Session 1

Microbiology

1

A Single Nucleotide Change in *Streptococcus mutans* Enhances Fluoride Resistance and Alters Virulence Factors

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Oral bacteria can become fluoride-resistant after exposure to a high dose of fluoride. Mechanisms of fluoride resistance have raised interest due to the wide application of fluoride. A recent study indicated that a mutation (–44 A→C) in the promoter region (*mutp*) of the chorismate mutase may be related to bacterial fluoride resistance. The aim of this study is to construct a clean mutant (UF35) of the wild-type *S. mutans* strain UA159 with only a single base pair mutation in *mutp* (–44 A→C) and to investigate phenotypes related to fluoride resistance and the virulence factors lactic acid production and acid tolerance of the mutant. The mutant strain UF35 was characterized for its growth with/without fluoride; lactic acid production at pH 7.0 and pH 5.5 with/without fluoride; its acid tolerance response (ATR) and the expression of three genes downstream of *mutp*. The strain UA159 was used as a control in all experiments. UF35 showed enhanced fluoride resistance, with an MIC of 400 ppm, as compared to UA159, with an MIC of 200 ppm. Lactic acid production without fluoride was similar for both strains. However, UF35 produced significantly more lactate when fluoride was present, irrespective of the pH. UF35 exhibited a lower survival rate than UA159 after exposure to the killing pH in the ATR. Expression of three genes downstream of *mutp* was found to be significantly up-regulated in UF35 compared to UA159 irrespective of the growth phase. Interestingly, two of these genes encode fluoride antiporters. In conclusion, a

single nucleotide mutation in *mutp* leads to enhanced fluoride resistance in *S. mutans*, through the up-regulation of genes coding for fluoride antiporters. Simultaneously, this mutation leads to a decreased acid tolerance, which can indicate a potentially impaired competitiveness in an acidic environment as compared to the wild type.

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Antimicrobial Activity of Essential Oil of *Cymbopogon citratus* (Lemon Grass) on *Streptococcus mutans* Biofilm

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The aim was to investigate the antimicrobial effect of the essential oil of *Cymbopogon citratus* (lemon grass – LGO) on *Streptococcus mutans* UA159 (ATCC 700610) biofilm (SM) developed on hydroxyapatite discs surface. Initially, we investigated the susceptibility of *S. mutans* to LGO by inhibition zone test and minimum inhibitory concentration (MIC) in planktonic suspension. SM was adjusted at 1×10^6 cell/ml and cultured on the discs for 5 days and immersed daily in the following groups: G1 – immersion for 5 minutes in LGO to 0.08 mg/ml (test group) and G2 – Brain Heart Infusion (BHI) + 1% sucrose (negative control). The biofilms were disrupted and subjected to colonies forming units

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counting (CFU) on blood agar plates with the data transformed into log₁₀. Triplicate of two different experiments were performed (n = 6). One-way ANOVA test with a level of significance set at 5%. The susceptibility test was positive indicating inhibition of microorganisms and the MIC value was 0.04 mg/ml. To biofilm test, the results showed a decreased on bacterial growth in G1 compared to G2 with a statistical significant difference (reduction of 0.56 log; P < 0.034). The daily immersion of lemon grass oil was effective in controlling the bacterial growth biofilms of *S. mutans*.

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Extracellular DNA Contributes to Dental Biofilm Formation: An *ex vivo* Study

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The extracellular matrix of dental biofilms plays an important role during caries development. It increases the mechanical stability of the biofilm, it prevents desiccation, it serves as a reservoir for nutrients and it contributes to the long-term preservation of acidic microenvironments. Research on the biofilm matrix in the field of dentistry has focused mainly on the synthesis, structure and function of extracellular polysaccharides. In recent years, studies conducted on biofilms from other habitats have shown that the presence of extracellular DNA contributes to biofilm formation and stability, and that the enzymatic removal of extracellular DNA might be used as a therapeutic approach to biofilm diseases.

Here, we investigate the effect of treatment with DNase I (100 Kunitz) on *in vivo* grown young dental biofilms. A total of 300 biofilm samples were grown on glass slabs placed on acrylic splints for 2.5, 5, 7.5, 16.5 and 24 h and subsequently treated with DNase I or heat-inactivated DNase I for 1 h. Biovolumes were quantified by confocal microscopy and digital analysis of 16200 images.

All samples taken together, DNase-treatment led to a strong reduction of the biofilm biovolume compared to negative control treatment, as determined by two-sample t-tests (p < 0.05). The effect decreased with biofilm age, with 94.2%, 85.0%, 52.0%, 46.6% and 15.8% reduction for 2.5, 5, 7.5, 16.5 and 24 h biofilms, respectively. The treatment effect was independent from the intra-oral location of the biofilm.

The present results make a strong case for an increased research focus on extracellular DNA in dental biofilms and might pave the way for new, non-bactericidal therapeutic approaches to caries control based on DNase treatment.

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Monitoring of Extracellular pH in Young Dental Biofilms Grown *in situ* in the Presence and Absence of Sucrose

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pH in dental biofilms is of central importance for the development of caries. We used the ratiometric pH-sensitive dye C-SNARF-4 in combination with digital image analysis to monitor extracellular pH in dental biofilms grown *in situ* with and without sucrose supply. 48-h dental biofilms from 10 individuals were collected on glass slabs mounted on intra-oral appliances. During growth, appliances were immersed extra-orally in either physiological saline or 4% sucrose for 2 min eight times per day. For each individual, eight biofilm specimens were analyzed for each experiment, yielding a total of 160 biofilms. Fluorescence emissions of C-SNARF-4 in deep layers of each biofilm were recorded *ex-vivo* with confocal microscopy for 15 min (3 sites) or for 1 h (6 sites) after exposure to a 100 µl salivary solution with 0.4% glucose in custom-made wells. The ratiometric pH data were analyzed using a mixed-effects linear regression procedure. Extracellular pH dropped rapidly in most examined sites after addition of glucose. Distinct pH microenvironments were observed within single biofilms. Variance components analyses showed similar variation between sites within the same biofilm (var = 0.02–0.04 (se = 0.014–0.025)) and sites from different individuals (var = 0.017–0.053 (se = 0.003–0.006)). The analysis indicated similar values of all variance components between the two treatment groups. pH drop patterns did not differ significantly between biofilms exposed to sucrose-free and sucrose-rich environments (sucrose-free group: pH_{3–4 min} = 6.39 (95% CI: 6.28–6.50), pH_{59–60 min} = 5.63 (95% CI: 5.50–5.77); sucrose group: pH_{3–4 min} = 6.38 (95% CI: 6.25–6.52); pH_{59–60 min} = 5.59 (95% CI: 5.45–5.73)). The present study is the first to apply the combination of pH ratiometry and digital image analysis to systematically record extracellular pH in intact dental biofilms from several individuals for up to 1 h. We observed heterogeneous pH landscapes and the presence of highly acidogenic microenvironments within the biofilms. The data suggest that pH drops in young dental biofilm are independent of the sucrose supply during growth.

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The Cariogenicity of Microcosm Biofilms Generated from Donors with Different Caries Experience and Its Detection Using QLF-D

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The aims of this study were to investigate whether individual oral properties have affect cariogenicity-related variables of dental microcosm biofilms and red fluorescence intensity changes on the Quantitative Light-induced Fluorescence-Digital (QLF-D) images in groups with various caries experience levels. Subjects were performed the oral examination and recorded the DMFT index. They were divided into three groups (i.e. caries-free, caries experienced, and caries active). Stimulated saliva was collected from subjects and inoculated into each bovine enamel specimens. The dental microcosm biofilms were matured in a cariogenic environment for 10 days. The numbers of total and aciduric bacteria, supernatant pH, and changes in the enamel surface microhardness were evaluated as cariogenicity variables. Fluorescence images were taken by QLF-D each day at 24 hours during the biofilm maturation. Next, the red fluorescence intensity of biofilm was analyzed to red/green ratio (R/G value) using image analysis software. Each experiment was repeated 3 times under same conditions. The collected data were assessed by one-way ANOVA to compare the differences in cariogenicity between the groups, followed by Tukey's test to identify statistically significant differences between groups. The aciduric bacteria exhibited more CFUs in the caries-active group than the other group ($p < 0.05$). However, the total bacteria CFUs, supernatant pH and red fluorescence intensity did not differ statistically between the groups ($p > 0.05$). The R/G values increased continuously with plaque maturation from 1 day to 10 days after inoculation, but there were no significant differences between the groups according to the donors' caries experience levels ($p > 0.05$). In conclusions, there were no significant differences in the cariogenicity-related variables and red fluorescence intensity on the QLF-D images of the microcosm biofilm between the groups in the same cariogenic environment.

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Type IV Collagen Promotes Internalization of *Streptococcus mutans* in Vein Endothelial Cells

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Streptococcus mutans, a major pathogen of dental caries, is also thought to cause infective endocarditis (IE). Recently, two 120-kDa cell surface collagen-binding proteins (CBPs) of *S. mutans* were characterized (Cnm and Cbm). Furthermore, CBP-positive strains have been shown to possess high levels of invasion of human umbilical vein endothelial cells (HUVEC). On the other hand, extracellular matrix (ECM) proteins, such as fibrinogen, fibronectin, collagen, and vitronectin, have been found in blood. In the present study, we analyzed the properties of CBP-positive *S. mutans* invasion of HUVEC by focusing on the presence of ECM proteins in blood.

We used an *S. mutans* blood isolate, TW295 (Cnm+), and its Cnm-defective mutant strain TW295CND, as well the oral isolate MT8148 (CBP-). Intergroup differences were analyzed using Student's t-test. All assays were analyzed in three independent experiments. ECM protein binding assay results revealed TW295 binding to type IV collagen and fibrinogen, but not to fibronectin or vitronectin. In a comparison of type IV collagen and fibrinogen, the level of binding of TW295 to type IV collagen was significantly greater ($P < 0.001$). On the other hand, CBP-negative strains showed no adhesion to any of the ECM proteins. In addition, TW295 showed a high level of invasion of HUVEC in the presence of type IV collagen that occurred in a dose-dependent manner. Furthermore, none of the CBP-negative strains showed invasion properties in the presence of type IV collagen. Confocal scanning microscopy observations of HUVEC infected with *S. mutans* in the presence of blood revealed an interaction of CBP-positive strains with type IV collagen. These results suggest that interaction between CBP-positive *S. mutans* strains and type IV collagen is involved in bacterial internalization in HUVEC, which may contribute to virulence for IE.

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Collagen-Binding Protein of *Streptococcus mutans* Promotes Proliferation of Pulp Fibroblast Cells

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Streptococcus mutans, a major pathogen of dental caries, possesses Cnm, an approximately 120-kDa collagen-binding protein that is considered to be an important cell surface antigen related to adherence of the bacterium to collagenous tissue. Proliferative pulpitis, also known by the term pulp polyp, is an inflammatory type of hyperplasia observed mainly in primary teeth with irreversible pulpitis, with prominent fibroblast cell proliferation seen in some

cases. In the present study, the adhesion, invasion, and cell proliferation properties of *S. mutans* to dental pulp fibroblasts (HDPFs) were investigated, with focus on Cnm.

We used the *S. mutans* blood isolate TW295 (Cnm+), its Cnm-defective mutant strain TW295CND and the oral isolate MT8148 (CBP-). HDPFs were obtained from a primary canine of an 8-year-old boy extracted during an expedient pulpectomy for orthodontic treatment and from the third molar of a 50-year-old man extracted because of pericoronitis. Prior to collection of these specimens, the subjects or their guardians were informed of the study contents and written informed consent was obtained from both. Data are expressed as means $\pm$ standard deviations of triplicate experiments. Intergroup differences were analyzed using Student's *t*-test. The rates of adhesion to and invasion of HDPFs isolated from primary and permanent teeth for TW295 were significantly higher as compared with TW295CND and MT8148 ($P < 0.001$). Furthermore, infection with strain TW295 stimulated proliferation of HDPFs isolated from the primary tooth, which was significantly greater compared to stimulation with TW295CND or MT8148 ($P < 0.01$ and $P < 0.001$). In contrast, TW295 infection did not cause prominent proliferation of HDPFs isolated from the permanent tooth, though the level of proliferation was significantly greater as that observed with TW295CND and MT8148 infections ($P < 0.05$). These results suggest that Cnm-positive strains have a capability to adhere to and invade HDPFs, which may trigger proliferative pulpitis, mainly in primary teeth.

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Contribution of Cnm-Positive *Streptococcus mutans* Strains to IgA Nephropathy

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IgA nephropathy (IgAN) is a chronic kidney disease that leads to terminal renal insufficiency, with approximately 30–40% of affected patients showing a poor prognosis, though its precise pathological mechanisms remain unclear. *Streptococcus mutans* is a known dental caries pathogen and it has been reported that 10–20% of healthy individuals harbor strains that possess Cnm, a cell surface collagen-binding protein encoded by the *cnm* gene. Furthermore, we previously found that the prevalence of Cnm-positive *S. mutans* in saliva specimens obtained from patients with IgAN was significantly higher as compared to a control group. The aim of the present study was to investigate whether nephritis is induced by Cnm-positive *S. mutans* strains inoculated into the oral cavities of dental caries model rats. The Cnm-positive *S. mutans* strain JD74, isolated from the oral cavity of an IgAN patient, was utilized to generate streptomycin-resistant strain JD74R. In addition,

we used another streptomycin-resistant strain, MT8148R, generated from the standard oral isolate MT8148. These strains were separately inoculated into the oral cavities of 15-day-old specific-pathogen free Sprague-Dawley rats fed a 56% sucrose-containing diet. At 24 weeks after inoculation, the animals were euthanized and their kidney tissues extirpated, then histopathological and immunochemical analyses were performed. All groups of rats showed dental caries, extending to the dentin. As for groups inoculated with the Cnm-positive strain, histopathological kidney evaluations revealed typical findings of nephritis, such as mesangial cell proliferation, while immunochemical staining demonstrated IgA expression in para-mesangial areas. These results suggest that Cnm-positive *S. mutans* bacterial organisms harbored in the oral cavity induce IgA nephropathy as well as severe caries in rats.

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Contribution of Cnm and PA of *Streptococcus mutans* to Aggravation of Non-Alcoholic Steatohepatitis in Mice

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Streptococcus mutans, a major pathogen of dental caries, can cause infective endocarditis following invasion of the bloodstream. Recently, specific *S. mutans* strains were shown to aggravate non-alcoholic steatohepatitis (NASH) in mice fed a high-fat diet. In the present study, the mechanism of that aggravation was investigated using NASH model mice by focusing on an *S. mutans* cell surface 120-kDa collagen-binding protein (Cnm) and 190-kDa protein antigen (PA). Intergroup differences were estimated using Bonferroni's method after analysis of variance (ANOVA). Mice intravenously administered with an *S. mutans* strain defective of Cnm (TW871CND) or a PA-defective strain (TW871PD) did not show clinical or histopathological signs of NASH aggravation, which was in contrast to those administered the parent strain TW871. Immunochemical analyses of liver specimens extirpated from mice administered TW871CND or TW871PD demonstrated significantly lower expressions of IFN- γ and metallothionein, whereas those expressions were found at typical levels in liver specimens from the TW871 group. Furthermore, assays of affinity to unsaturated fatty acids, such as oleic acid and linoleic acid, revealed that TW871PD infection resulted in a significantly higher binding level of binding as compared to mice infected with TW871 or TW871CND ($P < 0.05$, respectively $n = 5$). In addition, assays of bacterial adhesion to cultured hepatic cells revealed significantly lower levels with TW871PD infection as compared to with TW871 or TW871CND infection ($P < 0.05$, respectively $n = 3$), whereas bacterial adhesion with TW871CND infection was significantly lower as compared to infection with TW871 or TW871PD when unsaturated fatty acids were added ($P < 0.05$). These results suggest that *S. mutans* Cnm is related to bacterial adhesion to hepatic cells in the absence of fatty acid accumulation, while PA may enhance bacterial affinity to unsaturated fatty acids leading to elevated ad-

hesion to hepatic cells, both of which could be related to *S. mutans* aggravation of NASH.

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Serum Component Promotes *Streptococcus mutans* Agglutination Associated with Pathogenesis of Infective Endocarditis

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Streptococcus mutans, a pathogen responsible for dental caries, is occasionally isolated from the blood of patients with infective endocarditis (IE). Previously, we frequently detected bacterial DNA of *S. mutans* with 120-kDa collagen-binding proteins (CBPs: Cnm and Cbm) in heart valves extirpated from IE patients. More recently, we reported that *S. mutans* strains possessing CBPs frequently lack expression of the cell surface 190-kDa protein antigen (PA), known to be associated with sucrose-independent initial adhesion to tooth surfaces. Bacterial agglutination is considered to be important for the pathogenesis of IE, though that property of *S. mutans* in the presence of blood remains unclear. In the present study, we evaluated *S. mutans* agglutination in the presence of serum. In addition, an *ex-vivo* IE evaluation model using bovine heart valves and bovine serum was constructed.

Thirty *S. mutans* clinical strains were divided into CBP+/PA– (n = 10), CBP+/PA+ (n = 10), and CBP–/PA+ groups (n = 10), then their agglutination properties in the presence of serum obtained from a human volunteer were analyzed. Intergroup differences were analyzed using Student's t-test. The average rate of agglutination induced by the CBP+/PA– strains was significantly higher than that by the CBP+/PA+ or CBP–/PA+ strains ($P < 0.001$). Furthermore, agglutination was observed not only in the presence of human serum but also with bovine serum. Next, an *ex-vivo* IE evaluation model was constructed using bovine heart valves infected with various *S. mutans* strains in the presence of bovine serum. Gram staining showed that a prominent bacterial mass was formed by TW295 (CBP+/PA–) in the model, whereas that was not observed with the NN2094 (CBP+/PA+) and MT8148 (CBP–/PA+) strains. No bacterial masses were formed by TW295 infection without the presence of serum. These results were positively correlated with those previously obtained with a rat IE model and suggest that aggregation of CBP-positive *S. mutans* induced by serum may be involved in virulence for IE.

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Bovine Milk Osteopontin – Targeting Bacterial Adhesion for Biofilm Control

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Self-performed mechanical tooth cleaning does usually not result in complete biofilm removal, due to the complex oral anatomy and the strong adhesion of the biofilm to the tooth. Therefore, different supportive measures are employed, most of which aim at the chemical eradication of bacteria in dental biofilms. As their bactericidal action impacts the entire oral microflora, agents that inhibit biofilm formation without killing bacteria, such as the bovine milk protein osteopontin, have gained increasing attention. Here, we investigate the adhesion of 8 bacterial species associated with dental caries to salivary-coated flow-cells in the presence or absence of osteopontin or the control protein caseinoglycomacropeptide (0.32 mM/L). After 1 h of flow (9.45 mm/min) at 35 °C, adhering bacteria were quantified by digital image analysis in a total of 692 bright-field images. Experiments were performed in technical and biological duplicates. *Bifidobacterium dentium*, *Rothia dentocariosa* and *Streptococcus mutans* did not adhere to the flow cell, irrespective of the presence of osteopontin. Osteopontin reduced the adhesion of *Actinomyces naeslundii*, *Actinomyces viscosus*, *Lactobacillus paracasei* subsp. *paracasei*, *Streptococcus mitis* and *Streptococcus oralis* with 74.0%, 62.4%, 90.0%, 89.6% and 81.5%, respectively, compared to protein-free saliva. All reductions were statistically significant ($p < 0.05$) and significantly stronger than the reductions observed for caseinoglycomacropeptide ($p < 0.05$), as determined by two-sample t-tests. The broad range anti-adhesive effect of osteopontin on dental bacterial strains might explain the reduced biofilm formation observed and be exploited *in vivo* for increased caries control.

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Actinomyces Spp. Gene Expression in Root Caries Lesions

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The aim was to evaluate the functions of *Actinomyces* spp. in biofilms of root surfaces with and without caries. Natural biofilms from volunteers with exposed sound root surface (SRS; n = 10) or active root caries (RC; n = 30) were collected. Bacterial mRNA was isolated and the RNA-seq was performed (Illumina). Sequence

reads were mapped to *Actinomyces gerencseriae* DSM6844, *Actinomyces johnsonii* F0542, *Actinomyces naeslundii* NCTC10301, *Actinomyces odontolyticus* ATCC17982, *Actinomyces* sp. OT170, *Actinomyces* sp. OT178, *Actinomyces* sp. OT448, *Actinomyces oris* C505. Count data were obtained and normalized using DESeq2 to analyze gene expression and differential expression applying Benjamini-Hochberg correction and $p < 0.001$. The total number of reads per sample per organism ranged from SRS = 6,250–883,308 and RC = 2,461–2,015,578. Proportion of genes per genome expressed ranged from 49.3–75.9% (SRS) and 28.0–89.8% (RC). All *Actinomyces* spp. had a similar number of reads and transcripts (U-Mann Whitney; $p > 0.001$), except for *Actinomyces* sp. OT178 that had higher preference for the SRS samples ($p = 0.001$). Genes that code for stress proteins (clp, dnaK and groEL), enzymes of glycolysis pathways (including, enolase, formate-acetyltransferase, phosphoenolpyruvate carboxykinase and glyceraldehyde-3-phosphate dehydrogenase), adhesion (Type-2 fimbrial and collagen-binding protein) and cell growth (EF-Tu) were highly, but not differentially ($p > 0.001$), expressed in both groups. Genes with the most significant up-regulation in RC were those coding for hypothetical proteins and uracil DNA glycosylase (FDR = $2.61E-17$), which is an important protein for transcription and mutagenesis prevention. Two systems responsible for alkalinisation of the biofilm had up-regulated genes in RC: arginine biosynthesis, and urea catabolism. The gene with the most significant up-regulation in SRS was a peptide ABC transporter substrate-binding protein (FDR = $2.37E-05$). In conclusion the level of *Actinomyces* gene expression was very high and similar in healthy biofilms and root caries.

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Enamel Colonization and Caries in a Dual-Species Biofilm Model of *Streptococcus mutans* and *Streptococcus sanguinis*

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Caries has been defined as a dysbiosis attributable to imbalances in the dental biofilm ecology. Little is known about the dynamics of enamel colonization on caries. The aim of this study was to test whether the sequence of enamel colonization by a commensal and a cariogenic species would modify cariogenicity. Using a validated biofilm-caries model as the starting point, a dual-species

biofilm model was designed. Saliva-coated enamel slabs served as colonization substrate for *Streptococcus mutans* UA159 (Sm) and *Streptococcus sanguinis* SK36 (Ss). Strains were inoculated onto the slabs in sequences that gave origin to 5 groups: 1) Sm followed by Ss (Sm-Ss), 2) Ss followed by Sm (Ss-Sm), 3) Sm and Ss inoculated at the same time (Sm=Ss) and the controls 4) Sm followed by Sm (Sm-Sm) and 5) Ss followed by Ss (Ss-Ss). Once mature, biofilms were exposed to 10% sucrose, 3x per day for 5 days. Medium was changed twice daily and acidogenicity determined after each change. At day 5, slabs were recovered to assess demineralization by the percentage of surface hardness loss and biofilms were analyzed for biomass, protein and polysaccharide content. Each condition was assayed in triplicate and the entire experiment repeated twice ($n = 6$). Data were analyzed by one-way ANOVA test followed by Tukey post-hoc contrast for each pair of variables. Differences were considered significant with p -value < 0.05 . When compared with Sm-Sm, primary colonization by Ss (Ss-Sm) reduced demineralization more than 20% ($p < 0.05$), suggesting competition. Conversely, Sm-Ss decreased demineralization only around 5% ($p > 0.05$). Groups of Ss-Sm and Sm=Ss showed approximately 60% reduction in biomass ($p < 0.05$). Protein and polysaccharide formation followed a similar trend ($p < 0.05$). The lowest acidogenicity was observed with Ss formed before Sm biofilms ($p < 0.05$). In conclusion the initial enamel colonization with the commensal Ss seems to induce competition within the dental biofilm and reduce cariogenicity of Sm. Competition appears to be modified by colonization dynamics.

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Factors Influencing the Inhibition of *Streptococcus mutans* by *Streptococcus oligofermentans* in Dual Species Biofilms

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Streptococcus oligofermentans is considered as a potent probiotic candidate. Previous studies showed that it inhibited the growth of caries-associated *Streptococcus mutans* in dual-species biofilms. The current study aims to investigate the influence of various factors, including sugar types and the sequence of colonization in dual-species biofilms, on this inhibition. The biofilms were grown in buffered biofilm medium (pH 7.0) containing either 0.2% sucrose or 0.4% glucose in a 96-well active attachment model for

48 h. To form dual-species biofilms, *S. oligofermentans* LMG22279 or *S. mutans* UA159 was first inoculated to form 24-h single-species biofilms, and then the biofilms were subsequently immersed in a culture of the second species for another 24 h. All 48-h biofilms were analysed for viable cell counts and lactate production from glucose. Each group generally contained 4 biofilms replicates. The experiment was repeated 3 times. In the glucose groups, *S. oligofermentans* significantly inhibited the growth of *S. mutans* ($98\% \pm 0.3\%$ inhibition) and lactate production ($82\% \pm 6\%$ inhibition) in dual species biofilms irrespective of the sequence of colonization ($p < 0.05$, t-test). However, in the sucrose groups, the inhibition by *S. oligofermentans* could only be observed in the group where *S. mutans* was inoculated into 24-h *S. oligofermentans* biofilms. In the sucrose-group where *S. oligofermentans* was inoculated into 24-h *S. mutans* biofilms, the viable counts of *S. mutans* and lactate production in dual-species biofilms was not inhibited. They were similar to the single-species *S. mutans* biofilms. In conclusion, the inhibition of *S. oligofermentans* on cariogenic *S. mutans* is influenced by the sugar supplied and the colonization sequence in the biofilms.

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Effect of 1.5% Arginine on Cariogenic Biofilm

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The arginine deiminase system (ADS) is found in several oral *Streptococcus* species, as *Streptococcus sanguinis* and *S. gordonii*, and is responsible for the metabolism of the amino acid arginine resulting in ammonia, citrulline and ATP. This study evaluated the effect of arginine on the formation, composition and ADS activity of a cariogenic biofilm of four oral streptococci. Mixed biofilms of *S. mutans*, *S. sobrinus*, *S. sanguinis* and *S. gordonii* were formed on 24-well polystyrene plates in the presence of sucrose and supplied with 1.5% arginine, with exchange of media supplemented with 0.5% sucrose every 8 h and 16 h. After 1, 2, 4, 7 and 10 days, colony forming units were determined by differential counting and the citrulline production as well as the total amount of biofilm formed were quantified by colorimetric tests. The pH of the spent media was measured twice a day. Three independent experiments were performed in triplicate and data were analyzed by t-test ($\alpha = 0.05$). The citrulline production was significantly higher in the biofilms with arginine ($p < 0.01$) and the pH values for this group were always above 6.0, when exposed to a low concentration of sucrose. Arginine treated biofilms showed longer survival and higher concentrations of *S. sanguinis* and *S. gordonii* ($p < 0.05$) and lower quantity of biofilm after 1 and 2 days ($p < 0.05$). We concluded that arginine promotes the survival of ADS active *Streptococcus* species, increases the environmental pH by the production of ammonia from its metabolism and following, it might reduce the risk of caries.

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Antimicrobial and Anticariogenic Effect of 4% TiF₄ Varnish in a Dental Microcosm Biofilm Model

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The aim of this study was to evaluate the antimicrobial and anti-cariogenic effect of 4% TiF₄ varnish compared to 5.42% NaF varnish, 2% chlorhexidine gel (positive control), placebo varnish and control (negative control) using a dental microcosm biofilm model. The following parameters were analyzed: 1) the percentage of live and dead bacteria (Kit Live & Dead[®] cells viability assay); 2) The amount of colony forming units (CFUs) for total microorganisms (BHI medium), total streptococci (MSA medium) and mutans streptococci (MSA with 1% potassium tellurite); and 3) the degree of enamel demineralization (TMR). All experiments were performed in biological triplicate. A microcosm biofilm was produced on bovine enamel previously treated with varnish (6 h) using inoculum from human saliva, under 0.2% sucrose exposure, 5% CO₂, 37° C, for 14 days. The live and dead bacteria were observed by fluorescence using confocal microscopy and quantified using BioImage L 2.0 program. The colonies were photographed and the number of colonies was counted by three independent observers to calculate the total CFU. The enamel mineral loss and lesion depth were calculated. The data were analyzed using ANOVA/Tukey's tests ($p < 0.05$). Only chlorhexidine was able to reduce the number of live bacteria compared to negative control. For total streptococci, all treatments (TiF₄, NaF and chlorhexidine) showed a significant reduction of CFUs compared to the negative control, but only TiF₄ varnish significantly differed from the placebo. No significant differences were found among the treatments with respect to total microorganisms and mutans streptococci. TiF₄ and NaF varnishes were able to reduce the mineral loss and the lesion depth compared to negative control, while chlorhexidine was able to reduce the lesion depth only. TiF₄ varnish has no significant antimicrobial effect. Nevertheless, TiF₄ varnish was effective in reducing enamel demineralization.

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Antimicrobial and pH Buffering Activity by the Probiotic *Streptococcus dentisani* Strain CECT 7746

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The aim was to characterize the potential beneficial function of *Streptococcus dentisani* strain CECT 7746, isolated from supragingival dental plaque of caries-free individuals. Inhibition experiments, performed in duplicates, against oral pathogens were carried out by measuring their growth under solid and liquid media in the presence and absence of supernatants from *S. dentisani* cultures, using scanning electron microscopy to directly observe their antimicrobial effect. Peptidase and peroxidase treatments of the supernatants, affinity columns, HPLC and Mass Spectrometry were used to identify the compounds responsible for antibacterial activity. The growth and pH of *S. dentisani* in BHI medium enriched with L-arginine was measured for 24 h, and the expression of arginolytic genes measured by qPCR, performed in triplicates. We found that *S. dentisani* inhibited the growth of *S. mutans*, *S. sobrinus*, *Prevotella intermedia* and *Fusobacterium nucleatum*, and the proteomic analysis of its supernatant indicated the production of at least four antimicrobial peptides encoded in a 16-Kb genomic region. Growth experiments revealed that *S. dentisani* buffers acidic pH in the presence of arginine, over-expressing genes for the production of ammonia when the pH becomes acidic. Thus, *S. dentisani* appears to have a double probiotic action, as it inhibits the growth of major oral pathogens through the production of bacteriocins, and also buffers acidic pH (the primary cause of dental caries) through an arginolytic pathway.

We hypothesize that oral inhabitants associated to health conditions will be more effective than traditional, gut-associated probiotic species in key aspects such as colonization of the oral site where disease takes place or the possession of oral health promoting functions, as well as more practical issues like safety and toxicity, and establishing proper doses for administration. We propose the use of *S. dentisani* CECT 7746 as a promising probiotic against tooth decay.

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Oral Microbial Colonization of Babies during First Year of Life

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The aim of this study was to assess the colonization of *Lactobacillus* spp., *Streptococcus mutans*, and *Bifidobacterium* spp. in the oral microbiota of babies during the first year of life. Twelve

babies were evaluated at 0, 3, 6 and 12 months of age in the Family Healthy Center Parque dos Maias, Porto Alegre, Rio Grande do Sul, Brazil. Questionnaires recorded data on sociodemographic characteristics, type of birth, type of milk feeding (breast or formula), type of feeding, and oral hygiene, and was answered by the responsible for each baby. Oral examination assessed the number of erupted teeth, and collected samples of saliva from the oral cavity of each baby using calibrated sterile microbiological handles. The sample was diluted and spread onto plates containing the specific culture media, MSB agar for *S. mutans*, Rogosa SL agar for *Lactobacillus* spp. and MTPY agar for *Bifidobacterium* spp. One-way analysis of variance (ANOVA) was used for data analysis. Means of CFU/ml of *Lactobacillus* spp., (T1 = 4.05 ± 2.13 ; T2 = 2.67 ± 2.47 ; T3 = 3.13 ± 2.49 ; T4 = 1.95 ± 2.30) *S. mutans*, (T1 = 3.00 ± 2.40 ; T2 = 1.40 ± 1.92 ; T3 = 2.15 ± 2.39 ; T4 = 2.89 ± 2.62) and *Bifidobacterium* spp. (T1 = 3.11 ± 2.87 ; T2 = 3.24 ± 2.51 ; T3 = 4.27 ± 2.67 ; T4 = 3.06 ± 2.75) were not statistically significantly different during the periods of follow-up. Type of birth, breast or bottle milk feeding, sex, family income, type of feeding, presence of oral hygiene, and presence of erupted teeth was not significantly associated with a greater number of CFU/ml of *Lactobacillus* spp., *S. mutans*, and in each period of follow-up. In conclusion, the mean of CFU/ml of *Lactobacillus* spp., *S. mutans*, and *Bifidobacterium* spp. of babies remained stable during the first year of life.

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Metatranscriptomics of Dental Caries Lesions

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Introduction: New DNA- and RNA-based experimental approaches allow not only the identification of microorganisms involved in dental caries, but also the activities they perform during progression of the disease. **Aim:** The aim of this work is to use a metatranscriptomics approach through the study of RNA to (1) identify the microbial communities associated with dental caries at each stage and (2) characterize the gene expression profile of the disease progression. **Methods:** We collected 6 dental plaque samples from caries-free surfaces, 6 samples from dentin caries lesions and 6 samples from enamel, white spot caries lesions. We obtained a mean of 80×10^6 reads per sample by Illumina sequencing. Human and fungal sequences were filtered out by megaBLAST. We constructed a database with over 700 fully-sequenced and draft genomes of oral species against which the mRNA reads were mapped, quantified and normalized. TIGR and COG proteins were used to annotate the expressed genes, whose frequency ratios between groups were calculated. **Results:** Over 25,000 proteins were identified. We found differentially expressed genes at each of the three sample types. Dentin caries lesions showed a higher expression of aminopeptidases ($p = 0.03$) and biotin-carboxylase ($p = 0.005$),

suggesting an osmotic stress environment with a strong peptidase metabolism, including bacterial-encoded collagenases. Enamel caries samples showed an overexpression of genes related to sugar fermentation such as N-acetylglucosamine ($p = 0.002$), biofilm formation kinase ($p = 0.004$) and fibronectin adhesion genes ($p = 0.04$). **Conclusions:** Data support that dental caries is a tissue-dependent process where acid production initiates the lesion and protein degradation is instrumental to expand it. The list of molecules specifically associated with disease could be used to develop an oral marker to bio-indicate the specific location of caries activity.

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Anti-SM Monoclonal Antibodies: Production and Applications

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According to the extended ecological plaque hypothesis, Mutans-streptococci play still a major role in caries etiology. With the rise of molecular diagnostic techniques, the application of monoclonal antibodies for detection and quantification of Streptococcus mutans (SM) fall into oblivion. Here we report on advanced methods for the anti-SM SWLA1 antibody (IgG2a) isolation from ATCC HB-12559 mouse hybridoma cell cultures and various applications for *S. mutans* visualization and diagnostics. First, we tested the specificity of SWLA1, directed against a surface lipooligosaccharide, in a simple immunofluorescence assay subjecting *S. mutans* reference strains ATCC25175 and UA159, eight isolates from various European strain collections as well as four fresh isolates identified by Maldi-TOF and 16S sequencing. The secondary antibody was labelled with fluorophores (Alexa 350, 488, Cy5, Qdot 585) allowing for combination with Live/Dead or DAPI DNA-based stainings. All *S. mutans* strains showed reactivity with SWLA1 but strain specific differences in IF-intensities. Except *S. criceti*, representatives of closely related (*S. rattii*, *S. sobrinus*) or more distantly related (*S. gordonii*, *S. salivarius*, and *S. oralis*) streptococcal species showed very little or no cross-reactivity with the SM-specific antibody. This encouraged us to apply SWLA1 in several assays, such as advanced immunofluorescence testing, microarrays and confocal laser microscopy. We subjected triple- and six-species biofilms, the latter consisting of *Candida albicans*, *Actinomyces oris*, *Fusobacterium nucleatum*, *Veillonella parvula*, *S. oralis*, and *S. mutans*. Finally, native saliva and lesion-attached biofilms were investigated. *S. mutans* could specifically be identified in all samples but the exact procedure of the protocol was crucial. In conclusion, the application of anti-SM SWLA1 antibodies is

promising and could lead to a renaissance, especially as monoclonal antibodies harbor not only diagnostic but also therapeutic options.

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New Insights into the Arginine Metabolism of Oral Biofilms of Children with Caries

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Ammonia production via the arginine deiminase system (ADS) of oral bacteria can neutralize acids in a manner that reduces the cariogenicity of oral biofilms. This study investigated the relationship between arginine metabolism and microbial composition of oral biofilms with caries development in children. Seventy-nine children ages 2–7 years were assessed prospectively for 18-months. Children were grouped as caries-free (CF), caries-active with enamel lesions (CAE), and caries-active with dentin carious lesions (CA). Supragingival plaque samples were collected from caries-free surfaces (PF), and from enamel (PE) and dentin (PD) lesions. Plaque ADS activity was measured by monitoring citrulline production from arginine. HOMINGS and QIIME were used to obtain and analyze 16S sequence profiles from plaque DNA. At baseline, 37% of the children were CF, 34% CAE and 29% CA. At 18-months, 26% were CF, 41% CAE, 23% CA and 10% were caries-experienced (new restorations, no caries). Throughout the study, ADS activity of CF children was significantly higher ($p < 0.0001$) and considerably more stable compared to the other caries groups. ADS activity of PF samples was significantly higher compared to that of PE ($p < 0.0001$) and PD ($p < 0.0001$). There were no significant differences in beta diversity community when the plaque groups were compared at baseline and 12-months. When the time-points were combined, the CF microbial profile was significantly different from those of CAE and CA ($p \leq 0.0033$). Measures of beta diversity distance were highest for comparisons involving PF and PD. Arginine metabolism and the microbiome of healthy tooth surfaces differed substantially from those found during caries activity. Our findings provide fundamental information for early identification of children at greater risk for caries and for development of new therapeutic interventions based on moderation of oral biofilm pH.

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The Effect of Inoculum Type on the Development of Oral Biofilm Under Different Fluid Shear

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Saliva is typically used to develop representative *in-vitro* oral biofilm communities. However, saliva has a markedly different community composition compared to dental-plaque, even though plaque originates from the colonization of species within saliva. Differences in fluid shear may contribute to these differences. This work characterized changes in the biofilm architecture and microbial diversity of multi-species biofilms developed from either saliva or plaque inoculums under different shear in an *in-vitro* microfluidic system. Saliva and dental-plaque from four healthy donors were pooled and used as inoculum. Pooled filter-sterilized human saliva was used as medium. Biofilms (n = 16) were developed for 20 h and imaged using a confocal laser scanning microscope. Biomass, thickness and roughness were calculated from these images. Initial inoculums and developed biofilms were sequenced using IonTorrent PGMTM platform and community composition was analyzed. Data were compared by shear and inoculum using ANOVA. Shear force (0.1, 0.2 and 0.4-dyn/cm²) affected the biomass and thickness of saliva and plaque-developed biofilms (p < 0.05). The highest shear developed 2x thicker biofilms for plaque. Saliva and plaque biofilms developed under 0.1 and 0.4-dyn/cm² were not significantly different in biomass and thickness, but was significantly different compared to 0.2-dyn/cm² flow (p < 0.05). Plaque inoculum had greater alpha-diversity (Shannon index) than saliva inoculum. Both saliva and plaque-developed biofilms showed a significant reduction of diversity in comparison with the original inoculum. Increasing shear to 0.4-dyn/cm² decreased alpha-diversity of saliva-developed biofilms. Plaque-developed biofilms showed greater alpha-diversity when developed under 0.4-dyn/cm² but not different with slower run shears (p > 0.05). In conclusion, saliva and plaque can serve as precursors of dental biofilms developed *in-vitro*. Plaque biofilms developed under 0.2-dyn/cm² demonstrated the least variability in architecture. Within the microfluidic biofilm system, shear influences biofilm architecture and community composition.

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Relationship between Radiographic Depth and Dentine Infection of Approximal Caries Lesions in Primary Molar Teeth

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The aim of this study was to investigate the relationship between dentine depth observed on radiographs, and dentine infection of approximal caries lesions in primary molar teeth. Thirty-eight children aged 5–7 years old with approximal lesions that required operative treatment indicated by the general dentists were included in this study. A sterile bur collected dentine just pulpally to the enamel-dentin-junction. The material was vortex-mixed within 2 hours of collection, diluted and inoculated on agar plates with different pH (7.0, 6.5, 5.5, 4.5). The pH agars were incubated anaerobically for 7 days and evaluated twice regarding presence or absence of bacterial colonies. One examiner evaluated the lesions depth on bitewing radiographs twice regarding a) scores using the ICCMSTM 4-point classification system (R_{ICCMS}: 0=sound, 1=initial, 2=moderate or 3=extensive); b) measurement in millimeters using a software program (Dpx View Pro Image Management[®]). After this, a ratio between lesion depth in dentine and the distance to the pulp were obtained (R_{DEPTH}). The intra-examiner agreement of agar plates evaluation, R_{ICCMS} and R_{DEPTH} were assessed using percentage of agreement, weighted kappa and Bland-Altman analysis, respectively. The relationship between R_{ICCMS} and R_{DEPTH} and presence of infection were assessed using Spearman's correlation. The threshold in dentine was determined by the highest sum of sensitivity and specificity using the ROC curve. The percentage of agreement was 99% and kappa was 0.84. Bland-Altman analysis showed measurements difference nearly zero and not statistically significant (p = 0.91). Rank correlation was R_{ICCMS} = 0.75 and R_{DEPTH} = 0.73 (p < 0.001). The most suitable thresholds for depth was R_{ICCMS} = 1 and R_{DEPTH} = 0.5. The result for the latter was in terms of sensitivity/specificity of 80%/100%. In conclusion, there is a significant correlation between the radiographic lesion depth and presence of infection into dentine. Moderate/extensive lesions (R_{ICCMS} = 2–3) or lesions involving >50% of dentine from the enamel-dentine-junction to the pulp seems to be infected.

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The Type of Buffer Affects *Streptococcus oligofermentans* Biofilms

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Previously we demonstrated that a *Streptococcus oligofermentans* biofilm was able to produce higher amount of H_2O_2 under pH cycling condition (with a 16 h pH 5.5 period) than under neutral pH condition. To maintain pH 5.5 in the biofilm, we used a biofilm medium buffered by 30 mM MES. In the current study, we evaluated the influence of buffer types on HP production and biomass in a *S. oligofermentans* single-species biofilm. To this end, *S. oligofermentans* LMG22279 was inoculated in buffered biofilm medium in a 96-well active attachment model. The biofilms were grown under either constantly neutral pH or pH-cycling conditions. The latter includes two cycles of 8 h neutral pH and 16 h pH 5.5. Phos-

phate buffer was used to maintain neutral pH, while 30 mM MES or 100 mM sodium acetate was used to maintain pH 5.5. The 24 h biofilms were analysed for biomass and H_2O_2 production. The experiments were repeated twice, with quadruplicates in each experiment. The results showed that the biofilms grown under pH-cycling conditions produced significantly higher amounts of H_2O_2 (0.19 ± 0.04 mM or 0.28 ± 0.03 mM) than those grown under constantly neutral pH (0.07 ± 0.02 mM), irrespective of the type of buffer. However, the biofilms grown under pH-cycling conditions with MES buffer had significantly higher biomass and produced significantly higher amounts of H_2O_2 (0.28 ± 0.03 mM) than those grown with acetate buffer (0.19 ± 0.04 mM). No difference was observed in biomass between biofilms grown under neutral pH and those under pH-cycling conditions (buffered by sodium acetate). In conclusion, we proved our previous finding, the increased H_2O_2 production of *S. oligofermentans* biofilms under pH-cycling condition, was independent of the type of buffer used. However, the buffer itself can have a significant influence on the biofilm outcome parameters.

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Session 2 Epidemiology

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Caries Experience and Progression in the Primary Dentition in Colombians Using ICDAS-ICCMS™ Visual- and Radiographic Criteria

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This study aimed to describe the caries experience and progression in the primary dentition of Colombian children after a 2-year follow-up period. Three-age cohorts of children from Bogotá schools/kindergartens participated (2012): A) 2-; B) 4- and C) 6-yr. olds (200 children per cohort), reduced to 81, 131, and 140 children, respectively, after 2 years. Visual caries examinations with ICDAS-Epi were conducted by four trained examiners (inter/intra-reproducibility kappa values >0.70) at baseline, after 1 and 2 years. ICDAS-Radiographic was also conducted yearly, starting at the age of 3. The caries lesions were visually and radiographically staged (ICCMS™) as initial (ICDAS 1/2~d1), moderate (ICDAS 3~4~d2) or extensive (ICDAS 5~6~d3). If caries scores differed between scoring systems the highest score was used. Progression=higher follow-up scores than baseline caries scores, including filled-f/missing-m surfaces. Percentage of children with d2,3mfs ≥1 and d1–3mfs ≥1 were at baseline and final examination: Group-A: 33%–74%, 74%–90%; Group-B: 59%–89%, 89%–99%; Group-C: 67%–92%; 90%–99%, respectively. Caries progression was around zero in upper/lower-canine/lower-incisor teeth. In contrast, progression was noted in 17–51% of individual occlusal surfaces. In cohorts-B and C progression was recorded in 3–15% of approximal surfaces and 0–13% of smooth surfaces on the individual molar teeth, and in 3–21% of upper incisors in groups A and B. Two-year progression from sound at baseline to moderate/extensive stages was often recorded; e.g. in Group-A in occlusal on 75 (19%) and 55 (17%), and in 61 mesial (12%) sur-

faces. In Group-C 12% of the distal surfaces on 84 progressed from sound to moderate/extensive stages during the 2-year period. Concluding, lesion progression in the primary dentition was mainly confined to molar teeth, followed by upper-incisor teeth, and progression from sound to moderate/extensive stage can be rapid. The study was approved by Ethical Board, El Bosque University (UEB #77207-2011).

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Need for Orthodontic Treatment and Caries Experience in First-Grade School-Children

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Introduction: Orthodontic aberrations such as crowding and malocclusion seem to have an impact on caries prevalence and severity in adolescents and adults. Nonetheless, the epidemiological data linking caries experience and need for orthodontic treatment in children in a narrow sense are sparse. The few existing studies revealed a connection between orthodontic need for treatment and caries experience. **Aim:** We aimed to investigate the connection between caries experience and need for orthodontic treatment in the phase of the first year of school. **Experimental Approach:** Instructed examiners measured the d3mft index according to WHO criteria in 25,020 predominantly 6–7 year-old first-grade school children. Following a catalogue of criteria, need for orthodontic treatment was also determined. According to the hypothesis that children with orthodontic need for treatment have more caries experience, the data were analysed using the one-sided Mann-Whit-

ney U-Test. **Main Results:** In the collective, 50.8% of the children were male. Out of the examined $n = 25,020$ children, $n = 2,168$ (8.7%) showed a need for orthodontic treatment. In this group, the average d3mft was 1.47 versus 1.26 in the group without need for orthodontic treatment. The difference was not significant ($p = 0.098$). **Conclusions:** Even though the first-grade school-children with need for orthodontic treatment showed a tendency towards more caries experience in this study, this difference was not significant. In this age, normally the transition from primary dentition to the mixed dentition starts. Since there is a distinct connection between these two parameters in adolescence, we can assume that the impact of need for orthodontic treatment towards carious lesions is rather an attribute of the mixed and the permanent dentition and is not pronounced in the primary dentition.

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Association between Eating before Bedtime and Caries Prevalence in 4–6 Year Olds: Cross-Sectional Study

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Aim: The primary aim was to establish an association between eating in the hour before bedtime and caries experience, from preliminary data collected at baseline from a population based, longitudinal study. Secondary aims were to investigate associations of other factors, such as the amount of toothpaste used, or rinsing after brushing, with caries experience. **Experimental Approach:** In this epidemiological, cross sectional study, 1698 children aged 4–6 years from Cumbria were given a dental examination (caries scores were determined using BASCD criteria) and a short questionnaire about their dental behaviours was completed by parents/guardians. Children were considered to have caries if dmft ≥ 1 . The resulting data were analysed for associations between dmft, eating before bedtime and brushing behaviours. **Main Results:** Analysis revealed the percentage of children with dmft ≥ 1 was significantly higher in the group of children who did eat in the hour before bedtime (35.1%) than those who did not (27.4%) $\chi^2 = 11.774$ (1), $p = 0.001$, odds ratio: 1.44 (95% CI: 1.17 to 1.77). The percentage of children with dmft ≥ 1 was found to be significantly higher in the group who rinse after brushing (34.4%) than those who just spit (27.4%) $\chi^2 = 6.074$ (1), $p = 0.014$, odds ratio: 1.39 (95% CI: 1.07 to 1.80). **Conclusions:** There is a strong association between eating in the hour before bedtime and caries prevalence in 4–6 year old school children in Cumbria. There are further associations between tooth-brushing behaviours and caries prevalence. This builds on previous evidence and suggests eating in the hour before bedtime may be a potential risk factor for caries in children, and possibly be a focus of future targeted interventions.

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Dental Service Utilisation and Social Deprivation in Older Adults in the UK

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Oral health in older adults is in the midst of an epidemiological transition. Access to care for the elderly becomes increasingly problematic with increasing age, and unlike previous generations, 94% have their own teeth. This presents a potential burden to services. Predicting the future for oral health in the ageing population has been well described, but in order to plan dental services within the publicly funded health services it is helpful to consider the needs of the different age groups within the population. Our hypothesis is that caries is an issue in the ageing population and there is disparity between the treatments carried out across the socio-economic levels (IMD). To develop an understanding of service utilisation (demand and need), data extracted from NHS dental practices in North West of England have been analysed using descriptive statistics. The dataset provides the number of treatment claim forms (FP17s) submitted in a 15-month period for 794,502 adults aged <65, 65–74, 75–84 and ≥ 85 years, with additional detail regarding their IMD. We termed ‘older adults’ as those over 65 years old. Reporting on private dental treatment were not covered in the dataset. For older adults in the lowest quintile for deprivation, extractions appeared on 10.2 out of 100 FP17s submitted, 1.4 times more than those in the upper quintile. The highest number of upper acrylic dentures were given to adults aged 85 and over, at a rate of 11.9 per 100 FP17s for the most deprived compared to 3.8 for the least deprived, a 3-fold difference. Data regarding preventative treatment also may be indicative of caries experience. In older deprived adults best practice prevention was carried out 41.1 per 100 FP17s contrasting to only 31.9 per 100 FP17s for younger deprived adults. It was evident that more treatment claim forms were submitted for the most deprived patients across all age stratifications and the type of treatment provided is consistent with the patient’s IMD level. This research is part of PhD project to develop caries risk assessments in older adults. However, the application of this cross-sectional data is limited since reasons for treatment cannot be assumed, but it provides a good foundation for hypothesis generation.

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Relationship between MIH and DMFT/dmft Scores of 6–12 Year-Old Children in Chile

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Introduction: MIH is a clinical condition, in which there is enamel alteration of one or more first permanent molars, which may also involve the permanent incisors. The affected enamel can break down under forces of mastication, leaving unprotected dentin, favoring the development of carious lesions. In the literature it is reported that these patients have greater and more frequent needs for restorative treatment than patients without this condition. **Aim:** The aim of his study was to compare DMFT and dmft indices in 6–12 year-old children with MIH and children not affected by MIH, from Santiago's Metropolitan area in Chile. **Methods:** A sample of 851 schoolchildren aged 6–12 years were examined by two calibrated investigators (Kappa = 0.93). The clinical examinations were conducted for DMFT and dmft scores according to WHO criteria and the diagnosis of MIH was made according to European Academy of Pediatric Dentistry criteria. Data were analyzed using t-test for non-paired samples. **Results:** 108 (12.7%) children were diagnosed with MIH. Mean DMFT score of MIH children was (0.9 ± 1.21) , without MIH (0.41 ± 0.95) ($p < 0.001$). Comparison of DMFT's components median of MIH/without MIH children were: D = $0.27/0.11$, $p = 0.001$. M = $0.04/0.01$, $p = 0.03$. F = $0.59/0.30$, $p = 0.001$. Mean dmft score of MIH children was $1.93 (2.46 \pm 0.24)$ without MIH was $1.35 (2.15 \pm 0.08)$ $p = 0.006$. Comparison of dmft's components median of MIH/without MIH children were: d = $0.56/0.50$, $p = 0.329$. m = $0.08/0.07$, $p = 0.368$. f = $1.29/0.78$, $p = 0.002$. **Conclusions:** For the study sample DMFT scores of MIH children in all components were higher than children without MIH. dmft scores from MIH children's were higher than without HIM due to the f contribution.

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National Survey for Oral Health in Children in Germany 2015/2016: Design and Sample Selection

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Objective: The objective of this study was to highlight the study design and sample selection for the German Oral Health Study 2015/2016 in Children. **Methods:** The German Oral Health Study in Children has been performed regularly for 5 times since 1994/95 by the German Association for Dental Prevention in Children and Adolescents (DAJ) in order to monitor the caries experience in Germany. Besides 6–7- and 12-year-olds, the 2015/2016 survey includes 3-year-olds for the first time in 8 of 17 German regions. In 4 and 6 regions, resp., a full examination of all children is intended, in the others the sampling procedures have been changed to a randomized cluster selection. These regions provided the DAJ with lists of schools and kindergartens, out of which some were drawn for each region and age group depending on stratified random sampling with predetermined accuracy. **Results:** According to the expected drop-out rate from 5% of pupils for most states with compulsory school examinations to 45% for states with voluntary participation and to the variation between schools based on the previous studies (5% to 40% for schools with 6–7-years-old and 5% to 50% for schools with 12-year-olds) and an assumed drop-out rate of 5% for kindergartens a representative sample was drawn for each region and age group resulting in of the a total of 420 kindergartens for 3-year-olds, 1422 primary schools with first graders and 1374 schools for 12-year-olds. Thus a sample of about 85500 children will be examined for the school year 2015/16. **Conclusion:** Due to the legally defined structure of the DAJ and its regional subdivisions, the compulsory dental examination by the community health services in most regions, the organization of a representative in Germany on the oral health situation in school children is feasible with reasonable effort. The results for the 3-year olds are of special interest regarding response, representativity and variation in caries levels.

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National Survey for Oral Health in Children in Germany 2015/2016: Results from the Online Calibration for Initial Caries and dmft/DMFT

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Aim: The aim of this part of the German Oral Health Study in Children is to analyse the first nationwide online calibration for caries. **Methods:** The German Oral Health Study in Children has been performed every 3–5 years by the German Association for Dental Prevention in Children and Adolescents (DAJ) since 1994/1995 in order to monitor the caries experience in children and the success of caries prevention. For the 2015/2016 examination first graders (6–7 years of age) and 12-year-old 6th-graders are to be examined in all 17 German regions, 3-year-olds in 8 of 17 German regions. For quality assurance the examining dentists had to undergo an online calibration, which consists of 3 modules: A) Learning module B) Testing module C) Final calibration testing. The modules are designed for the online calibration of initial caries and caries experience according to WHO (dmft/DMFT) in the primary and the permanent dentition. The results from the online calibration were compared to the previous hands-on calibration in 2009. **Results:** From May to November 2015 a total of 450 dentists who were mostly public health dentists were calibrated successfully reaching at least 18 of the 25 possible correct diagnoses. The kappa values ranged from 0.65 to 1 with a peak at 0.85, matching 22/25 right answers. This mirrors the inter-examiner reliability of the previous hands-on calibration in 2009 of 265 dentists, even with the newly included initial carious lesions. **Conclusion:** An online calibration for caries including initial lesions shows high inter-examiner reliability and may be a convenient tool in large scale epidemiological studies.

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Decline of Dental Caries in Three-Year-Old Children in Greifswald, Germany, from 2003–2015

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Longitudinal data on caries in small children are very rare and, therefore, the aim of this study was to assess the oral health status of 3-year-olds in Greifswald and to compare this to caries data from the federal state Mecklenburg-Western-Pomerania (MWP). The data were extracted from the results of the community dental examinations in nurseries and kindergartens in Greifswald involving about 300 3-year-olds per year (~450 total population). Caries re-

duction was calculated from caries scores (deft) between 2003 and 2015 in comparison with the data of the community dental examinations for MWP generated by calibrated community dentists (deft, WHO standards). Greifswald started 2003 with 78% caries-free children and a mean deft of 1.05 (± 3.22). The d-component comprised 94% of the index. In 2015, 89% of children were caries-free. The mean deft was 0.37 (± 1.37) and the d-component was reduced to 57%. In 2003 and 2015 the community dental examinations of 7,453 and 7441 children (3-year-olds) in all 8 districts of MWP found a decreasing mean deft value from 0.74 (± 0.23) in 2003 to 0.52 (± 0.15) in 2015. The prevalence of untreated caries was reduced from 0.61 to 0.41. Caries reductions in Greifswald between 2003 and 2015 were calculated to be 65% and 30% in MWP. In conclusion Greifswald has achieved considerable caries reductions in 3-years-old compared to MWP. There is a lack of data for comparing this study on a national level in the 3-years-old age group in spite of a clear problem with Early Childhood Caries.

The study is funded by the public healthservice of Greifswald and was approved by the Ethics committee of the University of Greifswald (Vote Nr. BB48/10a).

Dental Caries among 12–17-Year-Old Athletes in Baku, Azerbaijan

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Aim: The aim of this study was to assess the prevalence of dental caries and its treatment among young athletes in Baku. **Methods:** Oral health examinations were performed on 240 young athletes (boys) aged 12–17 years were held among all of whom studied in 4 organizations, preparing professional athletes (boxing, kick-boxing and wrestling) in Baku city. Calibrated specialists ($n = 6$) used the WHO 1997 criteria for diagnosis and recording of DMFT index. Dental caries was diagnosed at the caries into dentine (D3) threshold, using a visual method with mouth mirrors, dental probes and daylight illumination. Ethical approval was obtained from the Ethical committee of the Azerbaijan Medical University. **Results:** Results demonstrated an average caries prevalence of $91.2 \pm 1.27\%$. The mean DMFT for all ages was 3.1 ± 0.13 of which the D component was 1.84 ± 0.08 and the F component was 0.87 ± 0.08 , resulting in a low Care Index of $28.1 \pm 2.9\%$. The mean M component was 0.39 ± 0.06 . Thus the mean number of missing teeth per subject accounted for $12.6 \pm 2.14\%$ of the overall mean DMFT. In general the subjects exhibited poor level of oral hygiene. **Conclusions:** In conclusion the study demonstrated a very high prevalence of caries among young athletes in Baku. Poor level of oral hygiene and most likely frequent eating of foods rich in carbohydrates resulted in the high prevalence of dental caries. In conclusion the athletes need to make better use of the available dental service or the services need to be improved.

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Caries Experience and Associated Factors among Pregnant Women Attending the Clinical Obstetric Department of Montpellier

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Introduction: Pregnancy entails major physiological changes which may upset oral health, but in France, pregnant women are not informed about specific preventive measures. **Aim:** The aim of this study was to evaluate caries experience relative to socioeconomic status among a set of pregnant women at the clinical obstetric department of the Montpellier hospital (France). **Material and Method:** This cross-sectional study combined a medical questionnaire with an oral examination. It concerned all the pregnant women attending their antenatal interview at the clinical obstetric department. Two groups of subjects, according to the EPICES index, were included: the deprived (D) and the non-deprived (ND) groups. During an oral health consultation, DMFT scores, salivary condition and oral hygiene indices (simplified Silness & Loe) have been collected. **Results:** A total of 203 patients were examined (mean age 31.7 years $\pm$ 5.2). The mean EPICES score was 35.1 (CI: 31.6;38.6). The caries prevalence was 76.3% and the mean DMFT was 7.73 ($\pm$ 5.36). The mean number of carious teeth was significantly higher in the group D (3.40) than in the group ND (2.35), $p = 0.02$ (Mann-Whitney test). But oral hygiene indices were not significantly different (1.39 vs. 1.52). Most of them (76.4%) did not know that the treatment of their carious lesions were free of charge during pregnancy. **Conclusion:** This study showed that caries experience was correlated with socioeconomic status among this sample of pregnant women. So, performing an oral examination at the same time than the antenatal interview could contribute to improve their oral health especially among the deprived subgroups of pregnant women.

Caries Knowledge of Pediatricians and Parents of 6–8-Month-Olds Involved in Punctually Applied Caries Prevention Program in Moscow

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Previous studies revealed that caries prevalence is moderately high in 3–6-year-olds in Moscow. Implementation of punctually applied prevention strategies at the earliest age has been initiated

in Moscow. The aim of the study was to describe baseline knowledge of pediatricians and parents of young children in Moscow. In 2015, 55 children aged 6–8 months were involved in the program, and their parents were interviewed about oral health habits and feeding patterns of their children. Ninety-five pediatricians in Moscow were surveyed regarding involvement in oral health education. Eighty-six percent of parents had middle socio-economic status; 76% completed higher education. Seventy-percent delivered the children naturally; 97% were healthy; Eighty-percent of parents breastfed their babies; 67% gave them pacifiers. Forty-two percent of children woke up during the night; 91% drank sugar-free drinks. Forty-eight percent of babies were fed 6–8 times daily; 79% had no feeding or weight problems. Fifty-eight percent of mothers visited the dentist regularly; 84% brushed their teeth every day; 39% had toothache. Seventy-nine percent of pediatricians gave advice to parents. Only 5% of pediatricians recommend a child's first visit to the dentist at the first tooth erupting; 50% recommended children brush teeth at this point; 98% supported breastfeeding; 54% recommended no sweet drinks; 56% proposed limiting night feeding. Seventy-eight percent believed that 'plaque' is a factor in caries development; 78% – 'food,' 68% – 'tooth brushing,' 48% – 'balanced diet,' and 20% – 'fluoride in toothpaste.' Results of the study showed parents require more oral health education, while involvement of pediatricians in oral health education is rather limited. The results give us important data to ensure a more effective punctually applied caries prevention program among young children in Moscow.

Social Gradients in Dental Caries Status in Italian Adults

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Objective: The objective of this study among Italian (Sardinian) adults aged 30–45 years was to assess whether there is a social gradient in dental caries experience in Italian adults and whether caries experience was associated with some behavioral factors such as smoking. **Materials and Methods:** 480 subjects, were dentally examined using the ICDAS index to record caries status. A simplified structured self-compiled questionnaire was administered and data on sex, educational level, employment and smoking habits were recorded. Multinomial logistic regression models were performed using decayed ICDAS scores, numbers of missing teeth due to caries and presence and type of fillings as the dependent variable. **Results:** Fifty two percent of the sample were males. Caries prevalence was 62.9%, 44.79% of the sample had at least one manifest caries lesion (ICDAS score 4–6). There was an educa-

tional gradient in DMFT; those with a higher educational level had lower caries experience (OR = 0.56 95% CI = 0.43–0.83 $p < 0.01$) and for presence and type of fillings (OR = 0.70 95% CI = 0.55 – 0.88 $p < 0.01$). Females were less affected by untreated caries (OR = 0.71 95% CI = 0.50–0.99 $p = 0.04$). Smokers had a higher risk of having more caries lesions (OR = 1.52 95% CI = 1.03–2.24 $p = 0.03$). A high number of missing teeth were positively associated with sex (OR = 1.44 95% CI = 1.02–2.04 $p = 0.04$) and smoking habit (OR = 2.41 95% CI = 1.64–3.55 $p < 0.01$). **Conclusions:** There was an educational gradient in dental caries experience. Educational level, sex, smoking habits were statistically significantly associated with caries experience in Italian adults.

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Caries Lesion Detection and Staging on Digital Photographs as an Assistance Tool for Caries Diagnosis

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The aim of this study was to assess the discriminatory power of standardized anterior clinical images to detect and stage caries lesions on buccal surfaces of anterior using the ICDAS criteria. Three hundred subjects enrolled in a previous study (where clinical ICDAS scores and clinical intra-oral photographs were acquired) were selected to provide a wide range of ICDAS scores. The images were integrated into a graphical user interface that randomized and blinded the images were displayed on a 32-inch flat screen monitor under controlled lighting. Two trained examiners scored the images of upper anterior teeth (canines, lateral and central incisors) using the same criteria in two opportunities (washout period of 1 week). Weighted kappa values were calculated to evaluate inter and intra-examiner reproducibility. Intraclass Correlation Coefficient (ICC) was used to correlate the clinical and digital examinations. The level of significance was set at 5% for all analysis. A total of 1800 teeth from 300 subjects were assessed. Weighted kappa values were 0.85 for inter-examiner reliability and varied from 0.75 and 0.78 for intra-examiner reproducibility. ICC values for both examiners were 0.63. Detecting and staging early and late caries lesions on digital photographs is a reliable and valid method to be used in terms of assessing the lesion severity according to the ICDAS.

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Dental Caries Lesions Activity Assessment among 18-Year-Old Lithuanian Adolescents

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Aim: The aim of study was to assess prevalence of active caries lesions, in association with gender, place of residence and oral hygiene status of 18-year-old Lithuanian adolescents. **Methods:** in 2014, a multistage sampling strategy was used to draw a representative sample of 1.063 18-year-old students of Lithuania. Dental examinations were performed following clinical Nyvad criteria for caries lesion activity assessment, under standardized conditions using portable dental equipment. Adolescents were examined by two trained and calibrated examiners to estimate surface-level dental caries experience. Active and inactive caries lesions (non-cavitated and cavitated) were recorded. Oral hygiene status was evaluated using Silness-Loe dental plaque index. Place of residence were classified as urban or rural. Kappa value for inter-examiner correlation was 0.92. The data were analyzed using t-test and χ^2 criterion; with threshold for statistical significance at $P < 0.05$. **Results:** 98% of all study participants were affected by caries; 83% of all lesions recorded were active (non-cavitated or cavitated). Mean DMFS was 25.0 (SD, 13.2) One third of the mean caries experience consisted of active lesions (9.3 (SD, 9.7)), most of them (8.4 (SD, 9.1)) were non-cavitated. Higher DMFS scores were estimated for boys than for girls (25.9 (SD, 13.9) vs. 24.2 (SD, 12.7)). No differences with respect to the mean numbers of active and inactive caries lesions were estimated between urban and rural residents. Boys had higher mean values of dental plaque index than girls (1.3 (SD, 0.7) vs. 1.1 (SD, 0.6)). Poor oral hygiene was associated with high number of active lesions. **Conclusion:** In conclusion, Lithuanian population of 18-year-olds was characterized by high prevalence of active caries lesions. Presence of dental plaque was associated with the number of active caries lesions.

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Prevalence of Dental Erosion among 5, 12, 18-Year-Olds in Cyprus

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The aim of this study was to assess the prevalence and risk factors regarding dental erosion among 5, 12, 18-year-old children of Turkish population in Cyprus. There is no data exist on the prevalence of tooth wear in Turkish Cypriot children.

Ethical approval was obtained from the Ethics Committee of Near East University Cyprus. Subjects were only included after receiving written positive consent from their parents. The study was carried out involving 339, 5-year-old children (188 boys and 151 girls), 627, 12-year-old (322 boys and 305 girls) children and 275, 18-year-old adolescents attending 34 schools in Cyprus. After tooth cleaning and drying, teeth were examined using the Basic Erosive Wear Examination Index (BEWE). General information, social background, dietary habits as well as brushing frequency were evaluated using a questionnaire. Pearson Chi-Square test was performed to verify the association between sex and dental erosion. We found that the prevalence of dental erosion in 5 year olds was 54.9% (n = 186). There was a statistically significant difference in prevalence between boys 60.1% (n = 113) and girls 48.3% (n = 73) (p = 0.031). In 12-year old subjects (n = 627) 57.6% (n = 361) showed dental erosion. No significant difference was observed between boys 56.4% (n = 172) and girls 58.7% (n = 189) (p = 0.56). The prevalence of dental erosion in 18 year olds was 70.5% (n = 194). There was no difference in prevalence between boys 70.2% (n = 92) and girls 70.8% (n = 102) (p = 0.91). High prevalence of dental erosion was observed in children that reported increased intake of acidic food and beverages. In conclusion the high prevalence of dental erosion suggests that risk factors should be addressed early, children as well as parents should be informed and educated about dental erosion and the acidic dietary habits.

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Prevalence of MIH in 6 to 12 Years Old Children from Santiago, Chile

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Background: The reported prevalence of MIH varies widely around the world (2.4–40.2%). Up until to day there is no epidemiological data about its prevalence in Chile. The aim of this study was to assess the prevalence of MIH in 6 to 12 year old schoolchildren from Santiago's Metropolitan Area, Chile. **Methods:** Two calibrated investigators (Kappa = 0.93) examined 851 schoolchildren aged 6 to 12 years. The dental examination included clinical (visual and tactile) inspection and dental photos. Diagnoses of MIH were made according to European Academy of Pediatric Dentistry Criteria. Socioeconomic status was determined according to Chile's borrow indexation and one school for each of the three levels was examined. Results were expressed in frequency. Data was analyzed using Chi² test. **Results:** 851 schoolchildren were included in the study; out of which there were 338 male and 513 females. Frequency at 6 years was 0% (n = 22). 7 years 12.38% (n = 92). 8 years 18.09% (n = 188). 9 years 16.57% (n = 181). 10 years 8.62% (n = 174). 11 years 9.92% (n = 131). 12 years 6.0% (n = 50). MIH frequency by gender was male = 14.79% (n = 50) female = 11.31% (n = 58) p = 0.135. By socioeconomic status, lower class children of 15.88% (n = 54), middle class 13.68% (n = 32), and higher class 7.94% (n = 22) p = 0.011. **Conclusions:** The prevalence of MIH was 12.7%. There was no significant difference according to gender. The prevalence was significantly higher in lower class children.

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Toothpaste Use and Caries Experience in Young Children: A Longitudinal Study

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The aim was to study caries experience in young children in relation to using toothpaste in a longitudinal. Children were examined during their visits at a pediatric clinic. Inclusion criteria were: children aged under 18 months, who had erupted teeth, were caries free, did not use toothpaste during teeth brushing and whose parents signed informed consents. Two toothpastes were recommended for the children and the parents decided which, if any, to use. The children were re-examined after 12 months. The mothers were interviewed about the children's toothpaste use. Caries prevalence (%), mean d₁₋₆mft/s values (ICDAS-II criteria, M±SE), Chi²

test, Student's t-test, relative risk ratio (RR), Number Needed to Treat (NNT) and P-values were calculated. 228 children (mean age 12.1 months) were included into the study. After 12 months 3 groups were identified according to the toothpaste use during this period. The toothpaste with 6% of xylitol was used in 79 children (X group), the toothpaste with 500 ppm F⁻ was used in 85 children (F group), and no toothpaste was used in 36 children (N group). Caries prevalence was 13.9%, 2.3% and 19.5% in X, F and N group respectively. Mean d₁₋₆mft/s values were $0.44 \pm 0.08/0.79 \pm 0.15$ in X, $0.02 \pm 0.09/0.02 \pm 0.09$ in F and $0.69 \pm 0.27/1.19 \pm 0.24$ in N groups. The differences were statistically significant only between X and F ($P < 0.01$), F and N ($P < 0.01$) groups. The chance to be caries free was higher in children from F group (RR = 8.26, NNT = 5.85, $P = 0.006$) than in X group (RR = 1.39, NNT = 18.1, $P = 0.448$). In conclusion caries prevalence, d₁₋₆mft/s and caries risk were significantly lower in the young children who used the toothpaste with fluoride than in children who used the toothpaste with xylitol or used no toothpaste.

The Ethical Approval for the study was given by the Regional Research Ethics Committee at the State Institution 'Volgograd Medical Research Center', registration number IRB 00005839 IORG 0004900 (OHRP), the approval number is №111 – 2010.

The study was funded by the Volgograd State Medical University.

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Dental Caries Profile in First Permanent Molars in Polish Children Measured with CAST Index

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The Caries Assessment Spectrum and Treatment (CAST) index is a novel tool developed for epidemiological surveys. Nine CAST categories are hierarchically arranged according to the severity of diagnosis. The aim of this study was to evaluate the dental caries profile in first permanent molars of 9-year-old children using the CAST index and WHO criteria. One-hundred and fifty children were selected by cluster sampling. The clinical examination of first permanent molars was conducted by one examiner in the school setting with artificial light, using a dental mirror and a periodontal probe. The lack of caries symptoms was found in 35.4% of children while 35.3% of subjects had a maximum CAST score of 3 and no child scored codes 7, 8 or 9. The average number of first permanent molars per child was as follows: 0.96 ± 1.34 for a sound tooth (CAST 0), 1.14 ± 1.51 for a fissure sealant (CAST 1), 0.79 ± 1.31 for a filling (CAST 2), 0.87 ± 1.23 for a caries lesion in enamel (CAST 3), 0.12 ± 0.4 for a caries lesion in dentin (CAST 4), 0.09 ± 0.38 for a cavity in dentin (CAST 5) i 0.02 ± 0.14 for a pulpal involvement (CAST 6). There were no differences in the joined CAST codes 3–7 and 5–7 according to gender and location ($p > 0.05$, U Mann-Whitney test). The correlation between CAST

categories 3–7 and the D component of DMFT was low ($r = 0.338$, Spearman's correlation coefficient), but it was very high between CAST categories 5–7 and D ($r = 0.925$). The intra-examiner reliability (unweighted Cohen's kappa coefficient) was 0.88 for CAST and 0.94 for DMFT. With the CAST index it was possible to conduct a comprehensive analysis of caries profile and a comparison with DMFT.

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Early Childhood Caries in Brazil: A 2-Year Follow-Up Study

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This longitudinal epidemiological study aimed to evaluate the incidence and progression of caries lesions after two years of follow-up in Brazilian preschoolers registered in basic health units. At baseline 163, average age of 3:41 (SD: $\pm 00:51$) years, children from twelve basic health units of primary health care (SUS – Public Health System of Brazil) were clinically evaluated using ICDAS index in their own houses. In the present study, after two years of follow-up, 119 children of baseline were re-evaluated also using ICDAS exactly as in baseline. The sociodemographic data of baseline were used in the analysis. The data were presented descriptively, with Poisson regression and generalized estimating equations (GEE). We found caries incidence in children was 2.5% (considered as any change from baseline to follow-up from healthy or non-erupted to caries or restored surface) and the prevalence of caries progression was 89.9% (it was regarded as progression any change in ICDAS scores towards an increase in severity). Permanent first molars were examined only at the follow-up and when analyzed separately from the other teeth showed a caries incidence of 38.6%. In general, the occlusal surfaces showed 7.4 times higher chances of progression followed by smooth surfaces (OR: 3.3 – CI: 1.9–5.50) when compared with the approximal surfaces. Posterior teeth presented 7.8 times higher chances of progression when compared to the anterior teeth. Sociodemographic factors that were associated with progression: marital status of mothers ($p < 0.040$) and the presence of cavitated lesions at baseline ($p < 0.001$) were significantly associated with a higher rate of progression. In conclusion the percentage of caries progression was very high in sample. The occlusal surfaces and the posterior teeth were those that demonstrated a higher progression of lesions. Marital status and a previous history of children's caries were significantly associated with the increased rate of caries progression.

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Training Examiners Prior to Calibration with the CAST Instrument

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Background: The CAST Manual was developed to assist the training of examiners prior to calibration in epidemiological surveys in which the CAST (Caries Assessment Spectrum and Treatment) instrument will be used. **Objective:** The aim of this study was to evaluate examiner's performance in assessing extracted teeth surfaces after reading CAST Manual (T0) and reading CAST Manual plus participating on a theoretical course (T1). A total of 20 examiners who would participate in an epidemiological survey assessed 30 extracted teeth, independently, after T0 and T1. Their scores were compared to reference scores determined by two experienced epidemiologists. Examiners (n = 18) also responded a questionnaire about their experience in oral health surveys, with CAST and personal information. **Results:** It was observed that it was the first time that 72.3% (n = 11) of the examiners were trained to participate on an epidemiological study. All participants had no prior contact with CAST, but after learning about the instrument would recommend it to be used in epidemiological surveys. Average age of the examiners was 35.7 years and average time working as a dentist was 11.9 years. Mean kappa coefficient value was 0.55 (0.28–0.73) after T0 and 0.64 (0.46–0.80) after T1. For 75% of the examiners, kappa coefficient value increased after T1, but it remained stable for 15% and decreased for 10% of the examiners (n = 2). Percentage of agreement among examiners at T0 was 62.8 (34.7–87.7) and 71.3 (45.0–87.4) at T1. Examiners improved their performance in scoring CAST codes 3–6 at T1. **Conclusion:** In conclusion the CAST manual assisted examiners in using the CAST instrument, but their performance could improve after the theoretical course. Further clinical training and calibration before starting an epidemiological survey might be required to achieve adequate agreement among examiners.

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Dental Attendance Pattern and Caries Experience Profile in Adult Belgian Citizens: Data from the National Oral Health Survey 2013

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In 2012–2014 a national Oral Health Data Registration and Examination Survey took place in Belgium. This included oral examination of a sample representative of the population aged

5 years and older and a self-administered questionnaire (dietary and oral health habits), collected by a dentist during a home visit. Caries experience was recorded according to WHO criteria and expressed using D₃MFT. Data were linked to oral care utilization in the previous 5-year period, as registered by the social security system. Dental attendance pattern was classified as regular (R, at least 3 dental visits in 3 different years), irregular (I, at least one dental visit) or avoidance (A, no visit registered). Data from 1340 dentate (≥ 1 tooth) individuals in age groups 25 years, and above were processed statistically. Statistical analysis was performed with t-tests with Bonferroni correction for multiple testing and Chi-square tests for categorical variables. We found 19% (n = 256) of the participants were avoiders, 30% (n = 405) irregular and 51% (n = 679) regular attenders. Regular attenders were more frequently higher educated. Absence of symptoms, anxiety and financial considerations were main reasons for avoiding a visit to the dentist. The overall D₃MFT (means ranging from 11.0–12.7) was not significantly different between attendance patterns (p = 0.052). Restored teeth were found more frequently in the I (5.8 $\pm$ 4.4) and R (5.2 $\pm$ 7.4) than in the A group (4.7 $\pm$ 5.0) (p < 0.001). On the contrary, A had more untreated decay (2.2 $\pm$ 3.5) than I (1.3 $\pm$ 2.2) and R (0.8 $\pm$ 1.6) (p < 0.001). There was no difference in self-reported tooth-brushing frequency or dietary habits.

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Oral Health among Adolescents from the Southeast Region of the FYROM

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Aim: The aim of this study was to assess dental caries in 15-year-old children attending regular public secondary school in Strumica. **Methods:** The study was conducted in 15 year olds during the year 2013. In this cross-sectional study, secondary school children from first grades (N = 476) were selected from two Secondary Schools in Strumica. Participants dental status was evaluated using the 1997 World Health Organization caries diagnostic criteria for Decayed, Missing or Filled Teeth (DMFT) by 2 calibrated examiners. For statistical analysis of DMFT scores to access the oral health among secondary school children, the SAS statistical program was used. T-test was used to find the difference in mean DMFT between gender groups and area groups. P value ≤ 0.05 was considered statistically significant. **Results:** The total number of children in the sample was 476, comprising 189 (39.71%) females and 287 (60.29%) males. The mean DMFT was 3.55 (standard deviation (SD) 2.99; 95% confidence interval (95% CI 3.28–3.82), the significant caries (SiC) index was 6.94. The prevalence of caries-free children was 17.25%. The percent-

age of untreated caries or the ration of DT/DMFT was 0.4060 (40.60%). **Conclusions:** Dental caries experience was seen to be moderate among secondary school children from Strumica city and its surrounding.

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Dental Management of Adult Patients with Special Health Care Needs in Slovenia

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Aim: The aim was to evaluate the dental management of adult patients with special health care needs (SHCN) in Slovenia. **Methods:** The database involved all the adult patients with SHCN treated at the Centre for dental diseases and endodontics, Dental Clinic, University Medical Centre Ljubljana, Slovenia, from January to December 2015. Medical charts were reviewed by one examiner and descriptive statistics performed. **Results:** A total of 110 adult patients with SHCN from all regions of Slovenia were treated by 9 specialists and residents. Age of patients ranged from 18 to 84 years (mean 34.5 ± 12.9), 61.8% were males. 47 patients had intellectual disabilities, 24 cerebral palsy, 36 seizure disorders, 14 psychiatric issues, 8 Down syndrome, 5 autism, 5 dementia, 11 head trauma and 2 strong gag reflex. 16 patients were successfully treated in the dental office in multiple visits. 40 patients had to be treated under general anesthesia (GA) in one visit. Half of the patients had already been treated under GA throughout their lifetime. Altogether 254 teeth were filled, 13 endodontically treated and 96 extracted. Waiting period for the treatment of acute dental problems under GA ranged from 4 to 184 days (mean 70 ± 70), for subacute dental problems from 0 to 11 months (mean 8.3 ± 4.3), and for chronic dental problems from 1 to 6 years (mean 3.0 ± 0.8). 54 patients had to be put on the waiting list for dental treatment under GA in following years. **Conclusions:** GA is often indicated to accomplish restorative treatment in adult patients with SHCN. Because of long waiting periods for dental treatment under GA education of parents and caregivers on proper oral hygiene and nutrition is mandatory.

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Trends on the Prevalence and Severity of Dental Caries in Portuguese Children and Teenagers

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The aim of this study is to evaluate the prevalence and severity of dental caries at 6, 12 and 18 years in Portugal and compare the data with those of previous studies. The III Portuguese Survey on Oral Diseases (PSOD) is a transversal, descriptive and analytical study, representative at national level. The target population consisted of 3,700 randomly selected young people, with 1326, 1309 and 1075 children of 6, 12 and 18 years-old, respectively. A questionnaire was applied and the visual record of caries followed the ICDAS II criteria later transposed to the WHO index, DMFT. For the statistical analysis the chi square with Rao-Scott correction was used and the results considered significant at a level of $p < 0.05$. At 6, 12 and 18 years-old the caries prevalence found was 45%, 47% and 67.6% respectively. The d_5mft was 1.62 to 6 years and the D_5MFT of 1.18 and 2.51 to 12 years and 18 years. At 12 years-old there was 0.35 teeth decayed and 0.74 filled and at 18 there was 0.75 teeth decayed and 1.53 filled. About 78.7%; 89.8% and 96.1% brush their teeth once or more daily. This habit was more frequent in girls, residents in urban areas, with mothers with high school level and employed ($p < 0.05$). At 12 and 18 years-old, 29.4% and 38.1% have less than 5 meals a day and 70.2% and 59.0% visited the dentist in the last year. At 6 years there has been an improvement in the percentage of caries-free children, from 33% in 2000 to 55%. At 12 there was a significant decrease in the D_5MFT , from 2.95 in 2000 to 1.18, probably related to the Oral Public Health Program.

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Effectiveness of Two Community-Based Fluoridation Programs in Preventing Dental Caries among 12-Year-Old South American Schoolchildren

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Aim: The aim of the study was to compare the effectiveness of two community-based fluoridation programs (water and salt) on caries prevention among 12-year-old schoolchildren. **Methods:** This study arises from the comparison of two population-based cross-sectional studies conducted with representative samples of 12-year-old schoolchildren receiving fluoride from different community-based methods: artificially fluoridated water in Porto Alegre (POA), South Brazil, and fluoridated household salt in Montevideo, Uruguay. Data on socio-demographic characteristics, mother's educational level and oral hygiene habits were collected. Dental caries was defined according to the WHO criteria (only cavitated lesions) and to the modified WHO criteria (active non-cavitated lesions and cavitated ones). The association between community-based fluoridation programs and dental caries was assessed using logistic regression (caries prevalence) and Poisson regression (caries extent). Estimates were adjusted for mother's educational level, school type and brushing frequency. Odds ratios (OR), rate ratios (RR), and their respective 95% confidence intervals (CI) were estimated and reported. **Results:** 2,682 schoolchildren were examined, being 1,528 from POA and 1,154 from Montevideo. Caries prevalence was similar between the cities irrespective of the detection criteria. Regarding caries extent, a significantly higher DMF-T index was found in Montevideo (2.80, 95% CI = 2.37–3.24) than in POA (1.96,

95% CI = 1.762.16) when the modified WHO criteria was used. Significant associations between caries prevalence/extent and the community-based fluoridation program were found with a higher caries risk in schoolchildren exposed to fluoridated salt (prevalence, OR = 1.62, 95% CI = 1.28–2.05); extent, RR = 1.33, 95% CI = 1.17–1.51). **Conclusion:** Fluoridated water conferred a greater protective effect against dental caries than fluoridated household salt among South American 12-year-old schoolchildren.

This research was supported by the Brazilian National Coordination of Postgraduate Education (CAPES), the Uruguayan National Agency of Research and Innovation (ANII), the Uruguayan Ministry of Education, the Uruguayan Ministry of Public Health, the Federal University of Rio Grande do Sul and the University of the Republic.

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Implementation of Minimal Intervention Concepts in General Practice in France

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Questionnaire surveys are often used to ascertain treatment philosophies in dental practice. This communication aims to present key findings of two questionnaire surveys undertaken in France to evaluate the implementation of Minimum Intervention (MI) concepts in cariology, namely that of caries risk assessment (CRA) and the use of dental sealants for caries prevention and as a non-invasive strategy to manage non-cavitated lesions. Two original questionnaires were developed to assess knowledge, opinions and practices toward CRA and dental sealants. They were submitted, in early 2015, to two simple random samples of general dentists (GDs) practicing in France. Descriptive analysis

and logistic regression analysis were performed. The response rates were 34.7% and 36.7% respectively for the CRA and the dental sealant surveys. Results showed that MI concepts had not widely entered clinical practice in France in 2015 and almost 12% of respondents admitted they did not know exactly what Minimal Intervention in caries management involved. 38.4% of respondents reported that CRA was not part of their routine practice and 32.3% reported no regular scheduling of preventive care based on the caries risk of their patients. Regarding dental sealants, while 89.9% of respondents use them preventively, only 42% reported to use them for non-cavitated caries lesion management. Practitioner socio-demographic characteristics also influenced practice patterns. For example, GDS who had recently participated in a Continuing Education course were more likely than others to assess the caries risk of their patients ($p < 0.001$) or to place preventive sealants ($p = 0.033$). Those studies, the first of their nature in France, showed the need to develop MI concepts in daily dental practice in France.

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18-Month Follow-Up Participant Retention in a Clinical Study of Early Childhood Caries

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Introduction: Conducting multi-year longitudinal clinical trials with diverse populations may be compromised due to unacceptable levels of subject attrition. **Purpose:** Evaluate retention efforts in an ongoing longitudinal multi-site clinical study to develop a self-administered caries risk tool to identify children at risk through medical settings. Preliminary data suggested retention rates without intermediate contact within a year of follow-up of approximately 75%. **Methods:** 1,326 primary caregiver/infant (12 months $\pm$ 3 months of age) pairs were enrolled at baseline across three study sites (Duke University, Indiana University, University of Iowa). Children were 49% female/51% male, 13% Hispanic/37% Caucasian/37% African-American/13% other or multi-racial, 61% on Medicaid, and 95% resided in urban communities. A follow-up clinic visit was conducted at 18 months post-baseline and a 36-month post-baseline visit is currently being conducted (2.5 and 4 years of age). To aid in retention, contact is attempted with the child's caregiver every 4 months. Study sites report number of contacts attempted, contact method, and whether successful (caregiver contact is achieved) or unsuccessful. Caregivers are sent \$10 for each successful contact. **Results:** Visit windows have ended for all study participants for the 18-month clinic visit, as well as all prior intermediate contacts. Successful contact was achieved for 95% of 4-month, 93% of 8-month, 91% of 12-month, and 90% of 16-month contacts. 18-month in-person dental exam visits had an 81% success rate across sites, and 7% of children had cavitated lesions. The most successful means of contact was by telephone (vs. email or

letter). **Conclusion:** Maintaining contact every 4 months with the primary caregiver in a very diverse population has resulted in reduced attrition at the initial follow-up clinical visit across sites versus preliminary estimates.

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Evaluation Efficacy of *Copaifera multijuga* Dental Varnish on Against *Streptococcus mutans*: A Randomized and Double-Blind Study

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The aim of the study was to evaluate the antimicrobial efficacy of a 1% *Copaifera multijuga* (copaiba) dental varnish against mutans streptococci (MS) in comparison with 1% Chlorhexidine and 5% Fluoride in children. Ninety high-risk caries-free children, aged 36–71 months were recruited and randomly divided into three groups to receive treatment with varnishes containing: copaiba, chlorhexidine (positive control) or fluoride (control). Varnishes were applied to occlusal surfaces of all deciduous molars once every 3 months, during 12 months. Salivary MS reduction was accessed before starting treatment (D1), 90 days after day 1 (D90), 6 months (D180) and 1 year after initiation of treatment (D360). For saliva collection, patients chewed on Parafilm[®] for 60 s. Samples were plated in duplicates (1:100 and 1:1000 mL dilutions) on Mitis Salivarius-Bacitracin (MSB). MS values were expressed as log (CFU/mL). Representative colonies of MS were counted, isolated and biochemically confirmed. Statistics were carried out by applying RM-ANOVA, Tukey's multiple comparisons, and paired t tests. We found the MS levels (mean $\pm$ standard deviation) were as follows: Chlorhexidine (D1: 0.58 $\pm$ 0.43; D90: 0.38 $\pm$ 0.23; D180: 0.33 $\pm$ 0.14; D360: 0.55 $\pm$ 0.52), Fluoride (D1: 0.86 $\pm$ 0.37; D90: 0.51 $\pm$ 0.33; D180: 0.41 $\pm$ 0.24; D360: 0.53 $\pm$ 0.44), Copaiba (D1: 1.32 $\pm$ 0.61; D90: 0.99 $\pm$ 0.57; D180: 0.39 $\pm$ 0.22; D360: 0.12 $\pm$ 0.19). Copaiba demonstrated MS reduction: D360 versus D1 ($p < 0.001$), D180 versus D1 ($p < 0.001$), D360 versus D90 ($p < 0.001$), D180 versus D90 ($p < 0.05$), D360 versus D180 ($p < 0.05$). Chlorhexidine varnish significantly reduced MS: D360 versus D1 ($p < 0.05$) and D180 versus D1 ($p < 0.01$). Fluoride reduced MS at D180 versus D1 ($p < 0.05$). Copaiba consistently reduced MS throughout the 360-day period. At D360, copaiba produced lower MS levels compared to fluoride ($p < 0.001$) and chlorhexidine ($p < 0.01$). In conclusion, four annual applications of the copaiba dental varnish showed substantial antimicrobial activity against MS for up to 12 months in high-risk

caries-free children. Further studies to identify the anticaries effect of this varnish are required to establish its use in caries prevention.

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Five-Year Survival of One-Time Application of Glass Ionomer Sealants versus Resin Sealants: A Randomized Controlled Trial

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The aim of this study was to compare 5-year relative retention performance and caries prevention effect of a resin sealant (RS) and a glass-ionomer sealant (GIS) in preventing fissure caries in permanent molars in a randomized clinical trial conducted in Marmara University, Istanbul (clinicalTrials.govNCT02063815). 80 GIS (Fuji Triage) and 80 RS (Ultrasal XT) were placed on the caries free first permanent molars in 40 children aged 7–10 years in a randomized manner by a pediatric dentist (EH). Fissure status of the molars in each group was evaluated clinically every year for 5 years and sealant status was assessed independently as total or partial retention and total loss by two pediatric dentists (SG, CD). In case of sealant failure the fissures were checked visually and with Diagnodent-pen for incipient caries. Scores higher than 20 and 30 were recorded as enamel and dentin caries, respectively. Kaplan Meier survival analysis was used to compare the sealants, followed by log-rank test to check the difference between survival curves. The chi-square test was used to evaluate differences in retention rates between the two sealants and new caries lesions at each evaluation period. The drop-out rates were 0%, 5%, 20%, 50% and 35% in GIS group and 0%, 5%, 15%, 30% and 50% in RS group for 1, 2, 3, 4 and 5 year examinations, respectively. For GIS group, total retention was 71.3%, 6.6%, 23.4%, 7.5% and 0%; partial retention was 27.5%, 42.1%, 68.8%, 67.5% and 51.9% and total loss was 1.3%, 1.3%, 7.8%, 25% and 48.1% for each year. For the RS group, total retention 90%, 85.5%, 58.8%, 39.3% and 20%; partial retention was 10%, 14.5%, 41.2%, 39.3% and 40% while total loss was 0%, 0%, 0%, 21.4% and 40% for each year. Survival analysis showed that sealant retention rate over 2 to 5 years for RS was significantly better than for GIS ($p = 0.000$). Caries incidence of molars was 1.6%, 10% and 9.6% for GIS and 0%, 21.4% and 35% for RS groups at 3, 4 and 5 years respectively. In conclusion the failure rate of the GIS was statistically higher than that of the RS at 5 years but the GIS was more effective at the prevention of caries development than the RS.

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Do Parents and Children Perceive Molar-Incisor Hypomineralization as an Aesthetic Problem?

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The present study aimed to identify whether parents and children perceive opacities associated to molar-incisor hypomineralization (MIH) as an oral health problem. A case-control study was designed, in which 131 children aged 7 to 13 years-old diagnosed with MIH in a previous epidemiological survey comprised the cases and 131 children without the condition were the controls, paired by age and gender. A validated questionnaire – ‘Child’s and Parent’s Questionnaire about Teeth Appearance’ – that is composed of 5 sections was used. Case and control children replied to the questionnaire at school premises under the evaluator supervision, while mothers were interviewed by phone by the same evaluator. Spearman correlation was used to assess children and mothers response from both cases and controls in relation to the variable ‘tooth appearance’. To compare the answers regarding tooth ‘pleasantness’, ‘alignment’, ‘staining’ and ‘health’, the scores distribution given by pairs of cases and controls were assessed using the Wilcoxon signed-ranked test ($\alpha = 0.05$). The sample was composed of 138 (53%) boys and 124 (47%) girls with a mean age of ± 9.61 years. For the variable ‘tooth appearance’, the children cases presented more difficult in ‘spontaneous smiling’ compared to controls, while the mothers from the cases showed to be more disturbed regarding the ‘appearance’ compared to the controls mothers ($p < 0.05$). It was also observed that the children cases were less satisfied with their teeth ‘alignment’ and ‘colour’ in comparison to controls, while the cases mothers showed to be displeased with their children teeth ‘colour’ compared to the mothers of controls ($p < 0.05$). It was concluded that opacities related to MIH are negatively perceived by children, who are affected by the problem, and their mothers.

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Presence and Activity of Root Caries in Community-Dwelling Elders

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Despite the increasing prevalence of root caries lesions, clinical characteristics and association with societal variables have not been fully understood. The purpose of this study was to characterize root caries lesions in community-dwelling elders and to associ-

ate their presence with sociodemographic variables. **Methods:** This study characterized root caries lesions of subjects who participate in a RCT of non-invasive therapies for root caries. The sample was constituted for 345 community-dwelling elders with at least 5 teeth present and one root caries lesion. Participants underwent a structured oral health interview and were orally examined to assess presence and activity of root caries using ICDAS criteria. The Root Caries Index (RCI) and percentage of Active Root Caries (ARC) were calculated and compared according to the tooth type. RCI and ARC were associated with sociodemographic variables, including, sex, age, educational level and socioeconomic status. Data were analyzed by T test and ANOVA, considering a significance level of 0.05. **Results:** The RCI was 52.1% and the ARC 31.7%. The highest presence and activity of root caries was detected in the molars and the lowest in the incisors. Both RCI and ACR were associated with educational level ($p = 0.0001$ and $p = 0.006$, respectively). When the group of subjects was divided into two age groups, older than 70 years showed higher ACR, but not RCI than 60–69 year-old older adults ($p = 0.01$). **Conclusion:** High RCI and ARC was observed among community-dwelling elders, with molars being more affected than other teeth. Educational level and age appear to be associated with an increased presence and activity of root caries in older adults.

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Randomised Clinical Trial on Caries Management Options for Primary Molars: 2-Year Outcomes

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The standard surgical approach to treat cavitated carious lesions in children has shown limited effectiveness in controlling the carious process with more conservative techniques involving partial or even no caries removal becoming more commonly used. The aim of this study was to compare the 2-year clinical efficacy and survival rates of three caries treatment approaches: Non-Restorative Caries Treatment (NRCT), the Hall Technique (HT), and Conventional Restorations (CR), for management of occluso-proximal caries lesions (ICDAS 3–5) in primary molars. In this multi-centre, secondary care-based, three-arm parallel-group, randomized controlled trial, 169 children, with one included tooth each, (3–8-year-olds; mean = 5.56, SD = 1.45) were allocated to: NRCT ($n = 52$; opening-up the cavity and applying fluoride), HT ($n = 52$; sealing caries with stainless steel crowns without caries removal), CR ($n = 65$; control arm, complete caries removal and compomer restoration), and treated by 12 paediatric dentists. Statistical analyses: Non-parametric Kruskal-Wallis analysis of variance, Mann-Whitney U test and Kaplan-Meier survival

analyses with Mantel-Cox statistics. 141 children (80.1%) had 23 months minimum follow-up. Patients' dropouts were censored. Nine patients (6.4%) experienced at least one 'Major' failure (irreversible pulpitis, abscess, unrestorable tooth): NRCT 3 (2.1%), CR 5 (3.5%) and HT 1 (0.7%). Twenty-five teeth experienced at least one 'Minor' failure (reversible pulpitis, caries progression, and secondary caries): NRCT 9 (6.4%), CR 14 (10%), HT 2 (1.4%). By independently comparing two samples, there was no statistically significant difference in failures between NRCT-CR ($p = 0.59$, CI = 0.40 to 0.65). Continuing the trend from one-year results, there were still differences between NRCT-HT ($p = 0.018$, CI = 0.015 to 0.022) and CR-HT ($p = 0.004$, CI = 0.004 to 0.007). The cumulative survival rate was 70.7% for CR, 74.4% for NRCT and HT 92.5% ($p = 0.045$). NRCT and CR outcomes were comparable. The HT performed better than NRCT and CR for all outcomes.

This study was funded by Greifswald University/Germany, Paediatric Dentistry Department (Trial registration no. NCT01797458).

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Resin Infiltration of Caries in Primary Molars in a Community Setting: 2-Year RCT Findings

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Aim: The aim was to assess whether resin infiltration of primary-molar proximal lesions is more effective than non-operative measures in controlling caries progression. **Methods:** A split-mouth randomized control trial: 90 children – each with 2 proximal lesions confined to the inner half of enamel or ≤ 0.5 mm into dentine – were included. Lesions were randomly allocated to test (infiltration: ICON-pre-product; DMG and fluoride varnish), or control (fluoride varnish). The primary outcome measure was 24-month radiographic lesion progression. The placement of a restoration during the study period was counted as lesion progression. The proportions of progressed lesions were compared using the McNemar test. Children also reported their acceptability of treatment. **Results:** Children in the study (46% female) ranged in age from 6 to 9 years. Their mean d_3mfs was 6.0 (SD 6.4); one tooth had been extracted due to caries. At baseline, 58% and 42% were moderate and low risk respectively. Test and control lesions presented with similar ICDAS, papillary bleeding and plaque stagnation scores at baseline. At 24-month follow-up, 6 children had moved away and fifteen teeth had exfoliated. In the test and control groups, 15/67 lesions (21.1%) and 30/69 lesions (43.5%) respectively had progressed ($P < 0.05$; McNemar). The 2-year therapeutic effect, based on pair-wise radiographic readings between infiltration and fluoride varnish, was 21.1%. Nearly all children (81.1%) responded that they had enjoyed their visit to the clinic and more than two-thirds (72.2%) responded that they were not anxious about returning for treatment. **Conclusions:** Infiltration is better than fluoride varnish

for controlling caries progression in proximal lesions in primary molars.

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Long-Term Surface Reflection Intensity Measurements of Artificial Caries Lesions on Native Enamel

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Aim: The aim was to measure surface reflection intensity (SRI) of native human molars using a hand-held sensor (Optipen) during caries development by a multi-species biofilm and after brushing/remineralisation. **Methods:** One-hundred unpolished human caries-free molars were included in the study. They were divided into 5 groups (n = 20) and then forwarded into a multi-species biofilm model for 2, 4, 6, 8, and 10 weeks, respectively, at 37°C in a microaerophilic atmosphere. During the experiment, the alternating media were brain heart infusion (BHI, 20 ppmF from NaF, 1% saccharose) for 6 h and buffer solution containing KH₂PO₄/Na₂PO₄ for 18 h and during weekends. The teeth were then placed in a remineralising solution and underwent controlled tooth brushing using fluoridated toothpaste slurry twice daily for 10 weeks. SRI measurements and visual caries assessment using with the International Caries Detection and Assessment system (ICDAS) were performed initially and throughout the duration of the experiment. The primary outcome parameter was %SRI reduction. Non-parametric ANOVA and Bonferroni-Holm correction were applied. **Results:** White spot lesions were visible after 6 weeks in most specimens (ICDAS score 1 or 2). Mean %SRIs dropped by 25% after 8 weeks of caries induction (p < 0.01). Cyclic brushing/remineralisation increased %SRI values of younger white spot lesions over 10 weeks in groups 1–3 (p < 0.01), but did not affect longer established white spot lesions in groups 4 and 5. Similarly, white spot lesions that developed up to 6 weeks in the biofilm model became visually undetectable after 8 weeks of cyclic brushing/remineralisation. **Conclusions:** Under standardized conditions Optipen was able to detect reflection intensity changes associated with undisturbed bacterial growth under cariogenic conditions. Influence of brushing/remineralisation was detectable for white spot lesions that developed during 6 weeks under cariogenic biofilm.

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The Role of Matrix Metalloproteinases (MMP2 and MMP3) Genetic Polymorphisms in Dental Caries Susceptibility

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The etiology of dental caries involves genetic factors. In the last decade it was proved that both pro- and active forms of host derived matrix metalloproteinases (MMPs) are present in the oral cavity and are potential participants in dental caries susceptibility. The aim of the present study is to estimate the role of genetic factors – *MMP2* and *MMP3* polymorphisms in dental caries susceptibility. Participants were 102 ethnic Bulgarian volunteers (42 males and 60 females), all students in Medical University of Sofia. All subjects were clinically examined DMFT data was recorded in forms. According to the DMFT the students were divided into 3 main groups – caries-free controls (DMFT = 0, n = 20), caries-resistant students (DMFT ≤ 5, n = 41) and caries-active (DMFT ≥ 9, n = 41). Each group was separated to males and females. DNA sampling procedure included collection of epithelial buccal cells and saliva in sterile Ependorf-type plastic containers (2 ml). The polymorphisms of *MMP2* (rs2287074) and *MMP3* (rs679620) genes were analysed by PCR-restriction fragment length polymorphism (RELP) in all volunteers. Data was processed with statistical program SPSS 16.0. Analysis of the *MMP2* gene showed significant differences (p = 0.007, p = 0.014) between the allelic and genotype frequencies of the caries free (DMFT = 0) and caries resistant (DMFT ≤ 5) groups. Significant differences were found also between caries resistant and caries active (DMFT ≥ 9) groups for the *MMP3* genes. In conclusion dental caries is probably under the precise control of several human genes that play a role in disease pathogenesis. *MMP2* and *MMP3* genes might be involved in caries susceptibility and progression.

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Infiltration of Proximal Caries Lesions – Efficacy after 7.5 Years

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Several studies with up to five years follow-up have shown efficacy of proximal caries infiltration to inhibit lesion progression. The aim of this randomized split-mouth placebo-controlled clinical trial was to assess the efficacy of resin infiltration of proximal

caries lesions after a longer follow-up up to eight years. In 22 young adults, 29 pairs of proximal caries lesions with radiological extension into the inner half of enamel or the outer third of dentin were randomly allocated to two treatments (split-mouth design). Test lesions were infiltrated (Icon, pre-product; DMG), mock treatment was performed for control lesions. All participants received instructions for diet, flossing, and fluoridation for the whole dentition. After approximately 7.5 years (range 86–98 months) 13 patients with 16 lesion pairs could be re-examined clinically and radiographically (two blinded examiners). Additionally one lesion pair had been restored after progression at 18 months follow-up. Pairwise comparison of radiographs showed progression in 1/17 test and 8/17 control lesions ($p = 0.016$; McNemar test). Although drop-out rate was rather high after 7.5 years and overall lesion progression rate was low, resin infiltration of proximal caries lesions was shown to more efficacious in reducing lesion progression compared with non-invasive measures alone.

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Caries Prevalence of 5, 12 and 15-Year-Old Greek Children Using the ICDAS II System. A National Pathfinder Survey

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The aim was to determine the caries prevalence and experience of 5, 12 and 15-year-old Greek children with respect to sociodemographic and educational level of parents and compare the findings to a similar survey conducted 10 years previously. A stratified cluster sample was selected according to WHO guidelines for national pathfinder surveys, from 24 sites (15 urban, 9 rural) with 100 children randomly selected from each site. All children were examined in a designated room of each school, with a head light, a #5 dental mirror and a CPITN probe by five calibrated examiners. Caries was recorded according to ICDAS₀₋₆ and caries experience according to WHO (MFT/mft) criteria and expressed using a modified d_{0-6mft}/D_{0-6MFT} index. The chi-square test and multivariable regression models were used to analyze the data and the threshold of significance was ≤ 0.05 . The percentage of $d_{3-6mft} = 0$ for the 5 yo and $D_{3-6MFT} = 0$ for the 12 and 15 yo children was 42.4, 27.7 and 16.3%, compared to 57.2, 37.1 and 28.9% examined 10 years previously. However, 17.7, 19.8 and 16.6% were recorded with ICDAS₁₊₂ with a mean of 0.93, 1.75 and 2.56 respectively. The mean d_{3-6mft}/D_{3-6MFT} score of the children was 1.48, 1.62 and 2.52 compared to 1.77, 2.05 and 3.19 scores 10 years previously. However, 86.8, 62.7 and 60.1% of the children presented with untreated caries, compared to 86.6, 45.3 and 55% 10 years ago. In conclusion, despite the decrease in the prevalence of caries in Greek children disparities still remain, necessitating in conjunc-

tion with the untreated needs and the identified significant number of incipient lesions, a more drastic intervention with better targeting of preventive programs at a national level.

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Assessment of Dental Sealants in a School Based Sealant Program in Children Seen >3 Years

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The clinical success and caries preventive effects of dental sealants (DS) depend on their retention. The aim of this study was to assess DS in children seen >3 years as part of a School-Based Sealant program (SBSP). Preventive oral health services, including DS, were provided by third or fourth year dental students under faculty supervision. The program returns to sites every 12 months to examine and provide services for children with parental consent, to check the integrity of DS previously placed by the program and repair or replace missing ones. Records from 7/01/2005 to 5/31/2015 were searched. Children examined >4 times (over at least 3 years) were included in the study. DS placement technique, case-selection criteria (sound surfaces and non-cavitated caries), and the DS brand remained consistent. Presence of dental caries or a restoration on previously-sealed teeth determined failure of that DS. 379 records of children ages 6–18 qualified for the study. 131 (35%) of the files were randomly selected and evaluated. Comparisons for survival of dental sealants placed in 1st permanent molars were made using t-tests. 430 DS were placed on 1st permanent molars out of 524 (82%); 21 (5%) DS converted into restorations and 22 (5%) DS developed caries (mean conversion time = 4 years, min = -3, max = 9). 100 (74%) of the children remained caries free. DS placed on lower-molars were significantly more retained versus DS on upper-molars ($p = 0.05$). Lower right molars had an increased risk of developing caries and/or receiving a restoration; upper left molars had the lowest risk. Sealants were able to prevent caries or placement of a restoration in this SBSP.

The study was funded by the Indiana University School of Dentistry.

Fluoride Varnish and Resin Modified Glass Ionomer Cement as Treatment for Active Occlusal Caries Lesions on Erupting Molars: A Randomized Clinical Trial

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Objectives: The objective was to evaluate the effectiveness of resin-modified glass ionomer cement for pit and fissures sealing and fluoride varnish on arresting active enamel carious lesions on molars in different stages of eruption. **Materials and Methods:** This randomized parallel longitudinal clinical study included 27 children (± 7.92 years old) with active lesions on 64 permanent molars (± 2.37 teeth per children). Children were examined for eruption stage, biofilm accumulation on site, whole mouth gingival bleeding index, whole mouth ICDAS scores and for activity status of the lesions. **Results:** Children were randomly treated. 33 teeth were sealed using Clinpro XT Varnish (3M ESPE) and 31 teeth treated with fluoride varnish (Duraphat, Colgate). Children were reassessed after 3, 6, 9 and 12 months. After 12 months, 97% of the lesions that were sealed and 74.2% of the lesions that received fluoride varnish applications ($p = 0.04$) were arrested. Children's age and type of treatment were significantly associated with lesion arrestment. **Conclusions:** Although both treatments were effective on caries arrestment, resin modified glass ionomer cement performed statistically better than fluoride varnish application after a 12-month follow-up period.

This research received material donation from 3M ESPE.

The Potential Dental Health Implications of Novelty Sweet Consumption

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Novelty sweets resemble or can be used as toys, are brightly coloured, with striking imagery. They encourage regular consumption as packaging can be resealed, leading to prolonged exposure. The aim of this study was to assess the most commonly available novelty sweets in the greater Cardiff area and their price. Furthermore, their sugar content, erosion potential and viscosity was also assessed. The most popular novelty sweets were assessed by undertaking scoping visits of shops in Cardiff city centre, 3 supermarkets and around the school fringe of 5 local high schools. Ten of each sweet were assayed. The pH was assessed using an electronic pH meter, the neutralisable acidity was measured by titration against 0.1 M NaOH and erosion of human enamel was assessed using

contact profilometry after a contact period of 1 hour. Viscosity was measured using a rotational viscometer and sugar content was measured using a refractometer. The ten most common novelty sweets were Brain Licker, Push Pop, Juicy Drop, Licked Lips, Big Baby Pop, Vimto candy spray, Toxic Waste, Tango candy spray, Brain Blasterz Bitz and Mega Mouth candy spray and their price ranged from 39p to £1. The pH of these ten novelty sweets ranged from 1.9–3.2 and their neutralisable acidity ranged from 9–202 ml of 0.1 M NaOH. The enamel erosion caused by the sweets ranged from 2.5–17.6 μm . The viscosity of these sweets ranged from 2–594 centipoises and their sugar content ranged from 31–73%. A wide range of novelty sweets were easily accessible and priced within pocket money range. The sugar content was found to be high and the erosive potential of these sweets was also high and they therefore have the potential for significant harm to teeth.

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SEAL Cambodia Caries Prevention Using a Modified Protocol for School-Based GIC Sealant Placement

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Background: Cambodian children have a high caries burden, with most cavitated lesions untreated. The SEAL Cambodia Project was initiated in 2012 with the goal of placing Glass-Ionomer Cement (GIC) fissure protection on the first permanent molars of 60,000 6 to 8-year-old children over three years. A revised sealant placement protocol (RP) was introduced in 2014 to address the putative impact of ambient temperature and clinical methodology on sealant efficacy. **Methods:** Sealants using hand-mixed GIC were placed by several trained operators in school premises following an original written and visual protocol (OP). The changes introduced in the RP involved cooling the GIC liquid in a cup of ice, timed hand-mixing (20 s), timed finger press (1 min), and occlusal adjustment following placement. Random cluster sampling was used at school level from RP participants for 1-year follow-up. The data were analysed against 1-year follow-up of a representative sample from OP group. The outcome measure was the presence or absence of new cavitated carious lesions in the occlusal fissure system on first permanent molars. Caries data (DMFT) was collected by four calibrated examiners. **Results:** RP ($N = 65$) 53.8% male, mean age 6.5 ± 0.5 years; OP ($N = 255$), 53.3% male, mean age 8.2 ± 1.2 years. The 1-year DMFT increment was: OP group 1.0 ± 1.2 ; RP group 0.5 ± 0.9 new lesions ($P < 0.001$; $\chi^2 = 10.917$). Within RP group, all participants with untreated cavitated carious lesions in first permanent molars at baseline ($N = 14$) developed new lesions, accounting for three-quarters of those with new lesions ($N = 19$). **Conclusions:** The RP is more effective at preventing carious lesion development than the OP.

The Study was funded by GC AsiaColgate PalmoliveCamKids Australia and New Zealand Society of Paediatric Dentistry.

Self-Efficacy Theory Based Intervention in Adolescents: A Cluster Randomized Trial

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This cluster randomized trial tested the efficacy of professional dental education for improving oral self-care skills (OSC-S) and oral self-care practice (OSC-P) in adolescents. All 15–16-year old adolescents from four public schools were invited and 206 agreed to participate. Schools were randomly allocated to the intervention group and to the control group. Five sessions were given for the intervention group and one for the control group. The OSC-S and OSC-P outcomes were measured as % Oral Cleanliness Scores at the baseline, 6-months and 12-month observations. Self-efficacy was measured as an adolescent's self-belief (likelihood) to perform oral self-care. We found OSC-S and OSC-P correlated significantly (Pearson's) at the baseline ($r = 0.777$, $P < 0.001$), at the 6-month ($r = 0.745$, $P < 0.001$) and at the 12-month ($r = 0.780$, $P < 0.001$) examinations. After the guided oral health care interventions, a significant time and group effect was observed (Repeated Measures ANOVA, $p < 0.001$ for the OSC-S (multivariate $\mu^2 = 0.355$) and for the OSC-P (multivariate $\mu^2 = 0.325$). There was a consistent improvement of oral self-care of all tooth surfaces in the intervention group after the discontinued intervention for another 10 months. In conclusion, OSC-S and OSC-P scores were significantly correlated. Self-efficacy theory guided intervention was superior to the conventional dental instruction to improve oral self-care in adolescents. Varying levels of oral self-care improvement were observed among the intervention group adolescents.

Variation in the Taste Gene TAS2R38 Is Associated with the Lactobacillus Counts and Caries Risk

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Genetic variation in taste receptors may account for differences in food choices and dietary habits. The aim of this study was to test the hypothesis that a single nucleotide polymorphism (SNP) in TAS2R38, which codes for a taste receptor for bitter, negatively impacts carbohydrate intake, and lactobacillus counts and, consequently, could increase the caries risk in adults. Participants were 154 healthy inhabitants of Isparta city in Turkey who were 20–60 years of age. Genomic DNA of 77 adults with 'high caries risk group' (DMFT ≥ 14) and 77 adults with 'low caries risk group'

(DMFT ≤ 5) was extracted from the buccal mucosa. The frequency of SNP in the TAS2R38 (A49P) was genotyped with PCR-RFLP method. Chair-side test was used to evaluate salivary counts of lactobacillus (Dentocult LB, Orion Diagnostica). The lactobacillus counts were scored in four classes (0–3) according to provided model chart, where 0=the lowest number of microorganisms. The patients were interviewed with a standardized structured questionnaire to elicit information on dietary intake between meals. The frequencies of normal homozygote (CC genotype) and C allele were 78.9% and 62.9% in high caries risk group, and 21.1% and 37.1% in low caries risk group, respectively. There were significant differences between the groups related to genotype and allele frequencies of TAS2R38 (X^2 , $p = 0.000$). The lactobacillus count was lower ($p = 0.000$) and dietary intake between meals was significantly less frequent ($p = 0.002$) in individuals in the low caries risk group (Mann-Whitney U). The presence of genotype CC and allele C was less frequent in individuals with the lowest lactobacillus counts. In conclusion, individuals with the normal homozygote genotype had higher carbohydrate intake, and higher lactobacillus counts, and variation in TAS2R38 (A49P) was negatively associated with caries risk.

The study was supported by the Suleyman Demirel University Scientific Research Projects Foundation.

Dental Caries Status of Institutionalized Young Adults with Mild Mental Disability and Multi-Problems – First Results

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The aim of this study was to describe the oral health of institutionalized young adults with mild mental disability and multi-problem behaviour. The study protocol was approved by the Medical Ethics Review Committee of VU University Medical Center (2015.340) and signed informed consent was obtained from all participants. Residents were excluded from participation when not mentally competent to give consent by themselves or when not yet 18 years old. Oral assessments (caries assessment: DMFT) were performed at 30 residents (16 female) with mean age of 22.2 years (SD 2.4, range 18–27). Individual oral hygiene instructions were given twice. Their IQ scores and diagnoses were obtained from their medical records. The data were compared with a study on 450 23-year olds with a low socio-economic status (reference group) in the Netherlands [Schuller et al. 2013]. The mean IQ score was 71.8 (SD 6.1, range 55–82). The mean DMFT was 7.6 (SD 5.8, range 0–18); with 10% DMFT 0, which was not significantly different from the reference group (DMFT = 6.1 (SD 5.5) with 11% DMFT 0). The need for dental treatment ($100 - \text{Restorative Care Index}$; with $\text{RCI} = (\text{FS}/(\text{DS} + \text{FS})) \times 100\%$) in this group was 42% (SD 34) which was significantly higher than the control group: 17% ($p <$

0.001, t-test). 20% of the participants brushed their teeth less frequently than twice daily (30% of 23 year olds in the Netherlands [Schuller et al. 2013]). This study indicates that the mean DMFT-value of this group is similar to a reference group within the Dutch population. The RCI is significantly lower in this group which indicates extra need for treatment and attention regarding oral health.

This study was performed at the Salvation Army – mental disability care, Amsterdam.

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Clinical Evaluation of Three Techniques for Treating Carious Lesions in Primary Incisors

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Aim: To compare the clinical results of three techniques for treating carious lesions in primary incisors in children. **Methods:** The study was carried out in Volgograd Pediatric Dental Clinic №2 with approval from the regional Ethics Committee. The parents' informed consents were obtained. In 34 children aged 12–51 months 69 smooth surfaces of primary incisors with caries (ICDAS-II, score 5) were treated by an experienced dentist. All cases were randomly selected in equal 3 treatment groups (n = 23): CT – conventional treatment (drilling and filling with glass ionomer cement (GIC), NIRT – non-invasive restorative treatment (sealing cavitated caries lesions with GIC without/with partial ART-excavation of carious dentin) and NRCT – non-restorative caries treatment (no caries removal, opening up the cavity, teaching brushing and fluoride application). The clinical results of caries treatment were assessed 12 months after the treatment by the same dentist. The criteria for assessment were: successful treatment' (retention of dental restoration/sealant without any signs of secondary caries/arresting caries lesions) or unsuccessful treatment' (restoration/sealant loss, caries progression). Chi Square test was used to compare the results at p-value 0.05. **Results:** After a 12-month follow-up 65.2% treated smooth surfaces of the incisors in CT group, 82.6% in NIRT group and 86.9% in NRCT group were evaluated as successful treatment' and 34.8% CT, 17.4% NIRT and 13.1% were evaluated as unsuccessful treatment' respectively. Main reasons of unsuccessful treatment were restoration/sealant loss' in CT and NIRT groups and caries progression' in NRCT group. The differences were not statistically significant between all groups (p > 0.05). **Conclusion:** The NIRT and NRCT were more successful clinically than CT technique for caries treatment in primary incisors in the children after 12-month follow-up but observed differences were not significant perhaps due to the low numbers of subjects/surfaces per group.

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Association of the Xerostomia Inventory with Saliva Properties and Root Caries in Older People

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The purpose of this study was to investigate the association of the validated in Greek Xerostomia Inventory (XI_Gr) with specific saliva properties (pH, salivary flow, quality) and the root caries index. One trained investigator examined 127 dentate subjects aged 60 years and over ($\bar{x} \pm SD: 72.4 \pm 6.9$), at the Open Care Community Centers for the Elderly in Athens. Clinical oral examinations included assessment of the state of root surfaces, both visual and tactile, using a WHO probe and a mirror, stimulated salivary flow rates (after chewing a paraffin wax pellet) and saliva pH measuring with specific pH indicator papers. Salivary quality was estimated by visual observation, as 'vesicular or serous'. The interview recorded sociodemographic data and the XI_Gr (7 items, range: 7–35) with higher values indicating more xerostomia complaints. Descriptive statistics were calculated and Mann-Whitney Tests and Pearson Correlation coefficients investigated the associations between variables ($P \leq 0.05$).

The mean XI_Gr value was 13.2 (± 4.8 SD) and the mean stimulated saliva flow rate was 0.8 (± 0.3 SD) ml/min. Root caries occurred in 26.8% of the subjects. Negative moderate correlations were recorded between the XI_Gr and salivary flow ($r = -0.412$, $P < 0.001$) and saliva pH ($r = -0.277$, $P < 0.001$). The XI_Gr was also significantly associated with hyposalivation ($p < 0.001$) with cut-off values set at 0.5 ml/min. A higher XI_Gr value was significantly associated with vesicular saliva quality (Mann-Whitney Rank Sum Test, $P = 0.019$). There was also a moderate positive correlation between XI_Gr and root caries index ($r = 0.333$, $P < 0.001$). In conclusion XI_Gr was significantly associated with hyposalivation and increased root caries prevalence. The diagnostic value of the index, particularly when applied in frail older persons with reduced access to dental care, requires further investigation.

The study was funded by the Department of Operative Dentistry, Dental School, University of Athens.

Effects of the Oral Health Education on Schoolchildren and Their Parents: Intergenerational Study

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The aim of this study was to assess if educational interventions toward children could allow to increase the educational level in oral health of their parents and/or to change their behavior regarding oral hygiene and thus to possibly highlight a transmission of the oral knowledge in the children to parent direction. The parents of 225 9–10 years old schoolchildren from 3 schools (in Rennes, France) were included in the study. Seventy four children came from a private school (48 in test group and 26 in control group) and 151 came from a public school (76 tests and 75 controls). The test group had explanations about tooth decay, link between oral and general health, oral hygiene, proper diet, fluorides. All parents were given a questionnaire (A) twice: before and after the children education sessions. They were questioned about their knowledge in oral health. A final questionnaire (B) concerning their change of behavior was also distributed. In the control group parents had mean 13.56 right answers for 20 questions before and 13.96 after (Student, $p > 0.05$). In the test group, mean number of right answers rose from 13.28 to 15.93 after the sessions (Student, $p < 0.0001$). The strongest improvements was for the questions about the link between oral health and general health, the definition of the dental plaque, the number of temporary teeth, the possible absence of caries thanks to prevention or the need to supervise children tooth brushing. Test group children spoke and tried to influence their parents about the oral hygiene, and that the latter put into practice some of these notions (especially taught tooth brushing technique, tooth brushing duration and child brushing supervision). In conclusion this study showed that the oral health education of the children can also allow to improve the knowledge and the behavior of their parents.

Toothbrushes and tooth paste provided by Unilever.

Is Deciduous Molar Hypomineralization a Predictor to Molar Incisor Hypomineralization?

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The aims of this cross-sectional study were to evaluate the prevalence and relationship between the deciduous molar hypomineralization (DMH) and the molar incisor hypomineralization (MIH) in a group of schoolchildren. The study population consisted of 1.971 6–11 year-old children from public schools of Paranoá/DF, Brazil. After a calibration exercise, three examiners performed the exams using the EAPD criteria for scoring DMH and MIH at schools premises. Only children with the 4 first-permanent molars and 8 incisors were considered for calculating MIH prevalence ($n = 859$), while for DMH prevalence, only children with the 4 secondary primary molars were considered ($n = 1592$). For evaluating the relationship between MIH and DMH, only children with both criteria cited above were considered ($n = 534$). Statistical analysis was performed using Chi² test and Odds ratio. The Cohen's kappa was around 0.61 to 0.69 for both variables. The prevalence for MIH was 14.78% (127 children from 859). For DMH, the prevalence was 6.41% (102 from 1592). A significant relationship was observed between MIH and DMH ($p < 0.05$). The odds ratio for MIH based on DMH was of 6.31 (95% CI, 2.59–15.13). From the results, it can be concluded that DMH may be used as a predictor to MIH, as children with DMH are 6 times more likely to develop MIH.

Session 4

De- and Re-Mineralization

73**Effect of Bovine Serum Albumin on the Subsequent Remineralisation of Enamel Subsurface Lesions in vitro**

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The aim of this study was to assess the effect of bovine serum albumin (BSA) on the subsequent remineralisation of enamel subsurface lesions. Artificial subsurface lesions were created on thirty enamel blocks that were subsequently sectioned into control and experimental halves. Experimental halves were randomly allocated to one of three pre-treatments: 1) 100 mM HEPES buffer for two days; 2) 100 mM HEPES buffer and 1 mg/mL BSA for two days; 3) 100 mM HEPES buffer and 1 mg/mL BSA for two days followed by two minutes immersion in 13.4 mM NaOCl. Localisation of BSA within the lesion was assessed independently with confocal microscopy and the use of a BSA-fluorophore conjugate. Intact BSA-fluorophore conjugates were observed inside subsurface lesions after pre-treatment with the BSA-fluorophore solution, however no BSA-fluorophore conjugate was observed in lesions following NaOCl pre-treatment. Each experimental half-block was then immersed in 1% (w/v) CPP-ACFP solution (pH 5.5) for ten days at 37 °C with a change of solution every two days. Experimental half-blocks were matched with their corresponding control half for analysis of percent remineralisation (%R) using transverse microradiography. %R of lesions pre-treated with HEPES and BSA ($31.44 \pm 6.01\%$) and lesions pre-treated with HEPES alone ($34.82 \pm 6.88\%$) were not significantly different ($p > 0.05$). %R of lesions pre-treated with NaOCl ($45.48 \pm 5.49\%$) were significantly higher than those pre-treated with HEPES and BSA ($p < 0.001$) and those pre-treated with HEPES alone ($p < 0.002$). In this study, BSA had no effect on subsequent remineralisation of enamel subsurface le-

sions, however pre-treatment with NaOCl did enhance remineralisation.

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74**Factors That Determine the Progression Pattern and the Conical Shape of Carious Enamel Lesions**

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Natural carious enamel lesions have a conical/triangular morphology with a wide base at the external tooth surface and the tip towards dentin-enamel junction. Despite extensive investigations, the factors that define the demineralization pattern of enamel are not fully clear. The aim of this study was to test the hypothesis that the pattern of lesions may be associated with the variations in the mineral structure of enamel and the differential demineralization resistance of enamel in outer and inner areas. First we studied the mineral density patterns through the whole thickness of enamel in nineteen sound extracted premolars using a high resolution micro-computed-tomography (XRMT) system with continuous mode exposures at 0.5 s intervals, a binning value of 2, an accelerating voltage of 60 kV and a current of 120 μ A. Image reconstruction, de-noising, colorization and creation of mineral density maps were performed in Nrecon and MATLAB. A calibration equation was used to transform the grey-level values into true mineral density values. At the next stage, the resistance of inner and outer enamel against demineralization was studied by immersing the specimens in demineralization solutions (0.1 M lactic-acid, pH 4.2) for 3-weeks at 37°C followed by XRMT scanning. Resultant XRMT mineral maps of sound enamel revealed that enamel has a gradient mineral structure which is in the form of layers of iso-

density zones with a gradient of decreasing densities from enamel surface (2.83 gr/cm³) towards DEJ (2.43 gr/cm³). The iso-density zones with lower density which were observed in the inner enamel extended to the fissural area allowing the evaluation of the effect of demineralization on inner and outer iso-density zones at the same time. The results of the XRMT scans of demineralized specimens showed a gradient of differential resistance against demineralization associated with variations in mineral density. Accordingly the internal iso-density zones extending to the fissure area have higher resistance and the external iso-density zones, have lower resistance against demineralization; indicating an inverse relation between the mineral density and demineralization resistance. The gradual increasing resistance of the enamel is a limiting factor in the progression of the lesion which can gradually decrease the width of the demineralization area from outside towards inside and therefore create a conical shape for the lesion.

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Model Parameters for Caries Initiation Adjacent to Composite by a *Streptococcus mutans* Biofilm

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The present study evaluated two factors for secondary caries formation using an artificial biofilm model in vitro. Within each bovine enamel sample (n = 44; 8 x 6 x 3 mm) a standardized cavity (6 x 3 x 2 mm) was created. Samples were randomly allocated to two groups: 1. Pre-treatment using phosphoric acid (37%) and an adhesive (Solobond M, Voco) at the cavity bottom (Bottom); 2. Adhesive application to all cavity walls without prior etching (All). Afterwards cavities were filled with a composite (Grandio flow, Voco). To create gaps at the restoration margins artificial aging was simulated [Storing of samples in distilled water (7d; 37°C), thermocycling (5000 cycles; ±5/±55°C)]. *Streptococcus mutans* (UA 159; approx. 10⁶ cells/sample) was grown on the samples to initiate caries. During the incubation process (7d) half of the samples of each pre-treatment group were either treated using a nutrition medium with (Bottom-S, All-S) or without 30% autoclaved human saliva (Bottom, All), respectively. The gap size and the formation of enamel caries lesions were analyzed using confocal laser scanning microscopy and transversal microradiography. Median gap size in enamel was 8.3 (interquartile range 7.1–8.9) µm and did not differ significantly between the four subgroups (p > 0.05; Kruskal-Wallis test). Median mineral loss next to restoration margins was significantly higher in Bottom-S [7254 (6571–9195) vol%×µm] and All-S [7860 (6866–8333) vol%×µm] compared to both Bottom

[3689 (3614–4411) vol%×µm] and All [3660 (3444–3994) vol%×µm] (p < 0.05; Mann-Whitney test), but did not differ significantly between the groups with different pre-treatments (p > 0.05). The combination of an aging procedure and the biofilm model seems to result in a reproducible formation of secondary caries. The results indicate that the addition of saliva, but not the type of pre-treatment chosen caused higher mineral loss.

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Effect of Zinc on Bovine Enamel Under pH-Cycling Conditions

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Enamel demineralisation is highly prevalent during orthodontic treatment. Newly developed Zinc doped phosphate-based glasses (Zn-PBGs) are controlled delivery agents for zinc ions that may be effective in reducing the incidence of white spot lesions. Zinc doped (3 mol% zinc and varying calcium concentrations, denoted as C11, C12 and C13) and control Zinc free PBG (C-PBG) rods (5 x 2 mm) were produced using a conventional melt quenching method, at 1100°C for 1 hour. Degradation studies (0–56 h) were carried out using a weight loss method in dH₂O at 37 ± 1°C and starting pH of 7 ± 0.1. pH analysis was conducted and calcium, zinc, sodium and phosphate concentrations remaining in solution were measured by inductively-coupled optical-emission spectrometry (ICP-OES). The effect of Zn-PBG on bovine enamel was investigated under pH cycling conditions, using non-contact surface profilometry, NCSP (Proscan 2000) and transverse micro-radiography (TMR). All tests were conducted in triplicate. Statistical analyses were conducted using the GraphPad software (San Diego, California, USA). Tukey-Kramer multiple comparison tests were used to compare values and p values < 0.05 were considered statistically significant. pH analyses showed no significant difference (p > 0.05) between Zn-PBGs and C-PBG (6.95 ± 0.25 for C-PBG, 6.87 ± 0.32 for C11, 6.71 ± 0.05 for C12 and 6.71 ± 0.05 for C13). But the degradation rates of the Zn-PBGs were significantly (p < 0.05) different (28.94 µg mm⁻² h⁻¹ for C11, 25.56 µg mm⁻² h⁻¹ for C12 and 19.72 µg mm⁻² h⁻¹ for C13) compared with C-PBG (10.17 µg mm⁻² h⁻¹). Zinc ion release rates were decreased as calcium concentration increased in the glasses, which correlated well with the weight losses of glasses. Controlled delivery of zinc ions from Zn-PBGs may have potential in oral applications.

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New Preventive Therapies for Remineralisation of Enamel in vitro

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The aim of this study was to investigate in vitro the effect of CPP-ACP containing products; Tooth Mousse (TM), MI Paste Plus (MI plus), in the remineralisation of enamel subsurface lesions when used supplementary to fluoridated toothpastes (FTP). Methods: 24 bovine enamel slabs with subsurface artificial lesions were used in each of five groups: 1- 0 ppm FTP; 2- 1450 ppm FTP; 3- 2800 ppm FTP; 4- 1450 ppm FTP+TM and 5- 1450 ppm FTP+MI plus. Each day the slabs underwent two 30 mins treatments in the toothpaste and one 30 mins treatment in the CCP-ACP containing products for groups 4 and 5: The enamel slabs containing artificial caries lesions were subjected to a pH cycling regimen and analysed by QLF images before and after 21 days. Data analysis was carried out using one way ANOVA. We found a statistically significant improvement in all groups for ΔF ($p < 0.000$) and ΔQ ($p < 0.01$) values. However, statistically significant reduction in the area values were observed in all groups ($p < 0.001$), except the 0 ppm F group. Comparison between groups showed that the ΔF in groups 1450 ppm F + TM, 1450 ppm F + MI plus and 1450 ppm F groups were statistically significantly higher ($p < 0.05$) than that for the 0 ppm F group. No statistical significant difference was found between the 2800 ppm F and the 0 ppm F groups. The ΔQ and area values of all tested groups were significantly higher than the 0 ppm F group. However, no significant difference was found between the test groups in the area values. In conclusion the use of TM and MI plus in conjunction with FTP showed increased efficacy in the remineralisation of enamel subsurface lesions; however, this did not reach a significant level in this in vitro model.

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Effects of Artificial Plaque Thickness and Saliva Flow Rate on Fluoride Efficacy in Caries Prevention

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This in vitro study investigated the interaction between artificial plaque thickness and saliva flow rate on fluoride efficacy in preventing caries lesion formation in enamel. The study followed a 4 (plaque thickness; 'no plaque'/1/2/3 mm) $\times$ 2 (saliva flow rate; 0.05/0.5 ml/min) $\times$ 2 (fluoride concentration; 0/383 ppm as sodium fluoride) factorial design. A continuous flow model was utilized. Agarose (1%) was used to prepare artificial plaque which' thickness was controlled by acrylic rings. Artificial saliva (1.5 mM

CaCl₂; 0.9 mM KH₂PO₄; 130 mM KCl; 20 mM HEPES; pH7.0) was injected into the chamber at 0.5 or 0.05 ml/min. The cariogenic challenge was: 50 mM acetic acid; 2.2 mM CaCl₂; 2.2 mM KH₂PO₄; 130 mM KCl; 0.2% Carbopol 907; pH5.0; 0.1 ml/min. Lesions were formed using a 5-day cycling model and characterized using Vickers microhardness. The three-way interaction gel thickness $\times$ flow rate $\times$ fluoride was significant ($p = 0.0006$). Fluoride: 383 ppm caused less softening than 0 ppm ($p < 0.0001$) regardless of gel thickness or flow rate. Flow rate: 0.5 ml/min caused less softening than 0.05 ml/min for 'no plaque' ($p = 0.0002$) and 1 mm plaque thickness ($p < 0.0001$) regardless of fluoride concentration, for 2 and 3 mm with 0 ppm F ($p < 0.0001$) but not for 383 ppm F (2 mm $p = 0.07$; 3 mm $p = 0.31$). Gel thickness: For 0.05 ml/min, softening was reduced as gel thickness increased from 'no plaque'-1-2 mm ($p \leq 0.001$), but not from 2-3 mm ($p = 0.54$). For 0.5 ml/min, 'no plaque' caused more softening than 1, 2, and 3 mm ($p < 0.0001$), but 1, 2, and 3 mm were not different from each other for 0 ppm F ($p = 0.08$ for 1 vs. 2 mm, $p = 0.11$ for 1 vs. 3 mm, $p = 0.85$ for 2 vs. 3 mm) or 383 ppm F ($p = 0.27$ for 1 vs. 2 mm, $p = 0.47$ for 1 vs. 3 mm, $p = 0.70$ for 2 vs. 3 mm). The anticaries efficacy of fluoride is modulated by both plaque thickness and saliva flow rate.

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Effect of Xylitol Varnishes on Remineralization of Artificial Enamel Caries Lesions in situ

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Aim: The aim was to analyze the effect of varnishes containing xylitol compared to commercial fluoridated varnishes on the remineralization of artificial enamel caries lesions in situ. **Methods:** Twenty subjects took part in this crossover, double-blind study performed in four phases of 5 days each. Each subject, worn palatal appliances containing four predemineralized bovine enamel specimens. Artificial caries lesions were produced by immersion in 30 ml of lactic acid buffer containing 3 mM CaCl₂·2H₂O, 3 mM KH₂PO₄, 6 μ M tetraethyl methyl diphosphonate (pH 5.0) for 6 days. The specimens in each subject were treated once with the following varnishes: 20% xylitol (experimental); DuoFluorid™ (6% NaF, 2.71% F + 6% CaF₂), Duraphat™ (5% NaF, positive control) and placebo (no-F/xylitol, negative control). The varnishes were applied in a thin layer and removed after 6 h. Fifteen subjects were able to finish all phases. The enamel alterations were quantified by surface hardness and transversal microradiography. The percentage of surface hardness recovery (%SHR), the integrated mineral loss and lesion depth were statistically analyzed by ANOVA/Tukey's test or Kruskal-Wallis/Dunn's test ($p < 0.05$). **Results:** Enamel surface remineralization (% median/IC) was significantly increased by Duraphat™ (12.9 (IC 9.3/23.6)), DuoFluorid™ 14.0 ((IC 6.9/18.6)) and 20% xylitol formulations (12.5 (IC 4.7/25.8)) compared to placebo (1.0 (IC -2.0/7.0)). Significant subsurface

mineral remineralization could also be seen for the experimental and commercial varnishes, except for Duraphat™, when the parameter 'lesion depth' was considered. **Conclusion:** 20% xylitol varnish seems to be a promising alternative to increase surface and subsurface remineralization of artificial caries lesions in situ.

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Root Caries Prevention via Sodium Fluoride, Chlorhexidine and Silver Diamine Fluoride in vitro

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Uncertainty exists as to how to best prevent root caries development. The aim of the present study was to compare sodium fluoride (NaF), chlorhexidine (CHX) and silver diamine fluoride (SDF) varnishes (V) and rinses (R) regarding their caries preventive effect in an artificial caries biofilm model. We hypothesized that mineral loss did not significantly differ between these preventive regimens. 140 bovine root dentine samples were cut, polished and embedded. Samples were allocated to seven treatment groups (n = 20/group): Four varnishes (applied once prior biofilm-challenge): 38% SDF (SDFV), 35% CHX-varnish (CHXV), 22,600 ppm NaF-varnish (NaFV), placebo-varnish (PV); two rinses (applied once daily during biofilm-challenge): 500 ppm NaF-solution (NaFR), 0.1% CHX-solution (CHXR); one control group (untreated). After pellicle formation, samples were transferred to a multi-station, continuous-culture *Lactobacillus rhamnosus* (LGG DSM 20021) biofilm model. Bacteria were inoculated 1× daily, 1% sucrose 8× daily, and artificial saliva 10 min after sucrose pulse. After 12 days, mineral loss (ΔZ) was measured in the effect area and adjacent to the varnished areas. Bacterial counts were assessed on DeMan-Rogosa-Sharpe agar. Mean $\pm$ SD ΔZ was significantly lower in the NaFR group (4687 ± 391 vol% $\times\mu\text{m}$) compared with all other groups ($p < 0.05$ /ANOVA, Turkey's post hoc). SDFV protected dentin against mineral loss significantly better compared with CHXV, CHXR and the control group ($p < 0.05$). Varnishes did not significantly prevent mineral loss in adjacent areas ($p > 0.05$). Bacterial numbers were significantly higher in the NaFR (1.78 ± 0.06 Log₁₀ (CFU+1)/ml) than all other groups except control group and areas adjacent to NaFV and PV ($p < 0.05$). In conclusion regular fluoride rinses showed highest root-caries preventive effect compared with antibacterial or fluoride varnishes, despite not reducing bacterial numbers within biofilms. Varnishes did not protect adjacent areas from demineralization.

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Impact of CO₂ Laser Irradiation on Enamel and Dentin Biofilm-Induced Mineral Loss

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The caries-protective effects of CO₂ laser irradiation on dental enamel have been demonstrated using chemical demineralization models. We compared the effect of CO₂ laser irradiation, sodium fluoride, or both on biofilm-induced mineral loss (ΔZ) of enamel and dentin in vitro. Ground, polished bovine enamel and dentin samples were allocated to one of four groups (n = 12/group): No treatment (C); single 22,600 ppm fluoride (F) varnish (5%NaF, pH = 4.5) application, single CO₂ laser treatment (L) with short pulses (5 $\mu\text{s}/\lambda = 10.6 \mu\text{m}$); combined laser and fluoride treatment (LF). Samples were sterilized and submitted to an automated mono-species *Streptococcus mutans* (ATCC 25175) biofilm model. Brain heart infusion plus 5% sucrose medium was provided 8× daily, followed by rinses with artificial saliva. After 10d, bacterial numbers in the biofilms were enumerated as colony-forming units/ml (CFU/ml) (n = 7/group). ΔZ was assessed using transversal micro-radiography (n = 12/group). Univariate ANOVA with post-hoc Tukey honestly-significant-difference test were used for statistical analysis. Bacterial numbers were significantly higher on dentin than enamel ($p < 0.01$ /ANOVA). On dentin, the combined treatment LF yielded significantly lower CFUs than other groups ($p = 0.03$ /Tukey), while no differences between groups was found for enamel. The lowest ΔZ in enamel was observed for L (mean/SD: 2036/1353 vol% $\times\mu\text{m}$), which was not only significantly lower than C (9642/2452 vol% $\times\mu\text{m}$), but also than F (7713/1489 vol% $\times\mu\text{m}$) (both $p < 0.05$). In dentin, only LF (163/227) significantly reduced ΔZ ($p < 0.05$). CO₂ laser irradiation did not increase adhesion of *S. mutans* to enamel and dentin surfaces in vitro. Laser treatment protected enamel against biofilm-induced demineralization, while a combined laser-fluoride application was required to protect dentin.

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Comparison of a Newly Developed Method Digital TMR with Conventional TMR Based on Glass Plates

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Transverse Micro Radiography (TMR) is the gold standard method to quantitatively assess the mineral profile. The classical method, TMR, comprises of X-Ray contact exposure of tooth sec-

tions onto, increasingly difficult to source, film or glass plates. Recently Inspektor Research Systems introduced a new method, TMRD, based on digitally capturing the X-Ray images. The aim of this study was to compare the quantitative parameters: Integrated Mineral Loss (IML) and Lesion Depth (LD) of both methods. TMR comprised of an X-Ray Generator operated at 20 kV and 20 mA using a Copper X-Ray source with an effective focus of Ø1 mm (Inspektor Research Systems). Glass plates were used. TMRD comprised of a Tungsten micro X-Ray focus source (Ø6 µm) operated at 45 kV and 45 µA. The images of the tooth sections were captured by an X-Ray detector with high sensitivity and high resolution (pixel size 9 µm) fitted with a machined input fibre optic scintillator. The distance between focus and tooth sections was 20 mm, the focus-detector plane distance was 100 mm providing a magnified image of 5X and a resulting image sharpness of 2–3 µm of the tooth section. Artificial caries lesions were created using a demineralising acid solution on the flattened surface of six extracted bovine incisors. With both methods microradiographic images were made and the sections were scanned at three positions with the same analysis software TMR 2006 (3.0.0.19). For TMR IML was 1760 µmVol% (SD 554 µmVol%) and for TMRD IML was 1601 µmVol% (SD 497 µmVol%), respectively. LD was 71 µm (SD 15 µm) for TMR and 73 µm (SD 17 µm) for TMRD. Correlation was found for IML ($r^2 = 0.95$) and LD ($r^2 = 0.95$). It is therefore concluded that digital TMR seems a promising alternative for classical TMR.

This study was funded by Inspektor Research Systems B.V.

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Effect of Highly Concentrated Fluorides Combined with a Saliva Substitute on Dentine Lesions in vitro

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Patients suffering from xerostomia lack oral comfort and are often afflicted with rampant caries in particular in dentine. Saliva substitutes are supposed to relieve the sensation of dry mouth. To increase remineralization additional use of fluorides is recommended. The aim of the present in vitro study was to evaluate the remineralizing effects of different highly concentrated fluoride agents in combination with a potentially demineralizing saliva substitute (Glandosane; pH = 5.1) being widely used in Germany. In each of 120 dentine specimens three artificial lesions were created. Subsequently, one of these lesions was covered with acid-resistant nail varnish for analysis of pre-demineralization (ΔZB). Treatments during pH-cycling (4×1 h demineralization [pH = 5.0] and 4×3 h Glandosane/day; 12 h 100%humidity) were: no treatment (NT), application (5 min) of 12.500 ppm F⁻ [pH = 6.04] (NaF-Gel1; ProSchmelz Fluorid Gelée), 12.500 ppm F⁻ [pH = 7.34] (NaF-Gel2; Paro Fluor Gelée), 12.500 ppm F⁻ [pH = 5.82] (AmF-Gel; Elmex

Gelée), 1450 ppm F⁻ [pH = 7.35] (KF-Gel; Fluor Protector Gel) and 5000 ppm F⁻ [pH = 8.14] (NaF-TP; Duraphat 5000 ppm Fluoride Toothpaste) for 7 days (E1) twice daily. Subsequently, from each specimen one lesion was covered, while the remaining lesion was cycled for another 7 days (E2). Differences in integrated mineral loss (ΔΔZE1/ΔΔZE2) were calculated between values before and after pH cycling (ΔZE1/ΔZE2). Mean (SD) ΔZB was 3851 (440) vol%×µm. Except for NaF-Gel2 and NaF-TP specimens of all other groups demineralized further. Only NaF-Gel2 induced a significant gain in mineral content ($p < 0.05$, t-test). Significant differences in the change of mineral loss [mean (SD)] were found between NT and all fluoride groups for ΔΔZE1 [NT: -1612 (553); NaF-Gel1: -935 (400); NaF-Gel2: 326 (505); AmF-Gel: -522 (631); KF-Gel: -704 (527); NaF-TP: -65 (492)] and ΔΔZE2 [-2667 (612); -326 (785); 580 (356); -460 (501); -405 (735); 53 (823)] ($p < 0.05$, ANOVA), but not among fluoride groups. In conclusion, slight remineralization was only observed for neutral NaF-Gel2 and 5000 ppm toothpaste under the conditions chosen.

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In vitro Real-Time 3D Monitoring of Artificial Lesion Progression in Human Enamel Using MicroCT

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We have previously reported the real-time progression of mineral loss in human enamel undergoing artificial lesion formation using scanning microradiography, a development of microradiography. However, such 2D studies require thin sections or small pieces of enamel. MicroCT is a 3D X-ray microscopic imaging method used to observe internal structures of materials including enamel. The aim was to use microCT to continuously monitor artificial lesion progression during demineralisation of a whole tooth in 3D, i.e. without the requirement to cut the specimen. A 2nd molar was coated with nail varnish except for a 2 x 2 mm window on the buccal surface. The tooth was mounted in a polycarbonate tubular environmental container (internal volume ~5 cm³), 1 cm diameter, and 2 cm tall. The container top included fluid entry and exit ports, through which standard artificial caries-like demineralising solution (0.1 M acetic acid, pH = 4.0) could be circulated from a 1L reservoir. The container was placed on the stage of the MuCAT (QMUL, UK) quantitative X-ray microtomography (XMT) scanner. A time-series of 3D XMT images of the entire crown were collected with a resolution of 30 micron voxels, for 1 week, as the demineralising solution circulated continuously, without disruption to the tooth. Seventy-seven quantitative 3D images of the crown were collected showing the time sequence of lesion formation. Each image took ~1 h to collect. Analysis of the reconstructed images showed that lesion progression was approximately linear with time as reported from previous 2D studies. The progression rate was 3.7 µm/h. There was evidence of surface layer formation, but only after some initial mineral loss from the surface. A technique has been developed that enables 3D real-time monitoring of lesion progression during artificial de- and remineralisation.

In-vitro Effect of Frequent Consumption of Green Vegetables on Demineralisation of Enamel

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Due to health promotion recommendations to consume daily five servings of fruit or vegetable, green smoothies are very popular. The aim of this in-vitro study was to evaluate the effect of frequent consumption of green vegetables on human enamel. Green smoothies (n = 10) and five freshly mixed vegetables (spinach, arugula, leek, parsley, mangold) were selected and pH and oxalic content were determined. Enamel samples were prepared from wisdom teeth and the prepared slices (n = 3 per food) were incubated with 3 selected foods (spinach, parsley, green smoothie with 50% apple juice) for up to 12 h. Control samples were incubated with a 0.9% sodium chloride solution and apple juice. The surface roughness (Ra) of the enamel samples was measured using an optical profilometric device (perthometer, Mahr, Göttingen, Germany). The quantitative analysis of Ca, P, O in the enamel samples in various depths ranging from 5–50 µm was carried out using an electron probe micro-analyser (Jeol JXA 8900RL). The surfaces of the enamel samples were also visually examined with a digital microscope. The pH-values of the green smoothies ranged from 3.6 to 4.2 and of the mixed vegetables from 5.6 to 6.3. Incubating the enamel slices with the selected foods caused only for apple juice a time-dependent release of different minerals. After 12 h of incubation, loss of calcium, phosphorus and oxygen was observed down to a depth of 45 µm. The measuring of the surface roughness confirmed these data. In this in-vitro study, frequent consumption of green vegetables showed no damage to the enamel surfaces. However, it must be considered that numerous modifying factors influence the enamel surface in vivo, therefore a direct translation to in-vitro conditions can only be done with caution.

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SnF₂ and CPP-ACP Synergism in the Remineralisation of Enamel Subsurface Lesions *in situ*

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The anticariogenic potential of sodium fluoride (NaF), stannous fluoride (SnF₂) and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been well-documented with SnF₂ showing superior results compared to NaF. The aim of this study was to compare the remineralisation efficacy of CPP-ACP with and without SnF₂ or NaF using a double-blind five-way

crossover randomised controlled *in situ* model. Eight participants wore removable palatal appliances with four recessed human enamel half-slabs containing subsurface demineralised lesions and rinsed with 5 mL of treatment solution for 60 sec four times a day for 14 consecutive days with a 7 day wash-out period. Five treatment solutions (pH 4) tested were: NaF: 290 ppm F as NaF; SnF₂: 220 ppm F as SnF₂ and 70 ppm F as NaF; CPP-ACP: 0.4% (w/v) CPP-ACP; CPP-ACP/NaF and CPP-ACP/SnF₂. After each treatment, the enamel half-slabs were removed, paired with their respective demineralised control half-slabs, embedded, sectioned and mineral content determined by transverse microradiography. Scanning electron microscope-energy-dispersive X-ray spectroscopy (SEM-EDS) analysis of the surface layer of the CPP-ACP/SnF₂ remineralised lesion was carried out. Mean (SD) percent remineralisation (%R) observed were: CPP-ACP/SnF₂ – 30.61% (1.57%); CPP-ACP/NaF – 24.51% (1.07%); CPP-ACP – 13.43% (0.76%); NaF – 10.81% (0.81%) and SnF₂ – 10.76% (0.79%). Differences in %R values were all significant (p < 0.001) except for the difference between the two controls (NaF and SnF₂; p > 0.05) using a linear model with a Sidak adjustment. SEM-EDS analysis of the surface layer of CPP-ACP/SnF₂ remineralised lesion showed a five-fold increase in F content with a typical Ca:P:O content of apatite. In conclusion the CPP-ACP/SnF₂ solution was superior to all other solutions including CPP-ACP/NaF solution at the same F concentration at remineralising enamel subsurface lesions *in situ*. A higher fluorapatite content at the mineral surface layer was observed.

The study was supported by the Oral Health Co-operative Research Centre. Dr CPC Sim is supported by the Singapore National Medical Research Council Research Training Fellowship.

Remineralisation Potential and Microstructure Investigations on Artificial Lesions after Treatment with Magnesium-Containing Sodium-Monofluorophosphate (MFP) Toothpaste

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The aim was to investigate the remineralisation potential of a magnesium-containing sodium-monofluorophosphate (MFP) toothpaste by characterising and visualising the remineralised artificial lesion using different complementary analytical techniques, in comparison to a placebo. Focus was on correlations between microstructural, mechanical and chemical properties of the entire volume of the lesion body.

Two treatment groups (verum, placebo), with 5 specimens each, were used. Verum toothpaste contained MFP (corresponding to 1450 ppm F⁻), magnesium sulfate and dicalciumphosphate; the placebo contained sodium fluoride (1450 ppm F⁻). Embedded, polished human enamel specimens were covered before deminer-

alisation and remineralisation, respectively, to obtain sound/demin/remin areas per section. Lactic acid based demineralisation was performed to depths of 100 µm. Remineralisation was a cyclically treatment with verum or placebo (6x2 min/d over 15 d). Scanning Electron Microscopy (SEM), Energy Dispersive X-ray analysis and nanoindentation were performed on surfaces and cross-sections of the treated samples. Further, X-Ray Micro-Computer-Tomography (µCT) was applied.

SEM showed a relative compact and even enamel surface after verum treatment, while nanoindentation revealed a slight increase in hardness and elastic modulus only for placebo-treated samples. On cross-sections a completely different progression was observed; hardness was significantly higher for verum treated lesions (0.8 GPa vs. 0.4 GPa for placebo; statistical significance is $p = 0.042$ using Mann-White U-Test) beginning at 40 to 100 µm. This was confirmed by SEM using line profiles of grey values from backscattering electron images. µCT on the entire sample volume further confirmed nanoindentation and SEM data.

The remineralisation potential of the magnesium-containing sodium MFP toothpaste has been examined and visualised: Crystallisation of potentially newly formed hydroxyapatite takes place at the bottom of the lesion. Outcomes of all applied investigation-al methods are in well agreement. µCT shows potential as an alternative to Transverse Microradiography. Further studies regarding remineralisation efficacy and clinical relevance are recommended.

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ISE Study of Effect of Silver Ions on Demineralisation of Hydroxyapatite Disc

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Silver has traditionally been used in dentistry as a bactericide. However, recently it has been suggested that Ag^+ influences enamel demineralisation directly. The aim was to understand the chemical interactions of Ag^+ with enamel's mineral components during demineralisation using hydroxyapatite (HAP) discs as isotropic enamel analogues. Three HAP discs (PlasmaBiotol, UK; 20% porosity), 14 mm (D) X 2 mm (H) were demineralised for 4 h in 50 ml, 1.0 M buffered pH4.0 acetic acid, 37°C. The discs were then removed and treated with either 3.16 M $AgNO_3$ solution; saturated KI solution; or 3.16 M $AgNO_3$ + saturated KI solution. The discs were then further demineralised for 4 h. ISEs (Ion Selective Electrodes) were used to monitor the rate of demineralisation by measuring Ca^{2+} concentration increases in the solutions at 1 min intervals. The changes in Ag^+ concentrations were also monitored contemporaneously. Similarly treated HAP powder samples were analysed using ^{31}P MAS-NMR (Magic-angle spinning Nuclear Magnetic Resonance) to detect formation of phosphate salts. The rate of Ca^{2+} release before and after treatments were:

$AgNO_3$: Initial increase in Ca^{2+} release rate from 0.11 mM/h to 0.17 mM/h for 1 h, reducing to 0.0266 mM/h thereafter. Overall decrease 24%. Staining observed.

KI: Decrease in Ca^{2+} release rate from 0.095 mM/h to 0.045 mM/h. Overall decrease 47%. No staining observed. $AgNO_3$ +KI: Decreased in Ca^{2+} release rate from 0.072 mM/h to 0.0175 mM/h. Overall decrease 24%. No staining observed. ^{31}P MAS-NMR indicated formation of Ag_3PO_4 for samples treated with $AgNO_3$, but not for KI treated nor $AgNO_3$ +KI treated samples. Topical application of $AgNO_3$ reduces demineralisation of HAP by forming a Ag_3PO_4 protective barrier, but stains the surface. Addition of KI prevents formation of Ag_3PO_4 , and also staining. However, KI does not detriment protection, possibly due to formation of an AgI protective barrier.

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Use of Zinc and Fluoride within an *in vitro* pH-Cycling Model of Post-Eruptive Enamel Maturation

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The present study aimed to determine the effect of zinc and fluoride when used within an *in vitro* pH-cycling model of post-eruptive enamel maturation (PEM). 80 polished bovine enamel blocks were prepared and subjected to pH-cycling for 0 or 20 days (30 min Demineralisation (pH 5.11, 2.25 mM $CaCl_2$, 17.65 mM KH_2PO_4 , 32.9 mM Lactic Acid, 4.25 µM Fluoride (As NaF)) at 9 am, 12:30 pm and 4 pm, stored in remineralisation solution (pH 6.58, 20 mM HEPES, 1 mM $CaCl_2$, 12.7 mM KH_2PO_4 , 130 mM KCl, 5.7 µM Fluoride (As NaF)). At each solution change, blocks were subjected to one of 4 treatment groups (untreated control, 231 µmol/l $ZnSO_4$, 12 mM NaF and Zn and NaF combined). Microhardness measurements were taken for both sound and cycled enamel. Blocks were then subjected to a lactic acid gel demineralisation and results analysed using Quantitative light induced fluorescence (QLF-D), Multispectral imaging (MSI) and Transverse microradiography (TMR). A significant increase in microhardness was observed compared to sound enamel for pH-cycled blocks treated with Zn/F ($P = 0.0087$, (independent samples t-test)). For all analysis methods significant decreases in fluorescence loss was observed for all pH-cycled blocks in comparison to un-cycled controls with the exception of Zn for QLF-D. ($P = QLF-D$: No treatment: 0.0004, F: 0.001, Zn/F: 0.001, MSI: No treatment: 0.0015, F: 0.001, Zn: 0.0308, Zn/F: 0.001). For TMR a decrease in mineral loss was observed for all pH-cycled blocks in comparison to un-cycled controls which was significant for untreated blocks and those treated with Zn/F ($P = 0.0004$ and 0.0184 respectively) These results indicate that exposure to plaque-fluid relevant pH-cycling conditions in the presence of zinc and fluoride may decrease susceptibility of enamel to subsequent acid challenges, simulating post-eruptive maturation.

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The Effect of Redbull® on Human Enamel Surface

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Aim: The aims were to measure the physico-chemical properties of Redbull® (pH and titratable acidity), and to study the changes of the mineral content and microhardness of the enamel surface after exposure to Redbull® and to study the remineralizing effect of saliva. **Experimental Approach:** 24 enamel specimens (4x3 mm) were randomly divided into 3 groups (control-A, Redbull®+saliva-B, Redbull®+deionized water-C). 16 specimens were exposed to 50 mL of Redbull® for 20 min after which 8 were submerged in artificial saliva (2 h and 40 h) and 8 in deionized water. The cycle was repeated 3 times a day for 7 days. The pH and titratable acidity of the beverage was measured. 4 specimens of each group were analysed with Raman Spectroscopy, 2 specimens with Vickers Microhardness tester and the remaining 2 were visualized with SEM. **Result:** The data were statistically analysed by ANOVA using software SPSS with a significance level of $p < 0.05$. We found that the pH of Redbull® was 3.49 ± 0.096 and 5.1 ml of NaOH (0.1 M) was required for 50 ml of Redbull® to reach pH 7. In group C there was an increased carbonate/phosphate ratio with decreased microhardness. In group B were no such significant changes observed however all differences were statistically significant in all the 3 groups. **Conclusions:** In conclusion Redbull® has a high erosive potential for the enamel, producing a loss of the mineral content and a decrease surface microhardness. Saliva has a protector/remineralizing effect when compared to deionized water.

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Enamel Demineralization Prevention by Different Fluoride Mouthwash Treatments Using an in-vitro Microbial Caries Model

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The aim of the study was to investigate the effect of essential oils (EO) on the anti-caries potential of hydroalcoholic or alcohol-free sodium fluoride (NaF) mouthwash formulations in an *in-vitro*

static monospecies biofilm caries model. Ground and polished 4x4 mm bovine enamel specimens ($n = 147$) were randomly distributed among the following parallel treatment groups ($n = 21$ /group) balanced by baseline surface microhardness values: (A) 100 ppmF/EO/hydroalcoholic mouthwash, (B) 220 ppmF/EO/hydroalcoholic mouthwash, (C) 220 ppmF/non-EO/hydroalcoholic mouthwash, (D) 220 ppmF/EO/alcohol-free mouthwash, (E) 220 ppmF/non-EO/alcohol-free mouthwash, (F) deionized water and (G) 0.12% chlorhexidine mouthwash. *Streptococcus mutans* UA159 was incubated for 144 hours on saliva-coated specimens suspended in 24-well culture plates. Growth medium was changed 2 times/day. Developed biofilms were exposed 3 times/day to 10% sucrose solution for 5 minutes and treated 2 times/day for 30 seconds with their respective treatment. After incubation, specimens were evaluated blindly for Surface Microhardness Loss (SML, VHN) and Enamel Fluoride Content (EFC, $\mu\text{gF}/\text{cm}^3$). Other efficacy variables were medium pH as an indicator of biofilm acidity, lesion depth and total biofilm accumulation. Between-treatment comparisons were at the 0.05 significance level, and accounted for plate, position, and baseline. We found SML and EFC results (Adjusted Mean $\pm$ SE) were as follows: A: 83.1 ± 5.1 , 321.0 ± 34.6 ; B: 60.6 ± 5.0 , 430.8 ± 34.6 ; C: 103.4 ± 5.0 , 380.7 ± 34.6 ; D: 54.5 ± 5.1 , 382.8 ± 34.6 ; E: 53.1 ± 5.0 , 411.9 ± 34.6 ; F: 138.3 ± 5.0 , 260.2 ± 34.6 and G: 23.6 ± 5.0 , 246.3 ± 34.6 . For SML, groups A and B were significantly better ($p \leq 0.008$) than C. Groups D and E were not significantly different ($p = 0.836$). For EFC, groups A and B versus C, group D versus E were not statistically different ($p > 0.05$). A Fluoride dose-response was demonstrated for SML and EFC ($p \leq 0.033$). In conclusion this *in-vitro* study suggests that the presence of antimicrobial essential oils in hydroalcoholic/NaF formulations enhances anticaries benefits against enamel demineralization in this laboratory model beyond NaF alone.

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Failed Bonded Interfaces Submitted to Microcosm Biofilm Caries Development

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This study aimed to evaluate the dentin wall carious lesion development of different composite-dentin interfaces in the presence of two adhesive bonding materials in the gaps, using a microcosm biofilm model. Dentin samples were prepared (10.4 mm^2), restored with composite resin using two adhesive systems (etch-and-rinse/self-etch). Different conditions with respect to composite-dentin interfaces were produced with a 200 μm gap: failed bond without ageing or after mechanical ageing, or non-bonded with or

without the presence of adhesive material on the dentin wall ($n = 10$). For cariogenic challenge, specimens were subjected a biofilm microcosm model for 14 days to create caries-like wall lesions. Before and after caries development, transverse wavelength-independent microradiography images were taken, and lesion depth (LD) and mineral loss (ML) were measured. Data were analysed with linear regression models ($p < 0.05$). The composite-dentin interface conditions significant influenced the caries development ($p = 0.019$). Lesion development was reduced by the presence of the adhesive material on dentin wall ($LD = 154.8 \pm 26.9 \mu\text{m}/167.4 \pm 45.7 \mu\text{m}$, $ML = 2946.5 \pm 559.5 \mu\text{m.vol\%}/2921.4 \pm 1117.7 \mu\text{m.vol\%}$), while lesion development was increased by the mechanical loading ($LD = 225.7 \pm 51.5 \mu\text{m}/241.5 \pm 37.9 \mu\text{m}$, $ML = 3482.4 \pm 632.7 \mu\text{m.vol\%}/3655 \pm 572.6 \mu\text{m.vol\%}$). There was no difference between the adhesive materials ($p > 0.05$). Different composite-dentin interfaces influence wall lesion development in gaps, with the interfaces submitted to mechanical ageing showing less carious protection than those interfaces with the presence of adhesive covering the dentin.

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Effect of Bamboo Salt on Remineralization of Subsurface Caries Lesion

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The aim of this study was to investigate the effect of bamboo salt on remineralization of subsurface caries lesion.

Incipient caries lesion was formed artificially in bovine incisors by applying pH 5.0 lactate carbopol buffer system for 72 h, and then divided into three groups of 7 specimens each by randomized. Specimens were treated with 2% sodium fluoride (NaF group), 3% bamboo salt (BS group), and the solution mixed with 2% sodium fluoride and 3% bamboo salt (NaF+BS group) for 24 h. After treatment, specimens were cut perpendicular, and then the cross-sectional chemical compositions and crystalline structure were assessed in subsurface in 150 μm depths. In energy dispersive spectroscopy (EDS) analysis, the difference intensity of before and treatment NaF group was increased phosphorus (14.25 ± 18.82), calcium (16.26 ± 17.58) and fluorine (1.42 ± 1.08) of at depths 150 μm . Also, the BS+NaF groups was increased phosphorus (8.88 ± 5.23), calcium (9.42 ± 5.84) and fluorine (3.34 ± 1.01) of at depths 150 μm . F content was significantly higher in the BS+NaF group than in the NaF group ($P < 0.01$). Laser Raman spectrophotometer (Raman) peaks of the NaF and BS+NaF groups exhibited weaker

peaks in the region vibration within the phosphate at 959 cm^{-1} than the BS group. Also, BS+NaF groups showed higher intensity compare to the NaF group at depths 50 μm . Such difference in the degree of Raman polarization allows for discrimination of penetration on fluorine between groups. The result of this study showed that bamboo salt helps with fluorine penetration deeply over to the subsurface caries lesion. Thus, it is suggested that bamboo salt with fluoride could be used as effective remineralization material on subsurface caries lesion.

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Long-Term Surface Reflection Intensity Measurements of Artificial Caries Lesions Before and after Brushing/Remineralisation

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The aim was to measure surface reflection intensity (SRI) of caries lesions using a hand-held sensor (Optipen) during caries development by a multi-species biofilm and after brushing/remineralisation. One-hundred enamel specimens were obtained by grinding and polishing of human caries-free molars. The specimens were divided into 5 groups ($n = 20$) and then forwarded into a multi-species biofilm model with *Streptococcus mutans* ATCC 25175, *Streptococcus sobrinus* ATCC 33478, *Actinomyces naeslundii* ATCC 12104 and *Lactobacillus acidophilus* ATCC 11975 for 2, 4, 6, 8, and 10 weeks at 37°C in a microaerophilic atmosphere. During the experiment, the alternating media were brain heart infusion (BHI, 20 ppmF from NaF, 1% saccharose) for 6 h and buffer solution containing $\text{KH}_2\text{PO}_4/\text{Na}_2\text{PO}_4$ for 18 h and during week-ends. The specimens were then placed in a remineralising solution and underwent controlled tooth brushing using fluoridated tooth-paste slurry (ratio 1:2 with water) twice daily for 10 weeks. SRI measurements were taken initially and throughout the duration of the experiment. At the same time-points the specimens were also assessed with the International Caries Detection and Assessment system (ICDAS). The primary outcome parameter was %SRI reduction. Non-parametric ANOVA and Bonferroni-Holm correction were applied. We found that white spot lesions were visible after 4 weeks in most specimens (ICDAS score 1 or 2). Mean %SRIs dropped by 50–90% after 6 weeks of caries induction ($p < 0.01$). Brushing/remineralisation did not increase %SRI over 10 weeks. After 8 weeks of brushing/remineralisation, white spot lesions that were formed during the first 2–4 weeks of the experiment were not visible any more (ICDAS score 0). In conclusion, under standardized conditions %SRI was able to detect reflection intensity changes that are associated with undisturbed bacterial growth under cariogenic conditions. The visible effects of brushing/remineralisation were not detected with %SRI.

In vitro Study of Enamel Surface Texture Changes from Erosion

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Previous qualitative and profilometric studies have reported unpolished enamel to have different responses to erosion. The aim of this study was to assess the change in surface roughness of unpolished enamel using the Laser Confocal Displacement Meter (LT-9010M, Keyence Corporation, Japan), which has a vertical resolution of 10 nm, following erosion and to compare different locations on the surface. Ten unpolished enamel sections, sourced from extracted human molars (REC: 12/LO/1836), were embedded in Prottemp4. Enamel sections were immersed in 100 mL of orange juice (pH 3.8, titratable acidity 27 mmol l⁻¹), agitated at 62.5 rpm for 15 minutes and the cycle repeated three times. Surface roughness (Sa) was measured at baseline and after erosion by scanning five areas in one central cluster compared to four peripheral clusters conveniently located (five located in each cluster) over the surface of the 4x4 mm enamel section, each cluster approximately 1.5 mm apart. All scans were analysed for Sa roughness using MountainsMap (DigitalSurf, France) with a gaussian cut-off filter of 25 µm. There was a decrease in mean (SD) Sa roughness after erosion from 0.72 (0.28) to 0.55 (0.15) µm within the central cluster and a decrease from 0.71 (0.35) to 0.68 (0.23) µm over the four peripheral clusters but the results were not statistically significant. The Sa roughness of the central cluster was not significantly different to clusters located over other parts of the unpolished enamel section. In conclusion using the current parameters there was no significant change in roughness after erosion. However, for future studies choosing a central location for measurement would be representative of the whole surface of unpolished enamel.

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Effect of Particle Morphology of Strontium Substituted Hydroxyapatite on Enamel Remineralization

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There is a lack of information regarding the effect of hydroxyapatite (HAp) particle morphology on enamel remineralization potential. Thus, our aim of this study is to evaluate Sr substituted HAp (SrHAp) particle morphology impact on caries inhibition. SrHAp were synthesized in Riga Technical University Institute of General Chemical Engineering. One part of the product was spray-dried (spSrHAp) and remaining part – left as paste (pSrHAp). Products were characterized by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) to verify the chemical similarity to enamel mineral phase. Scanning electron microscopy (SEM) was used for inspection of particle morphology. 15 bovine enamel slabs were prepared according to ISO 11609:2010 standard. During the experiment samples were demineralised in 34% H₃PO₄ for 15 seconds, stored in artificial saliva (KH₂PO₄ 0.33 g, KCl 1.27 g, NaCl 0.58 g, CaCl₂ 0.17 g, (NH₂)₂CO 0.2 g dissolved in 1 L deionized water). Two experimental groups were treated twice a day for 3 minutes with experimental pastes consisting of spSrHAp or pSrHAp, water, 2-hydroxyethylcellulose and glycerine. 5 enamel blocks were left as a control without treatment. After one week surface of blocks was examined with SEM, XRD and FTIR. Analytical methods confirmed, that synthesized products are chemically similar to natural enamel. Spherical agglomerates (up to 2 µm) for spSrHAp and needle-like particles (up to 500 nm) for pSrHAp were observed. SEM images showed, that spSrHAp agglomerates were deposited in the surface wells, but relatively uniform CDHAp layer was formed on the enamel blocks in the pSrHAp case. In addition, chemical analyses revealed formation of SrHAp layer with lower crystallinity in comparison with untreated enamel. spSrHAp could be used as desensitizing component in the dentifrice, while pSrHAp could be more resorbable due to its smaller particle size and induce enamel remineralization.

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Session 5

Clinical Studies

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Clinical Evaluation of Different Prevention Programs in Adults. One Year Results

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This study investigated the application of three preventive and minimally invasive protocols on early caries lesions. A total of 44 adult patients of high, moderate and low caries risk with 516 early caries took part in the present study. These patients were assessed for caries with ICDAS criteria and then were divided into 3 groups depending on their caries risk profile: A high risk (Group A) and a moderate risk (Group B) and a low caries risk group (Group C). Each group was divided into subgroups (A1, A2, B1, B2, C1, C2). In groups A1, B1 and C1 intensive preventive protocol was applied (A1: 118 incipient caries B1: 105 incipient caries and C1: 55 early caries) while in groups A2, B2 and C2 just instructions for oral hygiene were given (The control group; A2: 99 incipient caries, B2: 87 incipient caries lesions and C2: 52 incipient caries). The intensive protocol included the topical application of fluoride, brushing with 5000 ppm fluoride toothpaste, use of ACP-CPP at lesions on smooth surfaces, applications of sealants at lesions of occlusal surfaces (ICDAS code 2) and resin restorations at occlusal surfaces (ICDAS code 3 in high and moderate caries risk group). For the results the non-parametric Wilcoxon statistic test was applied in two different visits (baseline and after 12 months). There was no statistically significant difference in the number of lesions among high-risk and moderate-risk patients group where protocols were applied (Group A1, $p = 0.786$; Group B1, $p = 0.233$) while the control groups where statistically significant different (Group A2, $p = 0.024$; Group B2, $p = 0.041$). In low caries risk group there was no statistically significant difference in the number of lesions (Group C1, $p = 0.203$; Group C2, $p = 0.303$). In groups where statistical differences were found the actual change in levels of caries was: in Group A2 DMFS was 16.83 ± 1 (mean $\pm$ se) and after one year was

14 ± 1.15 (mean $\pm$ se) and in Group B2 DMFS was 12.43 ± 1.47 (mean $\pm$ se) and after one year was 14.7 ± 1.82 (mean $\pm$ se). In conclusion the different prevented protocols in high and moderate caries risk groups presented differences while in the low caries risk group no significant difference was demonstrated.

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The Influence of Chlorhexidine Mouthwashes on the Oral Hygiene in Adolescents Wearing Different Orthodontic Brackets

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The aim of this study was to investigate the influence of two chlorhexidine (CHX) mouthwashes on the oral hygiene in adolescents wearing two types of brackets (metal-stainless steel and ceramic). The research was conducted on 80 patients (61 female, 19 male; mean age 14.2 ± 1.4) who satisfied inclusion criteria: good general health, no use of antibiotic and mouthwashes three months before study, no periodontal disease, non-smoking. They were divided into two groups ($n = 40$) according to brackets type. Four weeks after brackets bonding each group were randomly assigned in two subgroups ($n = 20$) and each group were provided with different CHX mouthwashes. Patients rinse twice daily for 10 days; conventional CHX and CHX with anti-discoloration system (CHX-ADS) (conc. 0.2%). Following clinical measurements were done: oral hygiene index-simplified (OHI-S) and stimulated saliva flow (SS) were performed three times: prior to bracket bonding (T1), six weeks after bonding (T2), and eighteen weeks after (T3). The data were analysed using independent t-test and ANOVA, followed by the Bonferroni test. For SS between groups were found

no statistically significant difference ($p = 0.340$ at T1; $p = 0.141$ at T2; $p = 0.491$ at T3). Also, no significant differences in OHI-S were found between groups, but at T3 we found statistically significant increase of OHI-S in boys ($t = -3.05$, $df = 78$, $p = 0.003$). After that all data were divided depending to the gender. In both gender groups mean OHI-S were lower in patients using CHX-ADS; boys at T3 ($t = 2.74$; $df = 17$; $p = 0.014$) and girls at T2 ($t = 2.54$; $df = 59$; $p = 0.018$). In girls wearing ceramic brackets and using CHX, OHI-S values were lower at T2 ($t = -3.12$; $df = 31$; $p = 0.004$). The results revealed that wearing ceramic brackets as well as the use of CHX-ADS influences plaque reduction.

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Oral Health in Preterm Children

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Aim: Evaluating oral health in preterm children and analyzing child and mother related factors influencing oral health. **Material and Methods:** 128 children aged 3 and 4 years were included. 64 (27 male) preterm children were randomly selected from the pre-term registry of the Jena University Paediatric Clinic. 64 children with normal birth weight matching by age and gender were sampled out from patients visiting the Department of Paediatric Dentistry for regular recall. Dental examinations were provided by one dentist under standard conditions. Caries was scored by ICDAS II, developmental defects of enamel by DDE index and periodontal health by PSI. Mother and child related factors were collected by questionnaire addressed to parents, gynaecologists and paediatricians. **Results:** Caries prevalence was 50.0% (34.0% ICDAS 1, 16.0% ICDAS 2–3) in preterm children and 12.5% (7.8% ICDAS 1, 4.7% ICDAS 2–3) in the controls. Caries experience was higher in preterm children (1.0 ± 3.1 dmft; 2.5 ± 8.6 dmfs) compared to the controls (0.3 ± 1.0 dmft; 0.5 ± 1.8 dmfs). Significant higher prevalence of DDE ($p < 0.001$) and PSI ($p < 0.001$) were recorded in preterm children. Preterm children exhibited higher caries risk when their mothers had a lower socio-economic background (OR = 6.34) or had taken antibiotics (OR = 2.94) and higher risk of DDE when their mothers suffered from any disease (OR = 3.90) or from hypoxic-ischemic insult of the utero-placental unit (OR = 2.25). Children receiving protein supplements (OR = 8.75) or suffering from respiratory syndrome (OR = 6.17) had highest caries risk while children receiving antibiotics in the first year of age (OR = 2.84) or fluoride supplements starting at six month of age (OR = 2.14) had higher risk of developing DDE. **Conclusions:** Higher caries prevalence, dmft, DDE and PSI were observed in preterm children being influenced by various mother and child related factors.

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Effect of the New Synthesized Toothpaste (M4) On Salivary Oral *Streptococcus mutans* in High Caries Risk Adults

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The objective of this first phase trial was to introduce a newly synthesized toothpaste (M4) made from the mixture of Propolis extract, Miswak extract and a Probiotic strain of *Lactobacillus rhamnosus* which was isolated and serologically identified by dairy microbiological Lab., National Research Center., Egypt, and to assess its ability to reduce the salivary *S. mutans* counts in high caries risk participants over a 3 months period. Seven adult volunteers, with high caries risk, were selected to use the M4 twice daily. The participants were asked to brush their teeth with (M4) and leave it for 30 seconds then wash with water for 10 seconds and not eat or drink for 30 minutes after brushing. Saliva samples were taken on 3 occasions, before using (M4) (control) and 1 month and 3 months later. Salivary *S. mutans* count were determined. The selective media, Mitis salivarius-bacitracin agar (MSB), was used to isolate and grow *S. mutans*. *S. mutans* isolates were identified by its characteristic colony morphology (dark blue, small, irregularly margined and adherent). **RESULTS:** The data were analysed using the repeated measures ANOVA and Tukey's tests for comparison between Log₁₀ CFU/ml of salivary *S. mutans* at different time period. A statistically significant decrease in mean Log₁₀ CFU/ml of salivary *S. mutans* counts from baseline (8.350 ± 0.403) to one month (6.790 ± 0.159) and 3 months (6.157 ± 0.387). There was no statistical difference between counts at one month and 3 months. In conclusion the M4 toothpaste containing a mixture of Propolis extract and Miswak extract and a Probiotic strain showed a promising effect by significantly reducing salivary *S. mutans* counts in high caries risk individuals.

Methods of Funding: National Research Centre, Cairo, Egypt.

Long-Term Effect of Supervised Toothbrushing on Levels of Plaque and Gingival Bleeding among Schoolchildren

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The aim was to test the hypothesis that, in initial high-caries risk children, supervised toothbrushing (HR-STB) presents greater reduction in visible plaque levels and gingival bleeding compared to peers belonging to a no-supervised toothbrushing (HR-NSTB) and to an initial low-caries risk NSTB group (LR-NSTB) over 4 years. Schoolchildren assessed high-caries risk, aged 6–7-years, were allocated to three oral healthcare protocols using a cluster-randomized study design: (1) Ultra-Conservative Treatment (UCT), in which small cavities in primary molars were restored using ART approach and medium and large cavities were left opened and cleaned along with all other teeth under daily supervised toothbrushing at school premises (HR-STB group); (2) Conventional Restorative Treatment (CRT), in which primary molars were restored with amalgam and high-caries risk first permanent molars received composite resin sealants (HR-NSTB); (3) Atraumatic Restorative Treatment (ART), in which primary molars were restored according to the ART approach and eligible permanent molars received ART sealants (HR-NSTB). Low-caries risk children (dmft ≤ 1) excluded from the trial's interventions formed another NSTB group (LR-NSTB). 273 children were examined at baseline (T_0) and after 4 years (T_1) according to the VPI and the GBI index. Data were analysed using linear (dependent variable: mean VPI at T_1) and logistic regression (dependent variable: mean GBI at T_1). We found that the mean VPI and mean GBI scores were statistically significantly lower at T_1 than at T_0 ($p < 0.001$). Reduction in mean VPI scores in HR-STB children (58%) was statistically significantly higher than for the HR-NSTB children (34%) over the 4-year period ($p = 0.03$) but no difference was observed between HR-STB and LR-NSTB children (42%) ($p = 0.361$). No statistically significant differences in reduction of mean GBI scores (HR-STB:57%; HR-NSTB:73%; LR-NSTB:64%) were observed between HR-STB and HR-NSTB ($p = 0.62$) and LR-NSTB ($p = 0.74$). In conclusion, in high-caries risk children, the protocol based on supervised toothbrushing presented greater reduction in visible plaque levels than protocols based on restorations and sealants after 4 years.

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Establishment of a Dry Mouth Clinic at the Faculty of Dentistry, University of Oslo, Norway

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The symptom of dry mouth is an increasingly common complaint in several patient groups and may occur alone or together with dryness of eyes. A Dry Mouth Clinic (DMC) has recently been established at the University of Oslo. When applicable, this clinic will collaborate with the Nordic Dry Eye Clinic (NDEC), in Oslo. Patient groups may include those with primary Sjögren's syndrome (pSS), non-Sjögren's syndrome patients (dry eyes and dry mouth symptoms but no established pSS diagnosis), radiated cancer patients and patients with drug-induced dry mouth. After giving written consent participants will complete general and oral health related questionnaires. Participants will be examined at the DMC and NDEC. Objective and subjective clinical symptoms and findings of oral mucosa, tongue, gingivae, and teeth will be recorded at the DMC, as well as the secretion rate of stimulated and unstimulated whole saliva. Saliva samples will be stored for further analysis. Taste and smell assessments and candida tests will also be performed. Symptoms of dry eyes and quantitative and qualitative tear assessments will be recorded at the NDEC. Twenty female pSS patients (mean age 54 ± 12.7 y) and 32 healthy controls (mean age 50 ± 12.4 y) have so far been included in the study. Results indicate significant differences between patients and controls in clinical score of oral dryness (CSOD patients: 4.1 ± 1.9 ; controls 0.63 ± 0.94) and ocular dryness (Shirmer score patients: 4.7 ± 4.9 mm/5 min; controls 15.4 ± 11.8 mm/5 min). In conclusion, reduction in saliva and tear secretions may result in impaired oral and ocular health thereby reducing quality of life. A designated clinic for dry mouth patients collaborating with a dry eye clinic will aim to generate valuable research data.

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Oral Quality of Life, Salivary, Gustatory and Olfactory Status in Patients with Primary Sjögren's Syndrome

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Little is known about chemosensory functions in patients with primary Sjögren's syndrome (pSS). Aim: This study aims to record oral health quality of life in pSS, and to investigate salivary, gustatory, and olfactory status in this patient group. Material and methods: Twenty females with pSS (mean age 54 ± 13 y) and 32 healthy female controls (mean age 50 ± 13 y) were recruited to the preliminary study and after giving written consent were examined at the newly established Dry Mouth Clinic (Faculty of Dentistry, University of Oslo). Oral quality of life was assessed using the Oral Health Impact Profile 14 (OHIP-14) questionnaire. Saliva was collected using a standardized protocol and unstimulated (UWS) and stimulated whole saliva (SWS) secretion rates were measured. Gustatory function was assessed using 'taste strips' and olfactory function using 'sniffin' sticks' (Burghart Messtechnik GmbH). Differences in parameters between the patient and control groups, and correlation between OHIP-14 scores and the oral parameters were calculated using t-test and Spearman's rho, respectively. Results: The mean OHIP-14 score was higher in patients with pSS (15.2 ± 10.3) than in controls (2.7 ± 3.1), ($p < 0.001$). Mean UWS and SWS secretion rates were lower for pSS patients (0.1 ± 0.1 ml/min; 0.7 ± 0.4 ml/min) than for controls (0.3 ± 0.2 ml/min; 1.6 ± 0.7 ml/min), ($p < 0.001$). The pSS patients had a lower mean gustatory score (19.8 ± 6.6) than controls (25.4 ± 4.5), ($p = 0.002$), and a lower mean olfactory score (9.3 ± 2.7) than controls (10.7 ± 1.3), ($p = 0.036$). Within groups there was no correlation between the OHIP-14 scores and the oral parameters. Conclusion: Compared to the controls, pSS patients had a significantly reduced oral quality of life and significantly lower salivary secretion rates, as well as taste and smell dysfunctions. These results demonstrate that chemosensory functions should also be evaluated in dry mouth patients.

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Dental Health Status of Patients Diagnosed with Primary Sjögren's Syndrome

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This clinical study aims to assess some oral health parameters in patients diagnosed with primary Sjögren's Syndrome (pSS), and is part of a larger project studying groups of patients suffering from dry mouth. Material and methods: Twenty female pSS patients (mean age 54 ± 12.7 y) and 32 healthy controls (mean age 50 ± 12.4 y), were recruited to the study and examined at a newly established Dry Mouth Clinic at the Faculty of Dentistry, University of Oslo, after giving written consent. A questionnaire was filled out and clinical and radiological examinations were performed. Differences between the patient and control groups were calculated using t-test or Chi-squared tests. Results: Approximately 1/3 of patients considered their general and oral health as 'poor' or 'very poor'. No controls considered their general health as 'poor' or 'very poor', and only 1 control considered their oral health as 'poor' or 'very poor'. Ninety percent of patients and 75% of controls had visited the dentist within the last year. There was no between-group difference in the mean number of teeth present (patients 25.5 ± 2.7 ; controls 26.1 ± 3.8). The patients had higher mean caries experience: DMFT (patients: 18.0 ± 6.45 ; controls 12.9 ± 7.26 , $p = 0.014$), FT (patients: 15.35 ± 5.77 ; controls 11.28 ± 6.10 , $p = 0.02$), and $D_{3-5}T$ (patients: 0.60 ± 0.94 ; controls: 0.28 ± 0.58 , $p = 0.136$). Visible plaque was observed in 30% of patients and 9.4% of controls ($p = 0.056$). Signs of gingivitis were observed in 25% of patients and 6% of controls ($p = 0.054$). Conclusion: Compared to controls, pSS patients reported poorer general and oral health. They had significantly higher dental caries experience, and a tendency to more plaque and gingivitis. However, the pSS patients reported visiting their dentist more regularly than the controls, suggesting an awareness of the oral consequences of reduced salivary function.

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Clinical Study; Safety and Performance Evaluation of the Calcivis Caries Activity Imaging System

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Aim: The aim was to evaluate clinical Safety and Performance of the prototype Calcivis System in the assessment of caries lesion activity. **Methods:** Previous ORCA posters reported an assay for assessing caries lesion activity (2008/9/10/13/14/15). The Calcivis System combines a specialist intraoral camera and a calcium activated photoprotein. In this pilot study, 42 patients, who met the inclusion/exclusion criteria, were recruited at three UK, general dental practices. A minimum of one molar coded as sound (ICDAS 0) and one erupting or erupted third molar with a lesion identified ICDAS 1, 2 or 3, were imaged per patient using the Calcivis System. Investigators assessed the images for elevated luminescence. Adverse Events were collected throughout. **Results:** Safety; only two Adverse Events were recorded from 42 patients; bleeding gums and slight gum abrasion; both device related, non serious and asymptomatic with no action required. Performance; results from 31 patients showed statistically significant agreement between the dentist's interpretation of elevated luminescence from the Calcivis System images and their original scoring of the teeth by visual inspection (ICDAS scores) in 47 of 65 (72.3%) teeth corresponding to a Cohen's kappa of 0.45 (95% CI of 0.24 to 0.66; p-value <0.001). Images from 11 patients were deemed ineligible due to ambient light ingress and/or misapplication of the disclosing solution. Study results were of significant benefit in designing the second generation Calcivis System. **Conclusions:** The level of agreement between elevated luminescence with areas of expected lesion activity, is above 70%, is not due to chance and correlates well with published data on active non-cavitated lesions in molars at various stages of eruption. The Calcivis System is safe for use as determined by the low number and nature of adverse events.

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Pragmatic RCT on the Efficacy of Proximal Caries Infiltration: 36 Months Follow-Up

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Proximal caries infiltration has been shown to be efficacious to impede caries lesion progression when performed by dentists working in a university dental clinic after up to seven years as well as in a pragmatic study design after 18 months follow-up. The aim of this randomized split-mouth placebo-controlled clinical trial was to re-assess the efficacy of resin infiltration of proximal caries lesions being performed in private practice in combination with self-applied non-invasive measures compared with non-invasive measures alone to inhibit lesion progression. In 79 children and young adults giving informed written consent, 218 pairs of proximal caries lesions radiographically extending into inner half of enamel (E2) or outer third of dentin (D1) were randomly allocated to either one of two treatments (8 patients with 20 lesions were excluded). Test lesions were infiltrated (Icon; DMG). A placebo treatment was performed in control lesions by five German private practitioners. All patients received instructions for non-cariogenic diet, flossing and fluoridation. The primary outcome was radiographic lesion progression (pairwise comparison) evaluated by two independent evaluators being blinded to treatment allocation (SP and KB; kappa: 0.58). After approximately 36 months 165/204 lesion pairs (81%) in 64/77 included patients (83%) could be re-evaluated radiographically using individualized bitewing holders. No unwanted effects could be observed. Radiographic progression was recorded in 22/165 test lesions (13%) and 63/165 control lesions (38%) ($p < 0.001$; McNemar test; relative risk reduction = 65%); 10/165 test and 39/165 control lesions ($p < 0.001$; relative risk reduction = 74%) progressed from one stage to a deeper one. It is concluded that resin infiltration performed in a private practice-setting by various practitioners seems to be more efficacious in reducing lesion progression compared with self-applied non-invasive measures alone over a period of 3 years.

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Patient's Valuation of Effects of Dental Caries in the Netherlands: Results of a Discrete Choice Experiment

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The effects of dental caries affect people physically, socially and psychologically by causing pain, bad breath and functional and social limitations. Dental professionals have all knowledge and professional understanding of prevention, management and treatment of the disease, but how clinical outcomes are valued by patients is unclear. Since patient's preferences are becoming more and more important in health care, this study was designed to explore how different attributes of oral health related Quality of Life are valued by the general adult population in The Netherlands by accomplishing a Discrete Choice Experiment (DCE). In health care research DCE is used to find out what alternative maximizes utility. In a DCE, individuals are offered a series of choice sets. In every choice set individuals are asked to choose between two or more alternatives. He/she is assumed to choose the alternative containing the highest utility based on all presented attributes. The final sample used in the analysis comprised 604 participants of a national survey on oral health each providing 18 choice sets containing six of the most prevalent OHIP-49 attributes (toothache, bad breath, ability to chew, speech problems, bad looking teeth and feeling tense/embarrassed) at three levels (never, occasionally and often suffering from the attribute). People were also presented with an opt-out option, representing the solution of being edentulous, wearing a full prosthesis. This resulted in 29,913 observations. Mixed logistic regression analyses were performed to estimate the derived utilities. We found that having very bad looking teeth decreased the utility coefficient the most (-2.78) followed closely by often suffering from toothache (-1.92) and often having bad breath (-1.5). Often having problems with pronouncing words (-1.35), feeling tense or embarrassed (-1.12) and having problems with chewing (-1.1) decreased utility values less but still statistically significantly. Being edentulous resulted in a utility coefficient of -7.58. Since there is no anchored baseline, the derived utility values at itself should be converted into probabilities of patient's preferences; a total of 12.4% of the respondents would have chosen the full denture-option if they were to choose. When people were presented the best case scenario, this was only 0.05%. When they are presented with the worst case scenario, 89.9% would rather have a full denture than having their own teeth in that situation.

In conclusion dental health professionals should be aware that patient's preferences on dental health outcomes may vary and that these preferences should be addressed in clinical decision making to maximize utility in patients.

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Evaluation of Atraumatic Restorative Treatment Outcome in School Children Aged 9–15 in Rural Area of Romania

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Aim: The aim of this study was to evaluate the outcome of ART restauration as well as caries incidence following this procedure in children living in less developed rural areas of county Dolj, Romania, considering that these children have poor access to healthcare and hygienic education. **Material and Method:** A total of 141 children aged 9–15 with occlusal carious lesions on permanent teeth were selected for treatment. The treatment protocol included standardized oral hygiene instructions, professional brushing and caries treatment with glass ionomer cement (GIC) and compomers (COM). After 6 months 135 of the treated children reported for recall and were evaluated by a calibrated examiner and after 1 year only 122 children reported for reexamination. **Results:** After 6 months the fillings had a survival rate of 90.98% overall, 95.16% for GIC and 86.3% for COM. After one year the survival rate was 90.3% for GIC and 91.6% reported to the number of children presented for recall. There was no significant difference between the 2 materials tested ($p = 0.79$). After 6 months 95.74% children were caries free (considering only the permanent teeth) and after one year 89.36% children were reported caries free. **Conclusions:** We did not find any major differences between the 2 filling materials used in the study considering their short and medium outcome. The children respond positive to the healthcare instructions, having a low rate of caries incidence in short and medium time of recall.

Study was funded by the University of Medicine and Pharmacy Craiova.

Recording Caries in NHS General Dental Service Clinical Practice

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Aim: The aim was to quantify the data cleanliness of baseline and 24 month International Caries Detection and Assessment System (ICDAS) chartings in NHS General Dental Service (GDS). **Method:** Twenty-six general dental practitioners and their nurses within 6 NHS practices located in West Yorkshire had one hour face-to-face training after 90 minutes of self-directed learning

(ICDAS e-learning). Further training was provided as requested before each completed baseline ICDAS recordings for adult new patients and a repeat charting 24 months later. Pairs of charts were examined for caries transitions and completion errors. **Results:** 272 adults were seen at both baseline and 24 months: 79% of chart pairs were without errors ($n = 201$); 19 missing baseline ($n = 8$) or 24 month ($n = 11$) charts; partial missing data ($n = 14$) or illegal codes ($n = 3$); impossible transitions (e.g. missing to sound; filled to sound) ($n = 35$). Although the majority of charts were without error, even accounting for possible data entry errors such as right and left sided swaps or tooth number errors, there were numerous impossible transitions. The most common of which was charting dentine caries at baseline which was charted as sound at 24 months ($n = 22$). Data quality, even without assessing validity of coding, was relatively poor. **Conclusion:** Should caries be considered as a clinical outcome indicator within GDS in the UK, much work is required to ensure high quality data. Use of electronic recording whereby only legitimate codes could be entered would remove some issues along with extended training of dental staff in the accurate completion of charts.

Note: These findings are from the INCENTIVE study (Innovation in the commissioning of primary dental care service delivery and organisation funded by the UK NIHR Health Services and Delivery Research (HS&DR) Programme (Project 09/1004/04).

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Questionnaire on Current Clinical Practice for White Spot Lesions to UK Based Orthodontists

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Background: Enamel demineralisation or the so called 'white spot lesion' (WSL) is a constant risk of fixed appliance orthodontic treatment (FAOT) hence of great importance to both dentists and orthodontists. The aim is to investigate current clinical practice on problems related to WSLs. **Experimental Approach:** A nine-item questionnaire with open and closed questions was sent to the British Orthodontic Society. The Society emailed the questionnaire twice to all orthodontists members. **Main Results:** The response rate was 7.85% (115/1,464) and the fully completed questionnaires were 105/115 (91.30%). The mean age for all responders was 48 years old and the median 47. The median year for obtaining orthodontic qualification was 1997. Based on the responses the median risk for developing WSLs during FAOT was estimated to be 20% and the mean 42.86%. Early termination and need for restorative care were the main problems associated with WSLs. Responders would primarily use clinical examination and secondly photographs to diagnose and quantify severity of WSLs. Fluoride was at the core of the prevention protocol. Poor oral hygiene, diet and duration of FAOT were believed to be the main three risk factors for development of WSLs. Responders agreed with many risk factors (pre-existing WSLs, socio-economic status, duration of FAOT, DMFT and oral hygiene) but did not agree that age and/or

gender of the patient are risk factors as identified in the literature for development of WSLs. The most popular methods to treat/arrest WSLs are discussion with patient/parent, oral hygiene instructions and diet advice. **Conclusions:** For majority of responders the key factors to the problems related to WSLs are clinical examination and photographic documentation, employment of fluoride, oral hygiene practices, diet management and duration of FAOT.

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Reduction in Caries Risk Level of Patients in a Practice Based Research Network – CAMBRA-PBRN

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Background: In order to prove that Caries Management by Risk Assessment (CAMBRA) can be successfully implemented in dental practices outside of the university setting, dentists in the San Francisco Bay Area were approached to participate in a Practice Based Research Network (PBRN) study. **Methods:** 30 standardized and to a gold standard calibrated dentists were recruited to perform the CAMBRA-PBRN two-year study (IRB #10-02153) in their practices involving up to 900 patients. After initial screening, assessment of caries risk level, and treatment of cavitated caries lesions, patients were randomly assigned to either the intervention – an active preventive treatment according to CAMBRA rules, or a 'standard of care' control treatment. Newly formed caries lesions and changes in caries risk status were monitored as outcomes. Caries risk assessments (CRA) were performed at baseline and every 6 months. To minimize variations in caries risk assessment in the dental offices a validated, computerized caries risk assessment program (MyCAMBRA) was developed and provided to the dentists. **Results:** At this point 446 baseline CRAs were available with 232 subjects entering the study at high caries risk level (130 intervention, 102 control patients). At 12-month recall a significantly higher number of subjects in the intervention group had converted from high caries risk to moderate or low caries risk compared to the controls (58% intervention versus 29% control, Fisher's Exact Test, $P = 0.005$). At 18-month recall 83% of the intervention and 52% of the control group subjects improved to moderate or low caries risk (Fisher's Exact Test, $P = 0.03$). **Conclusion:** Significantly reducing the caries risk of subjects in the active intervention arm of a CAMBRA-PBRN study has shown that CAMBRA can successfully be implemented in dental practices outside of a university setting.

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Trials and Tribulations Encountered during the Implementation of a Public Oral Health Intervention in Grenada

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Aim: To prevent caries in the 26,000 children in Grenada where repeated surveys found extremely high caries rates. **Approach:** A daily, two-minute tooth brushing routine (1000 ppm fluoridated toothpaste) led by teachers was implemented in every classroom with toothbrushes remaining in school. Teachers provided classroom-based oral health and nutrition education and applied fluoride varnish to each child's teeth 3–4 times/year. Glass-ionomer sealants were applied to first molars of all 6–8 year-olds by teams of NYU dental students and local providers. **Results:** Despite repeated modification of the placement technique and repeated re-application of the sealants, few were retained over the 2.5-year course of this study. However, a 75% reduction in the number of new cavitations and new demineralizations in the first molars was observed. **Conclusions:** The trials and tribulations encountered in the implementation of this study were significant and numerous. Convincing the Ministry of Health, the Ministry of Education, the parents, teachers, principals and students to 'buy in' to the program proved most challenging. Obtaining consent from the parents proved to be a logistical nightmare. The hot humid tropical environment caused the sealant material to set extremely rapidly and caused extensive mold formation on the toothbrushes stored in schools. Distribution of toothpaste proved challenging requiring significant non-island manpower. When external manpower was no longer provided to train, supervise and distribute for the program, the program became non-operational. Despite these facts, this study confirms that in high caries-risk environments where improvements of public water supply may not be possible, school-based, public interventions implemented by a non-traditional lay workforce can result in significant oral health improvements. Even with these results, local 'buy-in' to the program was not adequate to sustain it after we left.

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Survival and Associated Risk Factors of Selective Caries Removal Treatment Performed in Primary Teeth

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Selective Caries Removal (SCR) is a minimally invasive technique that reduces the pulpal exposure and post-operative symptoms in deep caries lesions. The aim was to assess the survival and associated factors for failure of primary teeth restorations involving SCR. A retrospective study was conducted to evaluate the longevity of Composite and RMGIC restorations placed in primary teeth of children attending to a University Dental Service. The treatments were performed due to primary caries lesions by dentists (40) that attended a post-graduate course in Pediatric Dentistry (2005–2013). Factors potentially associated with treatment failure were investigated: gender, dmft, visible plaque and gingival bleeding indexes, tooth type, number of restored surfaces, capping and restorative materials used. Kaplan–Meier survival with log-rank test was used to analyze the longevity of restorations. Multivariate Cox regression analysis with shared frailty was used to assess the risk factors ($p < 0.05$). The time between the placement of the restoration and the last dental appointment, or and the failure event, if occurred, was considered. For analysis, data were censored at 36 months of follow-up. During the evaluation period no physiological exfoliation was observed. In all, 284 Restorations in 88 high caries risk children (5.2 ± 1.91 years) were analyzed. Three-year survival was 48.8%, representing an annual failure rate of 21.2%. Anterior teeth showed a risk of failure more than 4 times higher than posterior teeth ($p < 0.001$). Patients presenting visible plaque $>20\%$ had 4.55 times more risk of failure in their restorations ($p = 0.001$). In conclusions restorations placed in anterior primary teeth after SCR presented lower longevity compared to posterior restorations. Lack of oral hygiene was negatively associated with survival of the restorations.

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Comparative Evaluation of Infiltration and Remineralization of Non-Cavitated Smooth Surface Caries Lesions: 6 Months Results

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The aim of this clinical trial was to compare therapeutic effects of resin infiltration technique, self assembling peptide (P11-4) and fluoride varnish application for controlling white spot lesions (WSLs) on buccal surfaces. Among the patients that applied to university clinics, 113 of them with at least one visible WSL on buccal surface at each quadrant were invited for a second visit in order to score the lesions according to LAA-ICDAS (Lesion Activity Assessment Associated with ICDAS) and to assess the eligibility of lesions by means of laser fluorescence (LF pen) measurements. To be included in the study, participants were required to have at least 4 buccal white spot lesions, each at different quadrants, with LF pen score ≥ 8 . 21 patients were included in the study based on the laser fluorescence (LF pen > 8) values. A clinician blinded to baseline recordings randomly assigned the lesions into 4 groups ($n = 21$): GI (Icon), GCR (Curodont™ Repair), GD (Duraphat®) and GC (control) groups. The treatment protocols were applied by a trained investigator blinded to lesions' scores and control group received no treatment except regular brushing. Lesions were scored by LAA-ICDAS after 3 and 6 months and DIAGNodent pen after 1 week 3 and 6 months. Friedman and Wilcoxon signed-rank tests showed statistically significant decrease in the mean LF pen values in all groups ($n = 20$) after 6 months ($p < 0.05$). The therapeutic effects of four interventions compared with each other based on mean Δ LF pen values (Δ baseline-6. month) and the greatest regression was observed in GI (-23.25 ± 18.21) differing significantly from GCR (-8.15 ± 13.89 ; $p = 0.007$), GD (-10.1 ± 10.31 ; $p = 0.05$) and GC (-4.15 ± 9.72 ; $p = 0.001$) and followed by GD significantly differing from GC ($p = 0.005$; $p < 0.001$). McNemar Change test showed statistically significant differences in the activity status of the lesions scored with LAA-ICDAS between baseline and 6 month except GC. The study showed that resin infiltration and fluoride varnish have beneficial effects on arresting WSLs on smooth surfaces when compared with regular brushing alone at moderate to high caries risk individuals.

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Longevity and Associated Risk Factors of Adhesive Restorations after Complete and Selective Caries Removal

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Aim: The aim was to evaluate the longevity and factors associated with failures of restorations performed in permanent molars after complete (CCR) and selective (incomplete, partial) caries removal (SCR). **Materials and Methods:** The sample was composed of adhesive restorations performed in deep caries lesion of permanent molars. Factors such as gender, caries experience, visible plaque and gingival bleeding indexes, operator's experience, number of restored surfaces, type of capping and restorative materials were investigated. Kaplan-Meier survival with log-rank test was used to analyze the longevity of restorations. Multivariate Cox regression analysis with shared frailty was used to assess the factors associated with failures ($p < 0.05$). **Results:** 477 restorations placed in 297 children (9.14 ± 1.75 years) were included in the analysis. The survival of restorations reached 57.9% up to 36 months of follow-up with overall annual failure rate of 16.7%. There was no difference in longevity when CCR or SCR were performed ($p = 0.163$). The CCR presented more pulp exposure ($p < 0.001$). Patients presenting gingivitis have 2.88 times more risk of failure in their restorations ($p = 0.007$). Multi-surface restorations showed a risk of failure more than 3 times higher than single-surface ($p = 0.003$). Resin Modified Glass Ionomer Cement presented a risk of failure 4.11 times greater than Composite Resin ($p < 0.001$). **Conclusion:** The type of restorative material, number of restored surfaces and presence of gingivitis influenced the survival of restorations, independently of caries removal technique used.

No external funding was used in this study.

Dental Caries, Fluorosis, Oral Health Determinants and Quality of Life in Adolescents

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This study assessed the extent to which dental caries and fluorosis, in addition to socio-demographic and oral health behavior determinants, are associated with oral health-related quality of life (OHRQoL) of adolescents. All adolescents attending from 6th to 8th grades in the Region of Itapoã, Federal District of Brazil ($n = 1,122$) were screened and 618 10–15 year olds were selected. 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. The presence of cavitated dentine lesions (D3) within the dentition was observed in 39.5% of the adolescents and dental fluorosis was found in 48.5%. The outcome was a high score on OHRQoL (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 regression analysis showed that adolescents with toothbrushing frequency $\leq$ once per day and with moderate or severe cavitated dentine lesions were significantly more likely to report a high impact on their OHRQoL, respectively ($p = 0.002$; $p = 0.001$). Fluorosis did not impact daily life performances ($p = 0.545$). In conclusion, increased impact on OHRQoL was related to the severity of cavitated dentine lesions, but fluorosis resulting from combined fluoride exposure from early ages was not of concern for adolescents. Since, toothbrushing once or less than once per day predicted the outcome, daily twice brushing with conventional fluoride toothpaste should be recommended to control caries progression from early ages.

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Association between Metabolic Control, Salivary Status and Caries in Children and Adolescents with Type 1 Diabetes

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The aim of this study was to investigate the possible association between salivary dysfunction and incidence of caries, in relation to the level of metabolic control, in children and adolescents with type 1 diabetes (DM1). For the purpose of this study, a total of 150 children and adolescents (4–18 years old) were examined and allocated among 3 groups: 50 patients poorly-controlled (DM1-A, HbA1c $>8\%$), 50 well-controlled (DM1-B, HbA1c $\leq 8\%$) and 50 age- and sex-matched healthy controls (C). The study was approved by the Research Ethics Committee of University of Athens and parents signed written informed consent. All subjects were examined for dental caries, oral hygiene and salivary factors. Assessments of salivary function included self-reported xerostomia, quantification of resting and stimulated whole saliva flow rates, pH values, buffering capacity and saliva's viscosity. Caries prevalence was recorded using DMFT and dmft index, with further assessment of lesion activity at non-cavitated and cavity levels. Plaque index and gingival index were additionally evaluated. Data were analysed by Chi-square and Kruskal-Wallis tests. The results indicated higher caries levels and a decreased unstimulated salivary flow rate in poorly-controlled diabetics. The average caries indexes were DMFTDM1-A 3.6, DMFTDM1-B 1.2, DMFTC 1.5, $p \leq 0.05$). Salivary status and caries index were not found to be significantly different between well-controlled diabetics and healthy controls.

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Efficacy of Caries Infiltration in Primary Molars: 1-Year Follow-Up of Split-Mouth Randomized Clinical Trial

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The main purpose of this split-mouth randomized controlled clinical trial was to evaluate the efficacy of caries infiltration in arresting proximal caries lesions in primary molars. As secondary outcomes, dental anxiety and time required to caries infiltration procedure were assessed. Thirty-six healthy children with mean age 6.4 years (SD 1.2), presenting at least two primary molars with proximal lesion detected radiographically (in the inner half of enamel – E2 or outer third of dentin – D1) participated in the study. Lesions were randomly allocated to Test (caries infiltration with Icon[®], DMG, Hamburg, Germany) or Control group (brushing + flossing). A blind examiner assessed caries progression radiographically after 1 year. A Facial Image Scale (FIS) was used to assess dental anxiety. Fisher exact test was used to compare the proportion of caries progression between groups. The majority of the sample (93.3%) corresponded to medium/high caries risk. After 1-year, 30/36 participants were reassessed (drop out rate = 16.7%). The proportion with caries progression was 10% (3/30) in the Test group compared to 30% (9/30) in the Control group ($p = 0.05$). When only E2 lesions were analyzed, 4.5% (1/22) of test and 30.4% (7/23) of control lesions progressed ($p = 0.02$). No unwanted effects were observed. According to the FIS, the level of anxiety was low both before and after the treatment. The mean time required for the infiltration was 11.29 min (SD 1.16 min). The results indicate that caries infiltration is an applicable and well-accepted method to be used in children and efficacious in controlling proximal caries lesions in primary molars, particularly E2 lesions.

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Chemical and Physical Characteristics of Enamel Remineralised in vitro

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Transverse microradiography (TMR) is considered to be the 'gold standard' for remineralisation studies. However, while it gives an accurate measure of average mineral content as a function of depth it yields neither structural nor chemical information relating to mineral deposited. The aim of this pilot study was to investigate the utility of adjunctive physical and chemical techniques, in conjunction with TMR, to investigate the nature of mineral deposited during remineralisation in vitro. Human enamel lesions ($n = 2$), remineralised in the presence of fluoride during an in vitro pH-cycling study, were investigated. Focussed ion beam milling (FIB) lamellae were taken from the surface-zone maxima (SZmax), lesion bodies where maximum remineralisation had occurred as indicated by TMR, and from sound enamel. Lamellae were analysed for Ca, P and F using energy-dispersive X-ray spectroscopy (EDX). Ultrastructural detail was studied using transmission scanning electron microscopy (TEM). EDX Ca:P ratios were in the range 1.64–1.71, with a trend of slightly higher values in remineralised regions, compared to sound enamel, indicating that mineral deposited was predominantly apatitic in nature, and possibly slightly more crystalline than sound enamel. F concentration dropped markedly from average values of ca. 0.8% at SZmax to background levels in sound enamel. These F values are still an average, however, and localised values for deposited mineral may be higher. Further work is needed to characterise these localised regions. Overall, TEM showed that ultrastructural details were consistent with results from TMR, with a more compact crystallite structure where TMR indicated greatest remineralisation. In conclusion, these initial data support further work using FIB and chemical analyses to more fully understand the nature of remineralisation of carious enamel lesions.

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The Motivational Effect of Fluorescence Photographs Additional to Oral Hygiene Lessons at Primary Schools

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Aim: The aim was to investigate whether showing and discussing intra-oral fluorescence photographs as a complement to an oral hygiene lesson can motivate children to improve their oral hygiene. **Method:** The study was carried out linked to a classroom based oral hygiene lesson at primary schools based on a teaching program called 'Keep your mouth healthy' from the Dutch foundation for caries prevention 'Ivoren Kruis'. The study was approved by the Medical Ethics Review Committee of VU Medical Center (2014.048), informed consent from children and their parents was

obtained. Children (10–13 years) were randomly assigned to the test or control group. Locus of control (LoC), attitude, knowledge and behaviour regarding oral hygiene were assessed using a questionnaire before and after the lesson and 8 weeks thereafter. Fluorescence photographs of all children were assessed for simple plaque score (QA 1.25; Inspektor Research Systems BV, Amsterdam) after the lesson and 8 weeks thereafter. The test group was shown their fluorescence photograph on which matured plaque was fluorescing red. The control group was shown their white light photograph. **Results:** 493 children participated with a mean age of 11 years (SD 0.9 yr). No significant differences were found between test and control groups at baseline ($p > 0.05$, Mann-Whitney U). Knowledge and attitude had improved after the lesson and both remained stable ($p < 0.05$, Wilcoxon signed-rank). LoC had improved directly after the lesson but returned to baseline level after 8 weeks. Oral hygiene had not improved 8 weeks after the lesson ($p = 0.54$, Wilcoxon signed-rank). **Conclusion:** Oral hygiene lessons at primary schools improved children's knowledge and motivation towards oral hygiene, but did not improve oral hygiene. Showing fluorescence photographs has no added benefit compared to the oral hygiene lesson with white light photographs.

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Session 6

Diagnostics: Clinical and Laboratory-Based

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The Efficiency of Using Qscan in Improvement of Oral Hygiene and Oral Health Behaviors in Children

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The aim of the study was to determine oral hygiene, oral health behaviors of children and motivate to improve them by using portable QLF device such as Qscan. In addition prevalence, experience of caries were also determined. One hundred children aged 14–16 years were included in the study. The children were divided into 2 groups. To the control group of children hygienic lessons were provided as a traditional lecture-training (10 min). The experimental group were provided by a traditional method combined with the demonstration of plaque level using Qscan device (Inspector Research systems BV, Amsterdam, The Netherlands).

The children were examined every week, lessons were provided once in a two week. The total length of observation was 8 weeks. Oral hygiene level was evaluated by Löe and Sillness method, the oral health knowledge, behavior and attitude were assessed by the questionnaire developed by Matina V. et al (2014). Three dentists were calibrated by one professor. Inter-examiner and intra-examiner kappa values were higher than 0.7 in all periods of trial. Written informed consent was obtained from parents or caregivers of all participants before trial. T-student's test was used to calculate significance level between groups and periods.

At baseline oral hygiene status was 0.93 ± 0.03 , knowledge -19.73 ± 0.76 , behavior -20.68 ± 1.59 , attitude -16.45 ± 0.77 in control group and 0.83 ± 0.05 , 18.4 ± 0.81 , 20.52 ± 0.86 , 18.36 ± 0.58 in experimental group, respectively. After 2 weeks of examination both groups showed better significant changes in oral hygiene and oral health knowledge, behavior and attitude. However

at the end of trial after 2 months oral hygiene and oral health knowledge indices showed significant better changes in experimental group (0.07 ± 0.02 ; 29, respectively) ($p < 0.05$), however no significant changes were discovered in a control group (0.46 ± 0.04 ; 19) ($p > 0.05$).

It can be concluded that Qscan aided oral hygiene education was useful to train children and showed better and long lasting results.

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Detection of Caries Around Amalgam Restorations Using Four Different Modalities

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Aim: The aim of this study was to evaluate the ability of PTR-LUM (The Canary System, CS), laser fluorescence (DIAGNOdent, DD), LED fluorescence (Spectra), and visual inspection (ICDASII) to detect natural decay around bonded amalgam restorations.

Methods: 17 extracted human molars and premolars, consisting of 5 visually-healthy and 12 teeth with natural cavitated lesions were selected. For the carious teeth, caries was removed leaving some decayed tissues on the floor and wall of the preparation. For sound teeth, 3 mm. deep cavity preps were made and teeth were restored with bonded-amalgam. 36 sites (13 sound sites; 23 carious sites) were selected. CS and DD scans were performed in triplicate at 2, 1.5, 0.5, and 0 mm away from the margin of the restoration. Spectra images were captured for the entire surface, and dentists blinded to the samples provided ICDASII scoring. **Results:** Canary Numbers (Mean $\pm$ SE) for healthy and carious sites at 2, 1.5, 0.5, and 0 mm ranged from 12.7 ± 0.6 to 15.4 ± 0.9 and 56.1 ± 3.9 to $58.9 \pm$

3.9, respectively. DD peak values for healthy and carious sites ranged from 4.7 ± 0.5 to 13.5 ± 3.2 , and 16.7 ± 3.6 to 24.5 ± 4.3 , respectively. For Canary and DD, sensitivity/specificity for sites at 2.0, 1.5, 0.5, 0 mm ranged from 0.95–1.0/0.95–1.0, and 0.45–0.74/0.54–1.0, respectively. For ICDASII, sensitivity and specificity were 1.0 and 0.17, respectively. For Spectra, data and images were inconclusive due to signal interference from amalgam. **Conclusions:** CS and DD were able to differentiate between sound and carious tissue at the margin of the restoration, but larger variation, less reliability, and poorer accuracy was observed for DD. Therefore, CS has the potential to detect secondary caries around amalgam restorations more accurately than the other investigated modalities.

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Colour of MIH Defects as a Predictor of Enamel Breakdown: A Longitudinal 24-Month Study

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Objective: The objective was to investigate whether the colour of MIH defects is associated with enamel breakdown. **Methods:** a longitudinal 24-month follow-up was conducted with 163 children, aged 6–12 years, diagnosed as having MIH in an epidemiological survey using the EAPD criterion. Chi-square test was used to compare the proportion of white and yellow/brown opacities that progressed to enamel/dentine breakdown. To evaluate the survival rates of 1st permanent molars with MIH (dentine breakdown or extraction were considered as the outcome), Kaplan-Meier survival analysis was performed. **Results:** 48.5% of the sample was male, and 51.5% female (mean age 10.5 ± 1.41). The prevalence of 1st permanent molars with mild, moderate and severe MIH was 54.9%, 13.8% and 31.3%, respectively. The proportion of teeth with white opacities that progressed to enamel/dentine breakdown was 26.9% and with yellow/brown opacities 39.8% ($p = 0.02$). Considering only dentine breakdown, the proportion was 15.3% and 28%, respectively ($p < 0.01$). The mean survival rates for all 1st permanent molars affected by white or yellow/brown opacities were 22.4 ± 0.26 and 21.6 ± 0.49 months, respectively ($p = 0.02$). **Conclusion:** white and yellow/brown opacities associated with MIH have a tendency to progress to post-eruptive breakdown. Yellow/brown opacities run a higher risk of breaking down within 24-month follow-up.

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The Evaluation of Color Changes Due to Abrasion on Incisal Edges of Teeth at Different Time Periods: An *in vitro* and *in vivo* Study

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Discoloration is an important issue in dentistry and may be classified according to the location and etiology. The observed colour of a tooth depends on the thickness of the enamel and dentin. Dark colours may be result of secondary dentin formation, pulpal calcifications or decreasing enamel thickness due to aging. Colour scales are used as subjective methods to classify tooth colour while spectrophotometry and colorimetry, objective methods, are the main tools for measuring tooth colour. The aim of this study was to assess colour changes due to abrasion on incisal edges of teeth *in vitro* and *in vivo* using a colorimeter. In the *in vitro* study, 100 extracted human incisors were divided into two groups as 'enamel' or 'enamel-dentin' according to the 'Occlusal and incisal wear index'. Incisal edges were abraded 4 times (surface removal being 30, 100, 300 and 500 μm) using a modified mechanical surface grinder. Before and after each grinding cycle the colour of incisal edge was evaluated using the colorimeter. In the *in vivo* study, 20 teeth in 5 patients with bruxism [identified according to the ASDA Bruxism Criteria] used only toothpaste and an additional 20 teeth from 5 patients who used both toothpaste and occlusal splints were investigated. Maxillary central and lateral incisors assessed according to the 'Occlusal and Incisal Wear Index' were evaluated by the colorimeter [set to Vita mode] every month for three months. Each measurement was made twice by two independent observers. Each color in the Vita Shade Scala was numbered [from B1 to C4 shades were numbered as 1 to 16] and the Δ values were calculated. Data were analysed using Student t-test and Mann Whitney U Test. We found that the Colorimeter could detect colour changes in both groups (enamel or enamel-dentin) of extracted teeth and that there was no difference between the groups. According to *in vivo* results, Colorimeter could detect colour changes in both groups (splint or non-splint group). Also incisal colors of teeth in occlusal splint group were found to be lighter in colour than the other group due to less tooth wear loss.

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Evaluation of Dental Explorer and Visual Inspection for the Detection of Residual Caries among Greek Dentists

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Objective: The main purpose of this study is to determine the efficiency of tactile sensation through dental explorer and visual inspection in the management of the diagnosis and removal of re-

residual dental caries and the correlation of the results with the experience of the clinician. **Experimental Approach:** Ten freshly extracted human carious teeth were selected. The carious tissues were removed to a level selected in random. One hundred dental students, in the last 2 years of their training in the Athens Dental School, and 50 Dentists from 10 different regions were randomly contacted. Every tooth was examined by each person individually, initially only with the use of visual inspection and, consequently, with the use of a dental explorer for the need for further removal of dental tissues. In addition, a questionnaire inquiring about their dental experience, their familiarity with the Minimal Invasive philosophy and the technique which they use to detect and remove residual caries was completed. Mann-Whitney U-test and Kruskal Wallis tests were performed. ($P < 0.05$). **Main Results:** The mean value for correct answers based on the visual inspection, for all dentists, was 6.01 and, when the dental explorer was used 5.7. The majority of the students (86%) and dentists (83%) stated to be familiar with the Minimally Invasive philosophy. The use of carbide bar in a low speed handpiece was considered by the students as the best method to remove caries (60%); the same option is used by the most dentists (91%). **Conclusions:** Visual inspection without the use of dental explorer leads to the more precise detection of the existence or the absence of residual caries. Diagnosis based on visual examination, alone or with the assistance of dental explorer, was better in the more experienced dentists.

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Caries Experience Using ICDAS in Individuals with Cerebral Palsy Living in a Non-Fluoridated Water Supply Area

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The aim of this study was to compare the caries experience using the ICDAS (International Caries Detection and Assessment System) criteria in individuals with cerebral palsy (CP) and non-morreactive (NR) living in a socioeconomically poor area with a non-fluoridated water supply in Brazil. Sixty children aged 6–12 years, being 30 with confirmed diagnosis of CP and 30 NR were paired by sex and age. Clinical examination was carried out by one calibrated examiner using the two-digit ICDAS criteria, which were converted into components of dmfs/dmft and DMFS/DMFT indices in two diagnostic thresholds: enamel and dentine lesions (d_2mf_2/D_2MF_2 ; comprising ICDAS codes 1–6) and dentine lesions (d_3mf_3/D_3MF_3 ; comprising ICDAS codes 4–6). The f_2/F_2 component included fillings with neither an enamel nor a dentine carious

lesion, and the f_3/F_3 component included fillings with no dentine carious lesion diagnosed on the same tooth surface. Radiographic examination was not performed in this investigation. For the primary/permanent dentitions, the caries experience in NR was 88.9%/93.3% and in CP 92.3%/82.8%, respectively. The mean d_2mf_2/d_2mf_2t and D_2MF_2S/D_2MF_2T for CP were $17.0 \pm 16.8/7.5 \pm 4.3$ and $10.7 \pm 17.6/5.3 \pm 5.8$, respectively, and for NR were $17.2 \pm 16.9/6.9 \pm 4.8$ and $11.1 \pm 11.7/5.5 \pm 4.7$, respectively. The mean d_3mf_3/d_3mf_3t and D_3MF_3S/D_3MF_3T for CP were $10.1 \pm 16.7/3.0 \pm 4.1$ and $4.9 \pm 15.6/0.2 \pm 0.4$, respectively, while for NR the mean values were $9.8 \pm 13.0/3.5 \pm 3.8$ and $2.1 \pm 5.7/0.9 \pm 2.0$, respectively. There was no statistically significant difference between the caries experience in both groups (Mann-Whitney test; $p > 0.05$). In the primary dentition, the ICDAS score 6 was more prevalent in both groups, followed by ICDAS score 2. In the permanent dentition, the ICDAS score 2 was more prevalent, followed by ICDAS 1 in NR and ICDAS 6 in CP. In conclusion, dental caries was highly prevalent in CP and NR children in this Brazilian population, and the presence of enamel and dentine lesions was the most common condition.

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Visual, Radiographic, Separation and Laser Fluorescence for Detection of Proximal Caries in Primary Teeth

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Accurate detection and diagnosis of proximal caries in primary molars is challenging. Therefore, the aim of this *in-vivo* study was to assess the validity and reproducibility of four methods of proximal caries detection in primary molar teeth. Eighty-two children (aged 5–10 yrs) were recruited. Initially 1030 proximal surfaces were examined using meticulous visual examination (ICDAS), bitewing radiographs, and a laser fluorescence device (LF pen). Temporary tooth separation (TTS) was achieved for 447 surfaces and these surfaces were re-examined visually and using the LF pen. The teeth were subsequently extracted (due to caries or orthodontic reasons) and serially sectioned for histological validation. Intra- and inter-examiner reproducibility were assessed, the second examiner re-examined 10% of surfaces. At D_1 (enamel and dentine caries) diagnostic threshold, the sensitivity of ICDAS visual examination, radiographic examination, TTS and LF pen examination were 0.52, 0.14, 0.75, 0.58 and the specificity values at this threshold were 0.89, 0.97, 0.88, 0.85, respectively. At D_3 (dentine caries) diagnostic threshold, the sensitivity of the ICDAS examination, radiographic examination, TTS and LF pen examination were 0.42, 0.71, 0.49, 0.63 respectively, while the specificity was 0.93 for both ICDAS examination and TTS, and 0.98 and 0.87 for radiographic and LF pen examinations respectively. ROC analysis of the different methods showed the radiographic examination to be superior at D_3 level. For intra- and inter-examiner reproducibility the Kappa coefficient ranged

from $K = 0.75$ at D_1 to $K = 0.95$ at D_3 and $K = 0.73$ at D_1 to $K = 0.85$ at D_3 , respectively.

In conclusion meticulous visual examination (ICDAS) should be supported by radiographs for the detection of dentinal proximal caries in primary molars. The LF pen and TTS also provide additional diagnostic information at both thresholds.

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Caries Prevalence in Children with and without MIH: A Case Control Study

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The aim of this study was to compare the caries prevalence of children who presented with MIH with those who did not. A case control-study was designed in which 130 children aged 7–13 years old diagnosed as presenting MIH composed the cases and were matched with 130 children without the condition (controls) considering age, gender and school. Dental caries and MIH were assessed using the WHO and EAPD criteria, respectively, by one trained and calibrated examiner. Caries prevalence and the mean dmft/DMFT were calculated for both groups. An additional analysis was performed considering only the first permanent molars of cases and controls. Wilcoxon signed ranks test was used to determine differences between mean dmft/DMFT of both groups. McNemar test was performed to verify the effect of MIH in relation to the occurrence of caries lesions. Finally, odds ratio were performed in order to verify if MIH was a risk factor to dental caries development. The mean age of the children was 8.33 ± 1.26 years. Regarding the severity of MIH, 70.76% of the cases were classified as presenting mild, 8.46% moderate and 20.76% severe. The mean dmft/DMFT for cases and controls were $1.23 \pm 1.99/0.45 \pm 0.90$ and $1.71 \pm 2.22/0.07 \pm 0.25$, respectively, being statistically different only for DMFT ($p < 0.001$). Enamel and dentine caries prevalence for the 1st permanent molars were 48.46% and 23.08% (cases), and 28.46% and 5.38% (controls). Presence of MIH increased the risk of enamel [OR 2.53 (CI 1.44–4.43), $p = 0.0008$] and dentine caries lesions development [OR 8.67 (CI 2.62–28.63), $p < 0.0001$]. In conclusion children with MIH presented higher caries prevalence for the permanent dentition in comparison to those without the condition. MIH maybe considered a risk factor for caries development.

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Validation of the Visible Occlusal Plaque Index Estimating Caries Activity

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The present study appraises the construct and concurrent validity of the Visible Occlusal Plaque Index (VOPI) as a measure of the association between dental biofilm and caries occurrence and activity in permanent molars. A total of 618 10–15 year old Brazilian adolescents were included in the study. Data on adolescents' socio-demographic and oral health determinants were collected. The occurrence and distribution of occlusal plaque on first and second permanent molars were mapped and recorded at individual surface-anatomical sites of the groove-fossa-system. Caries activity and severity was assessed at surface level for the permanent dentition after plaque removal and air drying. On the occlusal surfaces of the molars, the occurrence and localization of caries were also registered at individual anatomical sites of the groove-fossa system. Caries outcomes were presence of active or inactive occlusal lesions or sound occlusal surfaces. At adolescent's level, the results showed that the higher the plaque score, the higher the caries activity on occlusal surfaces ($p < 0.001$, Fisher exact test). At surface-anatomical site level, sites with no or thin plaque had significantly greater chance of being sound rather than carious (RR range 1.9–22.4). Also, sites with no or thin plaque had a substantial chance of having inactive lesions whereas sites with thick or heavy plaque had a considerable risk of having active lesions (RR range 1.0–7.3). Additional GEE analysis showed that different scores of the Visible Occlusal Plaque Index were the most influential determinant for sound occlusal surfaces ($p < 0.001$), for inactive ($p = 0.018$) and for active occlusal lesions ($p < 0.001$). In conclusion, the Visible Occlusal Plaque Index, has construct and concurrent validity being therefore recommended as an additional tool to estimate caries activity and to support treatment decisions in daily practice.

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Caries Risk Assessment in Children Initially Aged 6–72 Months

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The aim of this study was to assess the relationship of a formalized pediatric caries risk assessment (CRA) procedure, and that of the individual CRA items, with clinically evident caries, cross-sectionally and longitudinally. CRA information was abstracted retrospectively from electronic patient records of children initially ages 6–72 months at a university pediatric dentistry clinic from the years 2009–2015 (N = 3810 baseline; N = 1315 with follow-up). The 17-item CRA form included caries risk indicators, caries protective items, and clinical disease indicators. Conditional random forests classification trees were implemented to identify and assign variable importance to CRA items independently associated with baseline high-risk designation, baseline evident tooth decay, and evident decay at first follow-up.

In the conditional random forests analysis, thirteen individual CRA items, including all clinical indicators and all but one risk indicator, were statistically significantly ($p < 0.05$) associated with practitioners' caries-risk designation after adjustment. Risk designation was strongly associated with follow-up decay, which increased by CRA category: low (20.4%), moderate (30.6%), and high/extreme (68.7%). Of individual baseline CRA items, before adjustment 12 were associated with baseline decay and 7 with decay at follow-up; however, in conditional random forests models, only the clinical indicators (evident decay, dental plaque, and recent restoration placement) and one risk indicator (frequent snacking) were statistically significantly associated with future disease after adjustment.

In conclusion, since practitioners consider a range of items in decision-making regarding pediatric caries risk assessment suggests that comprehensive CRA forms can aid individualized treatment planning, even if fewer items are essential for predicting disease status. In this predominantly high-risk child population, future decay strongly related to low, moderate and high caries risk levels assigned by a formalized caries risk assessment procedure. Baseline disease was the strongest individual predictor.

Diagnodent Validity and Pre/Post Test Probability for White Spot Lesions Detection in Orthodontic Patients

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The aim was to clinically validate the Diagnodent Pen (DP) for the detection of WSL around brackets during orthodontic treatment, using as reference the direct visual examination after debonding. 619 teeth in 31 orthodontic patients were evaluated for WSL before the removal of the brackets, with the diagnostic methods direct visual examination (DV), indirect visual (IDV) and DIAGNodent (DP); the reference method was DV examination after bracket removal. Examinations were performed by one examiner and WSL were recorded using the Gorelick index (sound, early, extended, cavitated). Intraexaminer reliability was found to be $k > 0.90$ for all methods.

When analyzing the results for all categories of WSL, accuracy was found as 0.97 for DV, 0.92 for IDV and 0.64 for DP; AUC did not differ between the different examination methods. For early WSL lesions, the DP had the smallest sensitivity and AUC. Pre-test probability was estimated using Bayesian theory as 0.11 for DV, 0.11 for IDV, and 0.39 for DP, while the post-test probability was 0.77 for DV, 0.66 for IDV and 0.55 for DD. In conclusion DP was not found superior to the visual methods for WSL detection. DV had the highest validity for the detection of WSL during orthodontic treatment and the highest probability to predict the white spot lesions presence after brackets removal.

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Use of the Calcivis Activity Imaging System for Activity Assessment of Caries Lesions

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Objective: Assessment of caries lesion activity is usually performed using visual criteria. A new approach is to use a disclosing solution specific for Calcium ions which are produced during active re- and de-mineralization processes and hence detection of free Ca^{2+} is specific to active lesions. The Calcivis Activity Imaging System applies a photoprotein to the tooth surface which binds calcium ions and emits a blue light signal proportional to the amount of calcium present. The Calcivis camera is able to visualize the elevated calcium ions as luminescence spots on the tooth surface. This study aimed to evaluate the ability of a prototype Calcivis System to assess caries lesion activity in-vitro. **Material and**

Method: Investigation sites of 46 extracted permanent posterior teeth were classified by two independent examiners using ICDAS and Nyvad criteria for lesion activity (activity yes/no). Consensus score of each site was determined as reference value. The sites were photographed using the prototype Calcivis System. The images were analysed by both examiners for presence or absence of luminescence spots. Correlation of methods was calculated using Spearman rank correlation coefficient (rs). Agreement between methods was assessed by kappa statistics. ROC curves were created for Calcivis and both visual methods and the areas under the Curve (AUC) were compared ($\alpha = 0.05$). **Results:** Significant positive correlation was found between Calcivis and the visual detection methods: rs ICDAS = 1.0, rs Nyvad criteria = 0.776 ($p < 0.001$). Kappa-values were: Calcivis/ICDAS = 1.0, Calcivis/Nyvad = 0.78. AUC for Calcivis was 1.0 (ICDAS as reference value) and 0.89 (Nyvad criteria as reference value). No significant differences were observed between the AUCs ($p = 0.30$). **Conclusion:** Calcivis showed good correlation and high agreement with visual criteria regarding lesion activity assessment in extracted teeth.

The Calcivis ActivityImaging System was provided by Calcivis-Ltd (Edinburgh, UK).

(0.96; 95%-CI: 0.83–0.99 and 0.89; 95%-CI: 0.56–0.99) and intra-examiner agreement (ICC) (0.69; 95%-CI: 0.28–0.97 and 0.74; 95%-CI: 0.35–0.97), respectively. D-enamel blocks presented significant higher roughness (Ra) (1.34 ± 0.69) than S (0.73 ± 0.21) (Student's t test; $p < 0.05$). Inter/intra-examiner reproducibility mean weighted kappa values were respectively for FCT: 0.66 and 0.61 and WHO: 0.53 and 0.71, with significant differences for inter-examiner reproducibility (paired Student's t test; $p < 0.05$). The performance parameters of the different probes in detecting presence of roughness did not present statistically significant differences using the McNemar test ($p > 0.05$). The sensitivity, specificity, and accuracy percentage values obtained with the FCT probe were respectively 77.8%, 90.6% and 82.2%. Using the WHO probe, corresponding figures were 77%, 84.6% and 79.4%, respectively. In conclusion, the designed force-controlled top probe showed similar reliability and validity to the WHO probe for tactile roughness in the enamel lesion activity assessment.

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Validity and Reliability of a Designed Probe in the Assessment of Enamel Tactile Roughness Related to Caries Activity

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The aim was to evaluate, using tactile roughness assessment, a designed force-controlled top probe (FCT) as an indicator of demineralized/sound enamel.

First, 4 FCT with different instrument designs in terms of handle width, weight, active part length and angulation, tip end form, and roughness and the WHO ball-ended probe were blindly tested for correlation between tactile feeling and roughness and inter/intra-examiner agreement among 5 experienced examiners in ICDAS-caries detection/activity assessment in 4 standard roughness plaques (0.10, 0.40, 0.80, 1.60 μm) to select the best design. Ten out of 20 4x4x2 mm human sound enamel blocks (S) were demineralized (pH demineralizing cycles) (D) and enamel roughness measured (surface roughness tester). Five-blinded examiners randomly tested roughness in the 20 S/D-enamel blocks with selected FCT and WHO probes. Inter/intra-examiner reproducibility was calculated for each probe. Considering the status of the tooth (S vs. D) as reference standard, overall sensitivity, specificity and accuracy (percentage of correct answers) were calculated for each probe and compared. All 5 probes presented a strong tactile roughness and roughness correlation (Spearman) (0.70 – 1.0), with FCT design Nr. 4 and WHO probes showing highest inter-

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Assessment of Uncavitated Carious Enamel Lesions by Optical Coherence Tomography and X-Ray Microtomography

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Aim: The aim was to evaluate the performance of spectral domain optical coherence tomography (SD-OCT) and X-ray microtomography (μCT) for detection and assessment of uncavitated carious enamel lesions. **Methods:** 28 extracted human teeth with 47 approximal carious lesions of ICDAS-II code 0–2 were selected. One region of interest (ROI) was marked by two drill-holes each in direction of maximum extension of the lesion, followed by imaging with SD-OCT (center wavelength 1310 nm) and μCT (100 kV). Transverse microradiographs (TMR, 20 kV/20 mA) of sections through the ROI (150–250 μm) and randomly scanning electron microscopy images (5 kV) were used for validation of OCT and μCT signals. Lesions were categorized according to the extent by a five-category scale: 0-sound; 1-lesion extended up to first, 2-second, 3-third and 4-fourth quarter of enamel. The lesion depth was measured (μm) and categorizing of lesions and lesion depths were statistically compared (weighted Cohen's kappa coefficient κ , Wilcoxon-test; Spearman correlation, $\alpha = 0.05$). **Results:** The agreement between the methods for categories 0–4 was moderate to substantial (TMR- μCT , TMR-OCT, μCT -OCT: κ : 0.47/0.48/0.69). For sound enamel (category 0) it was perfect to moderate (100%/67%/54%). In contrast, fair to substantial agreement was determined for category 1 (33%/60%/67%). Category 1 was detected 2.5 and 2.7 times more often with TMR and OCT than with μCT .

Combined categories 2+3+4 showed a moderate to almost perfect agreement (57%/52%/87%). The comparisons of methods TMR- μ CT, TMR-OCT and μ CT-OCT revealed a moderate to strong correlation regarding lesion depth (r : 0.515/0.614/0.597; all $p \leq 0.001$), whereby the values significantly differed in the comparisons of TMR- μ CT and μ CT-OCT, respectively (all $p < 0.005$). **Conclusion:** OCT seems to be more suitable to assess enamel carious lesions, limited to the outer quadrant of the enamel, than μ CT.

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Monitoring Caries in vitro by Visual Photographic and Diagnostent Methods

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This study aimed to monitor in vitro the demineralization of deciduous enamel tooth in 40 human deciduous submitted to demineralization cycles for 24, 48 and 96 hours by visual photographic (VP) and DIAGNodent methods (Dd). After demineralization, photographs of the specimens were analyzed blindly by three calibrated examiners who attributed scores for enamel appearance: 1-intact enamel, 2-initial white, 3-advanced white and 4-cavitation. The Kappa statistic showed an intra-examiner agreement of 0.85, 0.83 and 0.87, for examiners 1, 2 and 3 respectively. Examination with Dd was performed in triplicate also blindly, and were used concordant readings between at least two of the examiners. The evaluation of mineral loss (ML) was made by determining the difference between the phosphorus concentration in the demineralizing solution (DES) before and after the production of caries. The groups 24, 48 and 96 show respectively 0.916a; 1.458b and 1.922 c/ μ gP/ml in DES more after caries production. The evaluation of mineral loss (ML) was made by determining the difference between the phosphorus concentration in the demineralizing solution (DES) before and after the production of caries. Pearson correlation test showed a weak but significant correlation between the PM and the second examination methods ($n = 30$) with $p = 0.02$; for VP and $p = 0.01$ and for Dd, $p = 0.45$. Data were analyzed per group ($n = 10$), the VP showed statistically significant correlation with the (ML) in Group 96 ($p = 0.04$) and Dd 24 ($p = 0.00$). It was concluded that the Dd method was more effective in diagnosing caries lesions at its early stage than (VP) examination.

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Evaluation of the Calcivis Activity Imaging System in the Assessment of Caries Activity and Demineralisation

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The aim was to evaluate the capability of the Calcivis Activity Imaging Device as a method for assessing both caries lesion activity and demineralisation on enamel surfaces following exposure to acid challenge. Previous ORCA posters reported a luminescent assay for assessing caries lesion activity and erosive challenges (2008/9/10/13/14/15). The device consists of a sensitive intraoral camera capable of imaging very low level, transient luminescence when a calcium activated photoprotein is applied to enamel surfaces of teeth or enamel blocks. Using a dark box, images were obtained from 12 sample extracted teeth, showing visible evidence of active caries after demineralisation with a low pH gel, and 6 eroded bovine enamel blocks. The enamel blocks were exposed to erosive conditions, where approximately half of their enamel surface area was subjected to erosive conditions (30% phosphoric acid, 15 s), and a further image taken. The images obtained of the enamel blocks using the Calcivis Activity Imaging System clearly show visibly increased luminescence in areas exposed to erosive conditions. The increased luminescence is caused by an increase in free calcium ions liberated within the surface enamel layer, interacting with the photoprotein resulting in an increase in luminescence, which were then detected by the device. This increase in luminescence was evident in all 6 enamel blocks, which showed a clear distinction between the sound and eroded enamel surfaces. This increased luminescence was also observed in demineralised, extracted tooth samples exhibiting signs of active caries lesions. Of the 12 teeth, 10 showed increased luminescence in areas associated with signs of caries activity. In conclusion the Calcivis Activity Imaging System was able to detect caries lesion activity and areas of eroded enamel.

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Diagnostic Abilities of Light Induced Fluorescence – LIF with Soprolife Device

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The aim of this study was a validation of the diagnostic abilities of the Soprolife (SL) device which uses light induce fluorescence (LIF) with an excitation wave length 450 nm. Four in vitro studies, which included 328 human permanent teeth, were performed for 1. verification and detection of secondary caries and external defects (50 molars and 68 incisors were studies), 2. for fissure caries (45 molars), 3. for dental fluorosis (13 teeth from 116 teeth) and

4. for dental hypoplasia (11 teeth from 49 teeth). Clinical examinations using ICDAS II criteria and LIF were undertaken in the following diagnostic regimes: daylight and blue light with the manufacturer's software. Bitewing x-rays, histology of mirror images of sections and QLF Diagnodent Classic were used as positive control methods. The lesions were presented as images in both lighting regimes with magnifications x5, x30, x100. The defects identified were enamel hypoplasia, idiopathic white spots, hypomineralization of enamel, mild, moderate and severe fluorosis. Differences between external secondary defects and secondary caries are well visualized with SL diagnostic device in molars and incisors. Fissure caries was detected with SL with 75.6% accuracy, but in comparison with histology the differences between methods are $p < 0.001$ ($W = 342$). In 24.4% of the cases over-diagnosis was 15.6% and under-estimation was 8.9%. Differences between LIF and ICDAS $p < 0.1$ and between QLF and ICDAS $p < 0.05$. In conclusion: 1. For dental fluorosis and hypoplasia SL can act as intra-oral camera which helps for better visualization of stains on distal teeth. Clinical experience is necessary for accurate diagnosis. 2. SL distinguishes external marginal defects from secondary caries. 3. SL detected fissure caries and exhibited better diagnostic abilities than Laser fluorescence.

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Sealant Adaptation and Penetration on Questionable for Caries Occlusal Surfaces (ICDAS II Codes 1, 2 and 3)

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The effectiveness of pit and fissure sealants for questionable occlusal surfaces (having noncavitated, deep, stained, chalky pits and fissures and with no radiographic radiolucencies), is contra-indicated probably because of the different enamel composition of these surfaces. Alternative etching methods may improve their preparation and increase surface etching uniformity. The aim of this *in vitro* study was to test alternative methods of etching materials and times of application on pits and fissures of questionable occlusal surfaces. 40 occlusal surfaces of ICDAS II codes 1, 2, 3 were etched with an inorganic acid (H_3PO_4 37%) with or without previous preparation with NaOCl. Sealants were placed and teeth sectioned at the direction of the characterized lesions. Surfaces were studied by polarized light microscopy for estimation of sealant penetration and adaptation on fissure walls. Univariate and multivariate ordinal logistic regression and odds ratios and confidence intervals were used for statistical analysis. We found that the odds of sealant to get the maximum penetration was 16% lower for ICDAS code 2 compared to 1 (OR = 0.84, 95% CI: 0.28, 2.50, $p = 0.75$) and 73% lower for ICDAS 3 compared to 1 (OR = 0.27, 95% CI: 0.09,

0.84, $p = 0.02$). Respectively, the odds of sealant to get the maximum adaptation were statistically significant lower for ICDAS codes 2 and 3 compared to 1 (OR = 3.69, 95% CI: 1.47, 9.23, $p < 0.01$) and (OR = 20.02, 95% CI: 5.42, 74, $p < 0.001$) respectively.

In conclusion, sealant adaptation and penetration on questionable for caries occlusal surfaces are compromised and differ from sound enamel. Application of alternative etching methods with H_3PO_4 and NaOCl and different etching times did not improve the pattern of etching, especially on surfaces with ICDAS II codes 2 and 3. Further investigations with different methods of fissure preparation and etching are needed to improve sealant bonding on such sites.

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Effect of Resin Infiltration on Laser Fluorescence Measurements in Proximal White Spot Lesions *in vitro*

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The objective of this *in-vitro* study was an assessment of the influence of resin infiltration on laser fluorescence pen (LFpen) measurements of proximal enamel caries surfaces. The study was performed with freshly extracted human teeth (premolars and molars), with or without a visible proximal chalky white spot lesion without cavitation. After cleaning with prophypaste and after rinsing with tap water, one defined spot of each lesion was carefully measured with LFpen (DIAGNodent pen). Only teeth with laser fluorescence values below 30 were included ($n = 49$). The opposite sound sites acted as control sites ($n = 49$). Resin infiltrant (Icon) was applied on every white spot lesion and after one day the infiltrated surface was then reassessed with LFpen. The results were statistically analyzed with nonparametric tests (signed and Wilcoxon signed-ranked test). Before infiltration, the distribution of caries depths according to LFpen measurements was: D_0 : 4–6 (5 teeth), $D_{1,2}$: 7–17 (33 teeth), D_3 : 18–29 (11 teeth). After infiltration, the distribution was D_0 : 2–6 (24 teeth), $D_{1,2}$: 7–17 (23 teeth), D_3 : 18–29 (2 teeth). The results obtained from laser fluorescence device surfaces checked at the baseline showed that median (lower/upper quartile) was 13 (10/17). The median value for Diagnodent pen for resin infiltration-treated surfaces was 7 (5/9). LF pen readings before and after infiltration were highly significantly different and in comparison to the control sites ($p < 0.001$). It can be concluded that resin-infiltrated white spot lesions significantly reduce LFpen measurements compared to untreated control surfaces under *in-vitro* conditions.

Laboratorial Training with Extracted Teeth as an Approach for Training Students in Assessing Caries Lesions

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The impact of laboratorial activities on caries detection using extracted teeth has not been tested compared to conventional lectures. This unicentre study aimed to verify if laboratorial training for visual inspection using extracted teeth may influence on student's practical skills for detecting caries lesions and estimate the magnitude of this impact. This controlled randomized study is part of a multicentre study including other institutions and refers to the institution which designed the pre-clinical activity. First and last-years undergraduate students were invited to participate. They were randomized depending on the exposure to the laboratorial training with extracted teeth (control: no training; test: training). All students received a 90-min conventional lecture including basic concepts. For the training, the students were divided into smaller groups guided by graduate students as tutors. The training was based in scoring followed by discussion in groups using, firstly, photographic images, and then, extracted teeth. Multilevel Poisson models were used to test the influence of training on outcomes and Prevalence Ratios (PR; 95% CI) were calculated. The students' performance and the number of false positive and negative results in a practical test were considered as outcomes. 174 and 187 students were included in control and test groups, respectively. Students who received laboratorial training had a 10% higher percentage of getting the correct scores in practical test (PR = 1.10; 95% IC: 1.05 to 1.16). Training also reduced in approximately 30% the number of false positive results (initial: 0.68; 95% CI: 0.58 to 0.80; moderate: 0.68; 95% CI: 0.58 to 0.80; advanced: 0.69; 95% CI: 0.59 to 0.82). In conclusion, training with extracted teeth may improve undergraduate students' overall practical performance in detecting caries lesions and this pre-clinic activity reduces positive results.

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Caries Activity Assessment: Which Is More Dominant, Visual or Tactile Examination?

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The objective of this project was to evaluate which parameter is more dominant to determine caries activity; appearance (visual) or texture (tactile). Three dentists trained and calibrated with ICDAS participated. 91 approximal surfaces from 73 extracted human premolars that had white-spot lesions (ICDAS = 1 to 4) were examined. Examinations were performed independently with dental unit light, three-way-syringe, and explorer. Surface appearance (dull or shiny), texture (rough or smooth) and caries activity (active or inactive) were recorded. The majority across the three examiners was used for final judgment for each parameter. Logistic regression was used to predict caries activity from visual and tactile examinations to evaluate the relative importance of the two parameters. The total numbers of active and inactive surfaces were 49 and 40, respectively. The visual-tactile combinations by caries activity were: For active, dull-rough: 38 (79%), dull-smooth: 8 (17%), shiny-rough: 1 (2%) and shiny-smooth: 1 (2%); For inactive, dull-rough: 2 (5%), dull-smooth: 7 (18%), shiny-rough: 4 (10%) and shiny-smooth: 27 (68%). Overall, appearance of active surfaces consisted of 47 dull surfaces (96%) and 2 shiny surfaces (4%); and texture consisted of 39 rough surfaces (81%) and 9 smooth surfaces (19%). Appearance of inactive surfaces consisted of 9 dull surfaces (22%) and 31 shiny surfaces (78%); and texture consisted of 6 rough surfaces (15%) and 34 smooth surfaces (85%). The Wald chi-square statistic for appearance (visual) was 18.57 ($p = 0.00002$) and that for texture (tactile) was 11.85 ($p = 0.00058$). Within the limitations of this project, it can be concluded that appearance (visual examination) is more dominant than texture (tactile examination) for caries activity assessment.

Quantitative Analysis of Occlusal Tooth Surfaces for Distinction between Non-Cariogenic and Cariogenic Discolouration Using QLF-D

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Occlusal teeth frequently show discolouration of the pits and fissures. Non-cariogenic discolouration (NCD) refers to the attachment of staining chromogens to sound surfaces, whereas cariogenic discolouration (CD) represents the discolouration of porous structures due to bacterial metabolites and to mineral losses from the enamel surface. This study evaluated whether QLF-D can differentiate between NCD and CD via quantitative analysis of discoloured pits and fissures of occlusal tooth surfaces. A total of 62 extracted permanent molars and premolars, which had suspected discolourations on the pit and fissure areas, were selected and examined. Fluorescence images of each occlusal surface were captured by QLF-D, and then maximum values of fluorescence loss ($\Delta F_{\max}$) and red fluorescence change ($\Delta R_{\max}$) were calculated using the QLF-D software (QA2 v1.25, Inspektor Research Systems BV). After completion of all analyses, the teeth were sectioned and examined histologically as a gold standard. The sensitivity, specificity, and areas under receiver operating characteristic curves (AUROC) were obtained at the enamel level. Before the analysis, a sound tooth was defined as showing NCD, while enamel and dentine lesions were defined as indicating CD. Histological examination revealed that 12 teeth were sound, while 50 teeth had enamel and dentine caries. The $\Delta F_{\max}$ ($r = 0.76$) and $\Delta R_{\max}$ ($r = 0.80$) showed strong correlations with the histology ($p < 0.01$). At the optimum threshold of $\Delta F_{\max}$ (≤ 75.0) and $\Delta R_{\max}$ (≤ 105.0), $\Delta R_{\max}$ showed a higher sensitivity (0.96) and AUROC (0.94) and a lower specificity (0.83) than $\Delta F_{\max}$ (0.80, 0.91, and 0.92 respectively). The results confirm that the $\Delta R_{\max}$, or red fluorescence change, could discriminate between NCD and CD. QLF-D could therefore be used to distinguish non-cariogenic from cariogenic discolouration with high validity and reliability.

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Spectral Hyper-Vision as an Emerging Imaging Modality in Clinical Research and Management of Caries

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Introduction: The introduction of an innovative hyperspectral imaging solution as an aid for visual inspection to improve the accuracy of diagnosis, grading and follow up of dental caries. For this purpose, the ability of the system to detect and assess occlusal and proximal caries lesions on permanent teeth was validated histologically. **Experimental Approach:** Twelve adult premolars and molars containing lesions of various severity levels (ICDAS II range: 1–6) were utilized. Caries lesion distribution was assessed by the novel system configured as an advanced dental camera, through the construction of appropriate spectral maps. Specifically, the so-called Real-Time-Spectral-Mapper (RTSM), invented by the last co-author; a miniaturized, snap-shot spectral imager that captures and displays millions of spectra and spectral classes in real time, was utilized. The spectra were measured both in whole teeth and in histologic cross-sections, so that their pure and mixed profile comprised the training set of the system. Subsequently, spectral maps obtained from sound and carious dental tissues were constructed. Also, the teeth were histologically evaluated in a stereomicroscope (magnification 10x) for caries lesion depth. **Results:** The spectral characteristics of sound and carious enamel and dentin were established in a wide spectral range from ultraviolet to near infrared (360–1000 nm). The system's histological validation of 12 teeth showed that the clinical classes derived from acquired spectral profiles depict clearly the key features of the ICDAS II scale with high consistency (>90% using histology as reference) and 100% reproducibility. **Conclusions:** RTSM has the same operational complexity of a regular color dental camera, showing a very promising potential as a tool for early diagnosis, for the establishing of objective treatment-monitoring cut-offs and of a reliable, quantitative grading system for the clinical assessment of dental caries.

There are no funding sources associated with this scientific project.

Validation of an Automated Caries Detection System for ICDAS-II Classification of Occlusal Caries from Photographic Color Images in vitro

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The aim of this study is to validate the accuracy of the ACDS system [Berdouses ED, Koutsouri GD, Tripoliti EE, Matsopoulos GK, Oulis CJ, Fotiadis DI. A computer-aided automated methodology for the detection and classification of occlusal caries from photographic color images. *Computers in Biology and Medicine*, 2015;62:119–135.] on the detection of occlusal caries on permanent posterior teeth using their histological picture as the gold standard. There were used 18 teeth with 40 sections accounting for 53 pits or fissures classified clinically ICDAS 0–3 by 2 examiners. The ERK classified 18 surfaces as ICDAS 0 and 11, 16 and 8 as

ICDAS 1, 2 and 3. The areas were evaluated with the ACDS and the outcome was compared with the histological sections. The sections were evaluated twice by two experienced observers. Any disagreement between the observers was discussed to a final decision. This decision was used as the golden standard which was then compared with the classification of the ACDS. ACDS classifies the lesions according to ICDAS system and the histological samples were evaluated based on the Ekstrand-Ricketts-Kidd (ERK) classification. The examiner reliability that evaluated the histological sections was evaluated with weighted kappa statistics. The intra-examiner value of kappa was 0.87 and 0.89 for the first and second examiner, respectively, while the inter-examiner reliability was 0.87 for the first and 0.92 for the second evaluation. There used two cut-off points for every classification system, ERK and ICDAS that was used by the ACDS system. The best agreement between the ERK and the ACDS classification was found when caries was considered on both ICDAS (from the ACDS classification) and ERK systems, as code 3 and beyond. The un-weighted kappa for the two methods was found 0.76 while agreement, sensitivity and specificity was 92.5%, 100% and 91.1% respectively. In conclusion the ACDS system has very good agreement with the extent of the lesions into the tissue, offering an objective system to classify occlusal caries of posterior teeth eliminating the subjectivity of the dentist.

No external funding was received for this work.

Session 7

Erosion: Clinical and Laboratory-Based Studies

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Interim Results Investigating Timing of Dietary Acid Intake, Oral Hygiene Procedures and Dental Erosion

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The aim of this study was to assess the dietary behaviour of patients with and without dental erosion. Following informed consent, patients attending King's College London Dental Institute were recruited, from specialist clinics for erosive tooth wear and general clinics. A non-blinded single, trained interviewer questioned participants on their frequency and timing of dietary acid intake, in addition to habits associated with dietary acid consumption. The BEWE was used to differentiate both groups and those participants with an accumulative score >12 and a score of 3 in any one sextant were allocated into the erosion category. Comparisons between groups with and without dental erosion were done using non-parametric tests (Chi-squared and Mann-Whitney) using SPSS 22. Work is currently being done on multivariate regression analyses. Data from 528 participants consisting of 233 with erosive tooth wear and 296 controls were analysed. Groups were stratified into different age categories to limit age as a confounding factor and the median age of the sample was 43 (IQR 32.54). More males presented with erosive tooth wear than females (55.2% and 44.8% respectively; $p < 0.0001$). The erosion group consumed fruit a median of 2 times/day (IQR 1.3) compared to 1 times/day (IQR = 1.2) for controls ($p = 0.014$). Erosion patients consumed acidic drinks a median of 2 times/day (IQR 1.3) and controls 1 times/day (IQR 0.1) ($p < 0.0001$) and were more likely to sip, swish or hold drinks in the mouth than controls ($p < 0.0001$). No difference was observed between groups with regard to brushing the teeth before or after breakfast. However, erosive tooth wear patients were more

likely to self-report as brushing their teeth within 10 minutes of eating or drinking something acidic ($p = 0.006$). In conclusion, participants with erosive tooth wear had more frequent consumption of fruit and acidic drinks. They were more likely to have an alternate drinking habit and brush their teeth after consuming acidic foods or drinks.

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Protective Effect of Whole and Fat-Free Fluoridated Milk Against Dental Erosion

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This study analysed *in vitro* the effect of milk against dental erosion, considering three factors: type of milk (bovine whole/fat-free), presence of different fluoride concentrations and time of application (before/after erosive challenge). Bovine enamel ($n = 15$ /group) and root dentine ($n = 12$ /group) specimens were submitted to the following treatments: 0.9% NaCl solution (negative control); whole milk with 0, 2.5, 5.0, 10.0 ppm F; fat-free milk with 0, 2.5, 5.0, 10.0 ppm F; 0.05% NaF solution (positive control) (after first erosive challenge); whole milk with 0, 2.5, 5.0, 10.0 ppm F; fat-free milk with 0, 2.5, 5.0, 10.0 ppm F; 0.05% NaF solution (positive control) (before first erosive challenge). Specimens were submitted to demineralisation-remineralisation regimes, 4 times/day, for 5 days. The response variables were enamel and dentine loss (median $\pm$ interquartile range μm). Data were analysed using Kruskal-Wallis/Dunn's test ($p < 0.05$). For enamel, whole milk containing 10 ppm F (0.98 ± 0.25), applied before erosive challenge, was the most protective treatment, but with no significant difference com-

pared to the same treatment done after the erosive challenge (1.29 ± 0.23). For dentine, whole fluoridated milk (2.5 (2.09 ± 0.46), 5.0 (2.03 ± 0.36), 10.0 (1.83 ± 0.17) ppm F concentrations, after), fat-free 10 ppm F milk (after (1.95 ± 0.44), before (1.73 ± 0.34)) and whole milk with 0 (2.04 ± 0.54); 5.0 (1.96 ± 0.34), 10 (1.66 ± 0.54) ppm F all before significantly reduced dentine erosion. It seems that the presence of fluoride, especially at 10 ppm, is the most important factor in reducing dental erosion.

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Micro-Shear Bond Strength of Self-Etch and Etch-and-Rinse Adhesives to Eroded Primary Enamel

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The aim of this *in vitro* study was to evaluate the microshear bond strength of four adhesive systems to eroded and sound primary enamel. Primary molars were cut mesio-distally, embedded, grinded and polished. Specimens ($n = 96$) were randomly divided according to adhesive system: G1 (Adper Single Bond2[®]), G2 (Universal Single Bond[®]), G3 (OptibondFL[®]) and G4 (Bond-Force[®]). Half of the specimens were submitted to erosive/abrasive cycles, and the other half (control group; $n = 48$) remained immersed in artificial saliva. The erosive challenge consisted on specimen immersion in Coca-Cola[®] (pH 2.6, 50 ml, 1 min, 5x, 5 days). Once a day the specimens were brushed using an electric toothbrush with slurry (artificial saliva and NaF toothpaste, 1500 ppm) for 1 minute. Polyvinyl siloxane molds with a perforation of 0.9 mm diameter and 1 mm height were placed over the specimens to build the restorations with flowable composite resin. Molds were cut with surgical blades and removed for microshear bond strength test. Bonded specimens were attached to an universal testing machine and a shear load was applied with a thin steel wire at a cross-head speed of 1.0 mm/min until failure occurred. No statistical differences were found between any eroded and sound group for the same adhesive (two way ANOVA and Tukey's test). At factorial analysis for adhesive and erosion, the main factor adhesive was significant ($p < 0.001$). Also, the interaction between adhesive and erosive tooth wear was statistically significant ($p = 0.046$). According to the factorial analysis erosive/abrasive challenge did not influence microshear bond strength ($p = 0.395$). Erosive tooth wear does not affect the bond strength to primary enamel. Despite that, self-etch adhesives may benefit from eroded primary enamel surfaces.

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Characterization of *in vitro* Artificial Erosion Progression by Measuring Enamel Thickness Using OCT

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Aim: The aim of the study was to quantify the accuracy of a laboratory based Optical Coherence Tomography (OCT) system to measure dental enamel erosion utilising the Dentine-Enamel Junction (DEJ) as reference. **Methods:** 8 bovine enamel discs, were cut and polished into 5 mm diameter specimens, each comprising an enamel depth of approximately 1 mm overlying 2 mm of dentine. The specimen surfaces were painted with varnish to provide a 3x3 mm region accessible to dissolution. The samples were mounted in a custom-built sample holder for continuous OCT imaging while in solution, eliminating the need to move and dry specimens for imaging. 1% citric acid solution (pH 3.8) was pumped continuously over the enamel surface for 40 hours at room temperature (25°C). Average optical backscattering profiles were obtained as a function of depth into the enamel. Erosive depth was measured manually by measuring the change in the axial distances from A) the varnish surface to the eroded enamel surface and, B) from the exposed enamel to the underlying DEJ. These measurements were repeated at hourly intervals. **Results:** Measurement of the loss of enamel (method A) yielded an accuracy of $10.3 \pm 1.5 \mu\text{m}$ (95% CI) with a mean rate of erosion of $8.5 \pm 1.14 \mu\text{m/hour}$ (95% CI) determined from linear fitting. The mean enamel thickness measurement (method B) accuracy was $9.1 \pm 1.3 \mu\text{m}$ (95% CI) with the mean rate of erosion of $6.8 \pm 1.47 \mu\text{m/hour}$ (95% CI). **Conclusion:** This novel measurement method successfully measured the longitudinal loss of enamel under model erosion conditions. Crucially the technique was able to measure both non-destructively and continuously whilst the enamel specimens were submerged in acid, negating any drying artefacts.

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A New in situ Model to Study Erosive Enamel Tooth Wear, A Clinical Pilot Study

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Most in situ models used in dental erosive wear research have considerable limitations in modeling the clinical situation. A new in situ model, which would allow for more clinically relevant exposure circumstances was developed. This pilot study, including 6 healthy, edentulous volunteers wearing full dentures with 13 embedded enamel specimens (on occlusal, buccal and palatal/lingual surfaces), tested the erosive effects of orange juice on enamel by drinking or swishing for 30 days in addition to a habitual diet. The study consisted of two control runs: habitual diet only for 30 days, and two experimental runs: habitual diet plus 125 mL orange juice at 09.00, 11.00, 13.00 and 15.00 hrs (consumed over 4 minutes). In the first experimental run subjects were instructed to take the drink in their mouth and promptly swallow it. In the second experimental run subjects were instructed to take the drink in their mouth and hold each 25 ml mouthful for 30 sec, moving it around their mouth in a rinsing motion before swallowing. Sample enamel surface loss was measured using non-contact surface profilometry. The model showed significant differences in mean buccal (0.16 vs. 1.31 μm ; $p = 0.004$) and palatal/lingual (0.19 vs. 1.33 μm ; $p < 0.001$) tissue loss between control 1 and experimental run 1 (drinking). Swishing of orange juice (experimental run 2 compared to control run 2) showed a significant increase in mean tissue loss of occlusal (5.61 vs. 0.43 μm), buccal (2.68 vs. 0.97 μm) and palatal (4.10 vs. 0.54 μm) enamel samples. The model showed similar erosive wear distribution over tooth sites as seen clinically in erosive wear patients.

The study was supported by the Dutch association for Soft drinks, Waters, Juices (FWS).

A Modified in-situ Model to Evaluate the Anti-Erosion Properties of a Fluoride Dentifrice

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The aim of this study was to explore the effect of salivary fluoride in remineralizing acid-softened enamel and protecting against further acid-softening of enamel in the period shortly after brushing with a fluoride dentifrice. This was a randomised, controlled, single-centre, single-blind (analyst), two period, two treatment, cross-over, *in-situ* design to examine the ability of a fluoride dentifrice (1150 ppm fluoride as sodium fluoride) to remineralise acid softened enamel and render enamel more resistant to an extra-oral erosive challenge compared to a placebo dentifrice, as measured by

Surface Microhardness Recovery (%SMHR), Relative Erosion Resistance (%RER) and Enamel Fluoride Uptake (EFU). Each palatal appliance carried eight bovine enamel specimens. Each specimen had SMH measured before and after an *in-vitro* dietary erosive challenge (grapefruit juice for 25 minutes), at post-treatment intervals (remineralisation period) of 5, 10, 15, 30, 60, 120 and 240 minutes, and following a second *in vitro* erosive challenge at the above post-treatment intervals. %SMHR and %RER were analysed using ANCOVA and EFU by ANOVA. Both included factors for treatment, period and subject. %SMHR and %RER included co-variables for baseline and pre-treatment indentation length. Fifty subjects were randomised (59 screened). At intervals up to 30 minutes, %SMHR was numerically (no significant difference) higher for the fluoride dentifrice compared to the placebo. At 60 minutes, and all subsequent time-points, %SMHR was statistically significantly higher for the fluoride dentifrice compared to the placebo ($p < 0.02$). At all time-points, EFU and %RER were statistically significantly higher for the fluoride dentifrice compared to the placebo ($p < 0.0001$). This modified *in-situ* erosion study demonstrates protective effects of fluoride against a dietary acid challenge as early as 5 minutes post fluoride treatment.

Study funding was provided by GSK Consumer Healthcare.

In situ Evaluation of the Anti-Erosive Effect of Sodium Fluoride, Stannous Chloride and Sodium Polyphosphate

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The anti-erosive effect of solutions containing sodium fluoride (F: 225 ppm F), sodium fluoride + stannous chloride (F+Sn: 225 ppm F + 800 ppm Sn), sodium fluoride + stannous chloride + linear chain sodium polyphosphate (F+Sn+LPP: 225 ppm F + 800 ppm Sn + 2% LPP) and deionized water (C: control) was tested in a four-phase, single-blind crossover in situ clinical trial. Twelve volunteers (who met the inclusion/exclusion criteria) participated in this study, after signing an informed consent term. In each phase, volunteers used mandible appliances containing 4 specimens of flat and polished enamel, which underwent a 5-day erosion-remineralization cycle, consisting of 2 min extra-oral immersion in 1% citric acid (pH 2.6), 6x/day, with 1.5 h of exposure to saliva in situ between the challenges. Treatment with the test solutions were performed extra-orally for 2 min, 2x/day, 75 min after first and last erosive challenges. In the end of the experiment, enamel surface loss (SL, in μm) was evaluated with optical profilometry. SL data were analyzed using an ANOVA (which included a random effect for subject and fixed effects for treatment sequence, study phase, and solution) and Tukey tests ($\alpha = 0.05$). C (mean SL $\pm$ standard-deviation: 5.97 ± 1.70) and F (5.36 ± 1.59)

did not significantly differ between them, but they had significantly greater SL than F+Sn (2.68 ± 1.62) and F+Sn+LPP (2.10 ± 0.95). F+Sn and F+Sn+LPP did not differ from each other. Sodium fluoride alone did not exhibit a significant anti-erosive effect. The combination between sodium fluoride and stannous chloride reduced enamel erosion, regardless of the presence of the linear chain sodium polyphosphate.

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A Hand-Held Optical Reflectometer to Measure Initial Erosion on Dentine

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Previous studies have shown that a hand-held optical reflectometer can measure initial erosion on enamel, but this was yet to be tested on dentine. This *in vitro* study aimed to assess initial erosion on dentine with the optical reflectometer. We ground and polished premolar roots ($n = 30$) to obtain dentine specimens, which were sorted into 3 groups: tap water (TW), sodium fluoride (500 ppm F) solution (NaF) and Elmex® Erosion Protection (800 ppm tin and 500 ppm F) solution (EP). This cyclic experiment consisted of 5 erosion cycles. In each cycle, we immersed the dentine specimens in the respective solution (10 ml, 30°C, shaking; 2 min), washed them for 20 s, and then immersed them in citric acid (0.65%, pH 3.6, 30 ml, 25°C, shaking) for a total erosion time of 1, 2, 4, 6, and 8 min. Reflection intensity was measured using an optical reflectometer, and the amount of calcium released by the dentine specimens was quantified with an atomic absorption spectrometer. Between-group differences in relative surface reflection intensity (rSRI) and calcium release were tested using Kruskal-Wallis and post-hoc Mann-Whitney tests, association between the two variables was tested using Spearman's correlation. We observed dentine erosion in all groups to different degrees. After 8 min erosion, the lowest rSRI loss was observed in EP (median 85%), followed by NaF (91%) and TW (94%; $p < 0.01$). Likewise, the lowest calcium release values were observed for EP (median 38.1 nmol/mm^2), followed by NaF (55.0 nmol/mm^2) and TW (70.6 nmol/mm^2 ; $p < 0.001$). Additionally, the good correlation between rSRI and calcium release (correlation coefficient -0.70) shows that the optical reflectometer can measure and differentiate initial erosion on dentine.

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Modification of Acquired Pellicle with Cystatins Reduces Initial Enamel Erosion

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Cystatin-B was identified as an acid-resistant protein in the acquired enamel pellicle; it could therefore be included in oral products to protect against erosion. However, its cost is high. Recently, a cystatin derived from sugar cane (canecystatin-5) was cloned and recombinantly expressed. This study evaluated the effect of pellicle modification, by incorporation of cystatin-B or canecystatin-5, on the protection against initial enamel erosion *in vitro*. Seventy-five bovine enamel specimens (4x4 mm) were divided into 5 groups: 1) deionized water (control), 2) 0.5% mucin + 0.27% casein solution, 3) 0.025 µg/µL cystatin-B solution, 4) 0.025 µg/µL canecystatin-5 solution, and 5) 0.025 µg/µL canecystatin-5 solution applied before the formation of the acquired pellicle. Stimulated saliva was collected from three volunteers and used to form an acquired enamel pellicle on the specimens for 2 h. Specimens (groups 1–4) were exposed to the protein solutions with stirring at 30°C for 2 h. For group 5, blocks were exposed to canecystatin-5 solution before the pellicle was formed. All specimens were then incubated in 0.65% citric acid (pH 3.4) for 1 min at 30°C. Treatment was done once/day for 3 days. Surface hardness was analyzed at baseline and after days 1 and 3 and percentage of surface hardness change (%SHC) was calculated. Data were analyzed by ANOVA and Tukey's test ($p < 0.05$). At day 1, treatment with cystatin B ($35.1 \pm 9.9\%$) and canecystatin-5 ($35.2 \pm 6.6\%$) before pellicle formation significantly reduced % SHC compared with control ($46.9 \pm 6.7\%$). At day 3, all treatments with cystatins (54.5 ± 8.6 , 55.5 ± 10.7 and $53.1 \pm 9.3\%$ for cystatin-B, canecystatin-5 and canecystatin-5 before pellicle formation, respectively) significantly reduced % SHC compared with control ($67.6 \pm 9.4\%$). In addition, treatment with canecystatin-5 before pellicle formation significantly reduced % SHC compared with the combination mucin/casein ($64.4 \pm 9.4\%$). Thus, canecystatin-5 seems to be a good candidate to be added to oral products to protect against erosion.

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Professional TiF₄ Varnish and Home-Care TiF₄/NaF Mouthrinse Applications on Tooth Erosion Prevention in vitro

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The aim of this study was to investigate the isolated and combined effect of a professional application of TiF₄ varnish (single application) and home-care application of a mouthrinse containing TiF₄/NaF (daily application) against tooth erosion in vitro. Ninety bovine enamel and 78 root dentin samples were treated as following: Control (no treatment); Mouthrinse containing TiF₄/NaF (500 ppm F⁻, pH 4.4); NaF varnish (24,500 ppm F⁻, pH 5.0); TiF₄ varnish (24,500 ppm F⁻, pH 1.0); TiF₄ varnish+ mouthrinse; NaF varnish+ mouthrinse. The erosive challenges were performed 4x90 s/day (0.1% citric acid, pH 2.5) and, between them, the samples were immersed in artificial saliva. The tooth loss was measured using contact profilometry (µm) after 7 days. All treatments were effective in reducing tooth loss, except NaF varnish for enamel (ANOVA, $p < 0.0001$). The application of TiF₄/NaF mouthrinse and TiF₄ varnish were similarly effective in reducing enamel erosion (54–57% reduction compared to control). The combined use of TiF₄/NaF mouthrinse and fluoride varnishes improved the preventive effect of the isolated treatments (varnish or mouthrinse) on enamel erosion. For dentin erosion, TiF₄ varnish had the best protective effect. Isolated applications of TiF₄/NaF mouthrinse and NaF varnish were equally effective in reducing dentin erosion (70–75% reduction compared to control), but less effective than TiF₄ varnish and the combinations, which completely inhibited tissue loss. The combined use of TiF₄/NaF mouthrinse and fluoride varnishes improved the preventive effect of the isolated treatments on dentin erosion, except compared to TiF₄ varnish on its own. The application of TiF₄ varnish may be the best strategy to reduce dentin erosion, while both types of TiF₄ application were able to reduce enamel erosion for 7 days in vitro.

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Effect of Stannous Chloride on Matrix Metalloproteinases

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Background: Matrix Metalloproteinases (MMPs), enzymes, which are present in dentin and saliva, are, among others, responsible for the progression of dental erosion. In several in situ studies the protective effect of inhibitors of MMPs such as chlorhexidine (CHX) and sodium fluoride (NaF) has been reported. Whether stannous chloride (SnCl₂) can also inhibit MMPs and whether the protective effect of chlorhexidine and sodium fluoride is due to the inhibition of MMPs is the research question of the study. **Experimental Approach:** Human recombinant MMP-2 and MMP-9 were used at a concentration of 3 mM. SnCl₂ and NaF (both at 1% to 0.007%), and CHX (at 0.1% to 0.0007%), were either added to the zymography incubation buffer or directly added to the MMPs before loading the zymography gels. **Results:** Two bands with an approximate molecular mass of 72 kDa and 92 kDa, represented MMP-2 and MMP-9, respectively, appeared. SnCl₂, NaF and CHX added to the incubation buffer inhibited the enzymatic activity of MMP-2 at a concentration of 0.5%, 0.12%, and 0.012%, respectively. MMP-9 activity was inhibited at a concentration of 0.5%, 0.5%, and 0.05% of SnCl₂, NaF and CHX, respectively. Importantly, when SnCl₂, NaF, and CHX were added to the MMPs before loading the gels, only SnCl₂ caused an inhibition of MMP-2 at 0.007% and of MMP-9 at 0.12%. NaF, and CHX did not inhibit the MMPs in this experimental setting. **Conclusions:** SnCl₂, NaF, and CHX showed inhibitory potential on MMP-2 and MMP-9 when added to the zymography incubation buffer while SnCl₂ also inhibited MMP-2 and MMP-9 when directly added to the MMPs. The different inhibitory potential of the two settings requires further investigations.

Influence of Chitosans of Different Viscosity Added to a F/Sn Toothpaste on Enamel Erosion/Abrasion

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Chitosans increase the efficacy of F/Sn-containing toothpastes against erosion/abrasion but little is known about which type of chitosan molecules exhibits the best effect. The aim of the study was to investigate whether the viscosity of chitosan is a potential determinant for efficacy. Enamel specimens were cyclically demineralised (10 days, 6x2 min/day; citric acid, pH 2.8). In experiment (Exp) 1, specimens were exposed to toothpaste slurries (2x2 min/day), and in Exp2 additionally brushed (15 s). Groups (n = 16 each) in both experiments were: placebo, positive-control (500 ppm F-as amine-fluoride, 800 ppm Sn2+ as SnCl2) and four F/Sn-toothpastes with chitosan-additives (Ch) of different viscosity (0.5% each; 50, 500, 1000 or 2000 mPas). Tissue loss ($\mu\text{m} \pm \text{SD}$) was quantified profilometrically. Element (wt%) and morphological analyses of enamel surfaces and toothpaste abrasives were performed with energy-dispersive-X-ray-spectroscopy (EDX) and scanning-electron-microscopy (SEM). Tissue loss in the placebo groups was 4.96 ± 1.55 in Exp1 and 12.76 ± 2.45 in Exp2. Generally, all preparations with active agents (AA) reduced tissue loss compared to placebo ($p \leq 0.001$). In Exp1, all AA caused precipitates, thickest after Ch500. Carbon but not tin retention was increased by chitosan. In Exp2, no precipitation occurred except for Ch1000, which was the only chitosan increasing efficacy (-0.97 ± 4.48) compared to positive-control (2.98 ± 1.32 ; $p \leq 0.05$). EDX showed comparable values for carbon in AA groups; tin content was higher in Ch1000 (6.5 ± 3.4) compared to other AA groups (between 3.8 ± 0.3 and 4.3 ± 1.3). On abrasives, tin adsorption was decreased by all chitosans ($p \leq 0.05$). SEM revealed only minor structural differences on enamel surfaces. The viscosity of chitosan has an impact on efficacy. Under erosive/abrasive conditions best tissue loss reduction was found for Ch1000 with higher tin retention on specimens and lower tin absorption by abrasives, probably explaining the better effect.

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Comparison of Microindentation and Contact Profilometry to Measure Enamel Surface Loss

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This study compared the surface loss values after erosion and abrasion measured by means of microindentation and contact profilometry. Twenty enamel specimens were prepared from caries-free human molars. The teeth were cut in buccal and lingual halves, embedded in resin and a 200 μm layer of enamel was ground away. The resulting surfaces were polished, to obtain flat ground enamel specimens. Ultradent LC Block-Out Resin was applied to partly cover the enamel surface in order to maintain a reference area. Subsequently, the samples were individually immersed in 10 ml of citric acid (1%, pH 3.6) for 2 min (25°C, 70 rpm dynamic conditions). The samples were removed from the acid, indentations were made using a Knoop diamond indenter with a load of 200 g, and the length of their long axes measured. Specimens were brushed (250 strokes, 200 g) in an automatic brushing machine with toothpaste-slurry. Indentations were re-measured and surface loss calculated. The resin was removed from the reference areas and profilometry (Perthometer S2) was performed, after which Block-Out Resin was reapplied on the reference surfaces. The same procedure was repeated for a total of five cycles. Agreement between both methods was calculated with Intraclass Correlation Coefficient (ICC), and linear regression was also performed. Average surface loss per cycle was $0.87 \pm 0.19 \mu\text{m}$ and $0.41 \pm 0.16 \mu\text{m}$, while total surface loss was $4.37 \pm 0.64 \mu\text{m}$ and $2.04 \pm 0.28 \mu\text{m}$ for the indentation method and profilometry, respectively. The results of the two methods showed strong correlation with each other (ICC = 0.79; $p < 0.001$). In conclusion we can say that despite the different surface loss values, both methods delivered strongly correlated results.

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Differences in the Protein Compositions of in vitro Salivary Pellicles from Adult and Children's Saliva

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The aim of this study was to analyze qualitative and quantitative differences in the protein composition of salivary pellicles formed by different saliva pools on different substrates, namely adults and children's salivary pellicles formed on permanent as well as deciduous teeth. From 20 deciduous and 20 permanent molars, discs were prepared, the outer 200 μm of enamel were ground away and the surfaces polished. The area of the enamel

surface of each disk was determined and the disks were glued to bases for better handling. Each of these specimens went through three rounds of pellicle formation and harvesting, first forming a control (water) pellicle, then pellicles from a pool of adults and a pool of children's saliva. In between, the samples were cleaned by incubating them in NaOCl. Pellicles were formed by placing 15–20 µl of saliva onto the polished surface, followed by incubation at 37°C for 2 h. Saliva was removed and the samples were washed before adding 2 µl of 0.5% SDS onto each surface. Pellicles were then harvested by rubbing the surfaces with 4 cotton balls per group, which were then pooled. After elution from the cotton balls, the pellicles were loaded on an acrylamide gel and run for 15 min. The lanes were cut out and divided into four regions, which were submitted to tryptic digestion and analyzed by LC-MS/MS. Evaluation of the results of these analyses revealed qualitative and quantitative differences (>2-fold) of several pellicle proteins, depending on the type of saliva used to create the pellicle as well as the kind of tooth on which the pellicle was formed. These differences might explain differences in protection provided by the pellicles that have been shown before.

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Enamel Thickness Determination by Optical Coherence Tomography: In vitro Validation

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Optical coherence tomography (OCT) is a non-destructive diagnostic imaging tool that does not utilize ionizing radiation. This study investigated the agreement between polarization sensitive OCT (PS-OCT), micro-computed tomography (µ-CT) and histology, for enamel thickness measurements. Human enamel slabs (4×4×2 mm³) (n = 10) were obtained from extracted molars, flattened and polished. An orientation notch was created on the side of each slab. After 3D reconstruction, b-scans were located based on the orientation notch and selected for PS-OCT and µ-CT analysis. For histology, the slabs were sectioned at the b-scans location. Three pre-determined sites were chosen for enamel surface-DEJ distance measurements, which were performed with the aid of image software (ImageJ 1.48). A previously trained examiner performed all measurements independently, with at least a two-week period interval between each method. The agreement between the three methods for the determination of enamel thickness was assessed using intra-class correlations coefficients (ICCs) and Bland-Altman plots (α = 0.05). PS-OCT measurements were significantly higher on average than the µ-CT and histology measurements, by 0.064 and 0.088

mm, respectively (p < 0.05). No significant differences were found between µ-CT and histology. Other than the higher values for PS-OCT, no other patterns were identified in the Bland-Altman plots. All ICCs were 0.95 or higher, which indicates good agreement among the three methods. Moreover, PS-OCT was able to detect a relatively high range of enamel thickness. The results revealed significant high agreement between all tested methods, supporting the suitability of PS-OCT for the measurement of enamel thickness, indicating that it may be a valuable objective clinical tool for erosive tooth wear diagnosis and monitoring.

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Effect of Sodium Trimetaphosphate Combined or Not with Chlorhexidine on Eroded Dentin Biomimetic Remineralization

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The aim of this study was to examine the effect of sodium trimetaphosphate (STMP) and STMP combined with chlorhexidine (CHX) on erosive dentin biomimetic remineralization in vitro. Thirty-six root dentin specimens were prepared from human molars and demineralized by immersion in 1% citric acid solution (pH 2.3) for 60 min, in order to create a lesion of around 60 µm depth. The specimens were then divided into 4 treatments: Control (no treatment); 2.5 wt% STMP solution for 5 min; 10 mM CHX solution for 1 min and 2.5 wt% STMP (5 min) + 10 mM CHX (1 min). The specimens were placed into a plastic vial on white Portland cement discs. The lesions were remineralized in simulated body fluid system containing 500 µg/mL polyacrylic acid, for four months at 37°C, with the medium refreshed twice every month. After 1 and 4 months, the mineral content (ΔZ) and lesion depth (LD) were calculated using transverse microradiography (TMR). ΔΔZ and ΔLD (at 1st and 4th month) were calculated to compare the treatments using two-way ANOVA followed by Bonferroni's test (p < 0.05). The remineralization of dentin erosive lesions significantly enhanced after 4 months (ΔΔZ STMP 570.3 ± 163.6; STMP/CHX 409.4 ± 83.9; Control 405.4 ± 143.5; CHX 414.7 ± 149.9) compared to 1 month (ΔΔZ STMP 367.5 ± 118.3; STMP/CHX 187.5 ± 84.4; Control 248.6 ± 127.9; CHX 282.4 ± 160.8) regardless of the treatment (p < 0.0001). At the 1st month, none of the treatments was able to improve dentin remineralization compared to control. After the 4th month, STMP (ΔΔZ 570.3 ± 163.6) was the only treatment able to significantly improve mineral uptake compared to the control (ΔΔZ 405.4 ± 143.5), but not to reduce lesion depth. CHX and STMP+CHX did not differ between them and from control. STMP is able to improve erosive

dentin biomimetic remineralization after 4 months in vitro, which was not improved by the addition of CHX.

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The Effect of Some Remineralizing Products on Erosive Capacity of Different Commercial Sport Drinks

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The aims of the study were to evaluate and to compare the effect of three remineralizing products on erosive capacity of three commercial sport drinks by assessing the surface hardness of enamel and dentine. For this study Gatorade™ beverage (Pepsico), Isostar™ lemon tablets (Isostar) and CytoMax™ orange powder (CytoSport) were selected. One hundred and thirty specimens from thirtythree healthy extracted teeth were randomly split in seven groups. In groups 1–4 ($n = 10$) the specimens were maintained in artificial saliva (group 1, control), were immersed in Iso-star (group 2), in CytoMax (group 3) and in Gatorade (group 4) according to this protocol: five times immersion in beverages, three minutes each during two hours a day, fourteen days. In groups 5–7 ($n = 30$) the same acidic challenge was used, but before the first acid attack three topical remineralizing products were applied (subgroups a-c, $n = 10$): Colgate Total® toothpaste (Colgate Co.) for 3 minutes, a fluoride gel (Densell Co.) for 1 minute, MI Paste Plus (GC Corporation) for 3 minutes. The samples were analysed to evaluate the surface hardness of enamel (E) and dentine (D) using a digital device (CV 400 DAT, Namicon). In groups 1–4 the E/D Vickers hardness number (VHN) were: 203.05/57.23 (group 1), 113.80/36.56 (group 2), 110.30/37.13 (group 3) and 105.66/22.90 (group 4). In group 5 the VHN in E/D were: 186.96/41.26 (subgroup a), 202.16/56.53 (subgroup b), 234.66/66.13 (subgroup c). In group 6 the VHN in E/D were: 136.70/36.13 (subgroup a), 158.30/45.00 (subgroup b), 204.00/46.06 (subgroup c). In group 7 the VHN in E/D were: 130.76/33.70 (subgroup a), 138.96/38.83 (subgroup b), 146.26/39.53 (subgroup c). The three tested beverages significantly decreased the enamel and dentine hardness as a result of their erosive capacity (Mann Whitney test, $p < 0.05$). All remineralizing products significantly increased the enamel and dentine hardness (ANOVA and post hoc Bonferroni tests, $p < 0.05$) when used before acid challenge.

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Mucin5b and Albumin Mediate Greater Protection Against Dental Erosion Than Statherin

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Our previous data demonstrated that whole saliva (WS) provided better protection against erosion than parotid saliva (PS) after five erosion cycles. Using a profilometer, WS produced significantly less step height ($4.16 \pm 0.57 \mu\text{m}$) than PS ($6.41 \pm 0.71 \mu\text{m}$) ($p < 0.0001$). The responsible proteins for protection are unknown. This study aimed to measure four key proteins in an in-vitro erosion model comparing WS and PS. 30 human enamel samples were prepared and assigned to 2 groups: (WMS: $n = 15$) and (PS: $n = 15$); three subgroups each: control ($n = 5$), one cycle ($n = 5$), five cycle erosion ($n = 5$). Samples were immersed in the corresponding saliva for 24 h (control) followed by a further 30 min prior to exposure to a 10 min citric acid (pH 3.2) followed by 2 min water rinse (one cycle). This cycle was repeated five times. Enamel pellicle was eluted using filter papers for all groups. Proteins were immunoblotted for: mucin5b, albumin, carbonic anhydrase VI (CA VI) and statherin. Antibody binding was quantified using ImageLab software using purified protein standards of known concentration ($n = 3$) to assess quantity and reproducibility. Data were log transformed to attain normality and linear models and post hoc tests were used for the statistical analysis. We found albumin and mucin5b were more dominant in WMS pellicles than PS ($p < 0.0001$) whereas CA VI and statherin were dominant in PS pellicles ($p < 0.0001$). Mucin5b in WS pellicles, but absent in PS, at control [$57.5 \pm 33.3 \text{ ng}$] significantly increased after five cycles ($121.5 \pm 19.9 \text{ ng}$) ($p < 0.0001$). Statherin in PS pellicles increased after one cycle ($415.8 \text{ ng} \pm 43.6 \text{ ng}$) compared to control [$210.4 \pm 25.9 \text{ ng}$] ($p < 0.0001$) but returned to control levels after five cycles ($180.6 \pm 23.5 \text{ ng}$).

In conclusion The greater resistance of WMS pellicles to enamel erosion compared to PS pellicles relates to protein composition. Thus Mucin5b and albumin-rich pellicles gave better protection than carbonic anhydrase and statherin-rich pellicles.

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Efficacy of Toothpastes in Prevention of Severe Erosive Tooth Wear in Permanent and Deciduous Teeth

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Toothpastes are key vehicles for fluorides application. Studies have shown that various fluorides have different preventive effect on erosion/abrasion progression. Little is known about their effect on deciduous teeth. The aim was to evaluate the preventive effect of the toothpastes on surface loss (SL) in a severe erosion/abrasion model and to compare this effect between permanent and deciduous teeth. Material and Methods: Enamel samples of permanent (n = 85) and deciduous teeth (n = 85) were randomly divided into five groups according to toothpastes tested (n = 17). G1 – Placebo toothpaste, G2 – NaF toothpaste (positive control, Crest[®]), G3 – SnF₂ anti-erosion toothpaste (elmex[®]), G4 – SnF₂ toothpaste (Sensodyne Repair[®]), G5 – NaF anti-erosion toothpaste for children (ProNamelJunior[®]). Half of each enamel sample surface was covered with methacrylate-based resin to create a reference area. The samples were submitted to 30 erosion-abrasion cycles. In each cycle samples were incubated in artificial saliva (1 h), submitted to erosive challenge (3 min; 1% citric acid; pH3.6; at 25°C) and to toothbrush abrasion (2 min immersion in slurry; 50 strokes; 200 g). Tissue loss (µm; mean±SD) was quantified profilometrically with an optical, contactless measuring device. The effects of the two co-variables tooth and toothpaste were analysed by ANOVA and variables with significant effects were tested by Wilcoxon tests. Deciduous teeth (dt) showed significantly higher SL than permanent teeth (PT) in all groups (p < 0.001). The mean values of SL of each group were: G1 PT 18.18 (±3.98), dt 25.65 (±9.21); G2 PT 14.76 (±2.82), dt 18.11 (±3.92); G3 PT 12.62 (±5.29), dt 15.61 (±6.70); G4 PT 17.12 (±2.24), dt 23.41 (±7.9); G5 PT 13.24 (±1.29), dt 18.28 (±8.96). In conclusion, the SnF₂ anti-erosion toothpaste showed the best preventive effect against erosion-abrasion cycles in permanent teeth. In deciduous teeth NaF toothpaste, SnF₂ anti-erosion toothpaste and NaF anti-erosion toothpaste for children showed similar effect.

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Micro-Shear Bond Strength of Self-Etch and Etch-and-Rinse Adhesives to Eroded Primary Dentin

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The aim of this *in vitro* study was to evaluate the microshear bond strength of four adhesive systems to eroded and sound primary dentin. Primary molars were cut mesio-distally, embedded, grinded and polished. Specimens were randomly divided according to adhesive system: G1 (Adper Single Bond2[®]), G2 (Universal Single Bond[®]), G3 (OptibondFL[®]) and G4 (BondForce[®]). Half of the specimens were submitted to erosive/abrasive cycles, and the other half (control group; n = 10) remained immersed in artificial saliva. The erosive challenge consisted on specimen immersion in Coca-Cola[®] (pH 2.6, 50 ml, 1 min, 5x, 5 days). Once a day the specimens were brushed using an electric toothbrush with slurry (artificial saliva and NaF toothpaste, 1500 ppm) for 1 minute. Polyvinyl siloxane molds with a perforation of 0.9 mm diameter and 1 mm height were placed over the specimens to build the restorations with flowable composite resin. Molds were cut with surgical blades and removed for microshear bond strength test. Bonded specimens were attached to an universal testing machine and a shear load was applied with a thin steel wire at a crosshead speed of 1.0 mm/min until failure occurred. Two-way ANOVA and Tukey's test (α = 0.05) were performed. G4 showed less microshear bond strength in control group (7.01 ± 4.76) and G2 had the best results for microshear bond strength in both control (22.81 ± 4.80) and test (21.43 ± 4.83) groups. Besides, G2 test group also showed the best results when compared to the others groups. Erosive tooth wear affected the bond strength to primary dentin only for the self-etch adhesive containing fluoride (G4).

In vitro Evaluation of Erosive Potential of Various Drinks Marketed in Uzbekistan

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According to literature review the prevalence of tooth erosions is high and continuously growing within populations (Jaeggi T. et al., 2014).

The aim of the study was to investigate the erosive potential of 10 commercially available drinks in Uzbekistan. 100 bovine tooth samples with the square size of 2.5 mm and 2 mm thickness were prepared from freshly extracted bovine incisors and randomly di-

vided into 10 groups ($n = 10$). Before starting the experiment all specimens were weighed using a precision balance. The mean initial weight of specimens was 26.84 mg (SD-3.38). Statistical analyses were performed by using Student *t* test and no statistically significant differences were found between groups for initial masses ($p > 0.05$). Before specimen immersion pH values of soft drinks were determined: Coca Cola-2.5, Fanta-3.07, Sprite-2.94, Nestle-4.09, Hydrolife-4.56, Shaffof-4.47, Bliss (lemon)-2.96, Meva Juice (forest berries)-3.46, Redbull-3.44 and tap water-6.9. Then each specimen was placed in a 2 ml plastic tube that contains proper drinks. Drinks were kept in an incubator at 37° C for 2 days and then all specimens were weighed again and the mean mass loss was calculated. The mineral loss values were: Coca Cola-1.23, Fanta-2.96, Sprite-1.77, Nestle-0.51, Hydrolife-1.2, Shaffof-0.3, Bliss (lemon)-2.5, Meva Juice (forest berries)-0.49, Redbull-4.31 and tap water-0.08. Study showed that Redbull showed most erosive potential within all drinks studied.

It can be concluded that erosive potential of non-alcoholic drinks varies according to ingredients. The current results dictates that pH and erosive potential of drinks that was determined in the present study should be taken into account in planning a diet type to minimize tooth erosive risk.

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Interaction of Surfactants and Sodium Fluoride Over Enamel Initial Erosion

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This study aimed to evaluate if the interaction of surfactant agents, commonly used in toothpastes, with sodium fluoride (NaF) interfere with its ability to protect enamel in initial erosion. The agents tested were: SLS – Sodium Lauryl Sulphate, P20 – Polisorbate 20 and CAPB – Cocoamidopropyl Betaine; in two different concentrations (1% and 1.5%). Control group was distilled water, and NaF was used in the concentration of 275 ppm. Bovine enamel samples ($n = 12$) were submitted to erosion cycle (citric acid 0.3%, pH 2.6, 5 min, 4x/day), followed by remineralization in human saliva (2 h) and immersion in the surfactant solutions (2 min, 2x/day). Initial erosion was assessed by microhardness test before initial of the cycle (DS1), after the first acid (DS2) and after the treatment (DS3) and finally after the second immersion in the acid (DS4). The remineralization of enamel was calculated using the formula ($\Delta\text{rem} = \text{DS3} - \text{DS2}$); and the ability of NaF to protect enamel against new erosion was determined by the formula ($\Delta\text{prot} = \text{DS4} - \text{DS2}$). Student *t*-test (5%) showed that the different concentrations did not influence enamel remineralization or protection. Dunnett test (5%) showed no statistical difference for Δrem ($p = 0.375$); and for Δprot the values were: SLS-1% (-91.06 ± 15.2)A, SLS-1.5% (-85.71 ± 31.0)A, CAPB-1% (-57.35 ± 24.5)B, CAPB-1.5% (-58.77 ± 13.3)B, T20-1% (-50.97 ± 21.0)B, T20-1.5% (-62.51 ± 19.3)B and Cont (-57.30 ± 16.3)B. Therefore, only SLS in both

concentrations tested was able to decrease the protective effect of NaF in initial erosion conditions.

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Influence of Surfactants in Acquired Pellicle Formation

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This study aimed to evaluate the effect of surfactant agents commonly used in toothpastes, over acquired pellicle formation. The agents tested were: SLS – Lauril Sodium Sulphate, P20 – Polisorbate 20 and CAPB – Cocoamidopropyl Betaine; in two different concentrations (1% and 1.5%). Control group was distilled water. Bovine enamel samples ($n = 10$) were obtained and submitted to immersion in treatment solutions, followed by immersion in pooled human saliva (2 hr), for acquired pellicle formation. Protein adsorption over enamel was assessed by Fourier Transformed Infrared Spectroscopy (FTIR), using the difference in height of amid peak (1650 cm^{-1}) obtained from specimens before and after the treatment with the solutions and acquired pellicle formation. Kruskal Wallis and Mann Whitney (5%) tests revealed that: P20-1% (0.0 ± 0.0) < CAPB-1% (7.7 ± 10.4) = LSS-1% (5.2 ± 4.6) = Cont (1.59 ± 3.1) = P20-1.5% (9.3 ± 1.7) = CAPB-1.5% (7.2 ± 1.10) = LSS-1.5% (2.1 ± 2.2). Regarding the concentration, only T20-1% presented lower values than P20-1.5%. Therefore, only P20 in the lower concentration (1%) had a negative effect on acquired pellicle formation.

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Protective Effect of CPP-ACP Containing Varnish on Enamel Erosion Produced by a Soft Drink: An AFM Study

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The aim of this study was to investigate the *in vitro* effect of MI Varnish on preventing enamel erosion by Atomic Force Microscopy (AFM). A total of 21 enamel samples were prepared from extracted permanent molars, divided into 3 groups. Group-1: GC MI Varnish (5%NaF +CPP-ACP; GC Corporation, Japan),

Group-2: Positive Control Colgate® Duraphat® Fluoride Varnish (NaF 5%), Group-3: Negative Control (deionized water). Before enamel surface treatment, the samples immersed stimulated pooled human saliva for 1 hour. After treatment, varnishes was cleaned with cotton tip and deionized water; all of enamel samples exposed to 15 ml. Coca Cola for 10 min. The surface topography (Root-Mean-Surface Roughness (R_{rms})) as a measure of erosion was evaluated by AFM from three specimen in each group. Kruskal Wallis test were applied. Statistical difference was set at $P < 0.05$. We found that the mean R_{rms} was 33.78 ± 13.39 after remineralization with MI varnish, 57.05 ± 14.87 after Duraphat, 147.54 ± 69.15 after deionized water. MI Varnish leads to a significant reduction in R_{rms} values ($P < 0.05$) but there was no significant difference between the MI Varnish and Duraphat. AFM images of enamel surface treated with MI Varnish resulted in less morphological changes of the tooth substrate when compared with Duraphat; thus, indicating that MI Varnish promoted remineralization. In conclusion the results confirmed the use of MI Varnish had a protective effect on enamel erosion produced by soft drink. The morphological interpretation of AFM images suggested that this protective effect is considered more evident for MI Varnish.

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Efficacy of Fluoride Varnishes for Remineralization of White Spot Lesions

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Aim: The aim of this study was to evaluate the efficacy of different fluoride varnishes for remineralization of white spot lesions (WSL). **Methods:** 60 enamel specimens with 3 mm of diameter were obtained from bovine incisors using a diamond trephine mill. They were embedded in acrylic resin, flattened and polished. The initial Knoop microhardness (KM) was evaluated. Artificial carious lesions were created and the KM was measured again. According to KM values obtained after the demineralization, the samples were stratified into six groups, according to the kind of fluoride varnishes applied: C – Control without varnish application; BF – Bifluoride 12 (Voco – 6% sodium fluoride+ 6% calcium fluoride); DP – Duraphat (Colgate, 5% Sodium Fluoride); PF – Profluoride (Voco, 5% Sodium Fluoride); FP – Fluor Protector (Ivoclar, 9% Fluorsilane); CW – Clinpro White Varnish (3 M, 5% Sodium Fluoride + Tri-Calcium Phosphate – TCP). The varnishes were applied according to the manufacturer's instructions, and immersed in artificial saliva for 24 h. After that the pH-cycling (2 h in the demineralizing solution and 22 h in the remineralizing solution) was performed for 8 days. At the end of the pH-cycling the KM was measured again. The data were analyzed by one-way ANOVA and Tukey tests. **Results:** Non significant differences were observed among the groups at baseline ($p = 0.187$) and after WSL formation ($p = 0.999$). After the treatments, significant differences were observed among the groups ($p = 0.001$). The means (SD) and the results of Tukey test were: C-88.94 (23.68)a; PF-93.63 (23.38)ab; FP-97.95 (20.28)abc; BF-126.01 (27.01)bcd; CW-127.86 (17.07)cd;

DP-142.01 (37.54)d. **Conclusions:** Only the varnishes Bifluoride 12, Clinpro White Varnish and Duraphat showed a higher efficacy than artificial saliva in promoting the remineralization of WSL. None of the treatments was able to recover the baseline microhardness of sound enamel.

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Enhancement of Fluoride Retention in Oral Biofilm Using Extract from Experimental Toothpaste Containing S-PRG Fillers

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Enhancement of fluoride retention in oral biofilm, when fluoride is applied at the same time as other mineral ions released from experimental toothpaste containing surface pre-reacted glass-ionomer (S-PRG) fillers, was estimated by depth-specific analysis. Twenty subjects (age range, 18–34 years) wore in situ plaque-generating devices comprising a pair of enamel slabs (4 mm²) on their upper molars, and biofilm was allowed to form. A slurry of toothpaste containing S-PRG filler (ϕ 3 μ m, 5 wt%) was filtered. The filtrate contained Al (344.9 ppm), B (460.5 ppm), Sr (931.1 ppm) and F (202.0 ppm). Devices were removed after three days, immersed in the filtrate for one minute, and then reinserted at the same location. After thirty minutes, the device was removed and samples were serially sectioned, with 2×2 μ m sections taken first followed by 4×4 μ m sections for separation into outer, middle and inner layered fractions (300- μ m thick). This procedure was repeated until the required thickness was obtained. Samples treated with filtrate containing F (195.0 ppm) without S-PRG filler extract served as controls. Fluoride and the three other 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- μ m sections. The mean uptake ratios (S-PRG/control, ng/mm³) of Al, B, Sr and F were 186.6/53.7, 58.4/25.0, 456.9/125.7 and 43.6/12.0, respectively, in the outer layer. F concentrations in the outer biofilm treated by filtrate with S-PRG filler extract were significantly higher (*t*-test, *p* < 0.001) than those in controls, although both biofilms were exposed to the filtrates containing the same level of F. These results suggest that toothpaste containing S-PRG filler promotes fluoride retention in oral biofilms.

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Mechanism of Action of TiF₄ on Dental Enamel

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This *in vitro* study aimed to evaluate the mechanism of action of TiF₄ on dental enamel by analysing the morphology, the percentage of Ca, P, F and Ti as well as the alkali-soluble F deposition on sound and pre-demineralised bovine and human enamel treated with TiF₄ varnishes. The compounds produced by the interaction of TiF₄ and hydroxiapatite were also detected. Sound (S) and pre-demineralised (DE) bovine and human enamel samples were treated with TiF₄ or NaF varnishes (0.95%, 1.95%, 2.45% F) for 12 h. The treated enamel surfaces were analysed by scanning electron microscopy-EDX (*n* = 10, 5 S and 5 DE) and KOH-soluble fluoride extraction analysis (*n* = 20, 10 S and 10 DE). Hydroxyapatite powder was produced by precipitation method and then treated with the corresponding experimental fluoride solutions (0.5 g/10 ml of solution) for 1 min in duplicate. The formed compounds were detected by X-ray diffraction (XRD). ANOVA/Tukey or Kruskal-Wallis/Dunn's tests was applied for the EDX, and two-way ANOVA/Bonferroni tests for fluoride analysis (*p* < 0.05). The TiF₄ varnishes produced a coating layer on the enamel surface, rich in Ti and F, with microcracks in its extension. TiF₄ (1.95 and 2.45% F) also provided higher fluoride deposition than NaF varnishes, especially for bovine enamel, as well as a higher fluoride deposition on the demineralised samples compared to the sound, for both bovine and human enamel (*p* < 0.0001). The percentages of Ti and F elements were higher for bovine than human enamel. The XRD analysis showed that TiF₄ induced the formation of new compounds such as CaF₂, TiO₂ and Ti(HPO₄)₂·H₂O, which take part of the coating surface layer. We assumed that the promising effect of TiF₄ varnish against demineralisation, compared to NaF varnish, is related to the formation of a protective surface layer rich in Ti and F based-compounds on the enamel surface.

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Fluoride Concentrations in Dental Biofilm Fluid Vary Across Regions and Are Higher Than in Saliva

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The aim of this study was to estimate fluoride concentrations (F) in dental biofilm fluid obtained from six regions in the oral cavity and compare these concentrations with F-concentrations in unstimulated whole saliva. The null hypothesis is no difference in F in biofilm fluid from different regions and no difference between these concentrations and F in saliva. Unstimulated whole saliva samples were collected and dental biofilm was harvested from six intra-oral regions (posterior upper and lower, left and right; anterior upper and lower) in 42 subjects. Saliva and biofilm fluid were analyzed in triplicate for fluoride concentration (μ M) using a fluoride ion-selective electrode, adapted for microanalysis. Multilevel mixed-effects linear regression of log F of samples showed almost no variance between replicates (biofilm $\sigma^2(\text{repl}/\text{reg}/\text{subj}) = 0.010$; saliva $\sigma^2(\text{repl}/\text{subj}) = 0.020$), with most of the variance found between subjects (biofilm $\sigma^2(\text{subj}) = 0.29$; saliva $\sigma^2(\text{subj}) = 0.54$) and between regions within subjects (biofilm $\sigma^2(\text{reg}/\text{subj}) = 0.33$). The individual geometric mean (GM) of F in biofilm fluid in regions ranged from 9.4 (95% CI: [7.5–12.0])–16.0 (95% CI: [11.7–21.8]) μ M and was 1.6–2.7 times higher than the GM in saliva (6.0 (95% CI: [4.8–7.6]) μ M). Biofilm fluid F from all six regions were statistically significantly higher than the salivary F. Biofilm fluid samples from the upper anterior region had statistically significantly higher F than samples from the lower anterior region. Linear regression showed significant positive associations between biofilm fluid F from all regions and salivary F ($r^2 = [0.28–0.51]$, *p* $\leq$ 0.001). In conclusion, fluoride concentrations in biofilm fluid vary across oral regions, being higher in the upper anterior than in the lower anterior region. Biofilm fluid fluoride concentrations are higher than salivary fluoride concentrations. The mechanisms controlling the relationship between fluoride in saliva and biofilm fluid need further investigation.

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Available Fluoride Concentration in Local Toothpastes from Brazilian Regions

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Brazil is a continental country and in some regions there are local toothpastes available in the market. Nevertheless, their potential to control caries (given by their soluble fluoride concentration) is unknown. Given that the Brazilian (and Mercosur) regulations only determines the maximum total fluoride concentration that a toothpaste should have, we aimed to determine the total (soluble plus insoluble) and the soluble (as fluoride ion plus ion monofluorophosphate) fluoride concentration in brands of local toothpastes sold in the five Brazilian regions and to compare them with market leader toothpaste Sorriso®. Three samples of each of the following brands of toothpastes were obtained in each region: Even Dentes Fortes®, Even 2 em 1®, Ultra-Action®, Sorriso® (North); Even Dentes Fortes®, Sorriso® (Northeast); Contene®, Sorriso® (Midwest); Dentil®, Freedent®, Ultra-Action®, Sorriso® (Southeast); Anti-caries Dia®, Ice Fresh®, Ultra-Action®, Sorriso® (South). Total (TF) and soluble (SF) fluoride was determined in each toothpaste using a standardized method [Cury et al.: *Braz Dent J* 2010;21:396–400]. The TF concentration in the local toothpastes declared by the manufacturers ranged from 1200 to 1500 ppm F ($\mu\text{g F/g}$; w/w) and the found ranged from 1207.9 to 1638.2, mean $\pm$ SD ($n = 11$ brands) of 1402.9 ± 142.5 . However, the SF concentration found ranged from 502.3 to 1412.4, mean of 973.5 ± 296.0 ppm F. The percentage of insoluble fluoride in those toothpastes ranged from 0.9 to 61.2%, mean of $30.9 \pm 19.4\%$. In the leader toothpaste, the SF concentration found was higher than 1000 ppm F in all regions, 1227.2 ± 131.8 (mean $\pm$ SD; $n = 5$ regions). According to the best available evidence, toothpaste should contain a minimum 1000 ppm F of soluble fluoride to have anti-caries efficacy, but some local toothpastes sold in different Brazilian regions do not meet this requirement.

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Evaluation of Total and Total Soluble Fluoride of Toothpastes from Madagascar

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Evaluations of toothpastes from low- and medium-income countries have shown that they fail to achieve the 1000 ppm ($\mu\text{g F/g}$; w/w) total soluble fluoride concentration considered neces-

sary to control caries. As part of an overall policy in Madagascar to improve access to appropriate fluoride for caries control, this study aimed to evaluate the total fluoride (TF), total soluble fluoride (TSF) and fluoride ion of toothpastes in the country. A convenience sample of 26 toothpaste brands was purchased in the main market of the capital city Antananarivo, ensuring that they had not passed their expiry dates. Pertinent information on the packaging was noted including the indicated fluoride type, its concentration and other constituents. The concentrations of TF and TSF (MFP ion + fluoride ion) were determined with an ion specific electrode using a standardized protocol. The 7 samples where fluoride was not indicated on the labeling contained only trace amount of fluoride while the 2 'junior' toothpastes contained close to 500 ppm of TF and TSF. Of the remaining 17 toothpastes, where packaging indicated fluoride (expected from 1054 to 1450 ppm of TF), one contained negligible amounts, one contained less than 1000 ppm F of TF while 5 contained less than 1000 ppm F of TSF. Of these 5 samples, 3 were market leaders in the country. Furthermore, in 8 of the samples, the TSF was substantially below the TF declared and in one of them 43% of TF was chemically insoluble. The findings show that low TSF concentration of some fluoride toothpastes from Madagascar might lead to a compromised caries preventive effect. Global standards for testing combined with more stringent quality control systems and appropriate legislation are urgently called for.

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Biological Assessment of Three Experimental Toothpaste Additives

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Toothpastes may function as vehicles for antimicrobial agents that have a preventive role in helping to control dental caries. The aim was to study the inhibitory effect of two medicinal plants and a Probiotic strain (*Lactobacillus rhamnosus* included at 10^9 CFU/g) which was isolated and serologically identified by dairy microbiological Lab., National Research Center., Egypt, and their different mixtures in experimental toothpaste against *Streptococcus mutans* which was isolated and grown using Mitis salivarius-bacitracin (MSB) agar media, in addition to the survival profile of the probiotic strain in toothpaste. An experimental toothpaste base was tested for its passivity against the growth of *S. mutans* using agar well diffusion method as well as the incorporation of three active agents: Propolis extract, Miswak extract and the probiotic strain, each in the toothpaste base at three different concentrations. Four

different mixtures (M1; Propolis and Probiotic strain, M2; Miswak and Probiotic strain, M3; Propolis and Miswak, M4; propolis, Miswak and Probiotic strain), each in toothpaste base was evaluated against *S. mutans* growth. The viability of the probiotic strain was also carried out by counting *L. rhamnosus* (Log₁₀ CFU/g) in 1 gm of toothpaste which was mixed in (9 ml) of a physiological saline solution in test tube and further serial dilutions were made then 1 ml saline from final dilution of each tooth paste was seeded on triplicate plates of MRS agar (De Man, Rogosa, Sharpe agar with Tween 80). The inhibition zone diameter (in mm) was expressed as Mean $\pm$ Standard Error. One-way ANOVA followed by Tukey's post-hoc test showed that Propolis and Probiotic toothpastes had no significant at their different concentrations ($P \leq 0.05$, $n = 30$), and both had higher mean inhibition zone diameters than Miswak toothpaste. M4 had also the highest mean inhibition zone diameter (41.3 ± 1.1), followed by M1 and M3; both showed no significantly different (28.6 ± 1.6). In addition, M4 showed the highest mean (Log₁₀ CFU/g) of *L. rhamnosus* (2.41 ± 0.003) after 30 days of toothpaste storage. In conclusion the toothpaste containing mixture of Propolis extract, Miswak extract and Probiotic strain has significant influence against *S. mutans* growth and a survival potential for Probiotic strain.

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Masking Effect of Self-Assembling Peptide P11-4, Fluorides and Caries Infiltration on Artificial Enamel Caries Lesions in vitro

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Application of a self-assembling peptide on non-cavitated caries lesions is a novel approach to facilitate remineralization and mask their unfavourable appearance. The aim of this pH-cycling in vitro study was to compare recommended and novel treatment methods regarding their ability to mask artificial enamel caries lesions.

Bovine enamel specimens each with one artificial caries lesion were prepared and randomly allocated to eleven groups ($n = 22$). Treatments before pH-cycling were: application of a product containing self-assembling peptides (Curodont [C]); a low-viscosity resin (Icon [I]); two fluoride solutions (52,000 ppm F⁻; Tiefenfluorid [T] and 10,000 ppm F⁻; Elmex fluid [E]) and no intervention [N]. Specimens were pH-cycled for 28 days with 6 demineralization periods (6×60 min) per day and stored in remineralization solution in the meantime. Half of the specimens in each group were brushed with either fluoride-free (FF) (group name e.g.: C₀) or NaF (1,100 ppm F⁻) (e.g.: C₁) dentifrice slurry (10 s; 2x/day). In another subgroup specimens were pH-cycled but not

brushed (N_{NB}). Standardized digital images were taken and the CIE L*a*b*-values were measured. Differences between sound surface and artificial lesion were calculated using the equation $\Delta E = ((L_{\text{sound}} - L_{\text{caries}})^2 + (a_{\text{sound}} - a_{\text{caries}})^2 + (b_{\text{sound}} - b_{\text{caries}})^2)^{1/2}$ before (ΔE_{demin}) and after (ΔE_{treat}) treatments as well as after pH-cycling ($\Delta E_{\text{pH-cycle}}$).

For ΔE_{demin} no significant differences could be found between the subgroups [ΔE_{demin} (SD): 13.2 (3.6)] ($p > 0.05$; ANOVA). Significantly reduced ΔE_{treat} values indicating a masking effect were observed ($p < 0.05$; adjusted paired t-test) only after treatments with Icon (I₀/I₁). Except for N_{NB}, C₀, C₁ and T₀ all other treatments reduced the color difference between ΔE_{demin} and $\Delta E_{\text{pH-cycle}}$, but only being significantly for I₀ and I₁ ($p < 0.05$, adjusted paired t-test).

In conclusion, only the application of Icon reduced the color difference significantly, whereas Curodont and the used fluorides could not mask artificial caries lesions considerably.

Summary: Only the application of low-viscosity resin (Icon) could reduce color differences significantly, whereas the self-assembling-peptide-containing product (Curodont) could not mask artificial lesions considerably in vitro.

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

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Fluoride Content of Toothpastes Available to the South African Consumer

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Background: High dental caries burden coupled with a lack of water and salt fluoriation make it imperative that toothpastes available to the South African consumer demonstrate adequate caries inhibition. It is generally accepted that for toothpaste to be effective it should contain between 1000 ppm and 1500 ppm total fluoride (TF) and at least 1000 ppm F should be in free available/soluble form. Studies conducted in other countries have demonstrated that toothpastes often do not conform to this, indicating the need for quality assessments to be undertaken. **Aim:** To determine total and total soluble fluoride (TSF) concentrations in 26 adult, fluoridated toothpastes available to the South Africa consumer. **Materials and Methods:** Samples were purchased from a major pharmaceutical and food retailer located in the two metropolitan areas having greatest population density in South Africa. Total and total soluble fluoride concentrations were determined potentiometrically, in quadruplicate, following acid hydrolysis of the samples using a Combination Fluoride Ion Selective Electrode, calibrated with standards containing 0.0625 ppm F to 6.25 ppm F. **Results:** Although TF content on analysis was found to be statistically significantly lower than manufacturer declaration (1.2×10^{-7} ; $p \leq 0.05$), 78.6% of the sample still contained adequate free, available/soluble F levels. Relative mean available fluoride content for toothpastes formulated with a calcium-based abrasive was $85.5 \pm$

13.6% (n = 7); as opposed to $98.7 \pm 2.1\%$ (n = 19) for those containing silica. **Conclusion:** While most commercially available toothpastes are adequately formulated to provide anti-cariogenic activity, the TSF concentration and therefore the expected preventive and therapeutic value for the calcium-containing toothpastes was notably lower in contrast to the silica-based products. On the other hand commercial availability of toothpastes having sub-therapeutic fluoride concentrations indicates a need for more stringent regulation.

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Salivary Fluoride Concentration during Mastication of Food Prepared with Fluoridated Water or Salt

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Water and salt fluoridation are recommended approach of community fluoride use, and elevated fluoride levels may occur in saliva during the time that meals cooked with fluoridated water or salt are chewed contributing for their anti-caries effect. However, there are no studies comparing salivary fluoride concentration during mastication and following the ingestion of food prepared with them. In a double blind, crossover, 3-phase in vivo study, 12 volunteers ingested, during 15 min, a meal (rice, beans and meat) prepared with non-fluoridated water and salt (negative control group), fluoridated water (0.70 mg F/L, water group) or fluoridated salt (183.4 mg F/kg, salt group). Saliva was collected before (baseline), during chewing (after 5, 10 and 15 min), and up to 2 h after the meal. Fluoride concentration in the meals and in centrifuged saliva were determined with ion-specific electrode. The data were analyzed by ANOVA-two way, followed by Tukey's test. Fluoride concentration ($\pm$ SD; n = 4) in the meals was $0.039 (\pm 0.01)$, $0.43 (\pm 0.04)$ and $1.71 (\pm 0.32)$ μ g F/g for control, water and salt groups, respectively. During the meal mastication, the mean ($\pm$ SD; n = 12) of salivary concentration found was $0.02 (\pm 0.003)$, $0.35 (\pm 0.03)$ and $1.77 (\pm 0.26)$ μ g F/mL for control, water and salt groups, respectively, with significant difference among groups ($p < 0.0001$). Fluoride concentration in saliva returned to baseline values after 30 min in the water group ($p > 0.05$), but was still higher than baseline after 2 h in the salt group ($p = 0.0024$). Chewing food prepared with fluoridated water or salt increase fluoride concentration in saliva but the effect of fluoridated salt is greater due to the higher fluoride concentration in the meal prepared with it.

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In vivo Prolonged Retention of Fluoride Varnish Increases Reactivity with Enamel

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It has been shown in vitro that the reactivity of fluoride varnish with enamel increases with time (maximum at 24 h), but in vivo data is absent. We assessed in vivo the reactivity of fluoride varnish retained for 4 h (manufacturer's usual recommendation) or 24 h on enamel surface before thorough removal. Volunteers (n = 51, aged 18–30) were randomly assigned to 3 groups: a. no varnish application (negative control); b. varnish application (Duraphat®) and removal after 4 h; or c. after 24 h. Teeth were professionally cleaned and dried, and a thin varnish pellicle was applied on enamel surfaces of all teeth. Volunteers were instructed to avoid chewing hard foods and suspend brushing. After 4 or 24 h, remnants of varnish on the buccal surface of the central incisors were carefully removed. Fluoride reactivity with enamel (before, immediately after varnish removal and after 7 and 28 days) was assessed by an in vivo acid biopsy procedure, in which 5 μ L of a 1.6 M HCl solution prepared in 70% glycerol (v/v) was applied for 30 s on a 3.14 mm² exposed area of a central incisor, following by twice neutralizations with an equal volume of TISAB II. Fluoride concentration was blindly assessed using an ion-specific electrode. Immediately after varnish removal, enamel fluoride concentration increased significantly, being higher for the 24-h (before = 0.4 ± 0.1 ; after = 1.8 ± 0.5 μ g F/cm²) than for the 4-h group (before = 0.5 ± 0.2 ; after = 1.1 ± 0.3 μ g F/cm²) ($p < 0.05$, ANOVA and Tukey test). However, after 7–28 days, the F concentration decreased to baseline values, with no difference between the 3 groups ($p > 0.05$, ANOVA). The results suggest that prolonged varnish retention increases fluoride reactivity with enamel, which seems important in the first days after varnish application.

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Comparison of Fluorescence Changes in Enamel Fluorosis and Non-Fluoride Related Opacities

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Developmental defects of enamel (DDE) are changes from the healthy translucence and color of enamel caused by disturbances during amelogenesis. They have been associated to congenital defects, infectious diseases, nutritional deficiencies, and exposure to fluoride and other elements. Different types of DDEs have different clinical presentations and may have different fluorescence signatures. The current study aimed at comparing two types of DDE: Enamel fluorosis (EF) and non-fluoride related opacities (Non-F) using quantitative light fluorescence (QLF). As part of the Early Life Exposure in Mexico to ENvironmental Toxicants Project (ELEMENT), QLF images were obtained with a system that simultaneously acquires polarized white light and fluorescence images. Fluorescence images were analyzed by a processing program (ImageJ-1.49v, National Institutes of Health, USA). The average gray scale values of the region of interest (ROIG) and that of sound enamel (SoundG) were obtained. Changes in gray scale were assessed in two ways: 1) %Gray = (ROIG – SoundG)/SoundG × 100; and 2) ΔGray = ROIG/SoundG × 100. Results were compared using t-tests. 22 EF and 22 Non-F lesions from 44 children were analyzed. Mean (±SD) of ROIG, SoundG, %Gray and ΔGray in Non-F teeth were 64.8 ± 13, 64.7 ± 12.1, 1.4 ± 16.5, and 101.4 ± 16.5, respectively. Mean (±SD) of ROIG, SoundG, %Gray and ΔGray in EF teeth were 48.2 ± 10.8, 57.1 ± 14.6, and –14.9 ± 5.2, and 85.1 ± 5.2, respectively. Positive %Gray or ≥100 ΔGray values indicated brighter than sound enamel, while negative %Gray or ≤100 ΔGray values indicate darker than sound enamel. All values were significantly different ($p < 0.005$), except for measurements of sound enamel (SoundG). Significant differences were found in fluorescence signatures (ROIG) between EF and Non-F lesions; with Non-F lesions being either brighter or darker than sound enamel and EF lesions being darker.

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Abrasion Properties of Diamond Dentifrices on Dentine and Enamel

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This study aimed to analyze the abrasive wear of diamond-loaded dentifrices on dentine and enamel surfaces *in vitro*. Bovine dentine and enamel specimens ($n = 96$) were brushed with custom-made diamond-loaded dentifrices or with a reference dentifrice (CT, Colgate Total). Three diamond dentifrices were produced by adding diamond particles with ascending diameters of 1.0 μm (AMC-1.0), of 2.5 μm (AMC-2.5) or of 4 μm (AMC-4.0) to a dentifrice. Brushing was performed with a cross brushing machine ($F = 2.5$ N; 60 brushing strokes/min). Abrasive wear of all specimens (12 per group) was measured profilometrically (5 profiles per specimen). Dentine specimens were measured under moist conditions after 3600 and 7200 brushing strokes, while enamel specimens were measured after 21600 and 43200 brushing strokes. Data of enamel and dentine were each compared between groups using one-way ANOVA and post-hoc pairwise tests with Tukey correction, $\alpha = 0.05$. The diamond-loaded dentifrices (AMC-1.0, AMC-2.5, AMC-4.0) differed significantly on enamel with increased abrasion potential corresponding to particle size, while AMC-1.0-brushed enamel specimens did not differ from CT specimens. On dentine specimens, diamond-loaded dentifrices also showed diamond particle-size-dependent abrasion potential, while CT-brushed specimens exhibited significantly higher abrasion compared to all other groups. In conclusion diamond-added dentifrices show, according to their diamond particle size, similar or higher abrasion potential compared to the reference dentifrice CT on enamel, while CT dentifrice exhibits higher abrasive potential on dentine.

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Improved Resin Infiltration: Icon-Speed

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Reducing the application time for resin infiltration could improve safety and increase the acceptance of this treatment among practitioners and patients. Speed of resin penetration into the lesion pores correlates with the penetration coefficient (PC) of the

used infiltrant resin. It was the aim of the present *in vitro* study to evaluate the penetration ability of an experimental infiltrant resin (EIR; PC: 264 cm/s) compared to the conventional infiltrant (CIR; PC: 147 cm/s). Proximal active lesions (ICDAS 2) in 90 extracted molars and premolars were etched with 15% hydrochloric acid gel for 2 min and then allocated to one of the following treatments; EIR: lesions were infiltrated with EIR (DMG, Hamburg) for either 1, 2 or 3 min (n = 15/group), CIR: lesions were infiltrated with conventional infiltrant (Icon, DMG) for either 1, 2 or 3 min (n = 15/group). Subsequently, lesions were prepared and examined using dual fluorescence staining and confocal microscopy. Lesion depths (LD) and penetration depths (PD) were measured at three defined points along each lesion. Percentage penetration (PP) was calculated for each lesion ($PP = \text{mean PD} / \text{mean LD} \times 100$). Mean(SD) LD was 494 (236) μm . After 1 min application, PP of EIR [61 (28)%] was significantly higher compared to CIR [33 (15)%] ($p < 0.05$; ANOVA). After 2 and 3 min application PP of EIR in tendency was higher [2 min: 63 (22)%, 3 min: 83 (17)%] than control [2 min: 54 (14)%, 3 min: 68 (29)%] but differences did not reach significance level ($p > 0.05$; ANOVA). It can be concluded that the experimental infiltrant penetrated faster compared to the conventional infiltrant.

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Antibacterial Effect of Incorporation of Chlorhexidine Loaded-PLGA Microparticles and Chlorhexidine Salts into a Glass-Ionomer Cement

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Fluoride release from glass-ionomers (GI) may be important for the prevention of caries around restorations mainly due to its effects on mineral phases of teeth and on the remineralization process. This *in vitro* study aimed to evaluate the effects of chlorhexidine (CHX) salts (diacetate-DA or digluconate-DG) free base or encapsulated into poly(lactic-co-glycolic acid) (PLGA) microparticles – MP on physicochemical and antibacterial properties of a chemically activated glass ionomer cement (GIC). CHX was not incorporated into control specimens and experimental materials were prepared by adding 1% (w/w) of CHX loaded-PLGA microparticles or pure CHX salts into the GIC, constituting the groups: GIC (Control), DA, DG, MPDA and MPDG. Specimens were evaluated for determining CHX stability test, FTIR spectroscopy, setting time (ST), compressive strength (CS), flowability (F), CHX cumulative release (CR) and anti-biofilm effect against *Streptococ-*

cus mutans (AA). Biofilm formation on material surfaces was tested according to colony-forming units (CFUs) over freshly prepared or aged samples, in 3 independent biological replicates with 3 technical replicates each. Statistical analysis for physicochemical tests and anti-biofilm effect were performed by ANOVA and Mann Whitney test, both followed by post-hoc tests at a pre-set 5% significant level. Microencapsulated formulations enlarged, while DG reduced ST ($p < 0.05$). DG increased resistance to CS ($p < 0.05$); F was decreased by DA and DG ($p < 0.05$). Microencapsulated groups presented later and gradual profile of CHX-release when compared to CHX free base groups. Both CHX incorporating forms for DA and DG showed significant AA increase when compared to control. However, no statistically significant differences were found when comparing CHX free base or microencapsulated forms, as well as fresh and aged material. Experimental GICs have presented antibacterial activity, without detrimentally affect other material properties.

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Resin-Modified Glass Ionomer Containing 2-Methacryloyloxyethyl Phosphorylcholine with Dimethylaminohexadecyl Methacrylate and Silver Nanoparticles

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The aims of this study were to develop a novel resin-modified glass ionomer cement (RMGI) with orthodontic bonding applicability to prevent white spot lesions, and investigate the effects of incorporating protein-repellent and antibacterial agents into a RMGI on enamel bond strength, protein adsorption, and dental microcosm biofilm growth and acid production. Triple agents: 2-methacryloyloxyethyl phosphorylcholine (MPC), nanoparticles of silver (NAg), and dimethylaminohexadecyl methacrylate (DMAHDM), were incorporated into a RMGI. Enamel shear bond strength (SBS) (n = 10) and the adhesive remnant index (ARI) (n = 10) were determined. Protein adsorption was determined by a micro bicinchoninic acid method (n = 6). A dental plaque microcosm biofilm model with human saliva as inoculum was used to investigate metabolic activity (n = 6), colony-forming units (CFU) (n = 6) and lactic acid production (n = 6). One-way and two-way ANOVA were performed to detect the significant effects of the variables. Tukey's multiple comparison test was used to compare the data ($\alpha = 5\%$). The chi-square test was used to evaluate the ARI scores. Incorporating 3% MPC + 1.5% DMAHDM + 0.1% NAg (by mass) into RMGI, and water-aging for 30 d, did not adversely

affect the SBS, compared to control. RMGI containing MPC + DMAHDM + NAg had protein adsorption ($0.4 \mu\text{g}/\text{cm}^2$) that was 1/10 that of control ($4 \mu\text{g}/\text{cm}^2$). RMGI with triple agents (MPC + DMAHDM + NAg) had much stronger antibacterial effects than using a single agent or double agents ($p < 0.05$). Biofilm CFU counts on RMGI with triple agents were more than 3 orders of magnitude less than that of the commercial control. The incorporation of novel triple agents (MPC + DMAHDM + NAg) into a RMGI resulted in much greater reduction in biofilm growth and acid production, compared to double agents or a single agent. This was achieved without compromising the orthodontic bracket bond strength to enamel. Further studies are needed to assess the effects of new resin modified glass ionomer on in situ and in vivo biofilms.

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Effect of Adhesive Systems and Monomers on the Growth of Representative Caries-Associated Bacteria in vitro

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The aim of this in-vitro study was to evaluate the effect of three adhesive systems and their monomers on the growth of oral caries-associated bacteria. Three different adhesive systems (Optibond FL, Xeno V, Clearfil Protect Bond) and two monomers (HEMA & TEGDMA) were used. The unpolymerized components of each bonding system and their polymerized mixture were evaluated by using agar disc-diffusion test with *Streptococcus mutans* (DSM 20523) and *Lactobacillus casei* (DSM 20011). Additionally, the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) were determined. As positive control CHX 0.2% was used. The effect of ethanol 25% was additionally evaluated, as component of Optibond FL.

We found that for *S. mutans*, the biggest inhibition zones were found to be caused by the primer of Optobond FL and CHX 0.2%, followed by HEMA, Xeno V and the primer of Clearfil Protect Bond. In the case of *L. casei*, the inhibition zones were as follows: CHX 0.2% > primer of Optibond FL > HEMA > primer of Clearfil Protect Bond > polymerized form of Clearfil Protect Bond. TEGDMA and ethanol 25% showed no inhibition zones. The lowest MBC and MIC, among the materials, were found for Clearfil Protect Bond for both bacteria. HEMA and Xeno V showed higher MBC values compared to other substances for both bacteria. For *S. mutans*, Xeno V and HEMA showed higher MIC values than the other substances, while for *L. casei*, HEMA showed the highest MIC values. In conclusion the conventional bonding Systems

(Optibond FL, Xeno V) showed no antibacterial effect after polymerization in contrast to Clearfil Protect Bond. HEMA partially inhibited the growth of the two caries-associated bacterial species while TEGDMA shows no inhibition against these bacteria.

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Chlorhexidine-Releasing Ethylcellulose Film for Orthodontic Elastomerics

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The aim of this study was to identify the optimal combination of two casting solvents (ethanol; EtOH, dichloromethane; DCM) to sustain chlorhexidine diacetate (CDA) releasing on orthodontic elastomerics for prevention of dental caries in orthodontic patients. This study used ethyl cellulose (EC) as the polymer, and experimental groups were divided into five subgroups according to solvent ratio as follow: Group 1 (no EC in DCM only), Group 2 (EC in DCM only), Group 3 (EC in 70% DCM + 30% EtOH), Group 4 (EC in 50% DCM + 50% EtOH), and Group 5 (EC in 30% DCM + 70% EtOH). EC film was coated with 8 ml mixed coating solution by spraying method. Each side was sprayed twice then dried 5 min and this procedure was repeated 5 times. CDA release from the coated elastomerics was measured the absorbance using 254 nm wavelength with a UV-Vis spectrophotometer (UVD-3200, Labomed, inc., LA, USA). These procedures were repeated four times and analyzed by repeated measure ANOVA. In order to know that the original tensile forces of elastomerics are not disturbed by the coating process, the tensile forces of the coated elastomerics were measured using a Universal Testing Machine (Unite-O-Matic FM 20, United Calibration Corporation, CA, USA). To evaluate sustaining long-term antibacterial effect, the agar diffusion test was performed in petri dishes inoculated with *Streptococcus mutans*. The maximum release was observed from Group 3 whereas the minimum release was shown from Group 1. In the antibacterial test, all groups were shown long-term antibacterial effect over 7 days except Group 1. In addition, all groups were not affected when tensile force was tested in the coated elastomerics. Therefore the antibacterial effect of CDA can be adjusted according to combinations of polymers and solvents for orthodontic patients.

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***Streptococcus mutans* Biofilm Formation on Tooth Surface Infiltrated by Icon, in vitro Study**

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Streptococcus mutans due to its ability to synthesize extracellular polysaccharides takes part in biofilm formation, further is linked with dental caries. The aim of the study was to assess the formation of biofilm by *S. mutans* on the tooth surface infiltrated by ICON (DMG, Germany). The reference *S. mutans* ATCC 35668, originating from the collection of the Department of Microbiology of the Medical University of Białystok was used in this study. Caries-free extracted teeth, paired halves, cleaned with de-ionized water, infiltrated by ICON and not infiltrated (control) were sterilized in autoclave at 121°C for 15 min. The bacteria were cultured on a blood agar (Columbia Agar Base, BBL BD Biosciences Sparks + 5% defibrinated sheep blood PWWiU Pro Animal) and incubated at 37°C under microaerophilic conditions with the use of BBL Campy Pouch Microaerophilic System (Becton Dickinson) for 24 h. Then, the inoculum of *S. mutans* adjusted to approximately 2×10^8 CFU/ml was prepared. Moreover, 1% TTC (2,3,5-triphenyltetrazolium chloride) and the samples with ICON and control were added. The prepared samples were incubated at 37°C under microaerophilic conditions for 10 days to form a biofilm. After that time, the samples were rinsed three times in sterile PBS (pH 7.2) in order to eliminate bacteria forming plankton suspension. The formation of biofilm was observed with a confocal Olympus Lext 3D, Measuring Laser, Microscope OLS4000 (Japan) on the basis of the images of 1024x1024 pixels using different magnifications. Each sample was analyzed independently three times. The biofilm was formed on 1.12% of the surfaces infiltrated by ICON contrary to 9.05% of control surfaces, not statistically significant, $p < 0.333$ (exact Mann-Whitney test).

Within the limitation of the in vitro study and sample size, the formation of biofilm by *S. mutans* on infiltrated and control surfaces did not differ statistically.

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Protection of Longevity Action Against *Streptococcus mutans* by Incorporating Chlorhexidine in Glass Ionomer Cement

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Development of restorative materials with bioactive characteristics seems to be promising for prevention of dental caries. The study aim was to evaluate the effect of antibacterial action on *Streptococcus mutans* (SM) using two conventional glass ionomer cements (GIC) incorporated with chlorhexidine diacetate (DCHX). Antibacterial action was evaluated by agar diffusion, in *Streptococcus mutans* (SM) – ATCC 25175, replicated 3 times and analysis of eluates, as well as the influence of sodium fluoride in the antimicrobial efficacy of DCHX. The GICs Maxxion RTM and Vitro Fill RTM were tested, incorporating 0.5%, 1% and 2% of DCHX. Dimensions of the specimens were 2 mm diameter and 4 mm height. Statistical analysis was done by ANOVA and SNK tests. The inhibition capacity was proportional to the concentration of DCHX. Antimicrobial effect against SM reached 180 days. The fifteenth day showed higher antibacterial activity for both GICs. Maxxion RTM 1% and 2% showed the less differences over time. It was not observed SM grown to the 7 and 15 exhaustion days and bacteria grown in the agar surface appear only after 96 hours of incubation. It was not observed antagonistic effect in antibacterial action in the presence of sodium fluoride. In conclusion SM inhibition is dependant on DCHX concentration and fluoride solution did not showed ability to inhibit SM grown; the association of DCHX with GICs did not alter its antibacterial ability and the antibacterial action of DCHX, incorporated into GICs, remains effective for 180 days, regardless of the GICs tested.

Effect of Zinc-Doped Phosphate-Based Glasses on *Streptococcus mutans* NCTC10449

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

Zinc (3 mol%) doped (Zn-PBG) and control Zinc free PBG (c-PBG) rods (5x2 mm) were produced using conventional melt quenching, at 1100°C for 1 hour. For antibacterial assay, discs were placed on isosensitest agar previously inoculated with *S. mutans* NCTC10449, incubated for 24 h anaerobically at 37°C. Diameters of inhibition zones were measured in triplicate. Liquid broth assay was conducted in phosphate buffered saline using *S. mutans* suspension (OD₆₀₀=0.03) exposed to different Zn-PBGs (with increasing calcium concentrations of 11, 12 and 13 mol% denoted as C11, C12 and C13 respectively) and controls (a positive control of

0.2% chlorhexidine and a negative control of c-PBG). At 2, 4, 6 and 24 h, samples were removed, diluted appropriately in PBS, spread on BHI agar to assess viable colony-forming units (CFU) present. *S. mutans* biofilms were grown in a constant depth film fermentor on hydroxyapatite, using artificial saliva. At 6, 24, 48 and 120 h; discs containing biofilms were removed, subjected to 10 min exposure of Zn-PBG (C11) compared with 0.2% chlorhexidine or water. All the experiments were conducted in triplicates. Statistical analyses were conducted using R software (Vienna, Austria). Tukey-Kramer multiple comparison tests were used to compare values and P values <0.05 considered statistically significant. Inhibition zones decreased significantly ($p < 0.03$) as calcium concentration in the glasses increased (from 18 ± 1.7 , 16 ± 1.6 to 15.0 ± 1.1 mm for C11, C12 and C13 respectively) and in liquid broth assay ($p < 0.03$). The biofilm study Zn-PBG (C11) achieved growth inhibition of *S. mutans* NCTC10449 at 48 and 120 h compared with both controls, with a maximum of 0.95 log CFU reduction at 120 h ($p < 0.04$). Zn-PBGs inhibited *S. mutans* growth. Increasing calcium concentration in Zn-PBGs decreased antibacterial effects, C11 composition, may have potential use as an anti-caries agent.

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