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Abstracts

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Session 1

Fluoride and Erosion

1

Fluoridated Liquid Dentifrices: Effect on Enamel Demineralisation and Alkali-Soluble Fluoride Deposition in vitro

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This in vitro study analysed the ability of fluoridated acidic or neutral liquid dentifrices to protect against enamel demineralisation and on fluoride deposition on enamel. Bovine enamel specimens were randomly allocated ($n = 15$) to: experimental liquid dentifrices with 550 $\mu\text{g F/g}$ (pH 4.5); 550 $\mu\text{g F/g}$ (pH 7.0); 1,100 $\mu\text{g F/g}$ (pH 4.5); 1,100 $\mu\text{g F/g}$ (pH 7.0); 5,000 $\mu\text{g F/g}$ (pH 4.5); 5,000 $\mu\text{g F/g}$ (pH 7.0); placebo (pH 4.5); placebo (pH 7.0); and commercial toothpastes with 550 $\mu\text{g F/g}$ (Colgate Baby Barney, pH 7.0); 1,100 $\mu\text{g F/g}$ (Crest, pH 7.0); and 5,000 $\mu\text{g F/g}$ (Prevident, pH 7.0). The specimens were subjected to pH-cycles (demineralisation – 6 h/remineralisation-18h a day) and treated with one of the dentifrices 2×15 s/day for 7 days. The demineralisation was analysed by surface and cross-sectional hardness. Additionally, different bovine enamel specimens ($n = 9$) were demineralised for 6 h and then treated with one of the experimental dentifrices for 1 min. The deposition of alkali-soluble fluoride was determined. Data were tested using ANOVA/Tukey, Kruskal-Wallis/Dunn and two-way ANOVA/Bonferroni's test ($p < 0.05$). All F dentifrices significantly reduced the surface enamel demineralisation except the experimental dentifrice with 550 $\mu\text{g F/g}$ (pH 7.0). Regarding the subsurface demineralisation, all F dentifrices were able to reduce the hardness loss up to 90 μm ; however, in deep layers only the experimental dentifrices with 1,100 and 5,000 $\mu\text{g F/g}$ were different from placebo. All experimental F dentifrices promoted fluoride deposition compared to placebo. Higher fluoride deposition was found for 5,000 $\mu\text{g F/g}$ compared to the others regardless of the pH. Considering the experimental protocol, the effect of fluoridated acidic or neutral liquid dentifrices to protect against enamel demineralisation in vitro seems to be modulated mainly by the fluoride concentration.

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Enamel Crystals of Mice Susceptible or Resistant to Dental Fluorosis: AFM Study

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This study aimed to assess the surface roughness and width of enamel matrix apatite crystals of mice susceptible (A/J strain) or resistant (129 P3/J strain) to dental fluorosis through analyses by AFM (atomic force microscopy). Samples from the enamel matrix in the early stages of secretion and maturation were obtained from the incisors of both mice strains after sacrifice. All detectable traces of matrix protein were removed from the samples by a sequential extraction procedure. The protein-free crystals ($n = 13$ per strain) were analysed qualitatively in the AFM. Crystal surface roughness (Ra) and width were measured. The mean ($\pm$ SD) Ra of the crystals of A/J strain (0.58 ± 0.15 nm) was lower than the one found for the 129P3/J strain (0.66 ± 0.21 nm) but the difference did not reach statistical significance ($t = 1.187$, $p = 0.247$). Crystal width of the 129P3/J strain (70.42 ± 6.79 nm) was found to be significantly smaller ($t = 4.013$, $p = 0.0013$) than the same parameter measured for the A/J strain (90.42 ± 15.86 nm). The smaller width of enamel crystals observed for the 129P3/J strain might help to explain the resistance of this strain to the development of dental fluorosis.

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Plaque Fluoride Levels in Fluoridated and Non-Fluoridated Communities according to Dentifrice Fluoride Concentrations and pH

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This study evaluated the influence of dentifrice pH and fluoride concentration ([F]) on fluoride (F) uptake in dental plaque, in fluoridated and non-fluoridated communities. Forty-seven and fifty-nine 2-to-4-year-old children living in a fluoridated and in a non-fluoridated community, respectively, were randomly allocated into 3 groups in each community, according to the dentifrice they had been using for six months: A, liquid dentifrice (LD), 550 ppm F, pH 4.5 (n = 15, non-fluoridated/20, fluoridated); B, LD, 550 ppm F, pH 7.0 (n = 16/16); C, LD, 1,100 ppm F, pH 7.0 (n = 16/23). The LDs were loaded onto the toothbrush using the 'drop' technique. Plaque samples were collected 5 and 60 minutes after the last use of the dentifrices. Plaque [F] was analyzed with the electrode, after HMDS-facilitated diffusion. Data were tested by 2-way repeated-measures ANOVA and Bonferroni's tests ($p < 0.05$). Mean ($\pm$ SE, mmol/kg dry weight) plaque [F]s were 1.32 ± 0.23 , 0.80 ± 0.08 , 1.74 ± 0.24 for A, B and C, respectively, 5 min after the dentifrices use for the fluoridated community. Corresponding values for the non-fluoridated community were 0.53 ± 0.16 , 0.37 ± 0.08 , and 0.57 ± 0.11 , respectively. Sixty minutes after toothbrushing, corresponding figures were 0.95 ± 0.21 , 0.48 ± 0.07 and 1.03 ± 0.12 for the fluoridated community and 0.58 ± 0.16 , 0.41 ± 0.06 and 0.49 ± 0.10 , for the non-fluoridated community. For the fluoridated community, C was significantly different from B regardless the time, while A did not significantly differ from B and C. For the non-fluoridated community, the differences among the dentifrices or times did not reach statistical significance. The results suggest that the reduction of dentifrice pH tends to increase plaque F uptake. Additionally, in the non-fluoridated community, plaque F levels did not decrease with time, suggesting that F from dentifrice occupied more stable binding sites that were not previously filled by F originated from the diet.

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Chemically Soluble Fluoride is the Best Indicator of Fluoride Bioavailability from Toothpastes

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Since only bioavailable, absorbable fluoride from toothpastes can contribute to fluorosis risk, total fluoride (TF) may not be a good predictor of F exposure from toothpastes, especially in Ca-based toothpastes in which part of TF is insoluble. Therefore, we evaluated if chemically total soluble fluoride (TSF) in toothpastes would be better indicator of bioavailable F than TF. One NaF/silica-based (1,100 ppm F) and one MFP/CaCO₃-based (1,450 ppm F) toothpaste were tested, either fresh or aged, simulating the decrease of TSF that occurs with the latter after one year of manufacturing. In a blind, crossover study, 20 adults volunteers ingested both toothpastes in an amount sufficient to provide 49.5 μ g of TF/kg body weight. Saliva collected for up to 3 h after ingestion and 24-h urine were used to indicate F bioavailability. Spearman correlation between the area under the curve of salivary F concentration versus time (AUC) and amount of TF and TSF ingested by volunteers was determined. Also, the percentage of F excreted in urine was calculated in relation to TF or TSF ingested. TF concentrations in the fresh and aged toothpastes were in accordance to the expected values. All F in the NaF/silica formulation, either fresh or aged, was soluble, but in the MFP/CaCO₃ formulation TSF concentrations were $1,122.4 \pm 76.4$ and 869.3 ± 49.6 , respectively. Data showed a significant correlation ($r = 0.57$, $p < 0.001$, Spearman) between TSF ingested and AUC but not for TF ($r = 0.14$, $p = 0.20$). The expected percentage of F excretion, around 40–50% considering the studied population, was only achieved when TSF ingested was taken into account. In conclusion, the TSF fraction and not TF in toothpastes should be considered in the estimation of potential fluorosis risk.

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Dental Biofilm Fluid Fluoride Concentration after Use of Fluoride Toothpastes by Young Children

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Low-fluoride toothpaste (500–550 μ g F/g toothpaste) has been recommended for young children as an alternative to reduce the risk of dental fluorosis, but its anticaries efficacy when compared to the conventional toothpaste (1,100 μ g F/g) has been questioned. Since the capacity to maintain increased fluoride levels in the oral environment is an indicator of the anticaries effect of fluoride toothpastes, the aim of this study was to evaluate the availability of fluoride in biofilm fluid from these formulations used by children. In a double-blind, crossover study, 24 volunteers, 3–4 years-old, brushed their teeth 3 $\times$ /day with 0.3 g of the following tooth-

pastes: non-fluoride (negative control), 500 µg F/g or 1,100 µg F/g toothpastes (NaF/silica). On the last 3 days, they were instructed to brush only the occlusal surfaces, to allow biofilm accumulation. On the 7th day, dental biofilms were collected from buccal surfaces of posterior teeth before and 30 min after toothbrushing. The biofilm fluid was separated from the solids and fluoride concentration was determined using an inverted ion-specific electrode. Before toothbrushing, no statistical difference in fluoride concentration in biofilm fluid was observed among treatment groups. Although significantly higher fluoride concentrations were observed in the fluid 30 min after brushing with both fluoride toothpastes when compared to the baseline values ($p < 0.05$), fluoride concentration (µM, mean $\pm$ SD, $n = 24$) in dental biofilm fluid after brushing was significantly different from the control group (4.1 ± 3.6) only for the 1100 µg F/g toothpaste (12.0 ± 10.2), but not for the low-fluoride toothpaste (7.1 ± 5.8). The findings may suggest a rationale for the conclusions of the systematic review that a toothpaste containing at least 1,000 µg F/g has a greater anticaries effect than those containing 500–550 µg F/g.

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Urinary Fluoride Excretion by Children Drinking Fluoridated Water

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Drinking water fluoridation is considered a risk factor for dental fluorosis but the bioavailable fraction of fluoride ingested from diet that is responsible for its systemic effect has not been considered. In the present study, fluoride excretion in urine of children living in two communities with distinct levels of fluoride concentration in drinking water was determined as an indicator of fluoride bioavailability. Twenty-four h urine and all diet ingested by 51 schoolchildren, aged two to five years, living in communities with ($n = 27$) and without ($n = 24$) fluoridated water (0.65 ± 0.06 and 0.06 ± 0.01 ppm F, respectively) were collected for determination of fluoride concentration. Fluoride was extracted from dietary products (liquids and solids) by HMDS-facilitated diffusion and its concentration was determined using an ion-specific electrode as an indicator of total fluoride ingested. Fluoride concentration in urine was determined directly using the electrode, after appropriate buffering with TISAB II. Daily amount of fluoride intake from diet was 410.7 ± 191.7 and 27.9 ± 14.7 µg F/day, for communities with and without fluoridated water, respectively ($p < 0.0001$). Fluoride excreted in urine (µg F/day) was 263.0 ± 166.7 and 176.1 ± 174.2 , respectively ($p = 0.01$). The fluoride intake from diet was 93% lower in the non-fluoridated community but the difference in fluoride excreted was only 33% smaller. Although the results show that diet has an important contribution to the total amount of fluoride ingested daily by young children, the findings of urinary fluoride excretion suggest that only part of fluoride is bioavailable to contribute with dental fluorosis.

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Fluoride Released from Fluoride Varnish Three Hours after Application

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Fluoride varnishes prolong contact time between fluoride and enamel. Varnishes remain on tooth surfaces for 24-hours or more and act as a slow-releasing reservoir of fluoride. **Aim:** The aim of this study was to evaluate the rate of fluoride release from three varnishes: A = 3MTM ESPETM ClinproTM WhiteVarnish, B = PremierTM EnamelProTM Varnish, C = ColgateTM DuraphatTM Varnish, more than 3 hours after application. **Experimental Approach:** Varnish (0.042 $\pm$ 0.05g) was coated on a plastic slide ($n = 3$) over 2-inches² and submerged into an agitated TISAB/water solution containing an ion-specific fluoride electrode. The fluoride in the solution was measured every 10 seconds for 15 minutes, and thereafter every minute using a Mettler Titrator. MilliVolts were converted to ppm using a standardized curve, and percent fluoride released was calculated based on product label (5%NaF, 22,624.43 ppm) and initial sample weight. Fluoride released is reported in percent of total fluoride content. **Results:** More fluoride was released in the first minute than in any other time interval: A = 0.27%, B = 8.62%, C = 8.41%. After 3 h, all varnishes released fluoride according to a linear model, slope(R^2): A = 3.0%/h (0.993), B = 1.1%/h (0.955), C = 0.3%/h (0.995). At 24-hours, most of the contained fluoride was released from varnishes A and B, but not C. (A = 83.32%, B = 71.82%, C = 20.22%). **Conclusions:** This model assumes all of the varnish remains on the tooth surface. After the 3rd hour, Varnish A and B continue to deliver fluoride, varnish A delivers 11.5% more. In the mouth where saliva continuously bathes the varnish, the ability of varnish to continuously release fluoride is beneficial. The varnish that released low levels of fluoride initially, exhibited the highest sustained rate of fluoride release for the long term. This is likely related to the varnish's unique prolonged setting.

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Anticaries Potential of Products Formed/Retained on Enamel by Fluoride Gel or Varnish

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The anticaries effect of fast-versus slow-reacting topical fluoride products, such as acidulated F (APF) gels and fluoride varnishes (FV), has been demonstrated in clinical trials but the comparative mechanism of action of these products is still to be thoroughly explained. In the present study, the anticaries potential of products formed and retained on enamel after APF gel (DFL[®], 1.23% F, pH 3.5) or FV (Duraphat[®], 2.26% F) application was evaluated using a highly-controlled in situ model. Both products were

applied on enamel blocks according to the clinical recommendations: APF gel was applied for 4 min and FV was kept applied for 24 h before being removed with acetone. Half of the blocks were immersed in artificial saliva for 7 days to simulate the dissolution of CaF_2 -like products (' CaF_2 ') that occurs in vivo. This procedure caused a 96% reduction of ' CaF_2 ' on enamel treated with APF (42.3 ± 24.0 to $1.7 \pm 0.7 \mu\text{g F/cm}^2$), whereas for the FV the reduction was 79% (25.7 ± 2.8 to $5.5 \pm 2.1 \mu\text{g F/cm}^2$). In a double-blind, crossover, short-term in situ design, 12 volunteers wore palatal appliances containing blocks freshly-treated or exposed to saliva, kept in contact with a *S. mutans* test plaque and acid challenged by a sucrose rinse. Untreated blocks were used as control. ' CaF_2 ' products formed and retained on enamel by both gels were able to reduce mineral loss when compared to the control ($p < 0.05$). Although no significant difference on enamel demineralization among blocks freshly-treated with both F products was observed ($p > 0.05$), after aging the FV-treated blocks presented lower mineral loss than the APF-treated blocks ($p < 0.05$). The results suggest that the anticaries effect of slow-reacting topical fluorides is maintained for longer periods of time.

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Fluoride Intake from Infant Foods and Bottled Water in the US

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Excessive fluoride intake during enamel formation has been associated with increased risk for enamel fluorosis. Food and beverages have been identified as significant sources of fluoride intake for children. The current study assessed fluoride intake from infant foods and bottled water for one year old children in the US. FDA total diet study information was used to determine most frequently consumed amounts and types of infant foods and water used to reconstitute formula. Fluoride content was measured directly (for water) or using a modification of the HMDS microdiffusion method (for foods). Mean and standard deviations were calculated. Ranges of fluoride intake for different dietary combinations based on the typical US diet and 50th percentile weight for one year olds were also calculated. 458 water samples from 20 brands and 360 infant food samples from three brands were collected and analyzed for fluoride content. Fruit-based foods had a mean $0.059 \pm 0.018 \mu\text{g F/g}$; vegetable-based had $0.139 \pm 0.039 \mu\text{g F/g}$; non-chicken based had $0.164 \pm 0.065 \mu\text{g F/g}$; turkey dinners had $0.315 \pm 0.242 \mu\text{g F/g}$; and chicken-based had $0.578 \pm 0.257 \mu\text{g F/g}$. Chicken-based products had statistically significant higher mean fluoride concentration. For water samples, fluoride concentration ranged from $0.006 \mu\text{g/ml}$ to $0.740 \mu\text{g/ml}$. Variability between production lots was not statistically significantly different for foods or water. Calculated fluoride intake from infant foods and bottled water used to reconstitute formula ranged from 0.07 mgF/kg for dietary combinations using water and foods with

the highest fluoride content to 0.015 mgF/kg for calculations using the lowest fluoride content foods and water. Significant variation in fluoride intake was observed with some intake calculations being higher than the recommended optimal fluoride intake.

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Fluoride Retention on CO_2 Laser-Treated Enamel after an Erosive in situ Challenge

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The aim of the study was to evaluate the fluoride retention on CO_2 laser-irradiated enamel over a 5-days in situ erosive challenge. 10 volunteers participated in this in situ study, with a crossover design and 4 treatments: CO_2 laser irradiation, 0.3 J/cm^2 $2-5 \mu\text{s} - 226 \text{ Hz}$ (L); topical fluoride treatment – AmF/NaF , 1.25% F, 3 min (F); fluoride treatment + CO_2 laser (FL); and no treatment, as negative control (C). For each treatment the volunteers used palatal appliances containing 6 fixed sterilized bovine enamel samples during day and night except meals. For erosive demineralization the mouth appliances were immersed extra-orally in 80 ml of 0.05 M citric acid (pH 2.3) for 20 min twice daily. Individual oral hygiene was performed with fluoride-free products and without the appliance in situ. Before and between the treatment periods, a 1 week wash-out period was included. Two samples were collected from the appliances for analysis on days 1, 3 and 5 ($n = 20/\text{day/treatment}$). Acid-extracted fluoride was analyzed using Orion 96-09 ion-selective electrode and Orion EA-940 ion analyzer. Measurements were performed in triplicate, and the fluoride concentration in the enamel was determined in $\mu\text{g F/cm}^2$. Data were analyzed by means of repeated measure ANOVA and post-hoc comparisons (two-sided and at 5% significance level). On day 1 both groups FL (2.65 ± 1.43) and L (1.22 ± 0.61) presented statistically higher fluoride means than the control (1.63 ± 0.61), $p < 0.05$. On day 3 only F group (2.65 ± 1.22) presented significantly higher means than control (1.63 ± 0.61 , $p = 0.0087$), and on day 5 none of them ($p > 0.05$). CO_2 laser irradiation at 0.3 J/cm^2 combined with previous fluoride application significantly increased fluoride retention as compared to non-treated and solely laser-treated enamel up to one day in an in situ erosion model.

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Cola Drink Modifications and Their Effect on Initial and Prolonged Erosion Lesions: An in vitro Study

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This in vitro study evaluated the effect of exposure to Cola on initial and prolonged erosion lesions. Sixty bovine enamel blocks were randomly divided into 5 groups ($n = 12$): RC-regular cola, RCpH addition of base to increase regular cola pH, RCAS addition of aspartame to regular cola, LC-light cola, and LCpH addition of acid to decrease light cola pH. Two-thirds of the blocks surface was coated with nail varnish for reference. The samples were subjected to erosive challenges (30 ml/sample) for 2 min, 4×/day. Between the erosive challenges (2h) and overnight the samples were immersed in artificial saliva (30 ml/sample). After 1st experimental day, surface hardness test was performed in order to provide the percentage surface hardness change (%SHC). At the 5th day, enamel surface loss was measured by profilometry (μm). Data were tested using ANOVA and Tukey's test ($p < 0.05$). Independently of the cola modifications, all treatments promoted similar hardness change of enamel surface. RC promoted higher enamel loss ($6.69 \pm 0.71 \mu\text{m}$) than LC ($4.80 \pm 0.77 \mu\text{m}$). The acid addition to the light cola (LCpH: $6.60 \pm 1.78 \mu\text{m}$) significantly enhanced its erosive potential, which was comparable to RC. The base addition to regular cola (RCpH: $3.78 \pm 0.64 \mu\text{m}$) resulted in similar wear to ER and EL, respectively. The addition of aspartame to the regular cola (ERA $5.44 \pm 0.65 \mu\text{m}$) resulted in wear similar to LC, but also similar to RC. In conclusion, the present in vitro study showed that the pH modification of cola drink interferes with the erosive potential of prolonged enamel erosion, but not with initial erosion.

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Abrasion Protective Ability of Self-Adhesive Flowable Composites Applied to Eroded Dentin

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The aim of this in-vitro study was to evaluate the abrasion protective ability of two different self-etching, self-adhesive flowable composites applied to eroded dentin. 60 samples with an exposed dentin surface of $5 \times 5 \text{ mm}$ were prepared and polished up to 1,200 grid. The samples were eroded by using 1% citric acid for 5 min and

randomly divided among four groups. Four different materials were tested: Vertise™ Flow (Kerr), Fusio™ Liquid Dentin (Pentron Clinical), Seal & Protect (Dentsply DeTrey) and X-flow using Xeno V as adhesive (Dentsply DeTrey). The dentin surface of each specimen was covered with a layer of the respective material (diameter 5 mm, thickness: 1 mm). Then half of the surface of each specimen was covered with a tape and served as reference. Subsequently, the specimens were exposed to toothbrushing abrasion representing a six-month clinical situation [8,900 movements, load: 2N, in a solution of human saliva mixed with Elmex® Sensitive Professional™ (GABA) (3:1)]. Surface roughness and substance loss were measured using laser profilometry (μm). The samples covered with Seal & Protect did not survive toothbrushing. The X-Flow samples showed a higher surface roughness compared to the other two flowables. Among the three composite materials, X-flow showed the least substance loss ($0.39 \pm 0.32 \mu\text{m}$) followed by Vertise Flow ($0.51 \pm 0.18 \mu\text{m}$) and Fusio Liquid Dentin ($0.79 \pm 1.09 \mu\text{m}$). Tukey's Test showed no significant difference ($p > 0.05$) among the three tested materials with respect to substance loss. The two tested self-adhesive flowables showed similar performance compared to the conventional flowable composite. Within the limits of an in-vitro study it may be concluded they may protect eroded dentin from abrasion at least for more than six months.

The Effect of Daily Fluoride Mouth Rinse against Enamel Erosive/Abrasive Wear in situ

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It is unknown whether application of fluoride agents on enamel results in a lasting resistance against erosive/abrasive wear. The aim was to investigate if one daily mouth rinse with sodium fluoride (NaF), stannous fluoride (SnF₂) or titanium tetra-fluoride (TiF₄) solutions could protect enamel against erosive/abrasive wear in situ. The study was a prospective, paired, randomised and blind study. Sixteen human molars were cut into four specimens, each with one amalgam-filling (reference surface for measurements). Specimens from one tooth were mounted together on either left or right buccal side of the acrylic mouth appliances and worn by eight volunteers for nine days except during meals and oral hygiene procedures. Each morning the specimens were brushed ex situ with tooth brushes and water for thirty seconds and the fluoride solutions (0.4% SnF₂ pH 2.5, 0.15% TiF₄ pH 2.1, 0.2% NaF pH 6.5, all 0.05 MF) were applied by a pipette (one drop per second) for two minutes. At midday the specimens were etched ex situ for two minutes in 300 ml freshly made hydrochloric acid (HCl) 0.01 M, rinsed in tap water and reinserted in situ. The same etch procedure was repeated in the afternoon. Etch depths (μm) were measured by a white light interferometer. A paired t-test assessed the significance of differences between the treatment groups. The mean etch depths (SD) after 9 days of experiment were; SnF₂ 1.8 (± 1.9) μm , TiF₄ 3.1 (± 4.8) μm , NaF 26.3 (± 4.7)

μm , control $32.3 (\pm 4.4) \mu\text{m}$. In conclusion, daily application of SnF_2 , TiF_4 and NaF solutions reduced the etch depths by 94%, 90% and 18% respectively compared with the control ($p < 0.05$).

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Protective Effect of Fluoride Toothpastes or Fluoride Gel against Erosive/Abrasive Wear: An in-situ Study

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Background: High and low concentrated NaF toothpastes have shown caries protective properties, but the preventive effect against erosive/abrasive enamel wear is unclear. **Aim:** To measure the erosion-inhibiting effect of two fluoride toothpastes and a high concentrated SnF_2 gel on the development of erosion-like lesions in a double-blind, randomized in situ study, measured by a White Light Interferometer (WLI). **Materials and Methods:** 16 human molars were each divided into four specimens, mounted on acrylic mouth appliances and worn by 8 volunteers for 9 days. The specimens were brushed every day with fluoride-free toothpaste. Treatments; group 1: no treatment (control), group 2: SnF_2 gel 2,500 ppm (5 min) every third day, group 3: NaF toothpaste 5,000 ppm 5 min every third day and 2 min the other days, group 4: NaF toothpaste 1,450 ppm (2 min) daily. In order to mimic gastric reflux, the specimens were etched for 2 min twice a day (0.01 M HCl). Results. The mean etch depth in μm for the control specimens were $32.9 \text{ SD } 6.8$. The mean values for the other groups were $22.2 \text{ SD } 8.4$ (group 2), $30.8 \text{ SD } 7.8$ (group 3) and $31.4 \text{ SD } 7.7$ (group 4). Compared with the control, the SnF_2 treated enamel specimens showed significantly lower etch depths. The NaF toothpastes (5,000 and 1,450 ppm F) gave no significant protective effect, $p = 0.2$ and $p = 0.4$, respectively. **Conclusion:** Under the present experimental conditions it can be concluded that the application of SnF_2 gel every third day reduced the etch depth after acid exposures and that the daily application of both a low concentrated and high concentrated NaF toothpaste gave no protection when exposed to erosive and abrasive challenges.

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IA Survey of Norwegian Dentists' Opinions, Knowledge, and Diagnosis of Dental Erosive Wear

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An electronic questionnaire survey was sent to 1,262 public dental health-employed dentists in Norway in order to investigate their opinions, knowledge and diagnosis of dental erosive wear.

The response rate was 62% ($n = 783$, mean 43 years, 65% female). Nearly all dentists registered erosive lesions, 51% used a specific scoring system: 31% a two-graded system (enamel-dentine) and 14% a more detailed system. Erosive lesions were registered on the surface-level by 54% of respondents and on the tooth-level by 14%, while 26% registered on the patient-level. Lesions were reported located most often on the palatal surfaces in upper anterior teeth (79%), and occlusal surfaces of first mandibular molars (74%) or first maxillary molars (32%). Half the dentists used clinical photographs for documentation, 40% never used photos, 60% made study models and 34% never made models. While no gender differences were reported by 36% of dentists, 40% had the impression that there were more erosive lesions in males. Most respondents (77%) usually found a probable cause: carbonated beverages (97%), acidic juices (72%), citric fruits (46%), sports drinks (24%), acidic diet (20%) and reflux/eating disorders with vomiting (8%). Only 21% of the dentists recorded dietary history, and 73% reported that they never measured saliva secretion in patients with erosion. Regarding treatment, 78% treated erosive wear patients themselves, 9% referred to another dentist/specialist/faculty clinic and 13% referred only the more severe cases. **Conclusion:** The survey suggests that the dentists are relatively up-to-date regarding the clinical recording and diagnosis of dental erosive wear, although dietary and salivary analyses were not given priority. Documentation was not standardized, but the majority of dentists were confident of finding the cause of the erosive wear and treating their own patients.

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Ability of Antacid Suspensions to Counteract Enamel Loss by Gastric Acid

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Current recommended approaches to control dental erosion caused by gastric acid include the use of neutralizing agents. Rinsing with over-the-counter antacid medications has been shown to raise intraoral pH after erosive challenges [Lindquist et al.: Eur J Oral Sci 2011;119:301–304]. However, no data are yet available on whether such antacids can minimize enamel loss. This in vitro study was aimed to investigate the effect of antacid suspensions applied straight after an erosive event with HCl in controlling enamel loss. Forty-eight slabs from bovine enamel ($3 \times 3 \times 2 \text{ mm}$) were embedded, flattened and polished. After reference areas had been created with adhesive tape, specimens were divided into 6 groups ($n = 8$) to be exposed to a HCl challenge (0.01 M; pH 2) for 2 min and directly immersed in suspensions containing: (1) magnesium hydroxide (MH); (2) aluminum hydroxide (AH); (3) MH+AH; (4) sodium alginate, sodium bicarbonate and calcium carbonate (SA+SB+CC); (5) hydrated aluminum-magnesium sulfate (AMS). Suspensions were applied for

1 min and then rinsed from the surface with artificial saliva. In the control group artificial saliva rather than antacid was used. Specimens were subjected to further 4 sequences of erosion/antacid after which enamel loss (μm) was measured with a profilometer. Enamel loss (sd) observed as a result of applying MH, AH, MH+AH, SA+SB+CC and AMS suspensions was 0.719 (0.159), 0.685 (0.217), 0.751 (0.148), 0.660 (0.206) and 0.726 (0.172), respectively. Wear in control group was 1.270 (0.264). ANOVA and Tukey's test revealed that antacid suspensions significantly reduced enamel loss and that similar protection was afforded by all formulations. The findings indicated that antacid suspensions may to some extent counteract enamel loss.

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Abrasion of Sound and Eroded Enamel and Dentin: Effect of Brushing Force of Manual and Sonic Toothbrushes

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This study aimed to determine the brushing forces applied during in vivo toothbrushing with manual and sonic toothbrushes and to analyse the effect of these brushing forces on abrasion of sound and eroded enamel and dentin in vitro. Brushing forces of a manual and two sonic toothbrushes (Sonic complete, Oral B; Sensonic Professional, Waterpik; low and high frequency mode) were measured in 27 adults prior and after instruction of the respective brushing technique and statistically analysed by repeated measures ANOVA. Brushing forces determined in vivo were used for the in vitro experiment, where enamel and dentin specimens (each subgroup $n = 12$) were brushed in an automatic brushing machine with the respective brushing forces using a fluoridated toothpaste slurry. Sound specimens were brushed for 100 min (manual) or 128 min (sonic). Eroded specimens were brushed for a total of 150 s (manual) or 192 s (sonic) in a cyclic erosion-abrasion experiment (30 cycles, each: 30 s citric acid, pH 2.6, 15 min artificial saliva, and 5 or 6.4 s brushing, respectively). Brushing times were adjusted according to differences in the size of the toothbrush heads and, thus, to the contact time with the samples surface. Substance loss was determined by profilometry and statistically analysed by one-way ANOVA. Average brushing force of the manual toothbrush (1.6 N) was significantly higher compared to the sonic toothbrushes (0.9 N), which were not significantly different from each other. Brushing force prior and after instruction of the brushing technique was not significantly different. The manual toothbrush caused highest abrasion of sound and eroded dentin, while the Sensonic Professional induced highest abrasion of sound and eroded enamel. Brushing forces of manual and sonic toothbrushes are different and affect their abrasive capacity.

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Prevalence, Extent and Severity of Dental Erosions in Schoolchildren in Hamburg

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The aim of the study was to determine the prevalence as well as the average extent and severity of dental erosions in schoolchildren in Hamburg. The findings should be related to behavioural data. 1,580 schoolchildren aged 6 to 15 years were examined for dental erosions by two calibrated investigators. Erosions were recorded and rated on a scale of 1 to 3, using the index proposed by Lussi. Data about oral hygiene and diet were collected using questionnaires for parents and children. Statistical evaluation included prevalence, mean number of affected teeth and the distribution of scores. The prevalence of erosions was 17.8% and strongly related to the children's age with ranges from 5.0% in 6-year-olds to 29.9% in 15-year-olds. Boys (19.7%) showed a higher prevalence than girls (15.9%, $p = 0.051$, χ^2 -test). The mean number of affected teeth was 0.70 ± 2.27 in all children but 3.95 ± 4.01 in children with erosions which indicates a polarization of the erosion distribution. In total, 2488 tooth surfaces exhibited erosions. The majority (84.2%) was restricted to enamel (score 1), and in only 0.2% dentin was involved extensively (score 3). Further analyses were performed for 12-year-olds ($n = 341$), which had an erosion prevalence of 24.9% and a mean number of 0.90 ± 2.19 affected teeth. 36 schoolchildren (10.6%) had 74.4% of all erosion prone teeth in this age group in their mouths. However, in an analysis of relations to oral hygiene and diet, no single variable could be determined to be significantly related to prevalence or erosion extent. In conclusion, the study shows a considerable prevalence of erosions in schoolchildren, indicates a polarization, but reveals a low severity.

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Panel Study of Risk Indicators for Erosive Tooth Wear in Brazilian Preschool Children

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Aim: To assess the prevalence and risk indicators for ETW in children aged 3 to 4 years in the city of Diadema, São Paulo and to compare results from 2008 with those found in the latest survey (2010). **Design:** A total of 967 children (2008) and 995 children (2010) were randomly selected and examined during the National Children's Vaccination Day. Sixteen examiners were trained and calibrated to use a modified version of the O'Brien index (1994) for ETW lesions and nutritional status was assessed using the WHO criteria (2006). Data on socioeconomic factors, nutritional variables, dietary habits and frequent exposure to gastric

acid were collected using a questionnaire. Poisson regression model was used for data analysis ($p < 0.05$). **Results:** The prevalence of ETW was similar in 2008 (51.6%) and 2010 (53.9%) and most of the lesions found were confined to enamel. There were no significant associations between ETW and dental caries or socio-economic, environmental, and nutritional variables. Risk indicators for ETW were daily soft drink intake ($p < 0.001$), drinking methods causing prolonged contact with the teeth ($p = 0.007$) frequent gastroesophageal reflux ($p = 0.005$), frequent vomiting ($p = 0.011$), and an increase in age ($p = 0.003$). **Conclusions:** In conclusion, a high and similar prevalence of ETW was found in this sample of preschool children in 2008 and 2010 and risk indicators included the frequency and method of soft drink intake, vomiting, reflux and an increase in age.

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Impact of Abrasives and Chitosan on the Efficacy of Experimental F/Sn-Toothpastes against Erosion/Abrasion in-vitro

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Tin is a notable anti-erosive agent. In toothpastes tin might interact with abrasives possibly influencing its efficacy. Chitosan is a positively charged biopolymer with anti-erosive properties and able to bind to various surfaces. The effect of varying types and amounts of abrasives and of Chitosan in experimental F/Sn toothpastes on erosive/abrasive tissue loss was investigated. The study was conducted in two experiments. In both experiments (E1+E2; 8 groups each, $n = 15$) human enamel specimens were cyclically demineralised (10 days, 0.5% citric acid, pH 2.6; 6×2 min/day). Specimens were exposed to toothpaste slurries for 2×2 min/day and were brushed for 15 s within the exposure time (2 N, brushing machine). Negative control was erosion only. Substance loss was quantified profilometrically (μm). To a basic formulation (1,400 ppm F, 3,500 ppm Sn) varying amounts (wt%) of abrasives were added (5, 10, 15, 20% silica or 20% polyethylene, range of RDA: 56–81). One formulation contained no abrasives; placebo toothpaste contained 20% abrasives but no F/Sn. To all toothpastes in E2 Chitosan was added. Tissue loss (E1/E2) after erosion only was $11.7 \pm 3.2/11.0 \pm 2.5$. In E1/E2 highest tissue loss was found after brushing with placebo ($13.2 \pm 3.9/14.7 \pm 1.8$). Tissue loss reduction (%) compared to placebo ranged between 2% and 48% in E1 and between 48 and 84% in E2. In both experiments efficacy was not proportional to silica content. The best efficacy in both experiments was found with the formulation without abrasives (E1: 6.1 ± 3.3 ; E2: 3.1 ± 1.5 ; each $p < 0.001$ compared to placebo) and with 20% polyethylene (E1: 6.1 ± 2.8 ; E2: 2.4 ± 1.0 ; each $p < 0.001$ compared to placebo). Type and amount of abrasives may have an impact on efficacy beyond abrasiveness. Chitosan increased efficacy independent of the type and amount of abrasives.

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Mode of Action of Experimental F/Sn-Preparations: in-vitro and in-situ Tin Retention on Enamel after Erosion/Abrasion

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Tin anti-erosive properties are based on the incorporation of the ion into the dental hard tissue. This has been shown to occur with highly concentrated mouthrinses in-vitro. However, nothing is known about the mode of action of tin under in-situ conditions and for lower Sn concentrations as present in toothpastes. In contrast to mouthrinses, toothpastes have a complex composition, containing a multitude of ingredients including abrasives or efficacy enhancing additives, e.g. the biopolymer Chitosan. Therefore, their mode of action might be different to mouthrinses. The retention of tin on and in human enamel after corresponding in-vitro and in-situ use of three different F/Sn-containing preparations was investigated: one abrasive-free gel (3,000 ppm Sn, 1,000 ppm F), two experimental toothpastes (TP1: 3,500 ppm Sn, 1,400 ppm F; TP2: as TP1 plus Chitosan). All specimens were cyclically eroded (0.5% citric acid; 6×2 min/day) and treated 2×2 min/day with slurries. In-vitro specimens were brushed for 15 s (2 N, brushing-machine) within the exposure time. In-situ specimens were intraorally treated and brushed for 5 s within the 2 min exposure time (2.5 N, powered toothbrush). Tin retention was measured by Electron-Dispersive-X-ray-spectroscopy on surfaces and cross sections. Tin content (wt%) on in-vitro surfaces was (gel, TP1, TP2) 2.7 ± 0.3 , 0.9 ± 0.3 , 2.1 ± 0.4 ($p < 0.001$ between all groups), and on in-situ surfaces 5.6 ± 4.6 , 1.0 ± 1.0 , 1.1 ± 0.9 ($p < 0.01$ between gel and TP1/TP2). Significant differences between in-situ and in-vitro specimens were found for gel and TP2. Tin incorporation was deeper under in-vitro than under in-situ conditions. The use of the gel led to higher tin retention than the toothpastes, both under in-vitro and in-situ conditions, perhaps due to the lack of abrasives. Chitosan had a significant impact on tin retention under in-vitro, but not under in-situ conditions.

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Role of Abrasives and Chitosan in Experimental F/Sn Toothpastes: Tin-Uptake in Enamel after Erosion/Abrasion in-vitro

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Tin and Chitosan have promising anti-erosion properties. In toothpastes, however, tin might interact with abrasives influencing the efficacy of the preparation. Chitosan might adsorb to surfaces with negative zeta-potential, thus influencing the tin-uptake

in enamel. This study investigated the tin retention on eroded enamel surfaces treated with toothpastes having different amounts and types of abrasives with and without Chitosan. Human enamel specimens ($n = 15$ each) were cyclically demineralised (10 days, 0.5% citric acid; 6×2 min/day). Fourteen groups were immersed in toothpaste slurries for 2×2 min/day. Further fourteen groups were additionally brushed for 15 s within immersion time (2 N, brushing machine). Negative control was demineralised only. To the basic toothpaste formulation (3,500 ppm Sn, 1,400 ppm F), with and without Chitosan, varying amounts (wt%) of abrasives were added (5, 10, 15, 20% silica or 20% polyethylene, range of RDA: 56–81). One formulation contained active ingredients only, but no abrasives. Placebo toothpaste contained 20% silica, but no active ingredients. Tin-uptake (wt%) was measured by Energy-Dispersive-X-ray-spectroscopy on surfaces. All specimens treated with Chitosan formulations showed significantly higher tin-uptakes (range in slurry groups: $2.7 \pm 0.5 - 3.7 \pm 0.6$; range in slurry/brushing groups: $2.2 \pm 0.3 - 3.3 \pm 0.6$), than specimens treated without Chitosan (range in slurry groups $1.6 \pm 0.3 - 1.7 \pm 0.2$, range in slurry/brushing groups: $0.9 \pm 0.2 - 1.8 \pm 0.2$). Tin-uptake after polyethylene and after silica was similar. The formulations without abrasives, however, showed highest tin retention reaching up to threefold higher values than the corresponding toothpastes with abrasives (without Chitosan: slurry 3.2 ± 0.7 , slurry/brushing: 3.1 ± 0.7 ; with Chitosan: slurry: 7.3 ± 0.8 , slurry/brushing: 8.6 ± 1.9). Though there are effects from amount and type of abrasives, tin retention on eroded enamel surfaces depends mainly on its presence or absence, and is increased by the addition of Chitosan.

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Development of an Erosion/Abrasion in-situ Model and First Efficacy Data of Experimental F/Sn/Chitosan Toothpastes

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There are only few in-situ models with standardised intraoral brushing. In a pilot study simulating the clinical situation, such an in-situ model was developed. In a main study, including a larger group of test persons, the efficacy of an experimental F/Sn/Chitosan toothpaste was investigated in comparison to other fluoride compounds. Both studies conformed to GCP guidelines, using human enamel specimens worn in the lower jaw ($n = 3$ on both vestibular sides each). The enamel specimens were extraorally demineralised (7 days, 0.5% citric acid, pH 2.6; 6×2 min/day) and intraorally exposed to toothpaste slurries (2×2 min/day). Within the treatment time with the slurries, the specimens on one side were intraorally brushed with a powered toothbrush for five seconds (2.5 N). After each demineralisation and slurry exposure, specimens were rinsed with tap water. Substance loss was quantified profilometrically (μm). Pilot study ($n = 10$): Four toothpastes were tested (placebo, two experimental toothpastes

(TP1: AmF/NaF/SnCl₂, TP2: AmF/NaF/SnCl₂ plus Chitosan; both 1,400 ppm F, 3,500 ppm Sn), and a SnF₂-containing gel (3,000 ppm Sn, 1,000 ppm F)). In the main study ($n = 27$) experimental TP2 was tested against placebo and an experimental NaF-toothpaste (TP3, 1,400 ppm F). Pilot study: in the placebo-group, tissue loss was $11.2 \pm 4.6/17.7 \pm 4.7$ (slurry/slurry+brushing respectively); treatment with tin-containing slurries reduced tissue loss (TP1 $3.6 \pm 1.9/12.8 \pm 6.4$, TP2 $2.7 \pm 2.8/9.6 \pm 5.6$, gel $2.0 \pm 1.3/5.4 \pm 5.5$, each $p < 0.05$ compared to placebo). Main study: in the placebo-group, tissue loss was $12.5 \pm 5.9/20.2 \pm 7.8$. After NaF, tissue loss was $9.3 \pm 5.6/15.4 \pm 6.1$ and after TP2 $4.9 \pm 2.9/10.0 \pm 7.4$ (each $p < 0.05$ compared to placebo; TP2 vs. TP3 $p < 0.05$). The study model was able to differentiate effects of active agents and physical impacts and showed excellent reproducibility. F/Sn/Chitosan was superior to NaF.

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Interplay between Salivary Pellicle Proteins and Stannous-Containing Toothpaste on Dental Erosion-Abrasion

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Stannous-containing products have shown an ability to reduce the development of dental erosion; however, their interaction with salivary pellicle proteins is unclear and was therefore investigated. Ninety-six bovine enamel and root dentin specimens ($n = 8$) were submitted to episodes of demineralization (0.3% citric acid, pH 2.6, 5 min, $4 \times$ /day), remineralization (in between treatments) and toothbrushing ($2 \times$ /day), for a total of 5 days. The remineralizing solutions (S1-S5) contained 1.45 mM Ca, 5.4 mM PO₄, 0.1 M Tris buffer and sodium azide (S1), with the addition of 2.2 g/l of Mucin (S2), 2.2 g/l of Casein (S3), 1.1 g/l of Mucin + 1.1 g/l of Casein (S4) or 2.2 g/l of Albumin (S5). Human saliva (HS) was included as reference. Toothbrushing (2 min, 45 stk, 150 g) was performed using slurries of 1,100 ppm F (NaF) silica-based toothpaste suspended in deionized water (1:3 w/w), with (TP+Sn) and without (TP) the addition of 3,500 ppm Sn (SnCl₂). The pH of both slurries was adjusted to 4.5. Surface loss was determined using optical profilometry. Data were analyzed by ANOVA and Fishers LSD tests, at 5% significance level. When associated to TP, HS showed the highest loss, followed by S2. The other protein-containing solutions (S3-S5) did not differ from each other or from S1. Similar results were observed for dentin. In the presence of TP+Sn, significant surface loss reduction was observed for both substrates. HS still showed the highest dental loss, although no differences were found among groups S1-S4. S5 resulted in the lowest enamel loss, not differing only from S2. Similar trend was observed for S5 on dentin, although not as pronounced. **Conclusion:** The stannous containing slurry provided a strong protective effect against dental erosion-abrasion, which seemed to be enhanced by its association to albumin (S5), especially on enamel.

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Effect of Gallium on *Streptococcus mutans* NCTC10449 Biofilm and Bovine Enamel

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Antibacterial and biocompatible gallium doped phosphate-based glass (Ga-PBG) inhibits planktonic growth of caries-associated bacterium *Streptococcus mutans* NCTC10449 and was reported to exert no adverse effect on dental minerals. The aim was to evaluate effect of Ga-PBG on *S. mutans* NCTC10449 biofilm in an in vitro model and assess its effect on bovine enamel. Biofilms were grown in constant depth film fermentor (CDFF) on hydroxyapatite (HA), Ga-PBG and control (C) glass discs using artificial saliva. At 6, 24, 48 and 120 h; discs containing biofilms were removed, serially diluted in PBS and spread on BHI agar plates to assess the viable colony forming units (CFU) of bacteria. The effect of Ga-PBG on bovine enamel was investigated by exposing it in artificial saliva for 14 days and was compared with controls; C-glass and glass free sample. Surface profiling using Proscan 2000 Dental provided details of erosion, abrasion, attrition, surface porosity and surface roughness at pre- (0 day) and post-treatment (14 days). Likewise, mineralisation was assessed using TMR. All the experiments were carried out in triplicates. In the CDFF biofilm study Ga-PBG achieved a statistically significant ($p < 0.049$) growth inhibition of *S. mutans* NCTC10449 compared with both controls; HA and C-glass, with a maximum of 2.11 log CFU reduction at 48 h. Surface profiling and TMR analyses of bovine enamel revealed no statistically significant ($p > 0.05$) changes between samples treated with Ga-PBG and controls; C-glass and glass free sample. Gallium was effective in inhibiting biofilm formation by *S. mutans* NCTC10449 in an invitro model system. Further, Ga-PBG exhibited no adverse effects on the bovine enamel.

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Cariogenic Potential of Soy and Bovine Milk Beverages

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Soy beverages are water extracts of whole soybeans and are often promoted as a healthy alternative to bovine milk. Little analysis has been carried out to determine the effects of soy beverages on oral health, especially their potential cariogenicity. In this study we compared the potential cariogenicity of a range of soy with bovine milk beverages. In vitro acid production by *Streptococcus mutans* was measured in four soy and two milk beverages at a constant pH of 6.5 or 5.5, as was the fall in pH over a ten min period. The buffering capacity and calcium and phosphate concentrations (total and soluble) of the beverages were also determined. The rate of acid production at pH 6.5 by *S. mutans* in the milk beverages was ~ 90 nmol H^+ /mg dry weight cells/min which was five to six times lower than in the soy beverages. At pH 5.5 *S. mutans* acid production rate was ~ 114 nmol H^+ /mg dry weight cells/min in milk which was three to five times lower than in the soy beverages. Whilst the pH fall in the presence of *S. mutans* over ten min was negligible in the milk beverages there was a significant decrease of over 1.1 pH units in all soy beverages. This was also reflected in the lower buffering capacity of the soy beverages. The levels of soluble calcium in the soy beverages were lower than those in the milk beverages although total calcium contents were similar. In conclusion, soy beverages have a higher potential cariogenicity than bovine milk beverages and caution should be exercised in their frequent consumption by individuals at risk of caries.

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Biofilm Age and Thickness Determines the Amount of Red Fluorescence

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Red autofluorescence of dental plaque may indicate the presence of mature plaque. The aim of this study was to determine the effect of time and biofilm thickness on the amount of red fluorescence of in-vitro biofilm. Single donor saliva microcosm biofilms were grown in a constant-depth film fermentor (CDFF) with a constant supply of DMM (Defined Mucin Medium) and eight five minute 10% sucrose pulses per day. The depth of the PTFE (polytetrafluoroethylene) cylindrical holes were set at 100, 200, 500 and 600 μm . The samples were extracted 4, 7, 10, 14 and 17 days after inoculation ($n = 3$). Emission spectra of the biofilms were measured with a fluorescence spectrophotometer (λ_{exc} 405 nm) and photographed with a QLF-D SLR-camera (λ_{exc} 405 nm, Inspektor Research Systems, Amsterdam, The Netherlands). All the obtained spectra were normalized. After 4 days the biofilms (all depths) showed green fluorescence on QLF-photos but with the spectrophotometer also a peak in the red area of the spectrum could be measured. After 7 days and further, the biofilms on the QLF-photos showed red fluorescence. Red fluorescence peak intensities [arbitrary units] varied from 18.1 ± 4.6 at 4 days to 22.3 ± 3.3 at 17 days for 100 μm biofilm and 31.5 ± 12.0 at 4 days to 199.0 ± 75.0 at 17 days for 600 μm biofilm. At all thicknesses the intensity of the red fluorescence increased in time ($r^2 = 0.54$, $p < 0.001$), measured with the spectrophotometer as well as on the QLF-photos. With increasing thickness of the biofilm, a higher intensity red fluorescence was measured ($r^2 = 0.47$, $p < 0.001$, independent of the day of sampling), which was not directly proportional. We conclude that the age and the depth of a biofilm results in a higher intensity of red autofluorescence.

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Antimicrobial Activity of Plant Extracts from Brazilian Pantanal against *Streptococcus mutans* Clinical Isolates

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The antimicrobial activity of plants from Brazilian Pantanal had been recently screened [Brighenti et al.: Caries Res 2010;44:207]. The aim of this study was to evaluate the inhibitory effect of

these extracts against clinical *Streptococcus mutans* isolates. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined against 19 oral isolates of *S. mutans* in comparison to the reference strain (ATCC 35688). The plants used were: (1) *Buchenavia tomentosa*, (2) *Croton doctoris*, (3) *Mascagnia benthamiana*, (4) *Allagoptera leucocalyx* and (5) *Bactris glaucescens*. The extracts were prepared in the following conditions: A-Ethanol 70°, 72 h/25°C; B-Water, 5 min/100°C; C-Water, 1 h/55°C; D-Hexane, 72 h/25°C; E-Ethanol 99.5°, 72 h/25°C. All extracts were prepared using 20 g leaves/400 ml solvent. Ethanol and hexane were evaporated under reduced pressure; aqueous extracts were freeze-dried. The clinical samples were tested at 10^6 cfu/ml using the microdilution method in 96-wells plates. The concentration of the extracts varied from 0.1 mg/ml up to 50 mg/ml. The plates were incubated at 5% CO_2 /37°C for 24 h. Most of MICs were between 0.4 and 3.1 mg/ml; MBC values were 50 mg/ml or higher for most of the samples tested. Moreover, most of the samples (92%) showed MBC values greater or equal to the value found for the reference strain. In conclusion, most of the extracts were also active against clinical isolates. However, a greater concentration of the extract is needed to achieve this antibacterial activity.

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Effect of Sucrose Exposure on gtfB and dexA Expression in *S. mutans* Biofilms

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Bacteria into dental biofilm are subjected to dietary episodes of 'feast and famine' conditions that should be simulated when models of biofilm are developed because the expression of genes related to sucrose metabolism may change. Thus, the aim was to evaluate the effect of sucrose exposure constantly ('feast') or intermittently ('fast') on expression of glucosyltransferases and dextranases of *S. mutans* growing in a biofilm. *S. mutans* UA159 biofilms were formed on saliva-coated bovine enamel slabs in batch culture and they were grown in ultrafiltered tryptone-yeast extract broth at 37°C, 10% CO_2 during 5 days. The biofilms ($n = 4$) were exposed to 1% sucrose constantly or 10% sucrose 8 $\times$ /day and after 105 h of growth they were collected for analysis. The RNA was extracted from biofilms with acid phenol/chloroform, purified and the expression of gtfB, gtfC, gtfD and dexA genes were evaluated by quantitative PCR. The results were normalized related to 16S gene content and also expressed relative to the expression of control group, in which biofilms were grown in culture medium without sucrose exposure. The results showed that dexA gene expression of biofilm exposed to sucrose 8 $\times$ /day was higher than sucrose exposure constantly ($p < 0.05$) but the expressions of gtfB, gtfC and gtfD did not differ ($p > 0.05$). The findings

suggest that the condition of intermittent sugar exposure that occurs daily in the oral cavity should be simulated in vitro when models of biofilm are used to evaluate sucrose metabolism.

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Novel Dental Adhesive Containing Silver and Amorphous Calcium Phosphate Nanoparticles with Antibacterial and Caries-Inhibiting Properties

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Antibacterial bonding agents are promising to inhibit residual bacteria in the cavity preparations and invading bacteria along the margins. The release of calcium (Ca) and phosphate (PO₄) ions was previously shown to remineralize tooth lesions. Our objectives were to incorporate nanoparticles of silver (NAg) and nanoparticles of amorphous calcium phosphate (NACP) into a bonding agent, and to investigate the effects on dentin bond strength and dental plaque microcosm biofilm response. Commercial adhesive and primer were used as control. NAg were incorporated into the primer and adhesive at 0.1% by mass. NACP were mixed into adhesive at 10, 20, 30 and 40%. A microcosm biofilm model was used on composite disks with primer covering the adhesive on the top. Biofilm metabolic activity, colony-forming unit (CFU) and lactic acid production were measured. Bond strengths ranged from 26 to 34 MPa; adding NAg and NACP did not significantly decrease the bond strength ($p > 0.1$). Bonding agents containing NAg and NACP greatly reduced the biofilm viability and metabolic activity, compared to the control. CFU for total microorganisms, total streptococci, and mutans streptococci on bonding agents with NACP and NAg were an order of magnitude less than those of control. Lactic acid production for groups containing NACP and NAg were reduced to 1/4 of that of control. SEM examination showed long and well-filled resin tags in dentinal tubules, as well NACP infiltration in dentinal tubules. In conclusion, microcosm biofilm viability and acid production were greatly reduced on bonding agents containing NAg and NACP nanoparticles for the first time, without compromising dentin bond strength. The incorporation of remineralizing agent NACP and an antibacterial agent NAg may have wide applicability to other dental bonding agents.

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Comparative Study of Microorganisms in Dental Plaque from Caries-Active and Caries-Free Volunteers

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By using the Human Oral Microbe Identification Microarray (HOMIM), we are investigating the metagenomics of microorganisms in the dental biofilms of young adults. The aims of this study were twofold: (i) to determine the predominant bacterial species that are associated with health and dental caries of permanent teeth in young adults; and (ii) to describe the differences in bacterial profiles between different sites on those teeth. Plaque samples were collected from the buccal and lingual sides of the anterior and posterior teeth in 17 caries-free (CF) and 24 caries-active (CA), DMFS >8 , subjects. DNA was extracted from the samples and 16S rRNA genes from the DNA were amplified by PCR. The 16S rRNA were then hybridized with the HOMIM and the resulting data analyzed by dedicated software (MeV_4_8_1). We found 163 taxa in the 41 volunteers. Many taxa had high counts in both CF and CA samples. *Streptococcus anginosus* was more associated with disease and *Kingella oralis* was more associated with health ($p < 0.05$, Wilcoxon test; but not significant after adjustment for multiple variables). Based on presence, the following species were more associated with caries (%CA/%CF subjects): *Campylobacter gracilis* (35/8), *Cardiobacterium hominis* (53/25), *Dialister pneumonia* (41/17), *Leptotrichia hofstadii* (29/8), *S. anginosus* (65/25), *Streptococcus mutans* (18/0), *Veillonella* spp (41/25), *Streptococcus infantis* (29/17) and *Streptococcus mitis* bv2 (24/0). The following species were more associated with health: *Actinomyces gerencseriae* (29/50), *Bergeyella* sp. OT322 (14/33), *Granulicatella adiacens* (12/42), and *Streptococcus* sp. OT070 (17/42). However, there was no statistical significance ($p > 0.05$). Microbial profiling by HOMIM could be used to guide caries prevention, provided the above trends in microbial population attain significance with data from more subjects.

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Yeast Carriage on Chilean Preschool Children with High and Low Risk Caries

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Introduction: During the development of dental caries, species composition is altered in the oral cavity, predominantly fermentative microorganisms, aciduric and acidogenic. It has been

reported that *Candida* spp. are present in subjects with or without caries. However, it is unclear the role of these yeasts in such conditions. This study quantified and characterized the phenotypic and genetic diversity of yeasts present in the saliva of preschool children with and without caries. Method: We examined one hundred and thirty preschool children in the Metropolitan Region. We measured the prevalence caries and obtained saliva samples for microbiological analysis. The saliva was plated onto selective medium and incubated at 30 °C for 48 h. *Candida* CHROMagar was used for presumptive identification of yeast species. In addition, each isolate was identified by API ID 32C and genetic variability was assessed by RAPD-PCR. **Results:** The status of yeast carriage in healthy children was 27.3% and in children with caries of 48% (Fisher's Exact Test $p = 0.019$). The identification of yeasts was performed using API ID 32C and PCR. The species most frequently isolated in both groups were *Candida albicans* and *Candida dubliniensis*. RAPD-PCR analysis detected differences in similarity and genotypic diversity of species in both groups of isolates. **Conclusions:** The carriage of yeast was higher in children with caries than in those without caries. In both groups, the most frequent species were *C. albicans* and *C. dubliniensis*. Furthermore, there is genetic variability and genotypic diversity of yeast in both groups analyzed.

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Acidogenicity of Probiotic Lactobacilli Alone and in Combination with *Streptococcus mutans*

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Probiotics are considered to be able to improve oral health; e.g. by reducing counts of *Streptococcus mutans* (MS) in human saliva. However, data on their role in the caries process are scarce. As a surrogate for lower cariogenicity, a lower acidogenicity compared with caries-associated bacteria has been suggested. Thus, this in vitro study evaluated pH-drops induced by six commercial probiotic lactobacilli alone and in combination with *Streptococcus mutans* (ATCC 25175; MS) during fermentation of sugars and sugar alcohols. *Lactobacillus rhamnosus* GG (1), *Lactobacillus rhamnosus* LC705 (2), *Lactobacillus rhamnosus* LR32 (3), *Lactobacillus casei* LC11 (4), *Lactobacillus casei* 431 (5), *Lactobacillus fermentum* PCC (6) and MS were grown anaerobically overnight, washed twice in PBS and re-suspended in fermentation minimal medium (10^8 CFU/ml). pH-drops were monitored at 5 min intervals up to 30 min after the addition of 100 mM sucrose, glucose, lactose, xylitol, sorbitol or water (control) respectively ($n = 5-6$ /group). In an additional experiment the four probiotics, where pH did not drop below 5.5 after sucrose addition, were mixed with MS (1:1) and analyzed accordingly. Acidogenicity varied significantly between the tested probiotics ($p < 0.05$; ANOVA). pH-

drops were the highest with glucose for all seven single strains and the four probiotic-MS-mixtures ($< \text{pH } 4$) and the least for xylitol ($> \text{pH } 6$), followed by lactose and sorbitol. Although after addition of sucrose probiotics 1-4 showed no remarkable pH-drop, the final pH of their MS-mixtures did not differ significantly from those of pure MS, being lower than 4.5 ($p < 0.05$). It can be concluded that the acidogenicity of the tested probiotics varies considerably. Although probiotics 1-4 show only minor pH drops after addition of sucrose, they do not reduce the acidogenicity of MS under the tested conditions.

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Anticariogenic Properties of *Cymbopogon* Species Essential Oils

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The aim of this study was to evaluate the anticariogenic properties of *Cymbopogon citratus*, *C. flexuosus* and *C. martini* essential oils. Reference strains of *Actinomyces naeslundii* ATCC 19039, *Lactobacillus acidophilus* ATCC 4356, *Streptococcus gordonii* ATCC 10558, *S. mutans* ATCC 35688, *S. sanguinis* ATCC 10556, *S. sobrinus* ATCC 33478 and *S. mitis* ATCC 9811 and clinical LB isolates were also evaluated. Essential oils were obtained commercially and chemically analyzed for the evaluation of their main components. Citral was included in the tests. Preliminary antimicrobial tests were done by agar well diffusion test with concentration of 100 $\mu\text{l/ml}$. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for the reference strains were determined (concentration ranging from 0.20 to 100 $\mu\text{l/ml}$). Chlorhexidine digluconate was included as control. On agar diffusion test, the essential oils produced inhibition zones for all microorganisms tested with diameters ranging from 12.5 up to 23.5 mm. MIC values for the essential oils against reference strains ranged from 0.9 to 6.25 $\mu\text{l/ml}$. *C. citratus* showed the lowest MBC values (ranging from 3.12 and 12.5 $\mu\text{l/ml}$). Citral showed MIC values ranging from 1.2 to 4.6 $\mu\text{l/ml}$, and chlorhexidine 7.8 to 31.2 $\mu\text{l/ml}$. *C. citratus* also showed the lowest MIC and MBC values against lactobacilli clinical isolates. All the essential oils showed bactericidal activity against the tested cariogenic microorganisms.

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Cariogenicity Features of *Candida albicans* Biofilm in the Presence of Sucrose and Glucose

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The aim of this study was to evaluate *C. albicans* biofilm formation, metabolic activity and acidogenicity in the presence of sucrose and glucose. Reference strain and seven isolates from early childhood caries lesions were evaluated. Standardized suspensions containing 107 cells/ml were prepared in YNB supplemented with 10, 50, 100, 200, 500 mmol·l⁻¹ of glucose or 10, 50, 100, 200, 500 mmol·l⁻¹ of sucrose by using spectrophotometry. Biofilms were developed on polystyrene, flat-bottomed 96-well microtiter plates. The growth medium was refreshed daily. After 24, 48 and 72 h of incubation, biofilms were monitored using ATP bioluminescence and tetrazolium (XTT) reduction assays as well as the conventional colony forming unit (cfu) evaluation and pH measurements. All data were analyzed comparing the same concentration of sugar by Mann-Whitney test (5%). Significantly thicker biofilms were obtained in the presence of 50 mmol·l⁻¹ and 500 mmol·l⁻¹ sucrose when compared with the same concentrations of glucose, after 48 or 72 h of incubation ($p = 0.003$, $p = 0.002$). Nevertheless, higher metabolic activity was observed for biofilms formed under the presence of glucose after 24 and 48 h of incubation ($p < 0.02$). Also, the pH values obtained were lower for biofilms obtained in the presence of glucose after 24 and 48 h. Interestingly, when biofilms were formed for 72 h, higher metabolic activity and lower pH were observed in the presence of sucrose ($p = 0.0002$, $p = 0.04$). The results suggest that dietary sugars can modify candidal biofilm formation, metabolic activity and acidogenicity.

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Anticariogenic Properties of Fractions from *Croton doctoris* S. Moore Leaves Extracts

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The antimicrobial activity of *C. doctoris* S. Moore (Euphorbiaceae) crude extracts had been recently found [Brighenti et al.: Caries Res 2010;44:207]. The aim of this study was to find the most active fractions of hydroalcoholic (A) and ethanolic (B) extracts

against cariogenic bacteria. Reference strains of *Actinomyces naeslundii* ATCC 19039, *Lactobacillus acidophilus* ATCC 4356, *Streptococcus gordonii* ATCC 10558, *S. mutans* ATCC 35688, *S. sanguinis* ATCC 10556, *S. sobrinus* ATCC 33478 and *S. mitis* ATCC 9811 were used. The extracts were obtained by maceration of powdered leaves (20 g) in 400 ml of solvent in the following conditions: (A) 70% ethanol, 72 h/25°C; (B) 99.5% ethanol, 72 h/25°C (the powder was previously macerated in hexane 72 h/25°C). The fractions were obtained by liquid-liquid extraction resulting on hexane (HA), dichloromethane (DA), butanol (BA) and aqueous (AA) fractions for extract A; hexane (HB), dichloromethane (DB) and ethanolic (EB) fractions for B. The most active fractions were selected by the agar well diffusion test (concentration 50 mg/ml). Then, these fractions were submitted to the microdilution test (concentrations ranging from 0.1 to 50 mg/ml) to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). On diffusion test, HA, DA and DB showed inhibition zones for all microorganisms tested with diameters ranging from 10 up to 27 mm. MIC values were concentrated between 0.1 and 1.5 mg/ml for HA, 0.1 and 3 mg/ml for DA and DB. DA fractions showed the lowest MBC values (0.8 up to 25 mg/ml; except for *L. acidophilus* where no bactericidal activity was found). In conclusion, the most active fractions were the ones with lower polarity, present at the dichloromethane fractions for both extracts.

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Evaluation of Acidogenicity in Bifidobacteria Exposed to Different Carbohydrates

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Little is known about the influence of environmental factors, in particular dietary components, on bifidobacteria cariogenicity features. The aim of this study was to evaluate the acidogenicity of bifidobacteria exposed to different carbohydrates. *S. mutans* and *L. acidophilus* were included for comparative purposes. *B. dentium* DSM 20436 and *S. mutans* UA 159 were grown in SDMY, and *L. acidophilus* ATCC 4356, *P. denticolens* DMS 10105 and *S. inopinata* DMS 10107 were grown in MRS medium, anaerobically at 37°C. After growth, cells were washed twice and suspensions containing 10⁸ cfu/ml were obtained in McBain medium. Before addition of carbohydrates, or water (negative control) the bacterial suspensions were depleted of endogenous reserves of carbohydrates by incubation for 30 min at 37°C. Next, 10 mM of each carbohydrate (glucose, lactose, raffinose, sucrose) or water were added. Measurements of pH were performed at timepoint 0 and after incubation for 3 h. The experiments were repeated twice. Statistical analysis was carried out using Graphpad Prism 3.0 (Kruskal-Wallis and Dunn's test). The pH drop observed after

exposure to glucose, lactose and sucrose for all bifidobacteria species was similar to that observed for *S. mutans* ($p > 0.05$). For raffinose, *B. dentium* showed a significant higher pH drop when compared to *S. mutans* or *L. acidophilus* ($p < 0.05$). *B. dentium* and *S. inopinata* also showed a higher pH drop after exposure to raffinose when compared to *L. acidophilus* ($p < 0.05$). For glucose, *S. inopinata* showed a lower pH drop than *L. acidophilus* ($p < 0.05$). In conclusion, *B. dentium*, *P. denticolens* and *S. inopinata* could cause a pH drop similar to *S. mutans* and *L. acidophilus* for the majority of the tested microorganisms when exposed to different carbohydrates.

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Biofilm Formation by *Streptococcus mutans* Knockouts

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The ability to grow as biofilms is advantageous for bacterial survival. Functions necessary to this, such as glucosyltransferase production and others, are controlled by global regulator systems, mainly CovR and VicK. These systems also regulate genes encoding proteins with no assigned function and with unknown role in biofilm formation. In this study, we investigated the contribution of five genes, controlled by CovR and/or VicK, in biofilm growth and architecture. For this, single gene nonpolar deletions were performed by PCR ligation mutagenesis in five loci (SMU.609, SMU.1090, SMU.1437c, SMU.2146c, SMU.2147c). Mutants were then compared with parent strain UA159 (WT). Growth curves were carried out in BHI broth (37°C; 10% CO₂). Initial phases of biofilm were evaluated in polystyrene plates containing BHI (with or without 0.1% sucrose w/v) during 2 and 4 h of growth for scanning electronic microscopy (SEM), and 4 h for quantitative RT-PCR analysis. Total biofilm yield was evaluated after 18 h of growth. **Results:** All genes inactivation did not significantly increase or decrease biofilm mass after 18 h incubation. However, SEM of initial biofilms showed minor or major changes in architecture. Notably, absence of SMU.2147c leads to a long chain phenotype, only when grown as biofilm. Lack of SMU.609, SMU.1437c and SMU.2146c diminished microcolony numbers when compared with WT strain. Quantitative PCR in WT initial biofilms (4 h, 0.1% sucrose) showed increased expression of SMU.609, SMU.1090, SMU.2146c, SMU.2147c up to 2-fold when compared to cells growing as planktonic in same media, and up to 4-fold when compared to biofilms formed in absence of sucrose. **Conclusions:** Investigated genes may participate directly or indirectly in *S. mutans* biofilm development by yet unknown mechanisms.

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Understanding Acid Tolerance Response Mechanisms in Caries-Associated Bacteria

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Dental plaque is constituted by many bacteria which produce acids and change the de-mineralization/re-mineralization balance toward net mineral loss, leading to initiation/progression of dental caries. These microorganisms adjust its metabolism and tolerate acid pH. Clinical studies coupled with analysis in microbiology and molecular biology indicate *Streptococcus mutans*, aciduric strains of non-mutans Streptococci, Lactobacilli, Actinomyces and Bifidobacteria may become dominant. Streptococci strains have been extensively studied however little information exists about how other microorganisms caries-associated are able to tolerate acid environments. **Objectives:** The purpose of this study was to understand how members of two families of caries-associated bacteria handle acid tolerance response (ATR). **Methods:** In silico prediction of ATR genes and its regulation was carried out for *Bifidobacterium dentium*, *Bifidobacterium longum*, *Lactobacillus casei* and *Lactobacillus paracasei*, whose genomes are available on the websites of JGI (Joint Genome Institute) and NCBI (National Center for Biotechnology Information). **Results:** Bioinformatic analysis has revealed the presence of putative proteins typically involved in ATR in other microorganisms, such as malate, arginine and histidine transport systems. In addition, a candidate *fur* (Ferric Uptake Regulator) gene, that functions as a global regulator of iron homeostasis and ATR in most microorganisms has also been identified in *Lactobacillus* and *Bifidobacterium* genome and *Fur* regulatory sites have been predicted for a number of gene clusters including ATR related functions. **Conclusions:** The presence of ATR genes in *Lactobacillus* and *Bifidobacterium* genomes indicates that these bacteria are able to mount a comprehensive response to preserve homeostasis in acidic environments. Bioinformatic predictions also suggest that ATR regulation could be under *Fur* control in these microorganisms. Models derived from in silico analysis pave the way to improve hypothesis testing and well directed experimental research.

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Antimicrobial Efficacy of Medical Honey

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Various studies have shown that medical honey has an antibacterial effect on extraoral microorganisms. Aim of the present study was to investigate whether this effect could also be shown

with regard to oral microorganisms. The agar diffusion assay was performed with reference strains of 14 oral microorganisms (including *C. albicans*, *S. mutans*, *L. casei* and *A. odontolyticus*). Into wells in Balmelli agar mixed with the reference strains, 0.3 ml each of medical honey, chlorhexidine 1% and physiological saline were placed. Chlorhexidine and physiological saline served as positive and negative controls. Means and standard deviations of inhibition zones (IZ) were calculated and differences in IZ caused by medical honey or chlorhexidine were analyzed by using the Mann-Whitney Test setting $p < 0.05$ as statistically significant. A distinct antimicrobial efficacy was seen in all samples with medical honey as well as with chlorhexidine. The mean values of IZ were 20.2 mm (SD 3.9) for medical honey and 25.5 mm (SD 4.4) for chlorhexidine and this difference was statistically different ($p = 0.0152$). In all cases IZ caused by chlorhexidine was larger than those generated by medical honey. The IZ produced by medical honey reached values between 69.4 and 95.8% in comparison to those caused by chlorhexidine. It is concluded that medical honey has a distinct antibacterial and antimycotic effect against oral microorganisms. In further investigations the active components of the honey will be determined. Nevertheless, the data available to date, do not justify assessing the negative and positive effects of medical honey in the oral cavity.

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Osteopontin Reduces Biofilm Formation in a Multi-Species Model of Dental Biofilm

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Background/Aims: Combating dental biofilm formation is the most effective means of caries prevention. Among the chemical supplements to mechanical tooth cleaning procedures, non-bactericidal adjuncts that target the mechanisms of bacterial biofilm formation have gained increasing interest in recent years. Milk proteins, such as lactoferrin, have been shown to interfere with bacterial colonization of saliva-coated surfaces. We here study the effect of bovine milk osteopontin (OPN), a highly phosphorylated whey glycoprotein, on a multispecies in vitro model of dental biofilm. While considerable research effort focuses on the interaction of OPN with mammalian cells, no data investigating the influence of OPN on bacterial biofilms exist. **Methodology/Results:** Biofilms consisting of *Streptococcus oralis*, *Actinomyces naeslundii*, *Streptococcus mitis*, *Streptococcus downei* and *Streptococcus sanguinis* were grown in a flow cell system that permitted in situ microscopic analysis. Crystal violet staining showed a significantly lower biofilm formation in the presence of OPN

($OD_{585} = 0.26 \pm 0.06$ SD), as compared to biofilms grown without OPN ($OD_{585} = 1.0 \pm 0.30$ SD; $p < 0.001$) and biofilms grown in the presence of caseinoglycomacropeptide, another phosphorylated milk protein ($OD_{585} = 0.89 \pm 0.27$ SD; $p < 0.001$). Confocal microscopy revealed that OPN accumulated on bacterial surfaces and reduced mechanical stability of the biofilms without affecting cell viability. The bacterial composition of the biofilms, determined by fluorescence in situ hybridization, changed considerably in the presence of OPN. In particular, the relative biovolume of *S. mitis*, the best biofilm former, was reduced significantly from 78 to 14% ($p < 0.001$). **Conclusions:** OPN strongly reduces the amount of biofilm formed in a well-defined laboratory model of dental caries. If a similar effect can be observed in vivo, OPN might serve as a valuable adjunct to mechanical tooth cleaning procedures.

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Acidogenicity of Starch Hydrolisates by Dental Biofilm

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Starch hydrolisates of different dextrose equivalents (DE) are used in food industry, however their cariogenic potential is still controversial. The objective of this research was to study the acidogenicity of starch hydrolisates of distinct DE by dental plaque (biofilm). The study was a crossover, investigator blind and conducted in 6 experimental phases of 4 days each, in which 12 volunteers refrained from brushing their molar teeth and for 3 days rinsed with one of the following treatment solutions 5 times a day: water, glucose, sucrose and starch hydrolisates of DE 5, 20 and 40. The concentrations of the solutions was 20%. On the morning of the 4th day, in fasting condition, the pH of the biofilm was determined before (time zero) and 5, 15, 30 and 60 min after the volunteers rinsed with the respective treatment solution in use for each phase. The pH at each time of measurement and the area under the curve (AUC) of the pH versus time were considered response variables and evaluated by ANOVA followed by Tukey test (5%). The treatments did not statistically differ from each other with relation to the pH at times zero and 60 min. All treatments with carbohydrate resulted in pH drop after 5 min and statistically differed from water (6.9 ± 0.4), without any significant difference among glucose (5.7 ± 0.4), hydrolisates DE 5 (5.8 ± 0.4), DE 20 (5.5 ± 0.4) and DE 40 (5.5 ± 0.4), but sucrose (5.0 ± 0.5) was superior to glucose, hydrolisates DE 5 and DE 20 in relation to pH drop. With regard to AUC, the treatment groups sucrose (187.1 ± 20.6), glucose (200.8 ± 17.5), hydrolisates DE 5 (206.3

± 26.5), DE 20 (200.6 ± 22.2) and DE 40 (199.8 ± 21.7) did not differ from each other, but significantly differed from water (240.6 ± 18.8). The findings suggest that, irrespective of the DE, all starch hydrolysates tested are equally fermented by dental biofilm.

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Effect of pH on Calcium Release from Bacterial Surface Reservoirs

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Anionic groups present in the surface of oral streptococci are calcium ion binding sites, which could be replaced by hydrogen ions during pH drop, releasing Ca to the biofilm fluid. However, the importance of this release to function as a mineral buffer during a pH drop has not been explored. Thus, this in vitro study aimed to evaluate the increase in Ca concentration in biofilm fluid from Ca released from oral streptococci as a result of a pH drop.

Methods: Pellets obtained from cultures of *S. mutans* IB1600 pretreated with Ca solution containing 1 mM Ca (biofilm fluid-like Ca concentration) were exposed for 10 min to PIPES buffer, pH 7.0 (negative control), acetate buffer pH 5.0 (simulating pH drop after cariogenic challenge) or 0.5 M HCl pH 1.5 (positive control), all containing 1 mM Ca, at a proportion of 30% of treatment solution by wet weight of bacterial pellet (to simulate the proportion of fluid found in in vivo biofilm). Bacteria were separated from the test solution by centrifugation and Ca concentration in supernatant was determined using Arsenazo III. Ca concentration (mM, avg $\pm$ SD, $n = 3$) increased in acetate buffer and HCl treatments (2.77 ± 0.06 and 5.52 ± 0.22 , respectively), but not in the pH 7.0 buffer (0.95 ± 0.03). **Conclusion:** Ca bound to the surface of oral streptococci could act as an ion source to biofilm fluid during pH drop induced by a cariogenic challenge.

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Association between Red Fluorescence Emitted by Bacterial Plaque and Caries Activity: An in situ Study

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The aim of this two-phase crossover in situ study was to evaluate the influence of presence of red fluorescence emitted by bacterial plaque on the induction of carious lesions. We used 272

bovine enamel blocks, which were initially evaluated in relation to surface microhardness and green fluorescence ($\Delta F\%$) using the Quantitative light-induced fluorescence (QLF). Seventeen volunteers used intra-oral appliances containing eight specimens covered by a plastic net to promote plaque accumulation. Sucrose solution (20%) was dripped in the experimental group 8 times per day, and distilled water was dripped in the control group. Each phase lasted 14 days, with seven days of wash-out. After 4, 7, 10 and 14 days, bacterial plaque present on the blocks surface was analyzed using the QLF to quantify the red fluorescence ($\Delta R\%$). After careful cleaning, the block was evaluated again with QLF to assess the loss of $\Delta F\%$ and microhardness loss (%). Multilevel analyses were performed for comparisons, as well as multilevel linear regression analyses among the $\Delta R\%$ and other outcomes. Regarding the microhardness, we could observe a trend of hardness loss among the different periods only in the experimental group (mean $\pm$ SD, 4d = 27.3 ± 18.6 ; 7d = 41.4 ± 18.1 ; 10d = 52.5 ± 18.6 ; 14d = 63.8 ± 24.4), significantly higher than in control groups (4d = 4.7 ± 8.9 ; 7d = 6.8 ± 7.2 ; 10d = 10.7 ± 4.4 ; 14d = 13.0 ± 5.3). In relation to the $\Delta R\%$ of plaque, there was a gradual increase according to the different periods in both experimental (4d = 39.1 ± 11.0 ; 7d = 47.3 ± 23.0 ; 10d = 57.2 ± 22.1 ; 14d = 58.2 ± 19.9) and control groups (4d = 33.4 ± 6.6 ; 7d = 39.6 ± 10.7 ; 10d = 41.0 ± 12.9 ; 14d = 42.2 ± 10.9), being significantly higher in the experimental group only after 14 days. There was significant association between red fluorescence measurements and microhardness loss, independently of the groups. In conclusion, the red fluorescence of bacterial plaque is related to the mature plaque, independent of its presumed cariogenicity.

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16S rDNA Analyses of Dental Biofilm Microbiome of Caries Active and Caries Free Children

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Cross-sectional studies have suggested some bacterial species are associated more commonly with carious sites, while others with caries-free surfaces; however presence or absence of aspecific species does not predict future caries. **Objective:** Compare the biofilm, assessed by 16SrDNA, of caries active (C-A) versus caries-free (C-F) children in a subset of children enrolled in a 4 year longitudinal study in rural schools of Puerto Rico. **Methods:** 15 children (10–15 years old) were consented and assented. Children were well characterized by the International Caries Detection and Assessment System (ICDAS) in terms of caries lesions presence or absence (10 C-A and 5 C-F); and stage of severity, progression/regression or arrestment. Dental biofilm samples

were obtained after overnight fasting and brushing abstinence using a sterile toothbrush passed across all tooth surfaces. Dental biofilm was dislodged by agitation for 1' into tubes containing sterile PBS media and immediately stored at -80°C. The V3-V5 regions of the 16S rDNA of dental biofilm samples were sequenced. Subject specific barcode sequences were inserted into the 5'end of otherwise identical universal rDNA primers using the Roche 454 sequencing platform. High quality sequence reads (n = 589,935) (between 32,875 and 45,977, ave = 39,329 per sample) for each subject were generated. **Results:** The flora was variable across subjects; however 27 genera were present in at least

93% of subjects, with only 11 at greater than 1% of reads. *Streptococcus* reads were dominant (48% of all reads) followed by *Porphyromonas* and *Veillonella* (12.6% reads and 11.2% reads respectively). Streptococcus reads were clearly over-represented in C-A subjects, while Fusobacterial reads were over-represented in C-F subjects. When *Streptococcus* reads was atypically low (~10%), *Haemophilus* reads were often elevated. These data facilitate our ability to identify the most relevant phylogenetic groups to focus more intensive examination.

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Curricular-Perspective Analysis of Opportunities and Problematics around the New-Caries Paradigm in Colombian Dental Schools

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The Global Alliance-for-a-Cavity-Free-Future Colombian Chapter has embraced since 2010 to achieve by 2015 a national-wide cariology-teaching consensus on the new-carries paradigm as a disease continuum, with a series of stages from early to cavi-tated lesions. The aim of this study was to analyse the opportuni-ties and problematic situations around dental education in the new-carries paradigm from the curriculum perspective in the Co-lombian Dental Schools Association (ACFO). Two teachers from each school ($n = 23$) were invited to participate: the responsible of most-carries teaching and the representative of curricular design ($n = 46$). Discussion groups were conducted to analyse within the curriculum, aspects that can facilitate, or on the contrary limit, the new-carries paradigm incorporation process. These were com-plemented with a caries-management-guideline questionnaire answered by each school. **Results:** 44 teachers of 22 schools par-ticipated. Within the opportunity category, a relevant trend was found related with the presence of cariology as a transversal axis of the curriculum. In the problematic situations category, four trends were observed: (1) Cariology teaching continues being linked to the risk approach, without embracing the social dimen-sion of the pathology; (2) In the theoretical aspects related with cariology, there is an approximation to the new paradigm, but in the clinical practice the traditional pathology conception and the restorative emphasis predominate; (3) The current evidence of cariology is related to the adoption of the ICDAS criteria; and (4) There is a predominant caries-new paradigm management with-in the paediatric dentists. **Conclusion:** Caries cirricula delivered

in University dental schools in Colombia are fragmented and re-quire the incorporation of the new caries paradigm.

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Detection of Caries Lesions by Electrical Bioimpedance

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The purpose of this study was to investigate the in vitro ap-plication of a method based on bioelectrical impedance spectroscopy response in current to voltage step (BIS-VI) to the tissues of high impedance to detect changes in enamel and occlusal caries lesions which are difficult to diagnose. Initial studies have inves-tigated the potential of the method, set a protocol of data acqui-sition and establish the most appropriate electrical circuit that characterizes the experimental setup. Initial results indicate that there was a decrease in the resistance and increased capacitance proportional to the decrease of the mechanical thickness of the enamel and the presence and depth of the cavity. The most ap-propriate electrical circuit consists of a resistor (RS) in series with two parallel elements: a resistor (RP) and a constant phase ele-ment (CPE). From the theoretical estimate of the current response and its experimental counterpart, one can estimate, using a multi parametric optimization method, the four evaluation parameters of an electric model: RS and RP (resistive parameters) and C0 and α (CPE parameters).

We used 135 signals collected from 58 extracted healthy teeth (H) or with enamel caries (EC) or hidden cavities in dentin (CD).

Bioimpedance measurements were made at all points suspected of caries and these results were compared to the assessments of 6 calibrated experts volunteers. The teeth were investigated histologically to confirm their caries status by a single examiner. It was possible to find statistically significant differences ($p < 0.05$) between groups H and EC parameters for RS and between groups H and CD parameters for RS, Rp and α . The ROC (Receiver Operating Curve) was adequate only for the resistance and α (area under the ROC curve > 0.7) with better results to the RS (accuracy = 73.2, sensitivity = 77.9 and specificity = 60.7). The detection of lesions by bioimpedance was better than performed by volunteers. It was concluded that the method BIS-VI can be used as a method of detection of caries lesions of difficult diagnosis and represents an effective alternative to traditional clinical methods.

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Time of ICDAS-Caries Detection and Activity Assessment between Caries-Risk Groups in Young Children

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Eleven Latin American countries are jointly conducting an epidemiological study on caries using ICDAS criteria on young low SES children. The aim of this study was to relate on 1–5 years old Colombian children, the time of ICDAS-caries assessment with the caries risk status. Eight trained in-the-ICDAS-system examiners (Inter/intra-reproducibility-Kappa values: 0.70–0.77 and 0.60–0.85, respectively) examined 592 1 to 5-yr. olds (1-year: $n = 31$; 2-years: $n = 96$; 3-years: $n = 155$; 4-years: $n = 209$; 5-years: $n = 101$) in four schools in Bogotá. **Examinations Included:** caries risk assessment by means of the Cariogram program including 8/10 factors, caries visual assessment by means of the 'A'-ICDAS detection criteria (merging scores 1 and 2) and lesions ICDAS-activity assessment [Ekstrand et al: Oper Dent 2007;32:225–235]. Trained students assessed plaque presence on children and brushed their teeth, while caries-risk information was gathered from their parents. Then ICDAS-visual-caries and activity assessment were conducted using portable-dental chairs, head-lights, mirrors and WHO probes. Students timed the caries-examination process using chronometers. **Results:** Prevalence of caries experience (dICDAS4–6mf-s) was 85%, increasing to 96%, when including early lesions (dICDAS-1/2–3). Mean caries-experience data were 5.0 ± 7.7 (dICDAS4–6mf-s) and 9.7 ± 9.9 (d-s = 7.8 ± 8.4 ; f-s = 1.5 ± 2.2 ; e-s = 0.5 ± 2.4) (dICDAS-1/2–3). The caries risk was distributed as low (34.8%), moderate (35.6%), and high-very high (29.6%). The mean examination time was 217.0 ± 134.0

seconds, distributed according to caries risk as: low: 192.3 ± 122.1 , moderate: 222.9 ± 144.7 , and high to very high: 238.8 ± 130.0 , showing a linear correlation (ANOVA test; P-value: 0.01).

Conclusion: This study shows it is feasible for epidemiology studies and the clinical practice using these criteria in low-caries-risk groups as the process takes around 3 min/patient, while in high-caries-risk groups it takes around only 4 min.

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Two-Year Survival Rates and Secondary Caries of Amalgam and ART Restorations in Primary Molars

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The null hypothesis tested was that there is no difference between the survival rates and the occurrence of secondary caries of amalgam and atraumatic restorative treatment (ART) restorations in primary molars over two years. A controlled clinical trial using a parallel group design was carried out in six public schools from a deprived area of Brazil with 284 children aged 6–7-years. Two treatment groups were formed: conventional restorative treatment (CRT) as control group and ART. A total of 364 single- and multiple-surfaces amalgam restorations were placed in 126 children from two schools, while 386 single- and multiple-surfaces ART restorations were placed in 158 children from four schools. Restorations were placed by three pedodontists and were evaluated after 0.5, 1 and 2 years, according to the ART restoration criteria. The presence of secondary caries, defined as a dentine carious cavity alongside the restoration, was recorded. The survival analysis was conducted using the Proportional Hazard Rate Regression Model with frailty correction. Baseline mean dmft scores for children who received amalgam and ART restorations were 5.82 ± 3.18 and 5.72 ± 2.63 , respectively ($p = 0.78$). Cumulative survival rates for all amalgam (77.3%) and ART (73.5%) restorations in primary molars over two years were not significantly different ($p = 0.60$). Secondary caries was responsible for 36% and 38% of failures in amalgam and ART restorations, respectively ($p = 0.14$), and were predominantly associated with multiple-surfaces restorations: 100% and 94% for amalgam and ART restorations, respectively. In conclusion, amalgam and ART restorations in primary molars presented similar two-year survival rates, and no difference between the occurrence of secondary caries along the two types of restorations after 2 years was detected.

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Survival of Cavitated Primary Teeth Treated by Three Treatment Protocols

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Cavitated primary posterior teeth were treated according to conventional treatment using amalgam (CRT), Atraumatic Restorative Treatment using high-viscosity glass-ionomer (ART) and an ultra-conservative treatment protocol, in which small cavities were restored with ART and medium/large cavities regularly cleaned with toothpaste/toothbrush under supervision (UCT). The hypothesis tested was that the survival percentages of teeth treated according to CRT and ART were significantly higher than that of UCT. Teeth extracted because of toothache, sepsis or pulp exposure were failures. The (PHREG) test was used to estimate the survival curves. A total of 302, 6–7-year old Brazilian children were treated. The number of treated teeth were 341 (CRT), 244 (ART) and 281 (109 small ART, 166 open cavities and 6 combinations) for the UCT group. The number of teeth extracted was 17 for CRT, 12 for ART and 24 for UCT over the two years interval period. There was an age effect ($p < 0.0001$) but no gender ($p = 0.71$) and no d3mft effect ($p = 0.75$) observed among the three treatment protocol groups at baseline. After 2 years, the cumulative survival rate and SE of teeth treated with CRT was $94.0 \pm 1.6\%$, that with ART $94.5 \pm 1.6\%$ and that with UCT $90.1 \pm 1.9\%$. The PHREG test showed no statistically significant differences in cumulative survival rates for all treated teeth ($p = 0.13$) among the three treatment protocol groups. Over the two years interval period, PHREG test did not show an effect of age ($p = 0.08$), gender ($p = 0.63$), operator ($p = 0.19$) and d3mft ($p = 0.71$) for the three treatment protocols. In terms of tooth survival, the protocol consisting of restoring small cavities using ART and cleaning medium/large cavities with toothpaste/toothbrush under supervision was no different than restoring primary posterior teeth according to either the CRT or ART protocol.

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Handheld Mobile Devices for Radiographic Diagnosis of Approximal Caries

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Aim: Dental imaging is commonly used to diagnose caries, as well as plan treatment. Digital images can be reviewed on almost any computing platform and with more powerful devices and

higher band width, the use of mobile devices for examine dental radiographs seem possible. Consequently, our aim is to compare the radiographic diagnostic efficacy of handheld mobile devices for approximal caries. **Materials and Methods:** Fifty dental radiographs of approximal surfaces were examined on a conventional view box by a radiologist who diagnosed each surface in sound, enamel lesion or dentinal lesion. This diagnosis was considered as the true diagnosis. Then, we digitalized the radiographs with a Canon S90 digital camera in jpg files (compression level 6) and a resolution of $1,280 \times 960$ pixels (px). The pictures were sent to three handheld mobile devices: a Blackberry Bold (BB) 9700 (resolution 480×360 px), Ipad Touch (IT) (480×320 px) and Sony Ericsson T715A (SE) (240×320 px). Then, three last year students, three dentist and two radiologist scored every image in each device. Receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic performance of each device. **Results:** The areas under the ROC curves ranged from .552 (IC95% 0.490–0.615) for the IT, 0.555 (IC95% 0.491–0.620) for the SE to 0.623 (IC95% 0.554–0.692) for the BB. A statistically significant differences were found between SE in comparison with the BB-IT devices for detecting approximal caries. **Conclusion:** Radiographic caries diagnosis using handheld mobile devices maybe possible but improvements are still necessary.

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Validity of Visual and Visual-Tactile Detection of Proximal Cavitations in Primary Molars in vitro

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The aim of this in vitro study was to evaluate if gentle probing with a cow-horn-ended explorer (EXD3CH; Hu-Friedy) improves the ability to detect proximal cavitations. Extracted primary molars with proximal caries lesions were assessed using ICDAS. Each tooth was placed adjacent to another primary molar to mimic natural proximal contacts. Gingiva was simulated with soft silicone. Lesions extending radiographically up to the enamel-dentin-junction (EDJ) and into outer third of dentin (D1) were selected (ICDAS-codes: 2 $n = 34$, 3 $n = 8$, and 5 $n = 4$; cavitation prevalence: EDJ: 12% and D1: 35%). After reading the according radiographs, three trained and calibrated examiners (two experienced clinicians and one post graduate student) independently assessed proximal surface integrity (cavitation: yes/no) on dental simulation units in two settings [two weeks in-between: without (WOR) and with rubber dam (WR)]. Each examination was repeated after one week to determine intra-observer reliability. In

both settings surface integrity was at first assessed visually alone (visual) and subsequently in combination with probing, using a thin cow-horn-ended explorer (visual-tactile). In setting WR examiners were allowed to use flattened plastic wedges to push down the 'gingiva'. Sensitivity/specificity ranges for visual assessment were 0.08–0.58/0.94–1.00 in setting WOR and 0.25–0.67/0.88–0.94 in setting WR. According values for visual-tactile assessment were 0.67–0.83/0.74–0.91 (WOR) and 0.75–0.92/0.77–0.94 (WR). Youden's indices were significantly higher for visual-tactile assessment compared with visual assessment alone ($p < 0.05$; repeated measures ANOVA) while the setting (WOR/WR) had no significant influence ($p > 0.05$). Intra-examiner reliability (kappa) ranges were for WR: visual = 0.17–0.73; visual-tactile = 0.50–0.59 and for WOR: visual = 0.30–0.76; visual-tactile = 0.56–0.73. Within the limitations of an in vitro study it can be concluded that the use of a cow-horn-ended explorer improves the ability to detect proximal cavitations.

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Determination of Interfacial Gaps at Composite Restorations by Optical Coherence Tomography

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Objectives: To evaluate the ability of optical coherence tomography (OCT) to assess tooth-composite interfacial gaps. **Methods:** (A) 8 extracted human molars with standardized, box-shaped class-V cavities into dentin (4 × 3 mm, depth 1.5 mm, beveled enamel) were filled with composite (Grandio, Voco GmbH) without the use of an adhesive to produce circular gaps. In each tooth 3 regions of interest (ROI) were marked and imaged by Swept-Source-OCT (OCS1300SS, 1320nm, Thorlabs Inc.). OCT signals of the gaps were measured (length-%; ImageJ). The ROI were evaluated histologically. (B) Two additional groups of class-V-cavities (n = 8 each) were restored either with an experimental self-adhesive flowable (EF; DMG) or with Prompt L-Pop/Filtek Supreme XT Flowable (PLP; 3M ESPE). The length of the OCT signals were measured separately on 10 mesiodistal B-scans per tooth for enamel- and dentin-composite interfaces. Data were statistically analysed (U-test, adjusted $\alpha = 0.0125$). Additionally, these results were compared with those of a parallel microleakage study (Diegmann et al., unpublished). **Results:** A) Using OCT, 81% of gap length was detected at the enamel interface, 91% at the dentin interface. B) At the enamel interface, EF showed significantly fewer gaps than PLP (4 vs. 48%; $p < 0.0005$), at the dentin interface significantly more (75 vs. 11%; $p < 0.0005$). Microleakage indicated smaller value for EF at the enamel interface (9 vs. 82%; $p < 0.0005$) in contrast to dentin: 87 and 58% ($p = 0.129$). **Conclusions:** OCT and microleakage analysis were concurrent in detecting and quantifying interfacial gaps. OCT could be a diag-

nostic tool in the assessment of interfacial discrepancies that might be an indication of the development of carious lesions adjacent to restorations.

Thorlabs GmbH, Dachau, Germany provided the OCT equipment.

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ICDAS-II Implementation Study: Its Challenges in a Clinical Context in Japan

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The purpose of this study was to identify possible challenges upon integration of ICDAS-II into clinical procedures. The survey was conducted in collaboration with five regional dental associations. Participants were requested to fill out the questionnaires, before the workshop on their current caries treatment and after the workshop on the ICDAS-II integrated caries treatment and management. Participation was voluntary, and of 1092 dental clinics in these five regions, 117 (10.7%) clinics participated. One clinician took charge of four workshops, and another one workshop. The workshop was 60 min long, and an exposition of ICDAS-II and actual examples of ICDAS-II integration were presented. From these questionnaires, we learnt that 7 (6.0%) clinics 'Almost always take bitewing photos of patients between 5 and 20 years-old', and 27 (23.1%) 'Sometimes'; 14 (12.0%) 'Always consider drilling when there is radiolucency of any size in enamel'; and 'Consider drilling when there is wide radiolucency in enamel' 35 (29.9%). On preventive efficacy of brushing teeth with fluoride toothpaste twice a day over the long term, 6 (5.1%) answered 'Almost none' and 49 (41.9%) 'About 10%'. 64 (54.7%) answered, 'Would like to integrate ICDAS-II into daily clinical practice', but along with concerns; 66 (56.4%) 'ICDAS-II integrated diagnosis and treatment, to take effect, has to be covered by universal health insurance'; and 60 (51.3%) 'Staff training is necessary'. Further, 53 (45.3%) replied, 'Provision of health insurance points to ICDAS-II integrated clinical practice would lead to reduction of 'drill and fill' treatment.' There are problems with the current caries treatment procedures and invasive treatment oriented insurance system in Japan, but many clinicians appreciate and are willing to learn and integrate ICDAS-II into caries management in daily practice.

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

Impact of Different Treatment Protocols for Cavitated Dentine Lesions on Brazilian Children's Quality of Life

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To investigate the effect of three treatment protocols for cavitated dentine lesions on the oral health-related quality of life (OHRQoL) of Brazilian schoolchildren, cavitated dentine lesions of 302 children aged 6–7-year-old were treated according to different protocols (Conventional Restorative Treatment, Atraumatic Restorative Treatment, and Ultra-conservative Treatment). Children's parents completed the Brazilian version of the Early Childhood Oral Health Impact Scale (B-ECOHIS) at baseline and 1 year after the treatments. Only questionnaires that were completed by the same person at baseline and year 1 were analysed ($n = 161$). The outcome variable was the difference in mean scores of responses obtained at year 1 and baseline. Paired t-test was used to test for B-ECOHIS differences for child and parent sections, for individual child and parent domains and for all domains. ANOVA analyses were used to test the effect of the treatments on B-ECOHIS scores after 1 year. Results showed no statistically significant difference in B-ECOHIS scores over one year period for all domains ($p = 0.4$), and for child ($p = 0.218$) and parent ($p = 0.955$) sections. A statistically significant difference was found for the domains 'child symptoms' ($p = 0.024$) and 'child psychology' ($p = 0.018$). The magnitude of the mean difference was -0.24 (child symptoms) and -0.21 (child psychology). The treatment protocols did not influence the changes in B-ECOHIS scores significantly. For all dimensions ANOVA showed $p > 0.05$. In conclusion, the treatment protocols did not improve ORHQoL in general, but they were effective in reducing child's pain experiences (child symptom) and reducing their problems in sleeping and being irritable or frustrated (child psychology) after one year.

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Visual Detection of Caries Lesions on Oral White Light and Fluorescence Photographs

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A new fluorescence camera for the assessment of caries and plaque has been introduced, allowing the almost simultaneous capture of clinical white light and fluorescence photographs. The

aim of this study was to compare the visual assessment of white light (WL) clinical and fluorescence (F) photographs of the oral cavity for presence and extent of caries lesions. **Methods:** Subjects were recruited among the first year dental students of ACTA. Subjects were allowed to participate after informed consent. Series of WL- and F-photographs were taken of each subject with the QLF-D SLR-camera (Inspektor Research Systems, Amsterdam) from buccal and occlusal aspects of all teeth up to the first permanent molars in upper and lower jaw, as well as the lingual surfaces of the incisors in the lower jaw. WL-photographs and F-photographs were assessed independently and scored according to the International Caries Detection and Assessment System (ICDAS) with scores 5 and 6 collapsed and matching scores for F-images. Data were described by crosstabs and level of agreement between WL- and F-photographs was assessed by Spearman rank correlation. **Results:** A total of 822 surfaces in 20 subjects were assessed. Caries scores on WL- and F-photographs correlated significantly (all surfaces Spearman $\rho = 0.90$; buccal 0.89; lingual 0.93; occlusal 0.89, $p < 0.001$). For WL the ICDAS scores 0, 1, 2, 3, 4, 5 were found with the following frequencies 656, 70, 66, 12, 11, 7 and for F frequencies were 627, 95, 70, 11, 13, 6. **Conclusion:** Caries detection on F-photographs with the QLF-D SLR-camera has good agreement with caries detection on WL-photographs, but more early caries lesions (scores 1 and 2) are scored than on WL-photographs.

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Association of Red Fluorescence and Caries Activity Lesions in Primary Molars

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This study evaluated the association of red fluorescence emitted by occlusal caries lesions assessed by Quantitative Light-induced Fluorescence (QLF) and their activity status. Occlusal surfaces of 214 primary molars in 39 children aged 5 to 9 years-old were evaluated by QLF through the red fluorescence tool (ΔR) and by other tools: green fluorescence by QLF (ΔQ) and DIAGNOdent pen (DDpen). We also assessed the red fluorescence emitted by bacterial plaque on the lesions, prior to the cleaning procedures. For the evaluation of caries lesions status, two examiners independently assessed the surfaces by visual inspection using the ICDAS and additional lesion activity assessment criteria (weighted kappa values of 0.930 and 0.839 for ICDAS and activity assessment, respectively). Disagreements were solved by consensus. The analyses were performed separated for different ICDAS scores, considering active and inactive caries lesions. Comparisons for the different parameters were performed using multilevel analysis ($p < 0.05$). There was no difference between ΔR of the inactive and active caries lesions in any scores (mean $\pm$ SD; score 1 inactive = $19.4 \pm 15.4\%$ and active = $19.1 \pm 10.6\%$; score 2 inactive = $26.1 \pm 11.5\%$ and active = $32.3 \pm 18.1\%$; score 3 inactive = 30.1

$\pm 8.2\%$ and active = $35.4 \pm 9.7\%$; $p > 0.05$). Regarding the ΔQ , no difference was observed either (score 1 inactive = $13.3 \pm 17.0\%$. mm² and active = $3.5 \pm 7.1\%$. mm²; score 2 inactive = $38.1 \pm 33.8\%$. mm² and active = $26.5 \pm 32.5\%$. mm²; score 3 inactive = $128.0 \pm 149.6\%$. mm² and active = $90.0 \pm 175.6\%$. mm²; $p > 0.05$), as well as considering the red fluorescence emitted by the plaque. DDpen readings, however, exhibited statistically significant difference between inactive and active caries lesions, but only for lesions score 3 (score 1 inactive = 15.0 ± 15.0 and active = 8.3 ± 8.9 ; score 2 inactive = 34.9 ± 23.2 and active = 24.7 ± 22.6 ; $p > 0.05$; score 3 inactive = 29.1 ± 23.4 and active = 56.4 ± 35.6 ; $p < 0.05$). In conclusion, red fluorescence and other fluorescence parameters are not accurate tools to evaluate activity status of occlusal caries lesions.

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Estimating Pre-Test Probability of Non-Evident Approximal and Occlusal Caries Lesions in Primary Molars

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We aimed to estimate the pre-test probability of non-evident caries lesions in approximal and occlusal surfaces of primary molars. The participants were randomly selected from a pool of enrolment forms of children (4 to 12 years-old) who had sought dental treatment at our school. We were unaware of children's oral conditions. Then, we selected primary molars only with non-apparent caries lesions. First, two examiners independently evaluated 1,213 approximal surfaces in 126 children for the presence of cavities, through direct visual inspection after temporary separation with orthodontic rubbers for 7 days. Regarding the occlusal surfaces, 407 primary molars in 68 children were evaluated by two examiners using visual inspection and radiographic method. Then, presence of dentine lesions was verified after operative treatment in teeth tested as positive by visual and/or radiographic methods. The pre-test probabilities and 95% confidence intervals (95%CI) were calculated. Higher risk groups for non-evident caries lesions were identified using multilevel analyses, and pre-test probabilities were also calculated for these groups. The pre-test probability of non-evident cavitated approximal lesions was 4.20% (95%CI = 2.63 to 5.78%). For occlusal surfaces, the pre-test probability of non-evident dentine lesion was 5.16% (95%CI = 2.45 to 7.87%). The risk groups for approximal surfaces and their respective pre-test probabilities were: distal surfaces of first molars (pre-test probability = 7.84%; 95%CI = 4.62 to 11.07%), children with dmfs+DMF-S>5 (8.16%; 95%CI = 4.35 to 11.96%) and children older than 8 years (10.26%; 2.96 to 17.56%). For occlusal surfaces, the risk groups were second molars (pre-test probability = 9.36%; 95% CI = 4.46 to 14.26%) and children with dmfs+DMF-S>3 (12.71%; 95%CI = 5.47 to 19.95%). In conclusion, the pre-test probability of non-evident approximal and occlusal caries lesions of primary molars in children seeking dental treatment is low, even considering the risk groups.

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Optical Coherence Tomography to Assess Carious Lesions without Cavitation

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Objective: Display and assessment of carious lesions with intact surfaces using optical coherence tomography (OCT). **Methods:** 13 extracted human molars with approximal lesions grade 2 (ICDAS) were selected visually. A 'region of interest' (ROI) was marked and the lesion was displayed with swept-source OCT (3D-images; OCS 1,300 SS, 1,320 nm, Thorlabs Inc., USA). OCT signals of enamel, dentin and specific structures of the lesion were validated by light microscopy and categorized according to the lesion extent (score 1–4): 1 – first half of enamel, 2 – second half of enamel to the dentino-enamel junction (DEJ), 3 – into dentin, 4 – 3 with gap formation at the DEJ. The OCT image stacks were assessed in the bucco-oral (512 B-scans) and occlusal-cervical (296 B-scans) direction and the lesions were categorized accordingly. **Results:** Comparing histology and OCT 69% (9/13) of the lesions were classified the same. Three lesions were rated lower and one lesion was scored higher with OCT. Histologically caries-free areas under the enamel surface partly showed OCT signals, whereas in deeper parts of enamel OCT and histology were consistent. In 8 lesions a gap formation was visible histologically at the DEJ, which was confirmed with OCT in 6 cases (75%). Surface layer and variably mineralized zones in the lesion could be discriminated. Enamel cracks and dentin gaps were detected, sometimes even if they had been primarily inconspicuous in light microscopy. Structures generating strong signals partly masked deeper regions. **Conclusion:** In the assessment of carious smooth surface lesions without cavitation OCT provides additional information about extension and destruction, which can complement visual and potentially radiographic inspection. The sensitivity of the method can be increased by optimizing the signal evaluation.

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Detection of Secondary Caries on Approximal Surfaces of Amalgam Restorations

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The aim of this in vitro study was to evaluate the performance of visual examination (ICDAS), bitewing radiography (BW) and DIAGNOdent 2190 (LFpen) in detecting caries around amalgam restorations on approximal surfaces of extracted permanent teeth. Approximal surfaces (n = 136) of permanent posterior teeth

(n = 110) with Class II amalgam restorations were assessed twice by two experienced examiners using ICDAS, BW and LFpen, independently. Additionally, the occurrence of proximal overhangs and their possible association to secondary caries was also evaluated. The teeth were histologically prepared and assessed for caries extension after restoration removal. Intra- and inter-examiner reproducibility showed moderate to good agreement for all methods (WK = 0.40 to 0.87). The cut-off limits used for LFpen were based on a previous study [Lussi et al.: Caries Res 2006;40:97–103]. Sensitivity, specificity, accuracy and area under the ROC curve (Az) were calculated at D1 (enamel and dentine) and D3 (dentine) thresholds for each method and compared by the McNemar test ($p < 0.05$). The specificities at D1 and D3 were, respectively, 0.41 and 0.82 for ICDAS, 0.70 and 0.82 for BW, and 0.77 and 0.88 for LFpen. The sensitivities were 0.80 and 0.52 for ICDAS, 0.56 and 0.51 for BW, and 0.23 and 0.02 for LFpen at D1 and D3, respectively. At D1/D3 thresholds, the accuracy and Az values were similar and statistically higher for ICDAS (0.65/0.68 and 0.633/0.688) and BW (0.64/0.68 and 0.655/0.719), respectively, while LFpen presented lower accuracy (0.44/0.49) and Az (0.390/0.345) values. Overhang occurrence (26.8%) was not significantly related to the presence of secondary caries. It can be concluded that ICDAS and BW presented the best performance in detecting secondary caries at the D1 and D3 levels on approximal surfaces of amalgam restorations.

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Performance of Fluorescence-Based Devices in Detecting and Quantifying Smooth-Surface Caries Lesions in Primary Teeth

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The aim of this in vitro study was to evaluate the performance of two laser fluorescence devices (DIAGNOdent – LF e DIAGNOdent pen – LFpen) and a fluorescence camera (VistaProof – VP) in detecting and quantifying smooth-surface caries lesions in primary teeth. Two examiners evaluated 99 smooth surfaces in 65 extracted primary molars (approximal surfaces). Surfaces with cavitated lesions were excluded. Thus, we selected only sound surfaces or those with non-cavitated lesions. Mean of three measurements on each site was recorded. After the assessments, sections from the sites were evaluated by polarised light microscope and stereomicroscope. Lesions were classified according their relative depth in sound (D0), initial (D1) and advanced (D2) enamel lesions and dentine lesions (D3). Lesion depth was also measured in μm using the microscope images. Inter- and intraexaminer reproducibility was calculated using intraclass correlation coefficient (ICC). Pearson correlation analyses were performed among

the methods values obtained with the different devices and lesion depth obtained by microscopy. Sensitivity, specificity and accuracy were calculated for D2 and D3 thresholds, and comparisons among the methods were performed using McNemar test. All methods presented high ICC values for inter-examiner (LF = 0.687; LFpen = 0.828; VP = 0.873), and intraexaminer reproducibility (LF = 0.708; LFpen = 0.895; VP = 0.856). There was moderate correlation between the fluorescence-based devices and lesion depth (LF = 0.673; LFpen = 0.646; VP = 0.663). Sensitivities were similar among the devices at D2 threshold; however, the LFpen presented a significant superiority (0.84) than other methods (LF = 0.60 and VP = 0.52) at D3 threshold. VP exhibited significant higher specificity (0.97) than LF (0.80) and LFpen (0.80). In conclusion, although the fluorescence-based devices present good reliability, the correlation of the readings with the lesion depth is only moderate in the assessment of smooth-surface caries lesions of primary molars.

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Proximal Caries Lesions in Primary Molars Assessed Visually, Radiographically and Histologically

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Aim: Comparison of visual and radiographic scoring of proximal caries lesions in primary molars in relation to histological assessment. **Material and Methods:** 62 extracted primary molars with minor proximal lesions were mounted pairwise in casts simulating the clinical situation. All teeth were visually examined twice by two examiners according to UniViSS criteria (Kühnisch et al. 2010). Digital bitewing radiographs from each block were scored using the D0-D4 criteria. Sections 80–100 μm thick were assessed histologically in polarized light at 60 fold magnification. Analogous to the criteria used to assess lesions on radiographs, a 0–4 scale was used. Score 0 defines no enamel demineralization, 1–2 enamel lesion and 3–4 dentine lesion. Data was statistically analysed using BiAS9.05 and SPSS 19. **Results:** Comparing visual and radiographic examination with histological results 54.8 and 67.7% of the lesions were underestimated, 35.5 and 30.7% coincided, 9.7 and 1.6% were overestimated. At threshold D1 (caries detection) and D3 (dentine caries), highest specificity was observed for visual examination (1.00) and highest sensitivity for bitewings (D1: 1.00; D3: 0.96). Similar values of accuracy were calculated for visual and radiographic examination at D1 threshold (0.63 vs. 0.61) but higher values at D3 threshold for bitewings (0.69) than for visual examination (0.53). Receiver-Operating-Characteristics (ROC) revealed higher AUC for visual (0.88) than for radiographic caries detection (0.81), but higher AUC for radiographic (0.76) than

for visual dentine caries detection (0.60). **Conclusion:** Although both visual and radiographic examination showed the tendency to underestimate the depth of carious lesions, bitewing radiographs are an important diagnostic tool for early detection of proximal caries lesions in primary molars.

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Influence of Dental Plaque on Caries Detection and Activity Assessment Using Visual Scoring Indices

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We aimed to verify if the presence of dental plaque on occlusal surfaces influence on performance of visual indices (Nyvad (Ny) and ICDAS+LAA). The time spent and the child's discomfort were also assessed. Thirty-five children were selected and occlusal surfaces of 167 primary molars were evaluated by 4 examiners in different situations: Ny without cleaning, Ny after cleaning, ICDAS+LAA without cleaning, ICDAS+LAA after cleaning. The order of examinations was randomized. A reference examiner evaluated around 50% of the sample (15 children; 77 teeth) for concurrent validation regarding severity, cavitation and activity. ROC analyses were performed; sensitivity and specificity were calculated and compared by McNemar test. Duration of exams and discomfort were compared by analysis of variance and Friedman test. The sensitivity for enamel caries detection decreased, for both indices, when plaque was not removed (Ny: 0.86; ICDAS+LAA: 0.89, $p = 0.03$). ICDAS was more sensitive for detecting cavities than Ny with previous cleaning (ICDAS = 0.81; Ny: 0.71; $p = 0.01$) or without it (ICDAS: 0.95; Ny: 0.71; $p = 0.01$), but the plaque did not interfere in ICDAS+LAA sensitivity ($p = 0.25$). Regarding activity, ICDAS+LAA after cleaning presented higher sensitivity (0.97) than Ny without cleaning (0.73). Nevertheless, ICDAS+LAA specificity was higher for examination performed without plaque removal (without cleaning: 0.86; with cleaning: 0.54). No differences were observed regarding discomfort ($p = 0.12$). Exams with previous cleaning (Ny: 475 ± 37 s; ICDAS+LAA: 489 ± 34 s) spent more time than others (Ny: 326.6 ± 21.4 s; ICDAS+LAA: 403 ± 24 s; $p < 0.05$), but no difference was observed between indices when the cleaning was included ($p = 1.00$). Plaque interferes mainly on detection of initial caries on occlusal surfaces of primary teeth, but, when plaque is removed, it is important to take care specially with false positives when assessing caries activity using the ICDAS+LAA. Despite spending more time, previous tooth cleaning is well accepted by children.

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Occlusal Caries Lesion Detection and Association with Treatment Decision in Deciduous Teeth

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The aim of this in vitro study was to valuate the performance of visual (ICDAS-II) and radiographic examinations for occlusal caries detection and their correlations with treatment decision (TD). Deciduous teeth ($n = 77$) with occlusal surfaces varying from sound to cavitated were selected. Occlusal surface photographs (10x) were made and a site identified. Standardized bite-wing (BW) radiographs were taken. Dentists with at least 5 years experience ($n = 3$) analyzed all samples twice (one-week interval), giving scores for ICDAS-II (0–6), BW (1 = sound; 2 = caries restricted to enamel; 3 = caries in outer third dentin; 4 = caries in inner third dentin) and TD (1 = no treatment; 2 = sealant; 3 = microabrasion and sealant; 4 = round bur sealant; 5 = resin; 6 = amalgam). Validation was given by histological analysis observation under a light microscope with lesions classified on a five-point scale. Intra- and inter-examiner repeatability was assessed using weighted Cohen's kappa values (IC 95%). Comparisons between percentage correct, Specificity, Sensitivity and Accuracy were performed using McNemar test ($p < 0.05$) and area under the ROC curve were performed using a nonparametric test ($p < 0.05$). Kappa values for intra-examiner repeatability indicated good agreement for each examiner ranging from 0.66 to 0.79 and moderate agreement among examiners ranging from 0.50 to 0.58. At D3 threshold, the area under the ROC curve was significantly higher for ICDAS-II ($p < 0.0001$). The values of Accuracy, Sensitivity and Sensibility were similar at D3 threshold and statistically higher for ICDAS-II at D1 threshold. The TD score distribution showed that ICDAS-II and BW were available to the examiners when making the TDs. The visual examination showed better performance than radiographic examination for occlusal caries detection. However, both of them showed good correlation with treatment decision.

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How Strong is the Association between Clinical Parameters and Indices for Assessing Caries Lesion Activity?

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We evaluated the strength of association between visual indices (Nyvad (Ny) and ICDAS-II + LAA) and clinical parameters related with caries activity on occlusal surfaces of primary molars (plaque stagnation, staining, opacity, presence of cavities, depth and texture of the surface). 49 children (300 occlusal surfaces) were examined by three examiners: one using Ny, another using ICDAS-II + LAA and a reference examiner evaluating clinical parameters above. The association between the conditions assessed by indices and each parameter was analyzed by univariate and multiple logistic regression, and Odds Ratio (OR; 95% CI) was calculated. Other outcome was the divergence between two indices in assessing caries activity. When sound sites were analyzed in conjunction with inactive caries, lesions depth showed the strongest association to active caries for ICDAS-II + LAA (OR: 14.5; 4.2–49.8). Texture and pigmentation are similarly associated using Ny (OR: 5.0; 1.8–14.1 e OR: 4.2; 1.8–9.6, respectively). When sound sites were excluded from analyses, the strongest association for ICDAS-II + LAA was between active caries and cavitation (OR: 5.7; 2.5–13.0). For Ny, texture and pigmentation (OR: 4.0; 1.4–11.5 and OR: 3.8; 1.6–8.9) were associated with activity, as was depth (OR: 4.5; 2.4–9.1). Rough enamel and softened dentine were positively associated with divergence between indices in differentiating sound or inactive lesions from active ones (OR: 4.7; 2.1–10.3). For distinguishing active from inactive lesions, most divergences occurred when lesions were not opaque (OR: 0.5; 0.2–0.9). In conclusion, although the indices consider similar parameters for activity assessment, the weight of each clinical parameter is probably different for each index and this fact should be considered in clinical practice.

Suitability of Air-Abrasion in the Preparation of Enamel before Sealing: An in-vitro Study

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Objectives: In-vitro studies were conducted to assess the interest of air-abrasion to prepare fissures before sealing. Air-abrasion (alone or associated with acid etching) was compared with classical methods (diamond bur and acid etching). **Methods:** 90 sealed third molars were submitted to a method of dye infiltration. The depth of the fissure, the extent of microleakage and the resin penetration were measured with a digital-image analyser. Multivariate logistic regression models revealed the determinants of both microleakage and sealant penetration. SEM observations were conducted on 24 molars (6 for each method of preparation). In each group, 2 teeth were not sealed to provide direct observation of the prepared enamel surface. The sealant was applied on the other 4 teeth, 2 teeth completely demineralized (5N HCl) to observe the back of the sealant and 2 teeth partially demineralized to observe the enamel-sealant interface. Wettability tests were conducted on 60 flat enamel surfaces. Drops of distilled water were deposited on the treated surfaces. Contact angles were measured using a goniometer and duration of the spreading out was registered. **Results:** Sealants placed after air-abrasion alone displayed greater microleakage (73.3%) ($p < 0.0001$). They also showed the highest mean of dye infiltration in mm ($p < 0.05$). The main determinant of microleakage was the absence of acid etching (OR = 12.63; [7.35–21.70] IC95%) whereas the resin penetration was influenced by the fissure anatomy (OR = 117; [24.6–559.72] IC95%) and air-abrasion (OR = 3.08; [1.34–7.09] IC95%). SEM observations revealed smooth surfaces and rounded cavo-surface margins. Acid etching reduced the contact angle and the duration of the water drop spreading out ($p < 0.0001$). **Conclusion:** Air-abrasion preparation does not eliminate the need for etching the enamel before sealing. Yet, it provides soft preparation that enhances the penetration of the resin in the fissures.

Session 4 Epidemiology

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Caries Prevalence in Asthmatic Children in Four Geographical Regions in Cairo

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Link between dental caries and long-time exposure to anti-asthma medications has biological credibility. The objective of this study was to compare the caries experience among children diagnosed and being treated for asthma with that of non-asthmatic children. 400 children (200 with and 200 without asthma), 2–17 years old, were examined in El Shorouk City, Dar El Salam, El Nahdah and 10th of Ramadan regions of Cairo in Egypt. Inclusion criteria for the asthmatic children were, medically diagnosed asthma, current treatment for asthma, and asthma medication use for a minimum of one year. The control group are age-matched with the asthmatics but without any of the inclusion criteria. Caries status was determined by number of decayed, missing, and filled surfaces in permanent (DMFS) and deciduous (dfs) dentition through clinical examination by calibrated examiners, using International Caries Detection and Assessment System II (ICDAS-II) scoring criteria. For statistical analysis, the children were grouped into 4 age subgroups, 2–5, 6–9, 10–13 and 14–17 years. Mann-Whitney-U test was used to compare the D12MFS and d12fs of children with and without asthma ($\alpha = 0.05$) in each of the four age subgroups. The D12MFS and d12fs (D1 = non-cavitated caries, D2 = cavitated) were combined for children with mixed dentition. When the asthmatic and nonasthmatic children were compared, caries prevalence was significantly ($p < 0.001$) higher in asthmatic children (11.26 ± 5.42) than in their non-asthmatic counterparts (7.22 ± 4.34). Similar comparison in the 4 subgroups showed similar trend of significantly ($p < 0.001$) higher caries experience among the asthmatic children except for 14–17 years group: 2–5 (8.5 ± 3.5 vs. 4.6 ± 2.6), 6–9 (13.1 ± 4.5 vs. 7.0 ± 3.6), 10–13 (12.7 ± 5.7 vs. 10.5 ± 4.2) and 14–17 (7.1 ± 4.9 vs. 10.2 ± 4.2). The present study highlighted that treatment for asthma may place a child at high risk of developing caries.

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Erosion Risk Status of Urban and Peri-Urban Dwellers in Egypt

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This study investigated the dental erosion risk status of children in four regions of Cairo in Egypt. 500 children, age 2–14 years [250 (5–6 years), 250 (10–14 years)], were recruited from El-Shorouk (high Socioeconomic status), 10th of Ramadan (moderate SES), El-Nahdah (low SES) and Dar El-Salam (low SES) regions. Presence of erosion was determined by calibrated examiner using Basic Erosive Wear Examination (BEWE) index; no erosion (0), initial loss of surface texture (1), hard tissue loss $< 50\%$ of the surface area (2), hard tissue loss $> 50\%$ of the surface area (3). Erosion risk status was determined as recommended by BEWE i.e. the dentition was divided into sextant, and the highest score in each sextant is recorded, and the cumulative score matched to risk levels: Not-at-risk (≤ 2), low (3–8), moderate (9–13), and high (≥ 14). Dietary assessment questionnaires investigating dietary habits relating to consumption of acidic beverages and foods were used. Overall erosion prevalence was 31.2%, with 74.6% of children not at risk of erosion, while 8.8, 8.6 and 8% are at low, moderate and high risk respectively. Among 5–6 years old, 65.2% are not at risk, while 9.2, 11.2 and 14.4% are at low, moderate and high risk respectively. Among 10–14 years old, 84, 8.4, 6 and 1.6% are at no-risk, low, moderate and high risk respectively. In overall, 9.4 and 85.7% of children at low and high SES respectively, are at risk of erosion. Low intake of milk/water and high intake of citrus foods, juices and fruits are significantly (Chi-squared, $p < 0.001$) associated with erosion. In conclusion, among the Egyptian children examined in this study, erosion prevalence is 31.2%, and those at high SES are more at risk of dental erosion.

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Association of Caries Experience in 12-Year-Olds with Independent Variables in Areas with Low Caries Prevalence

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Aim: To assess the caries prevalence of 12-year-olds using ICDAS-II criteria and to investigate the influence of various independent variables on the findings. **Methods:** The study was approved by the ethics committee and informed consent was given by the parents and the participating children. The study was conducted in two regions in Hesse (Germany). In region 1 (Marburg-Biedenkopf) children receive regular school based prophylaxis (use of Duraphat 2× year from 1.-6. grade). In Region 2 (Vogelsbergkreis) there is no use of fluoride varnish in schools. 270 children were examined in each region using ICDAS-II criteria. Information about different factors influencing the outcome variable caries experience was collected using structured questionnaires. DF-S values were calculated at different ICDAS-II cut-off points. To compare the mean caries scores of the subgroups, non parametric tests were performed. Variables associated with caries were included in a binary stepwise backward logistic regression analysis. **Results:** Mean DF-S values were: Region 1: D1-6FS = 1.61; D1 + 2FS = 1.5; D3-6FS = 0.84; D4-6FS = 0.79, D5 + 6FS = 0.74. Region 2: D1-6FS = 2.8; D1 + 2FS = 2.3; D3-6FS = 1.1; D4-6FS = 0.84, D5 + 6FS = 0.72. At D1-6FS and D1 + 2FS level the differences between the regions were statistically significant ($p = 0.005$ and $p = 0.01$, respectively). Regression analysis identified the following variables as significant factors for prevention of caries at various stages of caries: use of fluoridated toothpaste, fissure sealants and ethnic origin. **Conclusion:** In a population with low caries prevalence significant differences between subgroups could only be found when initial lesions were included. Different factors were identified which significantly influence the prevention of dental caries either at the stage of enamel lesions or dentine caries.

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Face and Content Validation of the Caries Assessment Spectrum and Treatment (CAST) Index

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The aim of this study was to validate the CAST index [Frencken et al: Int Dent J 2011;61:117-123] for face and content. This hierarchical epidemiological index consists of ten codes that cover the spectrum of carious lesions in enamel and dentine, and that of sealants and restorations. Using the RAND e-Delphi modified consensus method, statements related to the content and description of the CAST codes, and those regarding its suitability for use internationally were scored on a scale of 1-9 by 15 senior epidemiologists from 15 different countries. Agreement of 75% or higher was required to reach consensus on a statement. After three rounds of assessing modifications to the text, consensus for face and content was reached by all panel members. After this initial validation, 41 epidemiologists from 25 different countries were requested to assess the validated statements as a feedback exercise and for testing external validity. Some minor changes to the statements' content and description were suggested. The CAST index, containing the altered statements, was then resubmitted to the original 15 epidemiologists for final judgment. Consensus was reached by all these epidemiologists on all ten codes: 1 code by 80%, 2 codes by 100% and the remaining codes by more than 86% agreement. In conclusion, the CAST index was validated for face and content. The participating epidemiologists found the Rand e-Delphi modified consensus method a good instrument for obtaining consensus.

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Evaluation of Oral Health Status of Workers: 7 Years of Follow Up by SESC-DF

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The objective of this study was to describe the oral health status of employees of a company determined using SESC-DF mobile dental units for 7 years. All workers examined during this period were included in the study. The oral examination was performed by four dentists trained and calibrated, seeking to identify the

number of teeth with caries in cavitated stage, lost or restored. These data were computed and the mean DMF-T index (WHO) determined, and the average of each component. All patients received dental treatment. A total of 472 patients were examined during this period. Only 25 (5.3%) subjects had DMF-T = 0. The mean DMF-T index was 11.97 (± 1.97). Furthermore, the decay component was 4.52 (± 0.84). From the above, it was concluded that few workers had excellent oral health. Thus, the program that provides preventive and restorative dental care should be strongly recommended for employees of companies.

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Scale of Oral Health Outcomes for 5-Year-Old Children (SOHO-5): Semantic Equivalence of the Brazilian Version

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The European instrument Scale of Oral Health Outcomes for 5-year-old children (SOHO-5) was developed to assess self-reported oral health-related quality of life (OHRQoL) for young children. Its use in Brazil requires prior cultural adaptation, and semantic equivalence is one step in this process. The objective of this study was to evaluate the semantic equivalence between the SOHO-5 and its Brazilian version. The methodology included eight steps: translation of the SOHO-5 into Portuguese, done by two independently translators; unification of the two versions by the Experts Team in São Paulo (SP); a first pilot test, in which the first version was tested in a group of 20 children of 5 to 6 years old and their respective parents; two back-translations done independently by two other translators; unification of the two back-translated versions by the Experts Team in SP; review of the back-translated version by authors of the original version in UK (both versions, original and the latter one, were very similar); a second pilot test in a group of 20 children of 5 to 6 years old and their respective parents, other than the first pre-test; and after completion of all steps the final version of the SOHO-5 was obtained. The use of translations and back-translations carefully evaluated by experts and incorporating suggestions from the target population allowed the development of a Brazilian version of the SOHO-5 that is semantically equivalent to the original instrument.

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The Natural History of Caries

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Aim: To explore whether time (tooth present in mouth) is a determinant of caries experience. **Background:** No studies have investigated the association of age and caries experience through the life-course (birth to age 38) **Methods:** Prospective cohort study of a complete birth cohort (N = 1037) born in 1972/73 in Dunedin, New Zealand. Dental examinations were conducted at ages 5, 9, 15, 18, 26, 32 and 38. Surface-level caries data were collected at each age (WHO basic methods). Only those teeth which had been lost because of caries are included in estimations of tooth loss due to caries and in the 'M' component of DMF scores. Statistical analyses and graphing of data were undertaken using Intercooled Stata Version 10. **Results:** Data are presented on dental caries experience in the permanent dentition at ages 5, 9, 15, 18, 26, 32, and 38. Percentile curves are charted and reported for caries experience at the mouth level. Data are also presented on the number of decayed teeth and tooth surfaces, (including root surfaces at age 38), as a function of the number of teeth and surfaces present (respectively). At the population level, the number of tooth surfaces affected by dental caries increased by approximately 0.8 surfaces per year, while the percentage of tooth surfaces at risk affected by caries increased by approximately 0.5% per year, with negligible variation in the rate of increase throughout the observation period. **Conclusion:** There is a strong positive association between age and caries experience. Time since eruption is a predictor of caries. The caries rate through life is relatively constant.

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Tooth Decay Experience and Oral Health-Related Quality of Life in Adults – A Hierarchical Approach

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The objective of this study was to evaluate the impact of tooth decay experience on oral health-related quality of life (OHRQoL) in adults. **Methods:** Cross-sectional household probability sample of 248, 20–64 year-old residents in Piracicaba-SP, Brazil. OHRQoL was measured using the OHIP-14 questionnaire. Socio-economic, demographic and dental services use data were collected. The household oral examinations followed the WHO criteria for caries (DMFT) and periodontal disease (CPI). An ordinal scale for tooth loss, based on tooth position and number of miss-

ing teeth, was created. The total OHIP severity score was the outcome for negative binomial regression and OHIP prevalence was the outcome for logistic regression. A hierarchical approach was adopted according to a validated conceptual model. **Results:** Mean OHIP score was 10.90 (± 10.56) with 46.4% ($n = 115$) reporting one or more impacts fairly/very often. Mean DMFT was 18.02 (± 8.89) and only 3.6% ($n = 9$) presented DMF = 0. Mean decayed teeth was 1.04 (± 2.07 ; range 0–13) and 36.7% ($n = 91$) had untreated caries. The final models for OHIP prevalence and severity showed that decayed teeth had a negative impact on quality of life (Prevalence Rate Ratio 3.46, 95% CI 1.90–6.29 and PPR 1.09, 95% CI 1.01–1.15, respectively). In both models, those who had lost up to 12 teeth, including 1+ anterior teeth, those who had lost 13–31 teeth, and the edentulous had a greater impact on OHRQoL compared with fully dentate adults. Dental care utilization due to dental pain or treatment needs, using dental insurance and personal income were also associated with OHRQoL. **Conclusion:** Our findings confirm that having untreated caries continue to negatively impact on the oral health quality of life of adults, adjusting for tooth loss, dental care and income.

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Dental Status of 6 and 12 Year Old Children in Mayotte Island

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A cross sectional epidemiological study was undertaken in Mayotte island (a new French overseas department with 204 000 inhabitants) in 2009 in order to determine the caries index for 6 and 12 year-old children and assess the need for dental care. Three hundred 6 year-old children and three hundred 12 year-old children were randomly selected (stratification variable: region and school) from the public schools pupils. They were examined in the sick room of the schools by two observers with a mirror, a probe, a transilluminating light and a frontal lamp. The observers were previously trained and calibrated by examining 6 children with senior examiners. The DMFT according to WHO and DMFS, dft and dfs were recorded as well as the hygiene index (OHI-S). Data were analyzed with EPI-INFO v 3.5.1. For 6 year olds, the mean dft was 3.94 ($dt = 3.87$; $ft = 0.07$) and the mean dfs was 8.95. For 12 year old, average DMFT was 3.74 ($D = 3.63$; $M = 0.05$; $F0.06$), and DMFS was 5.67. Only 24.7% of 6 year-old children and 15.3% of the 12 year-old children were caries free in mixed dentition. In both age classes, only 2% of decayed teeth had been treated. Compared to a study held in Rennes (French city with 207 000 inhabitants) in 2007, 6 year old Mahorian had about twice as many decayed teeth ($dfs = 1.58$ and $dfs = 3.48$ in Rennes). In Rennes, 10 year old children had DMFT = 1.13 and DMFS = 1.62; approximately 3 times less than 12 year old Mahorians. By the age of 6, 55.5% of the children were caries free, 40.1% at 10 years old: more

than twice the level in Mayotte. In Rennes, 25% of the caries had been treated for the 6 years children and 48% for the 10 years children. Dental status of children in Mayotte is significantly poorer than in metropolitan France, preventive measures have to be applied in Mayotte.

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Impact of Dental Caries Treatment and Intraoral Distribution on Quality of Life of Brazilian Schoolchildren

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Objective: To assess the impact of dental caries treatment and intraoral distribution on oral health-related quality of life (OHRQoL) of 12-year-old South Brazilian schoolchildren. **Methods:** This cross-sectional survey used a multistage probability sampling strategy to select a representative sample of schoolchildren from Porto Alegre, southern Brazil. Data was collected from September 2009 to December 2010, and 1,528 of 1,837 eligible schoolchildren attending public and private schools participated, yielding a response rate of 83.17%. OHRQoL was assessed by a self-reported 16-item Child Perception Questionnaire (CPQ11–14). After tooth cleaning and drying, clinical examination was conducted to assess dental caries experience (DMFT) and malocclusion (Dental Aesthetic Index). Parents/legal guardians answered questions on socioeconomic status. Survey Poisson regression models were used to estimate the effect of caries regarding treatment status (treated/untreated) and intraoral distribution (anterior/posterior) on overall and domain-specific CPQ11–14 scores (oral symptoms, functional limitation, emotional wellbeing and social wellbeing domains). Rate ratios and their respective 95% confidence interval were calculated. Estimates were controlled for gender, socioeconomic status and malocclusion. **Results:** Compared to schoolchildren without treatment needs, individuals with treated caries presented an improved OHRQoL (overall CPQ11–14, adjusted RR = 0.89, 95%CI = 0.84–0.96; functional limitations, adjusted RR = 0.85, 95%CI = 0.74–0.98) whereas those with untreated caries presented a poorer OHRQoL (oral symptoms, adjusted RR = 1.05, 95%CI = 1.01–1.09; emotional wellbeing, adjusted RR = 1.09, 95%CI = 1.01–1.18). Individuals with caries in anterior teeth experienced greater negative impact on oral symptoms (adjusted RR = 1.11, 95%CI = 1.05–1.17) and social wellbeing (adjusted RR = 1.27; 95%CI = 1.13–1.43) domains than students without treatment needs. **Conclusions:** Treated caries positively impacts OHRQoL; untreated caries and caries affecting anterior teeth negatively impacts OHRQoL of 12-year-old Brazilian schoolchildren.

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Proportion of 5-Year-Old Children Having Simultaneously Enamel and Dentinal Carious Lesions

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Most of International School Children Program assesses patient's risk of developing caries based on dentinal clinical sign. There is lack of epidemiological data showing correlation of carious lesion depth experience on same individuals. **Study Aim:** The aim of this study was to assess the correlation between enamel and dentinal carious lesion by individual and report the prevalence at population level in high caries risk territories. **Method:** We have been granted permission from three Quebec community centres to approach parents of 5-year-old children attending to provincial school children program. A total of 3 135 participants were examined for caries experiences and their parents completed a caries-prevention questionnaire. The defs (number of decayed, extracted [due to caries] and filled tooth surfaces) and its d1-4 variation (Burt, 1999) were used by 27 trained and calibrated dental hygienists for caries evaluation and measured using portable equipment. Enamel and dentinal carious lesions were summarized on each individual and then, analyzed for all sample. **Main Results:** Mean age of children involved was 5.6 years (± 0.4). The d1-4efs was in average, 10.4 surfaces (± 6.8) per child and 79% of children had caries experience. Weighted mean kappa values for inter-examiner lesion measurements were 0.89 and for intra-examiner reproducibility were 0.82. A total of 983 (31%) children had enamel lesions and 610 (19%) had dentinal lesions. Seventy percent of the time that a child had at least, one dentinal lesion, he/she also had at least, one enamel lesion. Twenty-two percent of the time that a child had at least, one enamel lesion, he/she also had dentinal lesions. **Conclusions:** This study suggests that at individual level, by capturing dentinal lesions, we may also capture the majority of initial lesions.

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Association between Overweight/Obesity and Dental Caries among Southern Brazilian Schoolchildren

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Objective: To evaluate the association between overweight/obesity and dental caries among 12-year-old Southern Brazilian schoolchildren. **Methods:** A cross-sectional study was performed

in Porto Alegre using a multistage probability sampling strategy. A sample of 1,331 schoolchildren was considered necessary to estimate a prevalence of 60%, with a precision level of $\pm 3\%$, 95% confidence interval, and a design effect of 1.3. A non-response error of 40% was added, resulting in a final sample size of 1,837. Clinical examination was conducted after tooth cleaning and drying, and decayed, missing or filled teeth (DMFT) were registered. Anthropometric measures (height and weight) were collected and schoolchildren were classified as normal weight, overweight or obese according to WHO categories for BMI-for-age Z-scores. Data on gender, socioeconomic status and oral hygiene habits were gathered using a questionnaire. Survey Poisson regression models were used to evaluate the association between weight status and dental caries. Prevalence ratios (PR, outcome – caries prevalence) and rate ratios (RR, outcome – caries extension/DMFT) were calculated. Estimates were adjusted for gender, socioeconomic status and brushing frequency. **Results:** 1,528 of 1,837 eligible schoolchildren were examined in public and private schools, yielding a response rate of 83.17%. The prevalence of overweight and obesity were 21.92 and 13.55%, respectively. Caries experience was observed in 55.23% (95%CI = 45.26–65.19) of the sample. Schoolchildren presented, on average, 1.39 (95%CI = 1.07–1.71) decayed, missing or filled teeth. No significant differences in caries experience or extent were observed among weight status groups. After adjusting for important co-factors, weight status was not associated with caries prevalence (overweight: PR = 0.99, 95%CI = 0.85–1.15; obese: PR = 1.00, 95%CI = 0.82–1.22) or caries extent (overweight: RR = 0.91, 95%CI = 0.67–1.22; obese: RR = 0.86, 95%CI = 0.66–1.11). **Conclusions:** This study found no association between dental caries and weight status among Brazilian schoolchildren.

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Occlusal Caries Activity in Permanent Molars as Function of Eruptive Stage and Biofilm Accumulation

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Objective: To estimate the independent effects of biofilm accumulation and eruptive stage on the occurrence of active caries lesions on occlusal surfaces of permanent molars. **Methods:** This study used a cross-sectional design. The sample consisted of 298 schoolchildren aged from 6 to 15 years, attending at a public school in Santa Maria, Southern Brazil. Schoolchildren were examined by a calibrated examiner at a dental unit, using artificial light, a dental mirror and a WHO probe. The occurrence of visible biofilm on occlusal surfaces and the eruptive stage of each permanent molar were recorded. After professional prophylaxis

and air drying, the occlusal surfaces were classified as sound, caries-inactive or caries-active. To evaluate the association of eruptive stage and biofilm accumulation with active caries lesions, a logistic regression model was used (sound and caries-inactive surfaces were combined in the same category to create a binary outcome). Since data were clustered, odds ratios were obtained using generalized estimating equations with a logistic link function. **Results:** Of the 298 sampled schoolchildren, 1,779 permanent molars were examined. All eruptive stages were associated with active caries lesions. Molars with the occlusal surface partially exposed to oral cavity were 85.5 times as likely as molars in full occlusion to have active caries lesions, adjusted for biofilm accumulation (95%CI = 28.9–252.6). Easily detectable biofilm were associated with active caries. Teeth with heavy biofilm accumulation were 15.8 times as likely as those without visible biofilm to present active caries lesions adjusted for eruptive stage (95%CI = 7.5–33.4). No association between active caries and hardly detectable biofilm was found in this population. **Conclusion:** The present study found that eruptive stage of permanent molars is strongly associated with active caries lesions, irrespective of biofilm accumulation.

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Caries Trends in Permanent First Molar Teeth Related to Surface and Age

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The aim of this study was to show the caries trends in permanent first molar teeth (P1M) in relation to surface and age. Of interest to this study was approximately 800 financially weak patients within the age range of 5–60 years were subject to a clinical examination at the Cooperative University of Colombia. The following age-groups within the total sample were selected: 5–7 (n = 47), 10–12 (n = 52), 15–17 (n = 45) 20–22 (n = 53), and 25–27 (n = 44) year-olds, giving a total of 241 patients. From the patients' records the D3MF-S status of P1M was investigated. The number of extracted P1Ms, was limited in all groups. At the surface level, 40% in maxillary and 52% in mandibular of the occlusal surfaces were decayed or filled in the age group 5–7; increasing to 69 and 75% in age group 10–12, to 99 and 95% within age group 15–17. In the age group 20–22 more than 83% of P1M maxillary and mandibular were decayed or filled. Other risk surfaces were palatal in maxillary and buccal in mandibular P1Ms. Less than 15% of the remaining surfaces were decayed or filled up to the age of 15–17. Between the ages 25–27, 25 and 34% of the mesial and distal surfaces on maxillary P1M were decayed or filled, similar number for mandibular teeth were observed. To conclude, the examined sample had a significant treatment need. Caries develops rapidly on occlusal and palatal surfaces in maxillary molar teeth

and on occlusal and buccal surfaces on mandibular teeth. By age 10 up to 12 years nearly 100% of P1M teeth were either decayed or restored on the occlusal surface.

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ECC Prevalence and Dental Treatment Attendance – One Year Study

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Aim: Determination of ECC prevalence in young unprivileged children, based in prevention and oral health education. **Method:** A prospective longitudinal study of dental caries was conducted along 2010 and 2011, with an initial sample of 150 children aged 24–36 months old, in the city of Mesquita, RJ. From the group, 116 children were finally selected due to some mothers' have similar classification of unprivileged social-economic characteristic. All children received an appointment card with 10 consultations scheduled for the year 2010. Mothers filled in a questionnaire at the first visit to the dentist, then being selected for the study those who stated their babies had sucrose ingestion higher than or equal to 3 times a day and up to 1 teeth brushing a day. There was painful oral symptomatology with mothers in the last year, and social-economic variables were similar for all mothers (kind of dwelling, family income and parents' or responsible's years of school education) Young children's caries were recorded according to WHO's criteria at baseline and after one year follow-up. All consultation proceeded to prophylaxis and, when needed, application of fluoride varnish to babies' teeth, with their mothers given oral health instructions. After one year the children were divided in two groups according to frequency to dentistry consultation. G1 mothers (n = 58) were given oral health instructions and their babies came often to the program. G2 mothers (n = 58) did not attend regular to the appointments and did not give a reason for that. Oral evaluation of the children and questionnaire filling were done in two moments: at baseline and after 12 months. **Results:** data were tabulated and statistically analyzed by Student's t Test (p = 0,001). Children from G1 attended 10 consultations a year had dental caries increment of 0.4 (± 1.34), while G2 showed 3.2 (± 2.18). The frequency of sucrose ingestion decreased 68% in G1 and there were differences in oral health habits, the presence of biofilm was 78% higher in G2 after 1 year. Initial dmf in G1 and G2 was 0.7 and final dmf was 0.9 and 2.7 respectively. **Conclusion:** Frequency of attendance dental appointment, focus in prevention and oral health instructions, are able to prevent ECC even under unprivileged population.

Dietary Based Caries Risk in the Belgian Population: Results of the First National Oral Health Survey

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Between 2008 and 2010, the first national oral health survey was held in Belgium. Its aim was to assess the oral health of a representative sample of the total Belgian population (age ≥ 5). It consisted of a health interview using a self-administered questionnaire and an oral examination, performed at home by a team of calibrated dentists using a portable headlight, mirror and probe. Moreover, the uptake of oral health care was evaluated by using social security records (Intermutualist agency). A total of 3,011 persons could be examined and from these, 2,162 complete data sets were available for dentate subjects. Dietary habits were assessed using a questionnaire reporting kind of foodstuff and drinks used as well as frequency and intake with or between meals. A caries risk profile was calculated based on the cariogenicity of foodstuffs and drinks and the reported frequency. The data showed that only 11 out of 2,162 persons were considered having no caries risk, 621 had a low, 134 a median and 1,396 a high caries risk profile. There was no clear association between dietary based caries risk and median DMFT (no risk:17.6, low risk: 7.4, median risk: 7.1, high risk: 8.3). Persons with no caries risk were less regular attenders (11%) in contrast to persons having a higher risk (low: 54%, median: 57%, high: 57%). Persons with no caries risk showed a lower use of restorative services than in the higher risk groups over the past 5 years (83% no filling versus 35 to 40%, respectively). Although cariogenic dietary habits were widespread, difference between a low or high risk showed no association with caries experience and a weak association with the use of oral health care.

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Caries Index and Oral Health Status of Students from Provence Alpes Cote D'Azur Region (France)

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Objectives: A report submitted to the French National Assembly in 2006 (L. Wauquiez) revealed a worrying situation for students in France. Their social coverage may be faulty and dental cares are often sacrificed. The aim of this study was to verify the alteration of the oral health status of students, especially as far as their caries index is concerned. **Methods:** Data were collected through a peer-led intervention in which our dental students (Dental School of University of Nice-Sophia Antipolis) were involved for three consecutive years from 2009 to 2011. Part of this intervention was composed of clinical screenings of dental caries and oral health status (free clinical examinations conducted on university campuses). DMFT score, presence of dental plaque or calculus and other dental care needs were recorded. **Results:** 496 students aged 21.64 ± 4.06 years were examined (males 224, females 272). 37.1% of students had never experienced dental caries but 50.0% showed poor oral hygiene with obvious presence of dental plaque or calculus. Overall, 69.8% of the observed students needed to visit a dentist. The mean DMFT score was 2.55 ± 3.07 , D was 1.06 ± 1.82 , M was 0.07 ± 0.39 and F was 1.43 ± 2.34 . Caries treatment needs affected 41.5% of the observed students. Only 46.6% had used dental services during the previous year. 17.9% do not benefit from complementary health insurance and this factor negatively influenced their oral health ($p < 0.0001$). **Conclusion:** the results of this study suggest that a specific preventive program could be useful to improve information on risk behaviours and prevention of caries among students.

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Caries Decline Related to Its Distribution in the Primary Dentition of Brazilian Children

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The aim of the study was to analyze the decline of dental caries in relation to its distribution in the primary dentition of Brazilian children. The subjects were 1 to 5 year-old children (Cohort 1996 = 1.465) and (Cohort 2006 = 2.511). The clinical examination determined whether the tooth surfaces were sound, presented non-cavitated lesions (active and inactive), cavitated lesions (active and inactive), fillings, were indicated for extraction or were

extracted. Caries experience was summarized in $d_{1,2}efs$ scores (d_1 = decayed at non-cavitated level and d_2 = decayed at cavitated level, e = indicated for extraction/extracted, f = filling, s = surface). Intra- and inter-examiner reliability of caries scores showed Kappa from 0.71 to 0.93. The percentage of caries-free children increased significantly in all age groups at $d_{1,2}efs$ level only ($p < 0.05$, χ^2 test); 1 year (6.2%), 2 year (26.2%), 3 year (13.7%) and 5 year (19.3%). Accordingly, significant decline in the mean $d_{1,2}efs$ scores of children aged 2 years or older was observed ($p < 0.005$, Mann-Whitney test). The mean $d_{2,efs}$ scores of children aged 2 years or older also reduced, but statistical difference was only observed at the age of 5 yr. In 5-year-old children the highest reduction in caries experience, $d_{1,2}efs$ scores, in maxilla was found at central incisors (21.1%) followed by second molars (19.0%). In the mandible, second molars showed the highest decline (44.1%) followed by first molars (30.2%). Occlusal surfaces showed significant decline in caries experience at $d_{1,efs}$ level in all age groups whereas the smooth surfaces at age groups of two years or older ($p < 0.002$). For proximal surfaces significance was observed at age groups of 3–5 years ($p < 0.002$). The same pattern was observed for tooth surfaces at $d_{2,efs}$ level, however significance was limited to the oldest group. In conclusion, caries decline in the primary dentition was related to both non-cavitated and cavitated lesions (active and inactive), to all teeth and surfaces with the molar teeth and maxillary incisors providing the main contribution.

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Association between the Sleeping Environment Characteristics and the Sleep Bruxism in Children: A Case-Control Study

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This study aimed to evaluate the association between the sleeping environment characteristics and the sleep bruxism in children. A 1:2 matched-pairs case-control study was conducted in the City of Belo Horizonte with 8 years-old scholars. It enrolled 120 children with sleep bruxism and 240 without sleep bruxism, all of whom were paired for gender and social vulnerability as well. The Social Vulnerability Index (IVS) drawn up by the City of Belo Horizonte was employed for social classification. Written consents and ethical approval were obtained. A pretested questionnaire based on the precepts of AASM (American Academy of Sleep Medicine) was applied to parents in order to gather information on the presence of sleep bruxism among the children. Parents were advised to register in a pretested form, for three consecutive days, the presence or absence of the 'audible nocturnal teeth grinding', the period of sleep, the use of medicines and the child sleeping environment characteristics. Data were analyzed using

the chi-square test and multinomial logistic regression were used with the significance level of 5% by the SPSS software (Version 15.0). It was observed that 51.6% of the children with sleep bruxism slept in an environment with the lights turned on ($p < 0.001$) and 53.3% slept in environments with noise ($p < 0.001$). The adjusted logistic model revealed that children who sleep in an environment with noise (OR = 2.8 IC 1.7–4.4) and with the light on (OR = 2.0 IC 1.3–3.0) have a greater risk of exhibiting the habit of sleep bruxism in comparison to those who do not sleep in such conditions. Light and sound stimuli in the sleeping environment might be risk factors for triggering the sleep bruxism among children.

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Dental Caries and Quality of Life of Preschool Children in Chile

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The aim of the work was to establish differences in quality of life among preschool children affected by dental caries. **Methods:** This is a cross sectional study approved by ethical committee of the faculty of dentistry of University of Chile. The sample consisted in 380 2–6 years old children attending to kindergarten in the north area of metropolitan region of Chile. A calibrated examiner did a clinical evaluation according to WHO criteria. After giving informed consent, parents or care givers answered a pre test Spanish version of the Early Childhood Oral Health Impact Scale (ECOHIS) to assess quality of life of children and parents. Prevalence of dental caries, dmft and dmfs were calculated. Differences among ECOHIS items among children with and without dental caries were evaluated using multiple comparison test (χ^2). **Main Results:** Prevalence of dental caries was 48.95%, dmft index was 2.48 and dmfs index was 3.98. Differences were found in items of ECOHIS related to pain, difficulties in eating, problems with attending to school, family economic impact ($p < 0.01$). Differences were not found in items related to interaction with others children, the need of missing work caused by dental problem of children. **Conclusions:** In this preschool population with a caries high prevalence differences in quality of life were related to dmfs scores.

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Correlation between ICDAS-Caries Active Lesions on Primary-Dentition Caries-Prone Surfaces and Caries Risk in Young Children

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The aim of this study was to correlate the presence of ICDAS-caries active lesions on primary-teeth caries-prone surfaces with caries risk on young children. Eight trained examiners examined 592 1–5 years old children (1–3 years: $n = 282$; 4–5 years: $n = 310$) in four Bogotá schools (Inter/intra-reproducibility-Kappa values: 0.70–0.77/0.60–0.85). **Examinations Included:** 8-factor-Cario-gram individual caries-risk assessment, 'A'-ICDAS-caries detection (merging scores 1 and 2) and ICDAS-caries-activity assessment using the modified 4-point-indicator combination: plaque-stagnation area (1-no/2-yes), visual appearance (1-brown-spot-lesion/3-white-spot-lesion/4-shadow/surface-breakdown/cavity), tactile feeling (2-smooth/4-rough), and papilla bleeding (0 = no/1 = yes), resulting an active lesion a point-sum ≥ 7 (occlusal-surfaces) and ≥ 8 (buccal-surfaces) [Ekstrand et al.: Oper Dent 2007;32:225–235]. The activity status of occlusal 1st- and 2nd-upper and lower-molar-tooth-pair surfaces and buccal of central-upper-incisor-tooth-pair surfaces (considering activity 1–2 active lesions) was compared with the patient-caries-risk classification (low/moderate-high) in two age subgroups: 1–3 and 4–5. **Results:** Prevalence and mean caries-experience data (dICDAS4–6mf-s) were 85% and 5.0 ± 7.7 , respectively, increasing to 96% and 9.7 ± 9.9 ($d-s = 7.8 \pm 8.4$; $f-s = 1.5 \pm 2.2$; $e-s = 0.5 \pm 2.4$), respectively, when including early lesions (dICDAS1/2–3). Frequency of active lesions on surface pairs in 1–3-year-olds was: 1st-lower: 45%, 2nd-lower: 32%, 1st-upper: 31%, 2nd-upper-occlusal-molar teeth: 28%, and central-upper-buccal-incisor teeth: 21%. Corresponding figures for 4–5-year-

olds were: 35, 51, 30, 46, and 19%. The caries-risk distribution was 32% low and 68% moderate-high in 1–3-year-olds and 38% low and 62% moderate-high in 4–5-year-olds. Statistically significant associations between presence of active caries lesions and individual moderate-high caries risk were found only for 4–5-year-olds on 2nd-upper and lower-occlusal-molar teeth (Fisher's-exact test; p -values = 0.0010 and 0.0016, respectively).

Conclusion: Among other caries-prone surface pairs in the primary dentition there was an age-related caries risk predictive association of progressing caries lesions on occlusal surfaces of 2nd-molar-primary teeth in the 4–5-year-old group.

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Association between Early Childhood Caries, Feeding, and Oral Hygiene in Children under 36 Months Old

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This study aimed to estimate the association between early childhood caries (ECC), feeding, and oral hygiene of under 36 months children in low-social-economical status hospitals in Pasto, Colombia. 'Cases' ($n = 132$) were children with type-II ECC diagnosis (upper-anterior teeth) and 'Controls' ($n = 264$); those presenting with no diagnosis of caries. With previous informed consent forms, parents were interviewed with a 49-dichotomous-question questionnaire (16 feeding, 8 oral-hygiene, and 25 demographic and behavioural questions). Association analyses between answers and ECC in cases/controls were conducted. **Results:** Up to December 2011 93 cases (70%) and 158 controls (60%)

had entered the study. The mean age of children was: cases 28.4 months (SD6.5) and controls 23.6 months (SD 9.4), and that of mothers: cases 25.5 years (SD5.4) and controls 26.9 years (SD7.1). Variables that showed association with ECC were: the mother smoking (OR 10.82; CI95%: 1.26–501.04); oral-hygiene procedure conducted only by caretaker instead of alternating with a parent/s (OR 2.84; CI95%: 1.13–7.41); a highly cariogenic diet (OR 2.16; CI95%: 1.19–3.90); going to sleep at night without oral hygiene after the bottle use (OR 2.14; CI95%: 1.15–4.00); using a feeding bottle (OR 2.13; CI95%: 1.13–3.99). Protective associations were found for combining breastfeeding with food products (OR 0.35; CI95%: 0.14–0.79), and for not using feeding bottle (OR 0.46; CI95%: 0.25–0.88). No association was found for gender, ethnics, breastfeeding duration, non-breast feeding, sweetened-pacifier use, spoon/cup feeding, mixed feeding (breast/formula, food products/formula), oral hygiene frequency, using gauze/cotton for oral hygiene. **Conclusions:** A very strong association between maternal smoking and ECC was found, as well as the feeding bottle use. Breastfeeding was not associated with type II-ECC.

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Caries Strategy Greenland for 5- to 9-Year-Olds with Focus on Risk Dental Ages: Principles and Results

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In 2008 a new strategy 'Caries Strategy Greenland' (CSG) was implemented in Greenland for all children. Goals were stated for different age cohorts, thus for the 9-year-olds in 2012: >80% with D3MF-S = 0; <5% with D3MF-S>4 and M-S = 0. **Aim:** This study describes 1) CSG for 5 to 9-year-olds and 2) caries status of 9-year-olds in 2008, 2009, 2010 and 2011. The 9-year-olds in 2008 had been offered the previous program with minor focus on risk dental ages whereas CSG had been offered to the 9-year-olds in 2009 from age 7–8, in 2010 from age 6–7 and in 2011 from age 5–6. CSG: Parents/child attend clinics related to risk dental ages of the child, thus focus is on eruption time of first permanent molars (1.M) and on when proximal contact has been established between 1.M's and second primary molars for 1½ year. All recalls are intended to be risk-related. Each visit/recall focus on education and training in toothbrushing using 1,450 ppm F-toothpaste. Topical fluorides are used on active caries and sealants are recommended on 1.M's. Method: d3ef-s/D3MF-S of 9-year-olds were recorded annually. Samples of 9-year-olds: A: 2008 (n = 689); B: 2009 (n = 698); C: 2010 (n = 727); D: 2011 (n = 570). From samples A, B, C National data of Greenland were used, from sample D data were computerized. **Results:** Mean d3ef-s: A = 9.54 (11.8); B = 8.87

(10.2); C = 7.94 (9.6); D = 7.85 (9.3). D3MF-S = 0: A = 57%; B = 59%; C = 64%; D = 64%. Mean D3MF-S: A = 1.72 (3.5); B = 1.48 (3.0); C = 1.37 (3.2); D = 1.25 (2.6) with p-values <0.05 concerning d3ef-s/D3MF-S between sample C and D versus sample A. D3MF-S>4: A = 12%; B = 11%; C = 10%; D = 9%. M-S: A = 0.32; B = 0.25; C = 0.20; D = 0.22. **Conclusion:** The mean caries experience dropped significantly from 2008 to 2010 and from 2008 to 2011; it is doubtful whether the goals for 2012 can be achieved because of the high def-s.

The work done by the personnel working in the Child Dental Health Service in Greenland is highly acknowledged.

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Oral Health Determinants and Caries Outcomes among Non-Privileged Children

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The study aimed to identify oral health determinants that are present in early childhood, and amenable to change through positive attitudes and behaviour and for which there is scientific evidence of their influence on modulating the rate of caries progression. While biological determinants focus on the child, non-biological determinants take into consideration socio-economic, demographics and parental attitudes on child's oral health related behaviour. Information on the role of child's institutional environments is lacking. The null hypothesis was that nurseries did not have significant influence on a child's oral health. The sample was formed by 2,511 Brazilian 1–5-year olds. Caries activity and severity were recorded. Parents were interviewed (n = 2,145) and nurseries (n = 31) answered a questionnaire. Caries occurrence (39%) increased mostly from 1 to 3 years of age while its severity at cavitated level was significantly pronounced from 2 to 3 years. The Poisson multivariate logistic regression analysis showed the importance of parents' attitudes on child's caries outcomes in terms of brushing assistance, visit to the dentist and feeding the child on demand during the night (p < 0.001). The highest likelihood of more severe outcomes was associated with daily sugar intake from 2 to 4 times at the nursery in children whose mothers had no formal education (OR = 13.72 95% CI 3.00–62.98) and whose family incomes were the lowest (OR = 6.98, 95% CI 4.46–10.96). Moreover, the absence of a preventive approach to oral health at the nursery influenced the likelihood of caries outcomes. Child's institutional environment played an important role on child's oral health. The null hypothesis was rejected.

Association between Prolonged Breastfeeding and Early Childhood Caries: A Hierarchical Approach

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No consensus exists regarding the potential association between prolonged breastfeeding and early childhood caries (ECC). Most previous studies did not consider potential confounding factors in a multivariable approach. Furthermore, few were guided by a theoretical framework, using a hierarchical approach. In this study, the association between prolonged breastfeeding and ECC was investigated with adjustment for important confounders, using a hierarchical model. **Methods:** this retrospective cohort study involved 260 low-income children (18 to 42 months). The number of decayed teeth was used as a measure of caries. Following a theoretical framework the hierarchical model was built in a forward fashion, by adding the following levels in succession: level 1: age, level 2: social variables, level 3: health variables, level 4: behavioral variables, level 5: oral hygiene related variables, level 6: oral hygiene quality measured by visible plaque and level 7: level of mutans streptococci. Sequential forward multiple Poisson regression analysis was employed. **Results:** prolonged breastfeeding was not a risk factor for ECC after adjustment for some confounders (Incidence Density Ratio = 1.15; 95% Confidence Interval 0.84–1.59, $p = 0.363$). In conclusion high sucrose consumption between main meals and quality of oral hygiene, as measured by the visible plaque, were significantly associated with ECC.

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Utilization of the Infant Oral Health Risk-Assessment for Prediction of Future Dental Needs in Saudi Arabia

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The Aim of the Study: to assess whether the utilization of the infant oral health risk-assessment will predict the future dental needs in a group of infants with their mothers in the Eastern Province of Saudi Arabia. The experimental approach: The study participants were mothers who attended the well baby appointments

at (3–9) months of age at the Pediatric department, King Fahd Military Medical Complex, Dhahran, Saudi Arabia. Response rate was 100% ($n = 50$). Caries was scored using WHO criteria. A bacterial count analysis using the Caries Risk Test (CRT) for both mutans streptococci (SM) and Lactobacilli (L) was performed. The caries risk assessment survey distributed included a dietary analysis for the infant. An educational pamphlet on home oral health care was distributed. Anticipatory guidance was further emphasized by direct demonstration with the study subjects. **Main Results:** Overall, 80% ($n = 40$) of mothers fed their infants at night, and 90% ($n = 45$) of mothers did not perform oral healthcare at home. Clinically, the mothers had a mean D of 7 which indicated moderate caries activity. The CRT showed that mothers had 75% of high counts for both SM and L, while 37% of the infants had a high count of SM and 25% for L. At the end of the study, most mothers ($n = 45$, 90%) reported that the intervention was helpful and would change the home oral healthcare. **Conclusion:** The infant oral health risk-assessment is a promising tool which pediatric practitioners could use as an 'add-on' caries preventive method for their infant patients.

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Predictor Factors for the Presence of Proximal Caries Lesions in Primary Molars

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This study aimed to analyze the association between the presence of proximal caries lesions in primary molars and caries risk factors (gingival bleeding, visible dental biofilm, hygiene habits and caries experience). Eighty-nine children, 5–9 years old, with at least two proximal sites with intact marginal crista were selected for this cross-sectional study. A total of 596 proximal surfaces were analyzed. Parents signed an informed consent and the Committee for Ethics approved the study. Clinical examinations were carried at a dental chair under artificial light. Compressed air was used to dry teeth before visual examination. A single examiner assessed papilla gingival bleeding and dental biofilm ($\kappa = 0.81$). A second examiner assessed dental caries based on Nyvad criteria ($\kappa = 0.80$). Proximal surfaces were reexamined after tooth separation. Bitewing radiographs were taken using film-holders and analyzed by the first examiner ($\kappa = 0.91$), who was blind regarding the previous collected data. Visual-tactile examination after tooth separation detected the greatest number of proximal lesions. Standard visual examination and radiographs detected 11 and 54% of the proximal lesions detected after tooth separation, respectively. No significant correlation could be established between the presence of proximal lesions and frequency of tooth brushing ($p = 0.71$; Mann-Whitney), flossing ($p = 0.30$; Mann-Whitney), visible dental biofilm ($p = 0.33$; Mann-Whitney) or gingival bleeding ($p = 0.87$; Mann-Whitney). Caries experience was significantly correlated with the presence of proximal carious lesions in primary molars ($p < 0.001$; Mann-Whitney). The chance of presenting proximal caries lesions was higher for children who had caries lesions at other tooth surfaces detected clinically (OR =

6.24; 95% CI = 1.98–19.68). In this study, the possibility of identifying children with proximal caries lesions using clinical predictors was limited. Only caries experience was significantly correlated to the presence of proximal caries lesions in primary molars.

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Comparison of DNA Extraction Methods to Assess the Genetic Risk of Caries in Preschool Chileans by PCR

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In our laboratory we have observed that, unlike in adult subjects, it is difficult to extract DNA from epithelial cells of saliva in young children. Therefore, the aim of this study was to compare the yield and quality of DNA purified by two different methods for the determination of the genetic risk of caries through genotyping by PCR. This study involved a group of 30 children with and without caries, from 34 to 59 months of age. DNA extraction was performed from buccal epithelial cells from saliva, using a salting out protocol (method 1) and a scraping with brush of the inside of the cheeks using a commercial kit (method 2). DNA concentration was measured by electrophoresis and DNA quality was assessed through genotyping by PCR of Db allele, associated with caries risk. The average DNA concentrations were 5.2 ± 3.3 ng/ul with method 1 and 45.6 ± 24.2 ng/ul with Method 2 ($p < 0.05$). The DNA obtained by method 2 was of high molecular weight, showed no signs of degradation and no trace of RNA, resulting in 97% of PCR positive samples, however only 56% of the purified samples with method 1 were positive. Genotyping of the samples obtained by method 2 revealed that 76% of the children were homozygous Db-/Db-, 21% had genotype Db+/Db- and only 3% were Db+/Db+. Method 2 makes it possible to get higher average concentrations, a higher DNA quality for PCR genotyping applications of alleles associated with caries risk.

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The Minimal Intervention Dentistry – Evaluation of Repercussions in Recent Years

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The Minimal Intervention Dentistry (MID) focus on patients' health and to treat the causes of dental diseases instead of only

addressing its symptoms. The purpose of this study was to evaluate the procedures performed during 2001 and 2010 and to verify the impact of MID. After approval of Ethics Committee of the Catholic University of Brasilia, a total of 959 patients' records of the dental clinic of post graduation in pediatric dentistry was analyzed. The following procedures were evaluate: pulpotomy, ART, pulpectomy, restoration, direct pulp capping (DPC), indirect pulp capping (IPC), steel crown, resin crown, extraction of deciduous and permanent teeth and sealant. Data base was created and analyzed in Microsoft Office Excel 2007 software. The data obtained were analyzed by the chi-square, Student t and Williams tests at a 5% level of significance. Accordingly to inclusion criteria a total of 499 patients' records were evaluated. Procedures such as ART, resin crown, deciduous extraction, IPC, pulpectomy, restoration and sealant demonstrate a significant statistically difference ($p < 0.05$) between the years analyzed. Steel crown, deciduous' extraction, DPC, pulpectomy, pulpotomy and restoration presents decreasing tendency curves. Unlike ART, resin crown, IPC and sealant presents increasing tendency curves. The results showed that OMI philosophy contribute in reducing the occurrence of pulp-otomys and pulpectomys. Besides, ICP and ART showed significant increasing in the last years. In conclusion, dentistry procedures practice is increasingly based on MID. This philosophy offers patients the possibly least invasive and most patient friendly treatment option and can also contribute to a better patient's quality of life.

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Concentration and Characterization of Saliva Proteins from Caries Free and High Caries Adults

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Objective: More than one thousand protein types have been described to be present in saliva. It has been reported that some salivary proteins may be caries-protective. Evidence supporting a link between protein profile and caries is scarce and inconclusive. We, therefore, aimed to determine whether adults with high caries or caries-free showed differences in the concentration or the electrophoretic pattern of saliva proteins. Method: High-caries Adults with at least 1 caries lesion coded as 5 or 6 ICDAS II ($n = 20$) and caries-free adults ($n = 20$) were asked to provide unstimulated samples of saliva. Each sample was centrifuged and the resulting supernatant was precipitated with acetone and frozen with protease inhibitors (-80°C). Samples were thawed, centrifuged and the pellet was washed. Protein concentration (ug/ml) was calculated by the Bradford method with a spectrophotometer. Denaturing polyacrylamide gels were resolved (30 min at 90 mV followed by 120 mV until completion) with 30 ug of protein per lane and the gels and analyzed using the Gel Analyzer Pro software. Using specific monoclonal antibodies, Western blot was performed to identify IgA. Protein concentration, number of bands from the electrophoresis and IgA bands from western blots were compared between the groups using student's t test ($p < 0.05$).

Results: Total protein concentration in caries-free was higher (50.65 ug/ml), than in high-caries individuals (26.91 ug/ml) ($p < 0.05$). There were more protein bands in the gels obtained from caries-free than in the high-caries group ($p < 0.05$), with a mean number of 12 and 7 bands, respectively. It was possible to identify more IgA bands in the Western blots from caries-free than in the high-caries subjects ($p < 0.05$). **Conclusion:** Total protein concentration, electrophoretic pattern and IgA concentration appear to be a protective factors for caries in adults.

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Association between Salivary Proteins and Dental Caries in Children

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Salivary proteins have a protective role in maintaining normal conditions of the oral tissues. Simultaneously, there is evidence salivary proteins interfere with bacterial colonization and also promote colonization of dental plaque. These proteins influence the enamel demineralization-remineralization process and dental caries formation. Variability in salivary proteins may play an important role in determining their protective features against dental caries. Knowledge of molecular composition of saliva in different populations is important to better understanding its protective properties. The aim of this study was to analyze electrophoretic patterns of protein composition in glandular and whole saliva of children and to correlate these data with deft/DMFT index. Parotid, Submandibular/Sublingual and whole saliva were collected from 40 children with deft/DMFT = 0 and 40 children with deft/DMFT >3. Individual saliva samples were analyzed by 10% SDS-PAGE stained with Coomassie blue and salivary proteins were scored as absent or present. The electrophoretic profiles were related to caries index. A parotid salivary protein of 35 kDa was detected in 78% of children from caries group and only in 25% of children from caries free group ($p = 0.01$). A polypeptide of 70 kDa from Submandibular/Sublingual saliva was present in 33% of children from caries group and only in 8% of children from caries free group ($p = 0.001$). A metachromatic submandibular/sublingual salivary protein of 24 kDa was present in 30% of children from caries group and in 87% of children from caries free group ($p = 0.001$). The salivary proteins of 35 and 70 kDa were related to the presence of caries, which might be risk marker for dental caries. The 24 kDa metachromatic polypeptide may play a protective role in dental caries.

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Effect of Fatty Acids on the Cariogenicity of an in vitro Biofilm-Caries Model

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Objectives: Fatty acids are commonly considered non-cariogenic, but this assertion is based on scarce evidence. The aim of this study was to evaluate the effect of different type of fatty acids on the cariogenic properties of *Streptococcus mutans* biofilms and on enamel demineralization. **Methods:** Biofilms of *S. mutans* UA159 were grown on bovine enamel slabs for 5 days. Slabs were exposed to 10% sucrose, 3 times a day for 5 min. After sucrose exposure, biofilms on slabs were treated with 0.9% NaCl (caries-positive control) or a panel of monounsaturated (oleic), polyunsaturated (linoleic) and saturated (stearic) fatty acids, in concentrations of 0.1, 1 and 10 mM for 5 min and washed. Biofilms were collected to analyze biomass, viable bacterial counts, proteins and intra and extracellular polysaccharides. Microhardness of the slabs was determined before and after the experiments by a Knoop microindenter to estimate demineralization. Two experiments in triplicate were performed and the comparisons were analyzed using ANOVA and Bonferroni ($p < 0.05$). **Results:** Biofilms exposed to 10 mM oleic or 10 mM linoleic acid showed less biomass than the caries-positive control and the rest of the groups ($p < 0.05$). 10 mM linoleic acid decreased extracellular polysaccharide production. Neither protein nor bacterial counts nor intracellular polysaccharides revealed differences among the groups ($p > 0.05$). 10 mM oleic and 10 mM linoleic acid exposure to the biofilms reduced sucrose demineralization as compared to the caries-positive control. Saturated stearic acid did not affect either cariogenicity of the biofilm or enamel demineralization. **Conclusion:** Mono-unsaturated and polyunsaturated fatty acids presented to the *S. mutans* biofilms after a cariogenic challenge appear to reduce caries formation on enamel.

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Preventive Measures in Early Childhood Caries: A Randomized Clinical Trial

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A randomised double blind clinical trial to compare the effect of three different combined preventive therapies was conducted in a simple-random sample of low-income 2.5-4 year-old children ($n = 447$) from a health institute in Medellín, Colombia. Children were randomly assigned to 3 groups: all received oral-health education, a toothbrush and professional prophylaxis.

Group 1 (n = 146) received non-fluoride toothpaste; Group 2 (n = 152), 500 ppm fluoride toothpaste, and Group 3 (n = 149) non-fluoride toothpaste plus fluoride-varnish application every 6 months. Clinical examination was carried out every year from 2007 to 2010 by two examiners trained in ICDAS (1/2–6). The inter-examiner agreement was rated as good (Kappa-values: 0.73–0.85). **Results:** The prevalence of untreated dental caries according to ICDAS was 74.7% with a caries-surface mean of 7.3 ± 9.1 at baseline and 59.7% (108) with a caries-surface mean of 3.7 ± 5.2 , after 3 years. At the end the caries-surface mean for each group was 4.6 ± 5.7 (Group1: n = 58); 2.9 ± 3.8 (Group 2: n = 60); 3.6 ± 5.8 (Group3: n = 63), without statistically significant differences (p-value = 0.205). The prevalence of treated and untreated dental caries was 66.3% (120), the def-s mean was 5.6 ± 7.3 and 40.3% (73) with no clinical signs of caries according to the ICDAS criteria. The prevalence of children with non-cavitated lesions decreased from 73.4 to 55.8%. Lesions were more frequently found on smooth than on occlusal (pits/fissures) surfaces, with statistically significant differences between the prevalence of affected smooth surfaces at the beginning (64.7%) vs. the end of the study (42%) (p < 0.02). **Conclusions:** In this population, with a high caries prevalence, positive dental caries control was obtained independent of the preventive therapy used.

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Randomized Dose-Response Trial on the Effects of Two Fluoride Toothpastes on Plaque-Retaining Enamel Lesions in situ

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The aim of this double-blinded, randomized, cross-over in situ study was to compare the effects induced by a NaF-toothpaste (1450 ppm) with those of an aluminium fluoride (AlF₃; 1,360 ppm)/chlorhexidine (0.05%) toothpaste on artificial caries enamel lesions. In each of four experimental legs of four weeks, 18 participants wore intra-oral appliances each with four pre-demineralized [Buskes, 1986 (pH 4.95; 7 days)] bovine enamel specimens (n = 156) in the vestibular flanges (surface inserted 1mm below acrylic under plastic mesh). Half of each lesion was cut before the in situ phase for analysis of baseline demineralization. The four randomly allocated treatments included the following toothpastes. A: NaF 1,450 ppm [Blend-a-Med ProExpert (BAM-PE); Procter&Gamble], B: NaF 500 ppm, C: F-free (both experimental, based on BAM-PE), and D: AlF₃ 1,360 ppm + chlorhexidine (0.05%; Lacalut, Dr. Theiss). Twice daily during tooth brushing participants applied slurry of toothpaste extra orally on the specimens for two minutes either with or without brushing (2 speci-

mens each). Differences in integrated mineral loss ($\Delta\Delta Z$) were calculated between values before and after the in situ period using transverse microradiography. Mean (standard error) baseline mineral loss was 2933 (26) vol% $\times\mu\text{m}$. Except for the brushed specimens of toothpaste D [$\Delta\Delta Z$: 260 (238) vol% $\times\mu\text{m}$] specimens of all other groups revealed an increased mineral loss after the in situ period [minimum $\Delta\Delta Z$ of all groups: C (non-brushed): -1,876 (355) vol% $\times\mu\text{m}$]. Significant differences (indicated by >; p < 0.05; ANCOVA) for $\Delta\Delta Z$ were C>B,A,D; B = A; B>D; A = D for the brushed and C = B; C>A,D; B = A; B>D; A = D for the non-brushed specimens. In conclusion, both toothpastes with fluoride concentrations around 1,400 ppm, one of them containing chlorhexidine, inhibited further lesion progression in a demineralizing in situ model.

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3-Year Follow-Up of QLF Advice versus Treatment in General Dental Practice: A Retrospective Study

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A fluorescence camera for caries assessment (Inspektor Research Systems BV, Amsterdam, the Netherlands) has been used in a dental practice in the Netherlands since 2005. Dental treatment decisions were based on both visual inspection and bitewing radiographs alone. In addition, QLF measurements of these teeth were obtained within 3 subsequent weeks. **Aim:** This study looked retrospectively at the level of agreement between the QLF data obtained and the dental treatment decision at baseline as well as with the dental treatment provided within 3 subsequent years. **Methods:** The QLF database Inspektor Pro, 1.0.0.45) was searched for patients who had one occlusal surface for which QLF had indicated that seal or restore would be appropriate. Furthermore, these patients had to have a matching occlusal surface considered healthy. For these patients and their teeth 3-year follow-up data had to be available. In total, 33 patients were found to have teeth that matched these inclusion criteria. Treatment decisions for the teeth included in this study were noted following retrieval from the patient database (Novadent, Complian Valens BV, Hoorn, Netherlands). QLF data were compared for level of agreement (kappa) with each dental treatment decision at baseline and treatment provided within 3 subsequent years. **Results:** Agreement between QLF data and baseline dental treatment decision was poor: kappa = 0.220, but demonstrated better agreement when compared with treatment provided within 3 subsequent years (kappa = 0.579). Agreement between baseline dental treatment decision and treatment provided within 3 subsequent years was also poor (kappa = 0.210). From the 33 surfaces scored as healthy with QLF at baseline 5 were sealed and 6 restored within 3 years. With regard to the 33 surfaces scored as carious at baseline, QLF data suggested to seal 7 surfaces and to restore 26 surfaces. After 3 years out of the 7 surfaces suggested to be sealed at baseline 5

had been sealed, 1 had been restored and 1 had not received treatment. Out of the 26 surfaces suggested to be restored 3 surfaces had not treated, 1 surface had been sealed and 22 had been restored. **Conclusion:** Data obtained with QLF seems to predict treatment outcome to a higher degree than baseline dental treatment decision.

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Ex vivo Evaluation of Caries Infiltration in Primary Molars

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Low viscosity resins (infiltrants) have been shown to penetrate the lesion body of natural caries lesions almost completely in vitro. The aim of the present study was to evaluate the penetration of an infiltrant into proximal caries lesions in primary molars after different application times under clinical conditions. The present study was designed as a randomized ex vivo study with a dose-response design and blind assessment. Fifty-nine proximal lesions from 34 children were infiltrated for either 60, 180 or 300 s. After extraction or exfoliation teeth were embedded in acrylic resin, transversal thin sections with 130 to 150 μm thickness were prepared and subsequently polished up to 100 μm thickness. Tooth sections showing the deepest part of each lesion were selected for analysis of lesion depths (LD) as well as lesion areas (LA) using polarized light microscopy. The same sections were etched with 10% HCl for 30 seconds and penetration depths (PD) and penetration areas (PA) were analyzed using scanning electron microscopy (SEM) in backscattered electron mode. Percentage penetration depth (PPD) and percentage penetration area (PPA) were calculated. Forty-eight teeth were available for analysis. Mean (standard deviation) LD and LA were 596 (203) μm and 4.03×10^5 (2.75×10^5) μm^2 , respectively. PPD ranged from 70% to 80% and PPA from 54 to 60%. Longer application times did not result in significantly deeper or more complete penetration ($p > 0.05$; ANOVA). In conclusion, proximal caries lesions in primary molars can be rather deeply and homogeneously infiltrated after one minute application time under clinical conditions.

The study was supported by DMG (Hamburg, Germany). HML and SP receive royalties for patents related to an infiltration technique for dental caries lesions.

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Treatment of Deep Carious Lesions with Photodynamic Antimicrobial Chemotherapy

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Previous in vitro and in situ studies have demonstrated that photodynamic antimicrobial chemotherapy (PACT) is effective in reducing cariogenic bacteria in demineralized dentine. The purpose of this study was to investigate the performance of a session of PACT using a light emitting diode (LED) associated with a photosensitization dye, as an alternative to remove microorganisms by use of drills. In a randomized, controlled, split-mouth, single blind clinical trial, 38 patients with at least two deep carious lesions on permanent molars were enrolled. Remaining dentinal samples of each deep carious lesion were treated either with non-PACT-control (application of sterile 0.9% NaCl solution) ($n = 38$) or PACT ($n = 38$). The PACT procedure was characterized by 0.01% toluidine blue ortho dye and irradiated with an LED ($\lambda = 630 \text{ nm}$; 94 J/cm^2). Standardized samples of dentin from the pulpal wall region were collected using a curette before and immediately after treatments and kept in a transport medium for microbiological analysis. Samples were cultured in plates of blood agar, Mitis Salivarius Bacitracin agar and Rogosa agar to determine the total viable bacteria, mutans streptococci and *Lactobacillus* spp. counts, respectively. After incubation, colony-forming units were counted and microbial reduction was calculated for different bacteria in each group. The data were transformed into logarithms and statistical analysis was performed using t test ($\alpha = 5\%$). PACT led to statistically significant reductions in mutans streptococci ($1.08 \pm 1.20 \text{ log}$), *Lactobacillus* spp. ($1.70 \pm 1.75 \text{ log}$), and total viable bacteria ($1.07 \pm 1.01 \text{ log}$) compared to the control, which showed log reductions of 0.05 ± 1.02 , 0.52 ± 1.75 and 0.47 ± 0.99 for the same microorganisms respectively. Dentin from deep carious lesions treated with PACT showed a decrease in cariogenic microbial albeit not clinically relevant.

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Video Analysis of Toothbrushing and Flossing Habits in Adolescents

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In Germany, prevention programs including oral hygiene education are well established. Structured programs are organized in form of group prophylaxis in kindergartens (age: 3–6 years) performed by dentists and for children between 6–12 years in schools performed by public health services. At the age of 12–18 years, children are regularly admitted to individual prophylaxis programs in dental practices covered by health insurances. Thus, the aim of this study was to investigate oral hygiene habits of subjects aged 18–19 at the end of the officially regulated preventive programs. The study population ($n = 101$) was a random sample of inhabitants of Giessen born in 1992 (age: 18–19). First, the plaque index (Silness and Loe) was recorded. Thereafter, each subject was filmed through a mirror while brushing and flossing without the investigator present. Then questionnaires about education level and oral hygiene habits were filled in. Video films were analyzed with the video coding-software INTERACT[®]. The variables of interest were: brushing duration, number of brushing events (defined as changes between sextants SI–SVI), brushing movements and flossing habits. Mean duration of toothbrushing was 162.4 ± 73.8 s (oral: 27.1 ± 27.8 buccal: 72.0 ± 31.7). Mean plaque index was 1.54 ± 0.37 (oral: 1.82 ± 0.36 ; buccal: 1.27 ± 0.54). Mean number of brushing events was 45.0 ± 22.4 (buccal: SI 4.8 ± 3.4 ; SII 7.2 ± 4.6 ; SIII 4.7 ± 3.7 ; SIV 4.4 ± 3.9 ; SV 6.0 ± 3.9 ; SVI 4.2 ± 2.9 ; oral: SI 0.6 ± 0.8 ; SII 1.6 ± 1.6 ; SIII 0.7 ± 1.0 ; SIV 1.0 ± 1.2 ; SV 2.2 ± 2.2 ; SVI 0.9 ± 1.2). Only 47% of the subjects have flossed. Predominant brushing movements were horizontal and circular. Mean brushing duration was in the order of general recommendations. Changes between areas were frequent and oral surfaces were brushed less than buccal surfaces. Flossing was performed only by half of participants, but only two subjects presented an adequate technique.

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Clinical Evaluation of the Carious Relationship between Adjacent Interproximal Surfaces of Permanent Molars

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Cariogenic dental plaque existing in the wedge-shaped space between two molars may result in enamel demineralization or carious cavitation on the two approximal surfaces. If one of the two approximal surfaces had a carious lesion and an approximal-occlusal cavity was prepared, the other approximal surface could be exposed and visual inspection (VI) could be made of any carious lesion in this surface through the approximal-occlusal cavity. For the two lesions were in the same wedge-shaped space, a relationship may exist between them. Our aim was to clinically evaluate caries on the interproximal surface adjacent to an approximal-occlusal cavity. The interproximal surfaces adjacent to approximal-occlusal cavities prepared in the carious molars which were treated for the first time were examined by VI. Approximal-occlusal cavities was divided into 3 categories and VI was divided into 5 degrees. 190 cases were enrolled. Approximal-occlusal cavities: category 1–71 cases (37.4%), category 2–65 cases (34.2%), category 3–54 cases (28.4%). VI results: degree 0–8 cases (4.2%), degree 1–12 cases (6.3%), degree 2–67 cases (35.3%), degree 3–52 cases (27.4%), degree 4–51 cases (26.8%). Statistical analysis (Nominal Regression and Chi-Square Tests and significance level, $p < 0.001$) revealed a positive correlation between the presence of an approximal-occlusal cavity and the development of caries on the adjacent tooth surface. In conclusion, caries between adjacent interproximal surfaces of permanent molars had an obvious positive correlation. The more serious the extent of cavitation resulting from an approximal-occlusal lesion on one interproximal surface, the more serious a lesion is likely to be on the opposing surface. Before the approximal-occlusal cavity was restored, the other approximal surface should be cleaned and carefully examined.

103A**Enamel Irradiated with Infrared Laser and Photo-Absorbing Cream following Cariogenic Challenge: Microhardness and Temperature Study**

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Caries lesions are the result of dynamic processes involving acid-mediated tooth structure and mineral loss. Admittedly high intensity lasers increase the resistance of the enamel but not the low intensity, due its poor interaction with the tissue. This interaction can be improved with photoabsorbing substances. The effects of laser on the enamel after a low-level infra-red diode laser therapy ($\lambda = 810$ nm, 100 mw/cm², 90 s, 4,47 J/cm²) and a photo-absorbing cream containing green indocyanine with or without 2% NaF was investigated using knoop microhardness (KNH) and surface temperature measurement after cariogenic challenge. Samples (n = 56) were divided into the following groups for microhardness study: negative control C(-)/no treatment; positive control C(+)/infra-red laser treatment (L); infra-red laser irradiation and photo-absorbing cream (IVL); photo-absorbing cream alone (IV); infra-red laser irradiation and fluoridated photo-absorbing agent (IVFL); and fluoridated photo-absorbing agent alone (IVF). Samples were analyzed using microdurometer before and after treatments and pH cycling cariogenic challenge. For surface temperature, samples (n = 30) were divided into 3 groups: C (+)/infra-red laser, IVL, IVFL. ANOVA was used to compare groups. After the treatment and challenge, all the groups with enamel laser irradiation had lower hardness decreases ($p < 0.001$). The combined use of cream and irradiation in IVFL, IVL groups had percentage of surface microhardness loss of 3.98 and 9.3%, respectively, while IV IVF, C + and C- presented 47,95, 30,06, 24,64 and 47,59%, respectively. As the laser effects are associated with the enamel structure and to the temperature alterations, the analysis demonstrated a higher increase an surface temperature of 74°C and average 45.25°C and 45.95°C for IVFL and IVL, groups, respectively. The results suggested that the combination of cream and laser irradiation promoted a high absorption of light with temperature changes that could promote protein denaturation of the enamel organic matrix that could act as blocking during demineralization process.

103B**Dental Enamel Irradiated with Leds and Photoabsorbing Cream after Demineralization Challenge: Microhardness and Temperature Study**

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Dental caries is a process in which tooth enamel is demineralized by organic acids causing changes to the enamel surface. While high intensity lasers alter the enamel structure improving its resistance but not light emitting diodes, due its poor interaction with the tissue. This interaction can be improved with photoabsorbing substances. The effects of light emitting diodes (LEDs) on the enamel after a LED therapy ($\lambda = 470$ nm, 190 mw/cm², 180 s, 31 J/cm²) and application of a photo-absorbing cream containing acridine orange, with or without 2% NaF, was investigated using knoop microhardness (KNH) and surface temperature measurement after cariogenic challenge. Samples (n = 56) were divided into the following groups for microhardness study: negative control C(-)/no treatment; positive control C(+)/LEDs irradiation (L); LEDs irradiation and photo-absorbing agent (ACL); photo-absorbing agent alone (AC); LEDs irradiation and fluoridated photo-absorbing agent (ACFL); and fluorinated photo-absorbing agent alone (ACF). Samples were analyzed using microdurometer before and after treatments and pH cycling cariogenic challenge. For surface temperature, samples (n = 30) were divided into 3 groups: C (+)/LED, ACL, ACFL. ANOVA was used to compare groups. After the treatment and challenge, all the groups with LEDs irradiation had lower hardness decreases ($p < 0.001$). It was demonstrated that the percentage of hardness losses were C (-) (48.46%) > AC (46.79%) > ACL (36.78%) > ACF (36.69%) = C (+) (36.69%) > ACFL (32.34%). As the light effects on enamel structure are associated to the temperature alterations, the analysis demonstrated the highest increase at surface temperature of 31°C and average 29.15°C and 28.85°C respectively for ACFL and ACL groups. The use of LEDs irradiation in conjunction with the fluoridated photo-absorbing cream, or not, reduced the percentage hardness loss in comparison to that produced by the non-fluoridated cream alone.

Session 6

Demineralization-Remineralization

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Effect of Professional TiF_4 Treatment on Enamel Demineralisation Process in situ

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This in situ study evaluated the effect of TiF_4 compared to NaF formulations on enamel demineralisation. Eleven subjects took part in this crossover, split-mouth and double-blind study performed in 3 phases of 14d each. Each 2 sound and 2 pre-demineralised bovine enamel specimens were worn intraorally while plaque accumulation was allowed. Each one sound and one pre-demineralised specimens were treated once with NaF-varnish or -solution (Phase A, 2.45% F), TiF_4 -varnish or -solution (Phase B, 2.45% F), placebo varnish, or no-treatment (Phase C). The initially sound enamel specimens were exposed to severe cariogenic challenge (20% sucrose, 8 × 5 min/d), while the pre-demineralised specimens were not. The enamel alterations were quantified by transverse microradiography and statistically analysed by ANOVA and Tukey's test ($p < 0.05$). Demineralisation of previously sound enamel was reduced by all fluoride formulations except NaF-solution. Both TiF_4 formulations were as effective as NaF-varnish. For the pre-demineralised specimens, all fluoride containing formulations significantly prevented further subsurface mineral loss, compared with placebo varnish and control that presented higher ΔZ and R values. Within the experimental protocol, TiF_4 was able to decrease enamel demineralisation as NaF varnish under severe cariogenic challenges, while all fluoride formulations were able to prevent further demineralisation of previously demineralised enamel compared to both controls.

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Validation of Transversal Wavelength Independent Microradiography

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Transversal Wavelength Independent Microradiography (T-WIM) is a modification of Transversal Microradiography (TMR), which in contrast to the original method, allows microradiographic analysis of thick sections (1,000–3,000 μm). The aim of this in vitro study was to validate T-WIM with the gold standard TMR and to compare the T-WIM measurements obtained at two centres. Fifty bovine enamel specimens were embedded in acrylic resin. The specimens' surfaces were polished and divided into three areas using nail varnish (sound control). Subsequently, specimens were demineralised in Buskes solution at pH = 4.95. Demineralised areas were covered with nail varnish after 42 days (area 1), 62 days (area 2) and 124 days (area 3) storage, respectively. Subsequently, 100 μm (TMR) and 1000 μm (T-WIM) sections were prepared from each specimen. Microradiographs were obtained using TMR (Berlin) and T-WIM (Berlin & Groningen). Mineral loss and lesion depths were analyzed and the values were correlated. The mean (SD) mineral loss (TMR) for areas 1, 2 and 3 were 3,409 (888), 8,435 (2,108) and 12,964 (4,266) vol% × μm , respectively. For mineral loss the Pearson linear correlation coefficient (r) showed a very good correlation between TMR and T-WIM Berlin ($r = 0.866$); the intraclass correlation coefficient was similarly good (ICC = 0.872). The correlation for TMR and T-WIM Groningen was good ($r = 0.861$ ICC = 0.859). A slightly better correlation was found between T-WIM Berlin and T-WIM

Groningen ($r = 0.922$ ICC = 0.924). Thus, it can be concluded that T-WIM is a valid and reproducible method for mineral loss measurement in vitro.

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Cerium Chloride Is Able to Reduce Carious Enamel Demineralisation

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This in-vitro-study aimed to determine the prophylactic potential of cerium chloride to reduce mineral loss and lesion depth development under artificial caries conditions. Eighty enamel samples were prepared from 20 bovine lower central incisors. Crowns were sectioned in four pieces, embedded in acrylic resin, ground flat from the labial aspect and one piece per tooth was randomly allocated to one of four groups (A–D; $n = 20$ per group). Samples were treated for 30 s with one of the following solutions: placebo (A), amine fluoride (Elmex fluid; B), cerium chloride (C) and combined fluoride/cerium chloride application (D). Specimens were kept in a demineralizing solution ((Buskes et al. Caries Res 1985;19:490–496) to induce artificial caries lesions. After 14 days, the integrated mineral loss (ΔZ) and lesion depth (ld) was determined by TMR and compared by Scheffé's post hoc tests ($p \leq 0.05$). The statistically highest mineral loss and lesion depth was observed for the placebo group A (5440 ± 2027 vol% μm and 196 ± 28 μm , mean $\pm$ SD). The significantly lowest values (ΔZ and ld) were observed for samples treated with the combined fluoride/cerium chloride (group D) ($3,101 \pm 1,059$ vol% μm and 128 ± 21 μm). The respective parameters following treatment with amine fluoride ($3,784 \pm 1,071$ vol% μm and 144 ± 27 μm) and cerium chloride ($3,409 \pm 1,337$ vol% μm and 134 ± 31 μm) were significantly lower as compared to the placebo group. The results among all treatment groups (C–D) were not statistically significantly different. Cerium chloride and its combination with fluoride are able to significantly reduce mineral loss and lesion depth development.

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In vitro Comparative Analysis of the Effect of Two Children's Fluoridated Toothpastes on Enamel Microhardness

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This in vitro study investigated the remineralization of artificial caries lesions by children's fluoridated toothpastes, through comparative analysis of superficial enamel microhardness

(Knoop Microhardness – KHN). Sixty enamel slabs of human third molars were divided into 4 groups: 1 – Tandy® (1.100 ppmF NaF, Colgate Palmolive); 2 – Aquafresh Kids® (1.100 ppmF MFP, Glaxo Smith Kline); 3 – Emoform® (Control – without fluoride, Andromed); 4 – Crest® ('Reference' – 1.100 ppmF NaF, Procter & Gamble). The KHN measurements were made in caries-free dental enamel (K1), after induction of artificial caries (K2) and after pH cycles (K3). The demineralized samples were immersed $3 \times$ / day in a solution of the respective dentifrice, for 5 min, under constant agitation. After each immersion, they were washed with distilled water and immersed individually, in artificial saliva (AS), during 4h and between the second and third fluoride exposure (FE), were immersed during 6h in a demineralization solution. After the last FE, they were immersed in AS until the next cycle. After 14 days, the KHN values (K3) and alpha remineralization (α) were analyzed through ANOVA and Student-Newman-Keuls method. The ANOVA test demonstrated a significant microhardness difference ($p < 0.0001$) among groups: 1 (228.9 KHN); 2 (215.2 KHN); 3 (147.8 KHN) and 4 (254.4 KHN), showing remineralization with the fluoridated dentifrices used. And for α values, there were differences ($p < 0.05$) among groups as shown in the following order: Crest® (54%) > Tandy® (33%) > Aquafresh Kids® (15.5%) > Emoform® (~40%). It was concluded that, the fluoridated toothpastes used in this study were able to remineralize the enamel, but NaF dentifrice showed major potential of remineralization, statistically significant, when compared with MFP dentifrice.

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¹⁹F MAS NMR Characterization of Fluoridated Apatite Phases Formed on Enamel during in-vitro Demineralization

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Fluorapatite (FAP) is more resistant to acidic challenges than hydroxyapatite (HAP) thereby slowing the progression of caries development. Fluoride is commonly formulated in dentifrices as an anti-caries agent and can be at concentrations as high as 5000 ppm. The aim of this study was to characterize the fluoride mineral phase formed on enamel surfaces in response to F-exposure in the demineralizing medium. Enamel blocks were immersed in demineralization solutions 0.1M acetic acid (pH 4.0) containing 0, 25, 300, 5000 ppm NaF respectively, for 96 hours at 37 °C. Samples were weighed before and after immersion to calculate the percentage weight loss of each. After grinding to powdered form, samples were analyzed using ¹⁹F Magic Angle Spinning Nuclear Magnetic Resonance (¹⁹F MAS NMR) spectrometry. The ¹⁹F MAS NMR spectra showed no fluoride containing mineral phases for samples immersed 0 ppm F. At 25 ppm NaF, the predominant

phase seen was a fluoridated apatite. At 300 and 5000 ppm NaF, the predominant phase was calcium fluoride (CaF_2) in addition to fluoridated apatite. A greater percentage enamel weight loss was observed at 5000 ppm NaF (16.1%) compared to 300 ppm NaF (10.6%). This ^{19}F NMR study suggests that at higher fluoride concentrations, CaF_2 is the predominant phase. The formation of CaF_2 may exhibit anti-caries like effects by forming a protective barrier on the enamel surface thereby lowering demineralization rates. However, its formation could compromise the structural integrity of enamel, whereas fluoridated apatite is closer in structure to enamel mineral than fluorite and therefore would not compromise the structural integrity, but in fact lower the solubility of enamel.

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Effect of Fluoridated Milk with Different Frequency of Consumption on Enamel Remineralisation in situ

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Aim: to evaluate the effect of fluoridated milk with varying frequency of consumption on enamel remineralisation under cariogenic challenge. **Methods:** 25 subjects were recruited to a controlled, randomised, 3-arm crossover, single-blinded clinical study. Subjects wore intra-oral appliances containing pre-demineralised enamel slabs for 21 days per study arm. The cariogenic challenge comprised 5 dippings (2 mins each) per day in 12% sucrose. Subjects also dipped their appliances in 50 ml 5.0 ppm F milk, either once, twice or once every other day for 5 mins and drank 200 ml 5.0 ppm fluoridated milk either once, 100 ml 5.0 ppm fluoridated milk twice or 200 ml 5.0 ppm F fluoridated milk once every other day immediately on reinserting their appliances. At the end of the study, mineral loss of the artificial caries lesions of the enamel slabs was measured to assess changes in mineralisation with transverse microradiography. **Results:** Both groups with the frequency of consumption once and twice per day using 5.0 ppm F milk were effective in promoting statistically significant remineralisation of pre-demineralised enamel in situ, compared with their respective baseline values. Mean differences from baseline (ΔZ) + S.E. were 299.53 ± 66.49 ($p < 0.001$, Wilcoxon test) for once per day group; 280.07 ± 82.67 ($p < 0.05$, Wilcoxon test) for twice per day group. There was a reduction of mineral loss in the every other day frequency of consumption group using 5.0 ppm F milk, however this was not significant when compared to baseline. There were no significant differences between groups. **Conclusion:** Using a caries-like in situ model and a randomised controlled single-blind crossover study design consumption of 5.00 ppm F in milk once or twice per day significantly promoted remineralisation.

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Effect of Stannous and Fluoride on Caries Lesion Mineral Distribution – A Preliminary Mechanistic Study

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Our understanding of possible mechanistic interactions of stannous and fluoride on lesion mineral distribution is rather poor. Therefore, the aim was to investigate the effects of stannous and fluoride on the changes in mineral distribution of caries lesions under plaque fluid-like conditions. Polished human enamel specimens were demineralized at 37°C for 7 day using an acid gel system (4% methylcellulose, 0.1 M lactic acid, pH 4.6). Lesions ($n = 15$ per group) were then imbedded in a similar acid gel system containing 4% methylcellulose, 30 mM lactic acid, 4.1 mM CaCl_2 , 8 mM KH_2PO_4 , 63 mM KCl at pH 4.9, 37°C for 11 day. The gels were supplemented with fluoride (0/50 μM ; as sodium fluoride) and stannous (0/0.1/1 mM; as stannous chloride) in a 2×3 factorial design. Lesion were analysed by transverse microradiography and changes in integrated mineral loss (ΔM), lesion depth (ΔL), ratio of mineral loss to lesion depth (ΔR) and maximum surface zone mineral density ($\Delta \text{SZ}_{\text{max}}$) were calculated. Data were analysed using two-way ANOVA ($p < 0.05$). Significant interactions between fluoride and stannous were found for ΔL and ΔR . Fluoride was a significant source of variation for ΔM (more remineralization), ΔR (greater reduction) and $\Delta \text{SZ}_{\text{max}}$ (more mineralization), but not for ΔL . Stannous did not affect any variable. Lesions exposed to fluoride in the absence of stannous exhibited laminations and extensive remineralization in the lesion body at the expense of mineral loss beyond the original lesion. Lesions exposed to stannous in the absence of fluoride exhibited only minor laminations. The addition of stannous to fluoride largely negated any fluoride effect with resulting lesion mineral distribution profiles being almost indistinguishable from the no fluoride/no stannous control. Stannous did not interfere with caries lesion de-/remineralization per se; however, stannous modified fluoride effects.

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Effect of Sr^{2+} on Hydroxyapatite Demineralisation Using Scanning Microradiography

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The literature on the cariostatic effects of strontium (Sr^{2+}) remains controversial, and the mechanism remains obscure. The aim was to study the effect of Sr^{2+} in the demineralizing solution on the kinetics of hydroxyapatite (HAp) dissolution using scan-

ning microradiography (SMR) under artificial caries and erosion conditions. Sintered hydroxyapatite discs (Plasma-Biotol, UK; 20 wt% porosity), 1mm thick, were used as enamel analogues. Each was coated with acid-resistant varnish leaving one surface exposed, and located in an SMR cell. Demineralizing solutions of 0.1% acetic acid (pH4) simulating caries conditions, and 0.3% citric acid (pH 2.8), simulating erosive conditions were circulated through the SMR cells. The rate of demineralization of the HAp discs (RDHAp) was measured using SMR. Further SMR measurements were carried out using identical demineralizing conditions, but with increasing Sr^{2+} concentrations of 5, 10, 20 and 30 ppm, and SMR measurements were continued for each case. The SMR measurements were then repeated at decreasing Sr^{2+} concentrations (30, 20, 10, 5 and 0 ppm). For caries-like conditions RDHAp decreased ($3.40\text{E-}4$, $2.73\text{E-}4$, $1.88\text{E-}4$, $1.44\text{E-}4$, $1.15\text{E-}4 \text{ g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$) at increasing Sr^{2+} concentrations. RDHAp also decreased ($1.47\text{E-}4$, $1.24\text{E-}4$, $1.04\text{E-}4$, $6.10\text{E-}5 \text{ g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$) at subsequent decreasing Sr^{2+} concentrations, except for $2.39\text{E-}4 \text{ g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ at 0 ppm. For erosive-like conditions RDHAp decreased ($4.22\text{E-}3$, $4.02\text{E-}3$, $3.58\text{E-}3$, $3.45\text{E-}3$, $3.12\text{E-}3 \text{ g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$) at increasing Sr^{2+} concentrations. RDHAp also decreased ($3.94\text{E-}3$, $3.55\text{E-}3$, $3.19\text{E-}3$, $2.58\text{E-}3 \text{ g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$) at subsequent decreasing Sr^{2+} concentrations, except for $3.65\text{E-}3 \text{ g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ at 0 ppm. In conclusion, Sr^{2+} decreased RDHAp under strictly controlled thermodynamic conditions relevant to caries and erosion. However, this decrease was not reversed when the Sr^{2+} concentration was subsequently decreased. This pattern of the influence of Sr^{2+} may result from the partial inclusion of Sr^{2+} into the HAp lattice.

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In vitro Evaluation of NaF Dentifrice-Mouthrinse Combinations for the Remineralization of White-Spot Lesions

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The purpose of this work was to evaluate remineralization of white-spot lesions in a pH cycling study comprising dentifrice-mouthrinse combination treatments. These treatments included: 0: 0 or 1: 1000 ppm F dentifrice (NaF, silica, GlaxoSmithKline) plus each of the following mouthrinses (NaF, functionalized tricalcium phosphate (fTCP), GlaxoSmithKline): A: 0 ppm F + 0 ppm fTCP; B: 225 ppm F + 0 ppm fTCP; C: 225 ppm F + 15 ppm fTCP; D: 225 ppm F 30 ppm fTCP; and, E: 225 ppm F + 60 ppm fTCP. White-spot lesions initially formed in bovine enamel specimens ($n = 10$ per group) via a carbopol-lactic acid solution were subjected to a daily pH cycling regimen consisting of four, one-minute dentifrice followed by one-minute mouthrinse treatments that were separated among immersions in a four-hour acid challenge (carbopol-lactic acid, pH = 5.0) and artificial saliva (pH = 7.0). After ten days, mean percent surface microhardness recoveries (SEM) were: 0A: 0.0 (0.7) and 1A: 5.6 (0.9)*; 0B: 2.8 (0.9) and 1B: 4.8 (1.0); 0C: 4.6 (0.8) and 1C: 12.3 (2.5)*; 0D: 3.5 (0.5) and 1D:

7.5 (1.7); and, 0E: 5.8 (1.4) and 1E: 5.8 (1.1). Additionally, lesion sizes (SEM) were estimated using cross-sectional microhardness: 0A: 396.0 (29.1) and 1A: 389.6 (43.4); 0B: 296.0 (25.6)* and 1B: 386.1 (27.3); 0C: 188.6 (18.7)* and 1C: 326.4 (33.1); 0D: 301.8 (24.8) and 1D: 242.1 (41.0); and, 0E: 232.3 (17.8) and 1E: 300.8 (39.7). In both analyses, * indicates significant differences (independent t-test comparisons, $p < 0.05$). These cycling results demonstrate sensitivity of white-spot lesions to NaF dentifrice-mouthrinse combinations. Overall, combinations involving NaF-fTCP mouthrinses produced the greatest surface recoveries and smallest lesion sizes, and provide support for continued evaluation of promising remineralization therapies.

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Approximal Caries Lesion Arrestment in Surfaces in Contact with Fluoride-Releasing Restorative Materials

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This study investigated the effect of different fluoride-releasing restorative materials on approximal caries lesions arrest. Eighty primary enamel blocks were covered with acid-resistant varnish, except for a 3×2 mm area, and submitted to artificial caries induction following a pH cycling model (demineralising solution, pH = 4.6, 8 h; remineralising, pH = 7.0, 16 h) for 10 days. A quantitative light-induced fluorescence (QLF) image was made of each block at baseline and after caries lesion development. Cylindrical blocks ($n = 20$) of composite resin (Z350), glass ionomer (Ketac Molar), resin-modified glass ionomer (Vitremar) and compomer (Dyract eXtra) were prepared and attached to an enamel block, simulating the contact point with a proximal restoration. These specimens (caries lesion + restorative) were submitted to a subsequent caries challenge by pH cycling (demineralising solution, pH = 4.5, 8 h; remineralising, pH = 7.0, 16 h) for 7 or 14 days ($n = 10$). New images of enamel blocks were then performed for the final QLF analysis. Comparison of lesion mineral content was analyzed through ΔQ ($\% \text{mm}^2$) and ΔF (%) values, using the QLF software. Normal distribution of data and equality of variances were confirmed using Kolmogorov-Smirnov and Levene tests, respectively, and values were compared using ANOVA ($\alpha = 5\%$). There was no significant difference among groups for the baseline values or those obtained after lesion development. Duration of cariogenic challenge had no significant effect on lesion progression or arrest, considering ΔF (0.854) or ΔQ (0.305), for any of the studied materials. Considering both parameters, a significant higher degree of demineralisation ($p < 0.0001$) was observed for enamel lesions in contact with the composite resin when compared to all other materials. In conclusion, different fluoride-releasing materials can prevent the progression of adjacent artificial enamel lesions when in approximal contact with them.

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Evaluation of Two Methods Used to Detect Enamel Demineralisation in Patients following Active Orthodontic Treatment

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Aims: To evaluate the detection of enamel demineralisation by Quantitative Light-induced Fluorescence (QLF) and clinical photograph (CP) images. **Materials and Methods:** Retrospective, observational study of QLF and CP images of 80 patients who were part of a demineralisation study. After a training session, four examiners individually assessed the QLF and CP images. The examiners had 15 s to screen each image for enamel demineralisation and record the result on the pro forma. The QLF and corresponding CP images were displayed separately and non-sequentially, in random order, so that each image for every subject was scored independently. The assessment session was repeated under the same conditions after four weeks and assessors repeated the visual assessment of the same images. **Results:** Significant differences in the mean number of teeth with WSL were detected using QLF and CP images during the first examination session ($p < 0.05$). Inter-examiner agreement for QLF improved between the first ($0.80 > \kappa > 0.60$) and second ($\kappa > 0.80$) session. While for CP images, there was moderate agreement in the first ($0.60 > \kappa > 0.40$) session with an improvement in the second ($0.80 > \kappa > 0.60$) session. **Conclusions:** QLF has a higher inter-examiner agreement, is more sensitive and specific than CP imaging in the visual detection of demineralised lesions. The validity and reliability of both techniques improves with training and calibration of the examiners.

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Protective Effect of a Low-Fluoride Gel Supplemented with TMP on Caries and Erosion in vitro

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This study assessed the effects of a low-fluoride gel (LFG) supplemented with sodium trimetaphosphate (TMP) on dental caries and erosion in vitro. Experimental groups comprised placebo gel

(P, no fluoride or TMP), LFG (4,500 $\mu\text{g F/g}$) LFG + 5%TMP (LFG+TMP), conventional neutral gel ('CNG', 9,000 $\mu\text{g F/g}$) and commercial acidulated phosphate fluoride ('APF', 12,300 $\mu\text{g F/g}$). Phase 1 involved pH cycling (de>remineralization) of bovine enamel blocks ($n = 10/\text{group}$) after topical application of gels, during five days. Following, blocks were evaluated by surface hardness (SH), integrated subsurface hardness loss (ΔKHN) and by polarized microscopy (PM). In phase 2, blocks ($n = 24/\text{group}$) were submitted to erosive challenges (ERO) by immersion in Sprite Zero (4 times/day, 5 min) during 5 days. Half of the blocks were also submitted to abrasive challenges using a brushing machine after the erosive challenges (ERO+ABR). Enamel blocks were analyzed by SH and by profilometry. Data were analyzed by ANOVA, followed by Tukey's or Student-Newman-Keuls' tests ($p < 0.05$). For Phase 1, no significant differences were seen among LFG+TMP, CNG and APF regarding SH and PM ($p < 0.001$). Moreover, significant lower values of ΔKHN were observed for LFG+TMP and APF in comparison with the other groups ($p < 0.001$). For Phase 2, a dose-response relationship was verified between fluoride concentration in the gels and SH. Treatment with LFG+TMP produced the lowest wear values when compared to the remaining groups ($p < 0.001$), for both ERO and ERO+ABR. The results indicate that the protective effects of LFG supplemented with TMP are equal or greater than those resulting from the use of conventional formulations after cariogenic or erosive challenges in vitro.

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Low-Fluoride Dentifrice with Trimetaphosphate on Enamel Caries and Erosion: An in vitro Study

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Although fluoride-containing dentifrices have promoted a marked decline in caries prevalence worldwide, they are regarded as a risk factor for dental fluorosis. This study evaluated the ability of low-F dentifrices (250 ppm F) associated to sodium trimetaphosphate (TMP) in inhibiting enamel demineralization and erosion in vitro. For the demineralization protocol, enamel blocks were submitted to a pH-cycling (5 days) and treatments ($2\times/\text{day}$ for 1 min) with slurries of dentifrices with 0, 250, 500 and 1,100 ppmF, as well as dentifrices with 250 ppmF and TMP (0 to 3%). For the erosion protocol, blocks were submitted to erosive challenges (Sprite Zero) $4\times/\text{day}$ for 5 min, followed by treatment (30 s) with dentifrice slurry with 0, 250 and 1,450 ppmF (Sensodyne ProEnamel), as well as dentifrices with 250 ppmF and TMP (0 to 1%). Following, surface and cross-sectional hardness were performed in all blocks. Enamel wear was measured by profilometry (erosive protocol). Enamel F concentrations were also assessed (demineralization protocol). Data were analyzed by ANOVA followed by Student-Newman-Keuls Method ($p < 0.05$). The

addition of 0.25% of TMP was sufficient to improve the F ability to inhibit mineral loss in the same degree to group 1,100 ppmF ($p = 0.719$). TMP groups (0.25 to 1.0%) showed enamel F concentrations similar to group 500 ppmF ($p = 0.662$). Fluoride dentifrices with TMP (0.25 to 1%) showed lower enamel wear than 1,450 ppmF ($p < 0.001$). However, the mineral loss was similar among TMP groups (0.5 and 1%) and 1,450 ppmF ($p = 0.098$). The addition of TMP (0.25 and 0.5%) to a low-F dentifrice (250 ppmF) inhibited enamel demineralization and erosion equivalent to that obtained with the 1,100 ppmF toothpaste.

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Caries-Like Lesion Prevention by S-PRG Filler Containing Toothpastes: Microhardness Evaluation

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The objective was to evaluate the ability of S-PRG filler containing toothpastes (S-PRG TP) to inhibit enamel demineralization. Sixty, 4 × 4-mm, sound bovine enamel blocks were prepared and equally divided into six groups, with different S-PRG filler particle sizes and concentrations: Group 1: 1 μm/5wt% S-PRG TP, Group 2: 1 μm/30wt% S-PRG TP, Group 3: 3 μm/5wt% S-PRG TP, Group 4: 3 μm/30wt% S-PRG TP, with the controls being Group-5: MI-Plus, and Group-6: 0wt% S-PRG TP. For each group, a slurry of the toothpaste was prepared by adding 5.0 g of toothpaste to 15 ml of deionized-water. The daily cyclic treatment regimen, repeated for 8 days [Queiroz et al.: Braz Dent J 2008;19: 21–27], consisted of two 5-min slurry treatments sandwiched around a 4-h demineralization challenge (acetic acid buffer) with the specimens stored in artificial saliva the remainder of the day. Before (baseline) and after pH cycling, Vickers hardness values (VHN) were obtained on all specimens by four indentations at a load of 200 g for 15 s. The differences between post-cycling and baseline hardness values (ΔVHN) were calculated. Data were analyzed using ANOVA. There was no significant difference ($p > 0.05$) in VHN among treatment groups at baseline. Post-cycling, there was no significant difference ($p > 0.05$) in ΔVHN (mean ± SD) among Groups 1 (−120.4 ± 20.7), 2 (−127.8 ± 18.0), 3 (−130.0 ± 31.5), and 4 (−128.1 ± 17.3); and between Groups-5 (−191.0 ± 27.5) and 6 (−200.3 ± 38.0). Groups-5 and 6 showed significantly more softening than Groups 1, 2, 3, and 4 ($p < 0.05$). The results suggested that S-PRG filler containing toothpastes appeared to inhibit enamel demineralization, independent of S-PRG filler particle size and concentration and compared to MI-Plus and a negative control (0wt% S-PRG).

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Surface Precipitation Can Obfuscate Measurement of Surface and Subsurface Mineral Deposition

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Aim: To compare the pattern and extent of remineralization produced by products containing unstabilized and stabilized calcium and phosphate ions. Experimental approach: Subsurface lesions were formed in human enamel (lesion depth ≈ 91 μm; dZd ≈ 2,200 vol%min·μm). The remineralization potential of four groups were tested ($n = 7$ per group) by exposing the enamel subsurface lesions to: (1) artificial saliva alone (AS, containing 0.5 mM CaCl₂, 0.5 mM Na₂HPO₄, 50 mM NaCl, 20 mM HEPES, at pH 7.0); (2) AS with 1,000 ppm fluoride toothpaste; (3) AS with Age defying toothpaste containing 1,100 ppm fluoride, calcium sulfate and dipotassium phosphate; and (4) AS with Tooth Mousse Plus containing 10% CPP-ACP with 900ppm fluoride. All products were diluted in the AS to standardize the fluoride concentration at 100 ppm. Remineralization was measured using surface microhardness, transverse microradiography (TMR) and scanning electron microscopy (SEM). **Results:** The remineralization (dZd-dZr, vol%min·μm) produced was: 4 (771 ± 54) > 3 (451 ± 47) > 2 (344 ± 58) > 1 (226 ± 37). The percent recovery of surface microhardness was: 3 (27.5 ± 12.1) > 4 (7.4 ± 1.5) = 2 (8.6 ± 1.9) > 1 (3.9 ± 1.4). Scanning electron microscopy revealed an inhomogeneous precipitate on the samples exposed to Age defying toothpaste. This layer resembled calculus and made surface microhardness testing invalid as the surface was not smooth. Additionally, if this layer was included in TMR analysis it led to an overestimation of the mineral 'returned' to the lesion. The pattern of remineralization in the CPP-ACP group was throughout the body of the lesion whereas Age Defying toothpaste promoted predominantly a surface change by the deposition of a calculus-like material. **Conclusion:** Products containing stabilized and unstabilized calcium and phosphate ions produced a different pattern and extent of mineral deposition.

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The Effect of Zinc on the Demineralisation of Sound Enamel in vitro

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The aim was to investigate the effect of zinc on enamel demineralisation at concentrations similar to those found in plaque fluid following application from zinc-containing toothpaste. Sound bovine enamel blocks were demineralised in five acidified methyl cellulose gels, partially-saturated with respect to enamel, at

37°C for 14d. All gels contained calcium, phosphate and fluoride at 4.10, 8.00 and 10.5 $\mu\text{mol/l}$ respectively; zinc was added to four gels at either 15.4, 77.0, 231 and 385 $\mu\text{mol/l}$, with the fifth a non-zinc control (nonZn). The degree of saturation with respect to Hopeite ($\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$) was calculated. After demineralisation, total mineral loss (ΔZ), average mineral loss (R), lesion depth (LD) and surface-zone maxima (SZMax) were measured using transverse microradiography. For nonZn, 15.4, 77.0, 231 and 385 $\mu\text{mol/l}$ Zn respectively, mean values were: ΔZ , 2720 (450) a, 2170 (555) b, 1350 (403) c, 1120 (415) c, 1070 (477) c; R, 26.1 (2.84) a, 19.4 (2.93) b, 14.4 (2.22) c, 13.1 (1.98) c, 14.5 (2.55) c; LD, 104 (15.5) a, 110 (15.5) a, 94.2 (25.8) a, 86.3 (31.7) a, b, 76.4 (34.6) b; SZMax, 61.5 (5.03) a, 67.3 (4.13) a, b, 71.9 (10.8) b, c, 76.6 (4.89) b, c, 72.7 (5.45) c. (Standard deviations in brackets, means with the same letter not significantly different, $p < 0.05$). All gels with added zinc were supersaturated with respect to Hopeite. The findings regarding effect of zinc concentration on ΔZ concur with those of previous work where similar concentrations were found to reduce the rate of hydroxyapatite dissolution markedly. The relatively small decrease in LD, but substantial reduction in R, with increasing zinc concentration, suggests that inhibited dissolution of more soluble mineral phases may have occurred. Chemical analyses of lesions may elucidate mechanism(s) involved. In conclusion, zinc reduced enamel dissolution in vitro, quantitatively and qualitatively, under simulated plaque-fluid conditions.

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Parallel Observations of Human Molar Enamel with XRD, Raman Microscope and Electron Microprobe

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Human enamel is surprisingly complex mixture of different apatite phases. Once it has erupted into the oral cavity, its surface constantly undergoes modifications. The changes concern the chemical composition, crystallographic phase, crystal orientation, mechanical characteristic. Knowledge of enamel structure interaction with the oral environment, and possible prerequisites for caries initiation, progression and arrest is still under investigation. **Objective:** The aim of the study was to collect the linear scans along the enamel of human molars. The interpretation of the results aimed in coupling of chemical with structural changes. **Material and Methods:** The studies were performed on sound human molar teeth with the XRD instrument to assess crystal structure. The same sites were scanned with Raman microscope, to observe specific chemical entities, and finally, the scans with the scanning electron microprobe were done at the same locations, to get the knowledge of the elemental distributions. **Results:** The results proved that the diffraction spectra differed very much inside the enamel. The variability resulted both from the differences in the orientation of the crystallites, from the variations in the sizes

of crystallites and from the chemical changes inside the crystals. The chemical changes were apparent from the electron microprobe measurements. The increased presence of Na, Mg and carbonates in locations closer to the DEJ corresponded with worse shaped microcrystallites. **Conclusions:** These techniques allowed the demonstration of the variability of apatite within the enamel. After subtraction of the orientation effects and the shape contributions, the chemical effects were taken into account. The rigorous stoichiometry and structure of the changes were considered, with involvement of Na, Mg, carbonate ions inside the crystallites.

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Remineralization of Early Caries Lesions by Theobromine

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This study investigated the anticaries potential of theobromine in comparison to a standard NaF dentifrice. 3 tooth blocks were produced from each of 30 teeth. Caries-like lesion was created on each block using acidified gel. A smaller block was cut from each block for baseline Scanning Electron Microscopy (SEM) imaging and Electron Dispersive Spectroscopy (EDS) analysis for surface Ca level. A tooth slice was cut from each lesion-bearing block for transverse microradiography (TMR) quantification of baseline mineral loss (Δz). Then baseline surface microhardness (SMH) of each lesion was measured. The three blocks from each tooth were assigned to 3 remineralizing agents: (1) Artificial saliva (AS); (2) AS with theobromine (0.0011 M); (3) NaF toothpaste slurry (0.0789M F). Remineralization was conducted using a pH cycling model with storage in AS. After 28-day cycle, samples were analyzed using EDS, TMR, and SMH. Intra-group comparison of Pre- and post-test data was performed using t-tests ($p < 0.05$). Intergroup comparisons were performed by post-hoc multistep comparisons (Tukey). SMH indicated significant ($p < 0.01$) remineralization only with theobromine ($37.6 \pm 31.9\%$) and toothpaste ($28.6 \pm 15.8\%$). With TMR, theobromine ($29.3 \pm 18.8\%$) and toothpaste ($24.5 \pm 19.0\%$) exhibited significantly ($p < 0.01$) higher mineral gain relative to AS ($6.3 \pm 16.5\%$). With SMH and TMR, remineralization produced by theobromine and toothpaste was not significantly different. With EDS, calcium deposition was significant in all groups, but more with theobromine ($12.8 \pm 8.3\%$) than toothpaste ($9.5 \pm 5.2\%$) and AS ($6.3 \pm 7.5\%$). Theobromine, at a molar level 71 times less than that of fluoride, has a remineralization effect comparable to fluoride.

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

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The aim of this study was to evaluate the effectiveness of herbal medicaments; ginger and rosemary on remineralization of white spot enamel lesion and assessment by a new detection device, FluoreCam. Demineralized human enamel specimens (White, 1987) were measured for baseline surface microhardness, QLF and FluoreCam. Ten specimens in each of six groups were used in this in vitro recycling study with the following treatments applied three times daily: (1) Casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) agent (GC Tooth Mousse), (2) hydroxyapatite and fluoride agent (Voco Remin Pro), (3) Sodium fluoride (NaF) toothpaste (Ipana Kalsi-Dent), (4) Ginger-

honey, (5) Ginger-honey-chocolate, (6) Rosemary. The recycling demin-remin and treatment regimens were continued for 21 days. The post-treatment data were obtained by measurements of surface microhardness, QLF and FluoreCam. Statistical analyses of the data included an ANOVA test with Tukey's HSD test. Significant differences between treatments were observed by microhardness; compared to the positive control group (NaF dentifrice) significantly greater remineralization was observed with the Remin Pro treatment and significantly less remineralization was observed with the ginger-honey treatment regimen. With fluorescence (FluoreCam) assessments significantly greater remineralization was observed with all treatments compared to the positive control (NaF dentifrice) and ginger-honey treatment was numerically more effective than other treatments followed by ginger-honey-chocolate. No significant differences between groups were observed using the QLF fluorescence assessments. Enhanced remineralization was observed with some treatment systems including rosemary, ginger-honey and Remin Pro using FluoreCam but not with QLF.

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ICDAS-Radiographic versus ICDAS-Visual Caries Detection in 6 Year Old Children

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This study aimed to compare the visual-ICDAS-caries detection ability with modified radiographic-ICDAS-caries detection on posterior-primary-tooth surfaces. Seventy-six 6-yr-old children from three Bogotá schools were visually examined with the primary/secondary caries ICDAS-epidemiologic detection system ('A'-ICDAS; scores 0, 1/2, 3–6), by four trained examiners (Inter/Intra-reproducibility-Kappa values: 0.71–0.77/0.70–0.85), also assessing filled and due-to-caries-extracted surfaces. Conventional-bitewing radiographs obtained using a portable-dental-X-ray system were assessed by a trained examiner (Intra-reproducibility-Kappa value: 0.71) with following caries-related (radiolucency) scores: 0-sound, 1-enamel-outer-1/2, 2-enamel-inner-1/2-to-dentine-outer-1/3, 3-dentine-middle-1/3, 4-dentine-inner-1/3, 5-filled-surface-plus-radiolucency, 6-filled-surface, 7-due-to-caries-missing-tooth. A total of 2736 posterior surfaces (36/patient) were assessed by both detection methods. **Results:** Prevalence of caries experience ($n = 76$) (dICDAS4–6mf-s): 53%, increasing to 90% when including early lesions (dICDAS-1/2–3 + 4–6). Mean caries-experience data were 5.3 ± 9.0 (dICDAS4–6mf-s) and 10.1 ± 9.9 (d-s = 6.6 ± 5.2 ; f-s = 1.3 ± 2.4 ; e-s = 0.4 ± 1.8) (dICDAS-1/2–3 + 4–6). Surfaces with highest ICDAS-caries frequency: occlusal-second-upper-molar teeth (70%), distal-first-upper and lower-molar teeth (65%), mesial-second-lower-molar teeth (57%). After excluding unerupted/non-assessable surfaces, data from 1651 surfaces assessed by both detection methods were compared. Of the 596 occlusal surfaces 66% (23% non-cavitated = ICDAS 1/2) were visually scored as carious. Radiographically only 42% (13% scores 1 and 2) were scored as carious. Of the 1055 proximal surfaces 5% (2% cavitated) were visu-

ally scored as carious. Radiographically 39% (14% scores 1–2) were scored as carious. In 5% of the proximal surfaces the radiographic examination disclosed deep dentine caries (middle 1/3 of the dentin or deeper), where 78% of the surfaces were scored sound with the visual examination. **Conclusion:** Data indicate that in the primary dentition on children aged 6, radiographical examination adds significantly to the detection of more lesions than those found during the clinical examination. Thus, its advisable to include radiographs along with the clinical examination in order to perform optimal cariological treatment.

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Clinical Performance of ICDAS-II with Lesion Activity Assessment (LAA) on Occlusal Surfaces of Permanent Teeth

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This study aimed to evaluate the clinical performance of the visual system ICDAS-II with the Lesion Activity Assessment (LAA) in detecting and assessing activity of occlusal caries in permanent teeth. The study was approved by the local Ethics Committee. A sample of 43 third molars was selected from 26 patients (aged 16–39 years) at the Clinical of Oral Surgery, Federal University of Minas Gerais, Brazil. One calibrated examiner ($K_w \text{ severity} \geq 0.67$; $K_{\text{activity}} \geq 0.70$) performed the visual exam using the ICDAS-II with LAA. The most advanced caries on the occlusal surfaces was recorded. After extraction, the teeth were sectioned and prepared for histological examination. A scoring system (Ekstrand et al.: Caries Res 1998;32:247–254) was used as the gold

standard for validation of caries severity. A 0.1% red methyl dye solution was used to validate the caries activity. Area under ROC-curves (A_z), sensitivity, specificity and accuracy for the systems were calculated at D1, D2 and D3 thresholds. The intraexaminer reproducibility for ICDAS-II was 0.60 (weighted kappa) and for ICDAS-LAA was 0.61 (unweighted kappa). The highest ICDAS-II sensitivity (1.0), specificity (0.82), accuracy (0.83) and (A_z) (0.91) were observed at the threshold D3 (dentine lesions). The system ICDAS-LAA showed sensitivity = 0.72, specificity = 0.37, accuracy = 0.46 and (A_z) = 0.55. The association of ICDAS-II with the histological examination was moderate ($r_s = 0.515$, $p = 0.01$). Under clinical conditions, the ICDAS-II is reliable and can detect caries on occlusal surfaces of permanent teeth, especially dentine lesions. The low specificity impacted the accuracy of the LAA system and lead to inferior performance in assessing caries activity.

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The Post-Amalgam Era in Norway: A Survey among Dentists

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Global 'phase down' of dental amalgams has been a recent WHO initiative. In Norway amalgam was banned in 2008 due to environmental reasons. This study aimed to explore dentists' experience after two years clinical dentistry without access to amalgam. Dentists in the county of Troms, Norway, were invited to participate in an electronically delivered questionnaire. Seventy eight dentists (67%) responded after one reminder; 29 were private practitioners and 48 were employed in the public dental health service. Seventeen dentists (22%) had no experience with amalgam therapy, while the others had on average 14 years of experience. More private practitioners supported the amalgam ban from an environmental point of view (55%) compared to public dental health employed dentists (48%) ($p = 0.134$). A total of 30% of dentists were against the ban and 20% were unsure. The dentists were asked to suggest treatment and choice of material of a lower first molar (photo provided) with a MODB amalgam filling showing secondary caries in dentine and a partial fracture. The 22 year old female patient preferred the cheapest alternative. 39% of all dentists would prefer amalgam as restorative material if it was legal to use. No statistically significant difference according to type of practice ($p = 0.704$). 64% of the respondents agreed to the statement 'Longevity of amalgam restorations was better compared to today's alternatives.', while 47% could not support the statement 'Secondary caries is not a greater problem now compared to before the amalgam ban was introduced'. **Conclusion:** The survey suggests that dentists in Norway are not convinced that alternative restorative materials can replace amalgam, although half of the dentists supported the ban from an environmental point of view.

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The Effect of a Single Fluoride Gel Application on Carious Lesions under Severe Cariogenic Conditions

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The aim of this randomized, double-blind, cross-over in situ study, conducted according to good clinical practice guidelines, was designed to evaluate the caries-protective effect of a single tray application of a fluoride gel (elmex gelée; 1.25% F-) on initially demineralized enamel specimens under high cariogenic conditions. 37 subjects wore appliances situated in the lower jaw over two periods of 4 weeks. Previously demineralized bovine enamel slabs were mounted to these appliances. The specimens were covered with a titanium grid and were stored in a 20% sucrose solution twice a day for 30 min to allow for plaque growth and to simulate severe cariogenic conditions. On day one of each period, a 1.25% fluoride gel or a placebo gel was applied for 4 min using the tray application method. During the treatment periods, subjects wore the appliances for at least 20 h per day, but not during eating or oral hygiene practice. The subjects cleaned their teeth with standard fluoride toothpaste (1,400 ppm F- from NaF) for the entire duration of the study. After the clinical phase, specimens were removed from the appliances and cleaned. Half of the specimens were sectioned perpendicular to the exposed surface for measurement of the mineral content and lesion depth using transverse microradiography. The remaining specimens were used to evaluate the fluoride content of the outermost 100 μ m. Specimens treated with the fluoride gel showed a significantly lower mineral loss ($p < 0.001$) and a decreased progression of lesion depth ($p < 0.001$) compared to the specimens of the placebo group. Regarding fluoride content no significant differences were found among the two groups.

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Effect of Iron Supplementation on Salivary Mutans Streptococci Count in Children with Early Childhood Caries

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Iron may have antibacterial effect reducing mutans streptococci (MS) but this has only been demonstrated in vitro or in situ experiments. This study was conducted to evaluate the effect ferrous sulfate supplementation on MS in saliva from children with

iron deficiency anemia and early childhood caries (ECC). Sixteen children diagnosed with anemia (hemoglobin <11.0 g/dl and serum ferritin < 30 µg/l) and ECC were treated with ferrous sulfate supplementation (5 mg/kg) during two months. Saliva samples were collected and MS were enumerated before supplementation and again 30 and 60 days after supplementation began. Children with ECC but without anemia were used as negative medicament control for MS. After application of the Shapiro-Wilk test, data showed non-normal distribution and they were log₁₀ transformed by the Box-Cox method. These were analyzed by t test with a significance of 5%. The ferrous sulfate supplementation reduced significantly the count of mutans streptococci after 30 and 60 days for iron-treated group ($p < 0.05$). The finding suggests that iron supplementation in anemic patients with early childhood caries may have an anticaries effect by reduction of salivary mutans streptococci.

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Compliance to Flossing and Association with Plaque Amount: Motivational and Manual Skills among 5- to 8-Year-Old Children

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We aimed to determine children's adherence to daily flossing and its association with overall amount of dental plaque. Secondly, we intended to identify variables and actual difficulties that might be related to children's negligence in daily flossing. 36 children were selected from those who looked for treatment at our School. The O'Leary index was adopted to evaluate plaque amount. Children answered questions concerning dental flossing (difficulties, self-reported motivation, previous orientation). After, the examiner observed and noted quietly how the patient flossed teeth and possible faults. Univariate and multiple logistic regression analysis were performed and Odds Ratio values were calculated (OR; 95% CI). Lack of adherence to daily flossing was regularly observed (69.4%) and a moderate mean O'Leary index (0.44 ± 0.32) was found. However, the amount of plaque did not differ between those that reported to floss daily or not ($p > 0.05$). Children's self-flossing negligence was strongly associated with self-reported laziness for flossing (OR: 18.80; 1.71–207.10) and negatively associated with their previous orientation by some dentist (OR: 22.36; 2.18–229.62), suggesting problems with children's motivation. Self-described difficulties in self-flossing also showed association with laziness for flossing (OR: 22.31; 2.70–184.03). Although some children (41%) did not report difficulties, none of them performed the flossing technique exactly as recommended, suggesting a lack of dexterity. The main faults observed were concerning curving the floss around the base of teeth (76%),

holding the floss tautly between fingers (73%). Concluding, the children's compliance to dental flossing is low, although the plaque amounts are not directly related to compliance. Low compliance and difficulties to use dental floss seem to be more related to lack of motivation, although problems concerning manual skills were also observed.

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Effectiveness of a Modified Toothbrush in Removing Dental Plaque from Occlusal Surfaces of Erupting Molars

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This study aimed to evaluate the short and long-term effectiveness of anteroposterior toothbrushing using a children's toothbrush with multilevel filaments vs. cross-toothbrushing technique using toothbrush with straight bristles in removing dental plaque from occlusal surface of erupting first permanent molars. Different outcomes concerning dental plaque were chosen: presence of visible plaque, presence of mature plaque, area of disclosed plaque and plaque fluorescence measured by a quantitative light fluorescence device (QLF). Two calibrated and blinded examiners performed the examinations using indices but only one assessed the biofilm using the QLF. Another examiner was responsible for explaining the techniques and checking children's compliance. Thirty-three children aged 5–7 years-old (mean = 6.23, SD = 0.56), who sought for treatment at our school, were included (92 erupting molars). These children were randomly assigned into two groups according to the toothbrushing technique. Teeth were evaluated by the methods at baseline and after 15 days, 1 month and 3 months. Multilevel analyses were performed to determine which technique was the most effective in removing plaque on occlusal surfaces of erupting permanent molars, considering different outcomes. After 15 days, no difference was observed between groups ($p > 0.05$). However, after 1 month, higher reduction in presence of mature and visible plaque were found using the toothbrushes with multilevel filaments (visible: 45%; mature: 29%) than using toothbrushes with straight bristles (visible: 28%; mature: 14%; $p < 0.05$). No changes were observed in area of disclosed plaque and plaque fluorescence using QLF. In conclusion, in a long-term analysis, the toothbrushing with multilevel filaments used in association with anteroposterior technique by children is able to disorganize the plaque on occlusal surface of erupting molars more efficiently than anteroposterior toothbrushing using toothbrush with straight bristles.

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Oral Health Conditions of Children Participating in an Infant Oral Health Program and Their Mothers

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Dental caries is a multifactorial disease that is still one of the biggest challenges in dentistry. The aim of this study was to evaluate oral health, dietary habits and oral hygiene of children undergoing an infant oral health program in comparison to the oral health condition of their mothers. Forty infants were evaluated at 6, 12 and 18 months of age. Gingival conditions and dental caries prevalence were assessed by a single examiner. Diet and oral hygiene were evaluated using a questionnaire. At 6 months of age, 75% of babies were already using baby bottles, 50% slept while nursing and 47% woke up to nurse. The consumption of foods with high content of sticky sugar was not reported at 6 months. However, at 18 months, this consumption was observed among 68% of the babies. Sweetened milk and dairy products such as yoghurt were already part of the diet of 25% of the babies at 6 months. After 18 months, this number increased to 79%. At 18 months, sweetened beverages were consumed by 76% of babies in a mean frequency of 10 times a week. Oral hygiene compliance increased 32% during the evaluation period, reaching 97% of the babies after 18 months. Nocturnal hygiene procedures increased 27%, reaching a total of 82% after 18 months. Only one child (3%) had carious lesions during the study period. There were no changes in gingival condition. At the end of the study, the DMFT of the mothers was 10.61 and 88% had no changes in gingival condition. In conclusion, although there was a considerable increase in high sugar content food consumption, caries prevalence was low due to a greater concern with oral hygiene.

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Comparative Evaluation of Noninvasive and Micro-Invasive Strategies in Improving Esthetics of Post-Orthodontic White Spot Lesions

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The aim of the study was to evaluate the stability of esthetic improvement and patient satisfaction on treating post-orthodontic White spot lesions (WSLs) using Micro-invasive {Resin Infiltration (RI)} and Noninvasive {ACP CPP toothpaste (ACP) and Fluoride varnish (FV)} strategies. 33 WSLs on labial aspect of an-

terior teeth were selected from post-orthodontic patients, who consented for participation. WSLs were randomly divided in three groups and were treated with different agents. Postoperatively, patients were reevaluated after 1 and 2 months. Standardized photographs were taken before, immediately after and at each recall. L*A*B* values were derived and ΔE was calculated for different time intervals. Following application, patient satisfaction scores were recorded ranging from Very Dissatisfied to Very satisfied. All the three materials exhibited shade change postoperatively with maximum change in RI Group ($\Delta E = 19.42$). At subsequent recall visits significant Shade improvement ($p < 0.001$) was seen, using Kruskal-Wallis test, with maximum change observed at 2 months recall as compared to baseline. Intergroup comparisons were done using Mann-Whitney U Test. Significant differences were observed when shade changes were compared between RI with either of the two materials, for preoperative Vs Post operative images at all recalls ($p < 0.001$) while Non-Significant (NS) differences were found between ACP and FV at any time interval. There was no significant difference in shade change between 1 and 2 month post-treatment images for any of the group comparisons. Patient satisfaction was significantly higher in RI group ($p < 0.001$) while Non Significant difference was observed between ACP and FV ($p = 0.702$). In conclusion RI technique immediately provides significantly improved esthetics as compared to other groups. For all the three groups esthetic changes achieved improved with time and at two months they all showed comparable results.

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Effectiveness of an Infiltrant and a Sealant in Artificial Dentinal Occlusal Caries in Primary Molars

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The minimal intervention approach advocates that dentinal occlusal caries lesions might be treated with conventional sealants. Recently, caries infiltration methods have shown that invasive treatments can be avoided by penetration of the lesion. The aim of this 'in vitro' study was to investigate the effectiveness of different treatments using an infiltrant (Icon, DMG) and conventional sealant (Fluorshield, Dentsply) on sound surfaces and on surfaces with cavitated dentinal occlusal caries lesions with different sizes of opening. Seventy-two sound primary molars were randomly assigned to three groups: sound, artificial dentinal caries lesion with 1mm opening and with 2 mm opening. The cavities were made using a spherical diamond bur in water-cooled high-speed handpiece. Artificial caries was induced in the cavitated

groups by pH cycling for 14 days. All groups were treated according to 4 experimental protocols (1 sealant, 2 infiltrant, 3 sealant before infiltrant, 4 sealant after infiltrant) and the sound group was used as control. After 24 h of sealing, the specimens were submitted to mechanical cycling (100,000 cycles, 80N). Subsequently, they were immersed in silver nitrate solution (50% by weight) in complete darkness for 6 h. All teeth were sectioned with two cuts along the central fissure and remained 16 h immersed in photo developing solution. Images ($\times 30$) were taken with the cut side facing up and dye penetration was appraised by two evaluators ($\kappa = 0.9$). Statistical analysis revealed that sealed caries molars showed significantly more dye penetration than sound molars (chi-square test, $p = 0.009$). No significant differences could be observed ($p = 0.05$) among the different experimental treatments. The use of caries infiltration before or after the application of the fissure sealant did not influence the effectiveness of the sealing treatment.

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Dentists' Caries Treatment Choices following Diagnosis Using the Nyvad or the ICDAS II Systems

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The aim of this study was to evaluate differences in operative and non-operative treatment decisions when dentists diagnosed caries lesions with two systems, the Nyvad (NY) and the ICDAS II (IC). Four volunteer dentists were randomly allocated to one of two groups. Both groups of dentists examined the same voluntary and purposeful sample ($n = 140$) of 18–20 year old caries active young adults using the NY and the IC criteria in different sequences. The first group used the NY criteria during period I, followed by IC criteria during period II; the second group did the examinations in the opposite sequence. There was a one-week washout period between the first and the second examinations. After each examination, the dentists made treatment decisions using the following options: background level care, non-operative treatment and operative treatment. The mean number of operative treatment decisions per surface amongst four dentists was consistently higher for the IC [$3.25(\text{SD} \pm 3.58)$] than for the NY system [$2.16(\text{SD} \pm 3.04)$]. The difference in the operative treatments per surface varied from 0.53 to 1.41 [mean $1.09(95\% \text{ CI } 0.70, 1.48)$]. The non-operative treatment decisions per surface were also consistently higher for the IC [mean $6.78(\text{SD} \pm 5.86)$] than for the NY system [mean $4.05(\text{SD} \pm 4.00)$]. The difference in non-operative treatments varied from 0.18 to 5.59 [mean $2.74(95\% \text{ CI } 2.15, 3.33)$]. This study suggests that the use of IC diagnostic system may result in more treatment, both non-operative and operative. We conclude that there is a need to carry out a long-term clinical study to compare the costs and health effects of both diagnostic systems.

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Loss of Sealants in First Permanent Molars of 6-Year-Old Chilean Children after 12 Months

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Objective: To describe the clinical behavior of pit and fissure sealants applied on first molars surfaces of children aged six in Chilean Metropolitan Region, after 12 months follow-up. **Methods:** With the informed consent of parents and ethics committee approval, 219 children (95 males and 124 females) received resin based sealant according to the manufacturing instructions. Baseline and final clinical exam was performed using ICDAS II criteria for first permanent molars surfaces. Information was collected on the baseline characteristics of children such as oral hygiene habits, diet, socioeconomic status and caries history (DMFT and dmft). **Results:** The average initial DMFT was 0.1 (CI95%: 0.04–0.15) and the average initial dmft was 3.3 (95% CI: 2.9–3.7). The global cumulative incidence for caries lesion was 19.2%. 33.8% of sealants were lost (totally lost = 16.4%; partially lost = 17.4%). 19.7% of molars with lost sealants presented with caries lesions after 12 months of follow-up (31% of surfaces with totally lost sealants and 15% of surfaces with partially lost sealants). **Conclusion:** There was a high proportion of sealant losses in this sample after one year despite the previous training of the operators. The technique of sealants placement is crucial and should be followed rigorously to achieve a better clinical behavior of this intervention across the time.

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Effectiveness of Fluoride Varnish for Caries Control on First Permanent Molars of 6-Year-Old Chilean Children

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Objective: To describe the effectiveness of fluoride varnish applied every six months, for caries lesions control on occlusal surfaces of first permanent molars in children aged six of medium-low and low socioeconomic status, living in Chilean Metropolitan Region, after 12 months of follow-up. **Methods:** With the informed consent of parents and ethics committee approval, 222 children (98 males and 124 females) received fluoride varnish according to the manufacturing instructions for three times in the study. Baseline and final clinical exams were performed using

ICDAS II criteria for first permanent molars surfaces. Information was collected on the baseline characteristics of children such as oral hygiene habits, diet, socioeconomic status and caries history (DMFT and dmft). **Results:** The average initial DMFT was 0.03 (CI95%: 0.008 to 0.06) and the average initial dmft was 3.2 (95% CI: 2.8–3.6). After 12 months, 16.4% of initially sound surfaces presented as new lesions and 16.1% of surfaces with pre-existing incipient lesions exhibited clinical progress, according to the ICDAS II criteria. **Conclusion:** In this population, fluoride varnish could be generally considered as an effective intervention for caries control on first permanent molars. Taking into account its technical facility when comparing with other preventive interventions, especially fissure sealants, and considering its advantages and costs, the use of this material should be recommended in a public oral health program context addressing the target population with similar characteristics. On the other hand, other basal measures of caries control must continue to be promoted, since 16.1% of pre-existing lesions progression was observed.

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Longitudinal Evaluation of Surface Integrity of Teeth Affected by Molar-Incisor-Hypomineralization

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The aim of this clinical follow up study was to evaluate the status of tooth surfaces affected by molar-incisor-hypomineralization (MIH). At the baseline of the study 196 first permanent molars from 49 children with MIH (6–9 years old) born in Araraquara, São Paulo, Brazil, were clinically examined by visual and tactile inspection, and data were compiled at baseline, 6 and 12 months using the European Academy of Paediatric Dentistry (EAPD) criteria for MIH severity. The evaluation was conducted by a trained and calibrated examiner of high reliability (Kappa coefficient = 0.88). All patients received preventive therapy: appropriate dietary and preventive advice and, topical fluoride varnish (Duraphat® 22,600ppm F, Colgate Oral Care) and, teeth with enamel breakdowns or restoration failures were restored with GIC (Ketac Molar Easymix, 3M ESPE). Data were assessed using descriptive statistics and Kaplan-Meier survivorship analysis. At 12 months, 90% of the teeth with demarcated opacities presented preserved enamel surface and only 23% of the restored teeth had breakdown, 50% in the restorative material; 43.7% had enamel and material loss associated and only 6.3% had only enamel loss. **Conclusion:** The majority of the molars affected by MIH had the integrity of the tooth surface preserved when only demarcated opacities were present.

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Effectiveness of 5,000 ppm Compared to 1,450 ppm F-Toothpaste when Used on Elderly Disabled Residents in Nursing Houses

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Background: Danish data show that root caries progression occurs frequently in elderly disabled residents living in nursing homes. **Aim:** To compare the effectiveness of brushing teeth with 5,000 ppm or 1,450 ppm fluoridated toothpaste on controlling root caries progression in residents living in nursing homes. Study duration 8 months. Sample: 176 disabled elderly residents in 3 nursing houses in the Copenhagen area consented to be enrolled in the study. They were randomly assigned to use either Duraphat 5,000 ppm F-toothpaste or 1,450 ppm F-toothpaste (Colgate Ultra Cavity Protection). Both groups had their teeth brushed twice a day by nurses. A total of 125 residents completed the study. **Material and Methods:** One trained examiner conducted a baseline and a follow-up clinical examination. Texture, contour, location and colour of root caries lesions were used to evaluate lesion activity. **Results:** No group differences were noted at the baseline examination concerning: Mean age (82.1/81.4 years; 1SD11.7/11.6 years), mouth dryness, number of participants wearing partial of full denture in one of the jaws, plaque and gingivitis and in active (2.61/2.67; 1SD = 1.7/1.8) or arrested lesions (0.62/0.63; 1SD (1.7/1.7) (p values >0.16). The mean number of active root caries lesions at the final examination were 1.07 (2.8) versus 2.66 (1.9) and the number of inactive caries lesion were 2.34 (1.7) versus 0.62 (1.6) in the 5,000 ppm F and the 1,450 ppm F group, respectively. A median test showed that the root caries status of participants in 5,000 ppm F-group had improved significantly, compared to the 1,450 ppm F-group (p values <0.001). **Conclusion:** The data from this randomised clinical trial suggest that 5,000 ppm F-toothpaste is significantly more effective in controlling root caries lesion progression compared to the 1,450 ppm F-toothpaste in elderly disabled people.

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Sealing Dentinal Caries Lesions in Primary Teeth

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The aim of this in vivo study was to assess the effect of a sealant in arresting dentinal caries lesions compared to conventional re-

storative treatment. Forty-two primary molars from thirty six patients with occlusal caries lesion reaching dentine with an opening in enamel not wider than 3 mm diameter have been selected. Radiographically, the lesion should be in the outer half of dentine. The patients were randomly allocated into two groups: partial caries removal and restoration with composite resin (control group) and sealant application (experimental group). The patients were submitted to clinical and radiographic (bitewing) evaluation after 6, 12 and 18 months. One tooth per patient was randomly selected for statistical analysis. Six exfoliated teeth were collected and processed for scanning electron microscopy (SEM) analysis. Kaplan-Meier survival analysis, Fisher's Exact test and linear logistic regression test were calculated in each evaluation period ($p < 0.05$). Control group showed better results in the clinical evaluation after 18 months ($p = 0.0025$). In both groups, all cases showed no signs of carious progression in the radiographic examination for all assessment periods. The tooth/restorative material interface analysis by SEM allowed a qualitative evaluation of the hybrid layer and adhesive system tags formation, as well as the visualization of bacteria in infected and affected dentin. The experimental group showed more areas of interfacial gaps between the sealant and dentin, while in the control group the formation of more homogeneous hybrid layer tags of adhesive system in affected dentin was observed. We conclude that although the control group has presented less restorations failures, both treatments are similar in arresting the caries progress.

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Radiographic Grading Systems for Assessing Approximal Caries Lesion Progression in Clinical Trials of Remineralizing Therapies

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In clinical trials of remineralising therapies it is critical to assess enamel approximal caries lesions. The aim of this study was to evaluate how three radiographic grading systems affect the determination of the number of decayed surfaces, progression and regression of lesions and examiner reliability. Methods Three grading systems (Pitts, Grondahl, & Morgan) were selected as they had comparable gradings of caries (two levels each of enamel and dentine caries) and define methods for the treatment of overlaps. Grondahl excludes all overlapped surfaces from further scoring. Pitts and Morgan allow scoring of caries in the presence of overlaps in certain situations. 100 baseline and final digital bitewing radiographs were sampled from those taken during a two-year caries clinical trial of adolescents. The radiograph reviewer was trained, standardised and calibrated in each grading system prior to its use. Scoring was undertaken in a dedicated room with controlled lighting, a calibrated monitor and custom-built database. Wash-out periods of at least 4 months elapsed between each grading system. Results Intra-examiner reliability kappa scores at calibration were at least 0.9 for each method. D1S

scores were 2.85 ± 0.23 , 2.01 ± 0.18 and 3.19 ± 0.23 ; D3S scores were 0.52 ± 0.07 , 0.33 ± 0.05 and 0.56 ± 0.08 for Pitts, Grondahl and Morgan, respectively. Pitts and Morgan were both significantly different to Grondahl at all caries thresholds ($p < 0.001$); no significant differences were found between Pitts and Morgan. Pitts and Morgan systems detected nearly twice as many progressions and regressions as the Grondahl system. Conclusions Reliability of all grading systems was excellent. The Pitts and Morgan systems provide for greater monitoring of caries progression and regression in approximal lesions. The Grondahl system, which excludes all overlapped surfaces, showed slightly higher reliability, but much lower caries scores and fewer transitions.

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Comparison between Stepwise Excavation and Indirect Pulp Treatment in Primary Teeth with Deep Carious Lesion

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Traditionally, Indirect Pulp Treatment (IPT) have been suggested for deep caries lesions in primary teeth. This study evaluated longitudinal clinical and radiographic performance of Stepwise Excavation (SE), compared to one-visit IPT, in primary molars with deep carious lesions. Also, dentin samples were analysed to compare microbial counts between dentin characteristics. Sixteen children (4 to 6 years old) were included. Radiographically, lesion depth was greater than two thirds of the dentin thickness, without preoperative signs and symptoms of irreversible pulpitis. Teeth were randomly selected and treated either with one-visit IPT ($n = 14$) or SE ($n = 15$). Dentin was classified according to color, consistency and wetness after cavity preparation in both groups at baseline and 6 months later in SE group, when cavity was reopened for tissue re-excitation and collection. In the first visit, results showed bacterial growth in all dentin samples (mean number of colony-forming-units were: total number, mutans streptococci and lactobacilli: 3.6×10^9 ; 1.5×10^7 , 2.1×10^6) and clinical evaluations revealed that 23 cavities (79.3%) were yellow-orange, soft and humid, with higher bacterial counts, being classified as active lesions. When cavities from SE group were reopened, the number of samples with positive growth had decreased (86.6%), characterized by a reduction of bacterial counts (total cfus: 1.9×10^9 , mutans streptococci: 8.2×10^5 and lactobacilli 7.7×10^5), and most of dentinal caries lesions (60%) were arrested. At 1year of follow-up, there was a higher success rate with SE [difference: 22.5%, 95% confidence interval (CI)], defined as an unexposed pulp or sustained pulp vitality without radiolucency or abscess. In conclusion, SE decreased the risk of pulp exposure and showed a higher success rate compared with IPT in primary molars. This approach should be recommended for managing deep caries lesions.

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In vitro Detection of Caries Beneath Dental Sealant with the Canary System

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Quantum Dental Technologies's infrared photothermal radiometry and modulated (CS), aids dentists in theTMluminescence based device, The Canary System detection and monitoring of dental caries. The aims of this study were to (1) evaluate the ability of CS to detect decay beneath dental sealant compared to other caries detections methods, DIAGNOdent (DD), and ICDAS II visual scoring, and (2) correlate the Canary Number (CN), an algorithm calculation based upon the PTR-LUM readings from the tooth surface, to carious lesion depth. 28 extracted human molars and premolars composed of 103 potential healthy and carious pits/fissures on their occlusal surfaces, as determined by ICDAS II scoring criteria, were used. After scanning marked pits/fissures with CS and DD, teeth were sealed with 3MTM ESPETM sealantTM by a clinician not involved in ICDAS II scoring. Following sealant placement, teeth were re-scanned at the same sites as before with CS and DD. Using polarized light microscopy (PLM), examined sites were scored as 'carious' or 'non-carious', and the lesion depth measured. With PLM as gold standard, sensitivities/specificities of CS, DD and ICDAS II before sealant placement were 0.92/0.70, 0.41/1.0 and 0.77/0.90, respectively, and after sealant placement were 0.83/0.79 and 0.64/0.46 for CS and DD respectively. Correlations (r^2) of lesion depth before/after sealant placement with CN and DD were 0.58/0.61 and 0.39/0.33 respectively. Correlation of CN with DD and ICDAS before sealant were 0.50 and 0.80 respectively, and after sealant was 0.44 with DD. There was no significant correlation between CN and sealant thickness ($r^2 = 0.05$). This study suggests CS has the potential to aid dental professionals to detect and monitor caries beneath sealants.

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Health Impact Profile (OHIP), Life Quality and DMF-t in Cidade de Deus, Brazil

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Aim: Evaluated through OHIP-14 questionnaire, own perception form a low social economic area in Rio de Janeiro-Cidade de Deus. It was conducting a Cross-sectional study with 615 indi-

viduals, from 5 to 44years old, divided by age groups. OHIP. Questionnaire was applied in all participants of the program after ethical approval and individual or parents authorization and χ^2 test was used. The results identify that the highest perception of life quality was founded in between the age 20 to 44 years in all impact dimensions, similar to self-oral health perception and high DMF-t scores while young children, had lower perception of oral health impact in life and low dmf-t. The perception increase with age as dmf-t/DMF-T. The psychological discomfort dimension, presented highest life impact between 15 to 19 years of old, and 25 to 44 years old, with extremely bad oral health (DMF-t of 4,2,with higher prevalence in caries cavities and 16,3,with higher prevalence of loose tooth due caries, respectively.) **Conclusion:** Lower impact and self-perception of oral health increase with age according the increase of caries in this population. Oral health policy strategies could be implementing during individuals life to achieve better oral health and quality life for the unprivileged population.

Occlusal Adjustment for Food Impaction on Teeth with a Normal Proximal Configuration

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Food impaction happens in 92.2% of Chinese population. It is a susceptible factor for proximal caries. Food impaction occurs at molar interproximal sites with a relatively normal proximal configuration is related to gap formation. An occlusal adjustment method, sequential occlusal adjustment (SOA) was developed to deal with such problems. The purpose of this study was to test the effectiveness of SOA for food impaction. 45 patients who complained of food impaction in the molar region and had normal proximal configuration, were selected from a clinical investigation of food impaction. All patients were consented to the term of this study. Gaps between the teeth were measured with a feeler. Moulds of the upper and lower teeth were taken for each patient, and occlusal contacts were studied both in vitro and in vivo. SOA was made to reduce any pestle- mortar-like cusp, to create a food escaping groove and to lower the mesial inclines of the tooth cusp. Interdental brushing was advised. Patients were recalled each week until their food impaction symptoms disappeared at a four week period. Food impaction was eliminated in all 12 patients with a proximal gap less than 0.1mm after the first SOE. Patients with proximal gaps between 0.1 and 0.2 mm had decreased symptoms after the first adjustment, 10 of 15 patients did not suffer from food impaction after the fourth appointment and the other 5 patients still had some degree of food impaction. For patients with gaps larger than 0.2 mm, only 4 succeeded in getting rid of food impaction, 5 improved and 9 had no change. **Conclusion:** The use of sequential occlusal adjustment results in an effective elimination of food impaction with small proximal gaps.

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Session 8

Clinical Diagnosis 2

144**Secondary Caries Assessment Agreement in Primary Teeth Restored with Composite Resin***D.P. Raggio^{*}, T.L. Lenzi, E.B. Gonzalez, C. Piovesan, M.M. Braga, F.M. Mendes**danielar@usp.br**School of Dentistry, University of São Paulo, São Paulo, Brazil*

Secondary caries lesions are the most common reasons for restoration replacement, and correct assessment is essential to avoid unnecessary replacements. The aim of this in vitro study was to evaluate intra and interexaminer agreement of secondary caries evaluation in primary teeth, restored with composite resin, using three different methods (visual, using International Caries Detection and Assessment System ICDAS-CARS, radiographic evaluation and quantitative induced laser fluorescence – QLF). Forty two restored and exfoliated primary teeth, obtained from the Human Tooth Bank of Dental School, University of São Paulo, were selected. Two trained and experienced evaluators performed the assessment independently. After one week, they repeated the assessments. Intra and interexaminer agreement were calculated using weighted Kappa. Interexaminer agreement for ICDAS-CARS was 0.8; for radiographic method was 0.87, and for QLF, 0.95. The examiner repeatability for all methods was comparable, varying between kappa of 0.82 and 0.96. All methods showed good reliability in detecting and assessing secondary caries in primary teeth restored with composite resin.

145**Effect of X-Ray Beam Angulation on the Accuracy of Subtraction Radiography for Detecting Occlusal Demineralization***S.R. Rollings^{a,b,*}, D.N.J. Ricketts^b, N.P. Innes^b, T. Macfarlane^a, K.R. Ekstrand^c, S. Martignon^d**samrollings@nhs.net**^aUniversity of Aberdeen Dental School and Hospital, Aberdeen,**^bUniversity of Dundee Dental School, Dundee, UK; ^cUniversity of Copenhagen, Copenhagen, Denmark; ^dUniversity of El Bosque, Bogota, Colombia*

The aim of this in-vitro study was to investigate the accuracy of subtraction radiography in the assessment of demineralization within occlusal cavities, using digital bitewing radiographs taken at varying horizontal and vertical angulations. Forty extracted molar teeth with occlusal cavities were selected and placed in anatomical relationship to each other. Baseline radiographs were taken with the x-ray beam passing tangentially through the contact points and at 90° to the x-ray film (0° angulation). Baseline radiographs were also taken altering the horizontal (7 and 15°) and vertical (–10° and +15°) angulations. Nineteen teeth were selected at random and underwent demineralization within the cavities. For all 40 teeth, 0° angulation radiographs were taken after 12 h. These 0° angulation radiographs were subtracted from the baseline radiographs at the different angulations to produce 200 subtraction images. Five examiners independently viewed the radiographs in random order and scored degree of demineralization using a five point certitude scale: 1 ‘definitely no demineralization’ through to 5 ‘definite demineralization’. The mean areas under the ROC curves (MnAUC) at 0 and 7° horizontal angulation were 0.87 (minimum = 0.82, maximum = 0.94) and 0.85 (minimum = 0.79, maximum = 0.9) respectively. When the hori-

zontal angulation was increased to 15° the MnAUC decreased to 0.67 (minimum = 0.61, maximum = 0.75). Any change made to the vertical angulation also led to a decrease in the MnAUC. At both +15° and -10° vertical angulation the MnAUC was 0.71 (minimum = 0.66, maximum = 0.77; minimum = 0.65, maximum = 0.78 respectively). These results indicate subtraction radiography can accurately detect demineralization within occlusal cavities, when x-ray projection geometry does not differ greater than 7° in the horizontal axis from 0° angulation.

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Performance of ICDAS in Detecting Caries Using an in vitro Methodology to Simulate Epidemiological Surveys

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We aimed to propose a methodology to simulate the examinations performed to detect caries lesions using the ICDAS in epidemiological surveys, evaluating the examiner's performance. A benchmark examiner conducted all training sessions. A total of 80 exfoliated primary teeth were set in arch models to simulate a complete mouth in primary dentition (4 lower and 4 upper arches). Sixteen examiners (graduate students) evaluated the teeth under illumination using buccal mirrors and a ball ended probe, using only coronal primary caries scores of the ICDAS. As a reference standard, two different examiners scored the approximal surfaces by direct visual inspection, classifying them in sound, non-cavitated or cavitated lesions. Afterwards, teeth were sectioned in bucco-lingual direction, and the examiners assessed each section using stereomicroscope, classifying the occlusal and smooth surfaces according to lesion depth. Inter-examiner reproducibility was evaluated using weighted kappa. Sensitivities and specificities were calculated at two thresholds: all lesions and advanced lesions (cavitated lesions in approximal surfaces and lesions reaching the dentine in occlusal and smooth surfaces). Regarding the reproducibility, the mean (range) of kappa values was 0.781 (0.529–0.927) for occlusal surfaces, 0.568 (0.191–0.881) for smooth surfaces, and 0.844 (0.698–0.971) for approximal surfaces. At all lesions threshold, sensitivity and specificity mean values were respectively 0.724 and 0.844 for occlusal, 0.635 and 0.943 for smooth and 0.658 and 0.927 for approximal surfaces. For detecting advanced lesions, sensitivities and specificities were 0.563 and 0.920 for occlusal, 0.670 and 0.985 for smooth, and 0.838 and 0.985 for approximal surfaces. In conclusion, the ICDAS exhibits good validity and acceptable reliability and the methodology used could be used to train and calibrate prior to epidemiological surveys, possibly improving the performance of the examiners.

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Restorative Treatment Decisions Reported by Chilean University Teachers

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The aim of this study was to assess caries management strategies among Chilean university teachers. The study population consisted of 130 teachers of operative dentistry, paediatric dentistry and/or integrated dental practice, of 9 dental faculties of the Metropolitan Region, Chile. A validated Spanish version of a pre-coded questionnaire (Espelid, 2001) concerning approximal and occlusal caries diagnosis and restorative treatment decisions was applied to the respondents. The questions were related to knowledge and beliefs about caries, treatment strategies and choice of dental materials. **Main Results:** For the case question, 'In an adolescent with low caries activity and good oral hygiene', 34.62% of the teachers stated that they would automatically restore a primary approximal caries lesion confined to the enamel or that had reached the enamel dentin border. Moreover, 52.30% of the teachers would only consider immediate restorative treatment of an occlusal surface if obvious cavitation and/or radiographic signs of dentin caries could be observed. Composite resin was the material of choice selected for restoring both, approximal and occlusal surfaces. More than half of the teachers would monitor a lesion detected radiographically near the enamel dentin border for six months in order to determine whether it was active and to evaluate its rate of progression. **Conclusions:** The results illustrate a wide disparity among Chilean university teachers concerning diagnosis, restorative treatment thresholds for approximal and occlusal caries and knowledges about the rate of caries progression and the need for monitoring incipient lesions.

Colgate-Palmolive Chile funded the printing of the questionnaires.

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Comparison between DMF/dmf and ICDAS Indexes in Silva Jardim, RJ

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Epidemiology is essential to determine clinical need and to evaluate oral health professional interventions and treatments. According to the index used, dental treatment planning could be

different. **Aim:** To compare ICDAS with the DMFT/dmft (WHO) in 220 schoolchildren aged 3–12 years and attending public schools in the Municipality of Silva Jardim. **Methods:** A cross-sectional survey was undertaken between September 2009 to December 2010. After obtaining ethical approval and signed parental consent, a single examiner (RV) recorded the presence of cavitated and non-cavitated caries lesions, after tooth cleaning and drying, using the two different criteria. **Results:** dmft was 2.85 in the 3–5 year-olds and DMFT was 3.47 in the 6–12 year-olds. ICDAS was scored in 1389 lesions of which 279 lesions were scored 1/2 (20.1%), 303 were scored 3 (21.8%), 121 were scored 4 (8.7%) and 686 lesions were scored 5/6 (49.4%). Based on the WHO, DMF/dmf criteria only 703 lesions (86.6%) would not be considered carious requiring treatment compared to 13.4% of those diagnosed using ICDAS. **Conclusion:** Caries prevalence increased as the diagnostic criteria became more sensitive. ICDAS, besides providing information on non-cavitated caries lesions, detected a significantly higher dental caries prevalence compared to DMF/dmf but can also generate data comparable to that obtained using internationally accepted WHO criteria.

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Impact of Activity Assessment on Caries Parameters in Epidemiological Surveys of Preschool Children

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This study evaluated the magnitude of the reduction in caries associated parameters after inclusion of dental caries activity assessment considering cavitated and non-cavitated caries lesions in an epidemiological survey with preschool children. The survey was carried out in Santa Maria, Brazil, during the National Children's Vaccination Day, and 639 children aged 12 to 59 months were included. Fifteen examiners calibrated using ICDAS and additional lesion activity assessment criteria performed the examinations. Dmft, dmfs, caries prevalence, and 95% confidence intervals, were calculated, firstly considering all lesions using different thresholds of ICDAS. Afterwards, caries activity status was considered and inactive lesions were classified as sound in this second analysis, and the same caries parameters, at the same thresholds, were recalculated. The reduction and the number need to be assessed were also calculated. When including all visually detectable lesions (non-cavitated and cavitated), the dmfs, dmft and prevalence increased (6.6, 4.0 and 69%, respectively) compared to the values when only active lesions were considered (5.7, 3.5 and 63%). At cavitated threshold, the reduction was lower: 10% for dmfs (all lesions = 2.3; active lesions = 2.1), 9% for dmft (all lesions = 1.4; active lesions = 1.2) and 2% for caries prevalence

(all lesions = 38%; active lesions = 37%). The number of children who needed to be assessed in order to change their status from decayed to sound was 15 considering all lesions, but this value was around 106 if we included only cavitated lesions. In conclusion, the inclusion of activity assessment in caries epidemiological surveys has little impact on the dmfs, dmft and prevalence of dental caries when only cavitated caries lesions are included. The impact, however, is higher at non-cavitated thresholds.

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Dental Caries Lesion Severity and Activity in a Belarusian Adult Population

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The aim of the study was to assess the prevalence, severity and activity of carious lesions in adult population living in Borisov city, Belarus. A voluntary sample of 18–34 (G1), 35–54 (G2) and 55–64 (G3) years of age adults was generated. Dental examinations were performed by the trained and calibrated examiner with intra-oral mirrors, CPI and sharp probes, using dental equipment with standardized drying and light capacity. Before the examinations, supervised tooth brushing was carried out for each patient. For caries lesion detection and severity assessment the ICDAS II diagnostic criteria were used. Lesion activity was assessed by the Nyvad criteria. For the 164 (G1), 262 (G2) and 65 (G3) age groups participants the mean ($\pm$ SD) DMFS values were: 57.38 ($\pm$ 27.5); 94.30 ($\pm$ 32.48) and 112.12 ($\pm$ 29.65) respectively. The proportion of the D component out of DMF index was reduced with age [60%, G1; 30%, G2; 20%, G3]; the F component was approximately stable (25; 32 and 30% respectively) while the M component was increased with age (14; 39 and 53% respectively). The prevalence of active caries lesions was high in all age groups and varied from 77% (G3) to 89% (G1). The mean D active component in the three age groups was 9.20 ($\pm$ 11.16); 7.16 ($\pm$ 11.65); 5.91 ($\pm$ 7.07) respectively. Among all active lesions the proportion of non-cavitated active lesions was reduced with age from 39% (G1) to 13% (G3) while the proportion of dentinal/cavitated lesions increased from 61% (G1) to 87% (G2 and G3). This study suggests that the caries lesion severity in Belarusian adults tended to increase with age and the activity of carious lesions was constantly high in all age groups.

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The Caries Assessment Spectrum and Treatment (CAST) Index among Children in Northwest Russia: Pilot Study

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The aims of the pilot study were to assess the caries experience of children aged 3 to 4 years old and to test usefulness of CAST index [Frencken, et al., *Int Dent J* 2011;61:117–123] in Russia. In December 2011, a cross-sectional survey was conducted in two (out of 8) randomly selected kindergartens by one examiner. Total sample comprised 157 children (49% boys) with mean age of 3.5 (SD 0.5) years old. The study was approved by the Ethical Committee of the Northern State Medical University, Arkhangelsk, Russia. All parents gave informed consent. Prior to examination, the child's teeth were cleaned by toothbrush/toothpaste under supervision. Each coronal tooth surface was assessed using the CAST criteria. The caries experience of primary teeth was expressed as d(3–5)mft/d(3–5)mfs values (presented as mean $\pm$ SD). Clinical consequences of untreated carious lesions were diagnosed as 'code 6' (pulpal involvement) and 'code 7' (abscess). Chi-squared tests and Mann-Whitney tests were used for the dichotomous and numerical data, respectively. The prevalence of dental caries (including enamel and dentine lesions) was 81.5% (95% CI: 75–87). Mean values of d3–5 mft/d3–5 mfs, d3 mft/d3 mfs and d4–5 mft/d4–5 mfs were $4.8 \pm 3.7/7.8 \pm 7.6$, $1.4 \pm 1.5/2.3 \pm 4.0$, $4.2 \pm 3.6/7.0 \pm 7.8$, respectively. No gender differences were observed. The number of enamel and dentine carious lesions increased significantly with age ($p < 0.001$). The prevalence of pulpal involvement was 38 (32%) (95% CI: 24–40) and that of abscess was 21 (15%) (95% CI: 10–22) with mean scores of 0.4 ± 0.8 and 0.3 ± 0.9 , respectively. **Conclusion:** Dental caries situation is not under control in the city Onega and urgent preventive and cure measures are needed to improve the situation. CAST index was easy to use.

Impact of Different Diagnostic Criteria on the Assessment of Risk Indicators for Dental Caries

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Objective: To assess the impact of different diagnostic criteria on the assessment of risk indicators for dental caries using a representative sample of 12-year-old South Brazilian schoolchildren.

Methods: A cross-sectional survey was undertaken in Porto Alegre, Southern Brazil, from September 2009 to December 2010. A multistage probability sampling strategy was used to select a representative sample of 12-year-old schoolchildren attending public and private schools. After tooth cleaning and drying, a single examiner recorded the presence of cavitated and non-cavitated caries lesions. A questionnaire gathered socio-demographic information. Three different thresholds were used to define the decayed component of DMFT: WHO, WHO + white spot lesions, and ICDAS. The association between socio-demographic variables and dental caries was assessed using Poisson regression models. Prevalence ratios (PR), rate ratios (RR) and 95% confidence intervals were estimated. **Results:** 1,528 of 1,837 eligible schoolchildren participated, yielding a response rate of 83.17%. Caries prevalence and DMFT increased as the diagnostic criteria became more sensitive (WHO: 55.23% and 1.39; WHO + white spot lesions: 63.33% and 1.95; ICDAS: 79.82% and 3.78). A significant association was found between caries prevalence and socioeconomic status, mother's and father's education, crowding and number of siblings, which remained unaltered irrespective of the diagnostic criteria ($p < 0.05$). The association between caries prevalence and gender was the only one influenced by the diagnostic criteria (WHO: PR = 0.90, $p = 0.009$; WHO + white spot lesions: PR = 0.91, $p = 0.01$; ICDAS: PR = 0.95, $p = 0.15$). When the DMFT was used as the outcome, the diagnostic criteria exerted no effect on the associations (all of them presented $p < 0.05$). **Conclusion:** The inclusion of non-cavitated caries lesions did not promote major impact on the association between dental caries and socio-demographic variables.

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Stepwise, Partial or No Caries Removal versus Complete Dentinal Caries Removal: A Cochrane Systematic Review

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Objectives: To compare stepwise, partial or no dentinal caries removal with complete removal for management of dentinal caries, in unrestored primary and permanent teeth. **Methods:** MEDLINE, EMBASE, CCRCT (CENTRAL) and Cochrane Oral Health Group Trials Register databases were searched to July 2011 for randomised control trials comparing stepwise, partial or no dentinal caries removal with complete caries removal. Primary outcomes were: exposure of dental pulp; signs or symptoms of pulpal disease; progression of caries and restoration failure. Study eligibility for inclusion, quality assessment and data extraction were carried out independently by three authors. **Results:** 608 titles/abstracts were initially identified. Eight trials were eligible for inclusion (934 patients with 1,372 teeth). Four studies investigated primary teeth, three permanent and one included both. There was variation in the extent of initial caries lesions in the studies and

clinical heterogeneity from the descriptions of caries removal even within intervention groups. The spread of interventions was: Stepwise in three studies, both partial and stepwise in one, partial in two and no caries removal in two studies. For exposure of dental pulp; stepwise and partial caries removal (six studies) resulted in significantly fewer pulpal exposures than complete caries removal (risk ratios 0.44 [95% CI 0.33, 0.6] and 0.23 [95% CI 0.08, 0.69] respectively). The interventions did not result in increased signs or symptoms of pulp disease in any of the eight studies. Similarly, there was no difference in restoration failure, apart from one study showing an improved outcome in the no caries removal arm (preformed crowns on primary teeth). **Conclusions:** Stepwise and partial excavation reduced the risk of pulpal exposure and no interventions were associated with increased pulpal signs or symptoms or restoration failure.

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Resin Infiltration of Fissure Caries with Different Techniques of Pre-Treatment in vitro

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Resin infiltration of the enamel components of pit and fissure caries might be a novel approach to arrest lesion progression. The aim of this in vitro study was to evaluate the influence of various modifications of a commercially available infiltration set on the penetration of the infiltrant into fissure caries. Extracted human molars and premolars (n = 140) with fissure caries (ICDAS code: 2) were allocated to seven groups. Group A (control) specimens were etched with 15% HCL gel (Icon etch, DMG) for 120 s and subsequently resin infiltrated for 180 s (Icon infiltrant). Groups B to G were treated accordingly, but 1% sodium-dodecyl-sulfate and 15% HCL solution (1:1) were added to the etchant to reduce surface tension and viscosity. Additionally, in group C the etchant and in group D the etchant as well as the infiltrant were applied using an oscillating tool. In groups E, F and G instead, the etchant, mixed with 2% pumice, was applied using either a conventional (F) or a modified tooth brush (E, G). Moreover, in group G the time of etching was shortened to 30s. Lesion depths (LD) and penetration depths (PD) were analyzed using dual fluorescence staining and CLSM. Percentage penetration (PP) was calculated as 100xPD/LD. PP [median (Q25/Q75)] was significantly higher (p < 0.05; Mann-Whitney test) in groups E [92 (85/98)%] and G [91 (76/98)%] compared with the control group (A) [56 (29/72)%]. In contrast, groups B [33 (12/67)%], C [73 (35/91)%], D [78 (45/99)%] and F [57 (41/72)%] showed no significant differences compared with group A. It can be concluded that micro-abrasion of the surface using a modified brush was most effective to enhance resin infiltration into enamel parts of fissure caries lesions in vitro.

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Effect of the Chinese Medicine Beijing Propolis on Biofilm-Mediated Enamel Demineralization in vitro

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Beijing propolis is a medicine derived from honeycomb. In solution the material can inhibit the growth and adherence of cariogenic bacteria. The objective of this work was to study the effect of various preparations of Beijing propolis on human enamel demineralization. Forty extracted sound human premolars were cleaned, sterilized and randomly divided into 5 groups. The buccal surfaces were treated with aqueous solutions containing (A) 0.125% water-extracted propolis, (B) 0.5% water-extracted propolis, (C) 0.16% ethanol extracted propolis and (D) 0.16% chlorhexidine respectively, and a water control (E). Next, artificial caries lesions were formed by immersing each tooth in a liquid culture of *S. mutans* and *S. sobrinus* for 48 h. The treatment/immersion sequence was then repeated. Lesion depths and demineralization extents were determined by microradiography and analyzed through a computer graphic analysis system. Mean demineralization extents (SD) of artificial caries lesions were (Vol%.um): (A) 1884 ± 481, (B) 932.7 ± 295.9, (C) 1966.6 ± 449, (D) 1408.9 ± 871, (E) 2628 ± 376. Values for the groups treated with the two kinds of propolis solution (A, B and C) were significantly less than those in the control group E (p < 0.05; ANOVA followed by t-test), with that for the group treated with 0.5% water-soluble propolis being the least. Mean lesion depths gave similar significant differences between treatments. Pre-treatment of enamel blocks with Beijing propolis reduced biofilm-mediated demineralization.

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Effects of Mouthwashes on Human Enamel and Restorative Surfaces

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The impact of long-term exposure of mouthwashes containing ethanol and essential oils or hydrogen peroxide on human enamel and restoratives is of interest to dental professionals and researchers. This in-vitro study compared three mouthwashes and

a control for their effects on enamel and restoratives, using various exposure regimens. Listerine® Coolmint, Listerine® Total Care (100 ppm fluoride) and Listerine® Whitening were compared with a control (hydroalcohol solution). All enamel and restorative surfaces (nanoparticles composite resin and feldspathic ceramic) were immersed in the mouthwashes for three cycles of 10, 30 or 60 min each. Between treatment cycles the samples were immersed in artificial saliva for 10, 30 or 60 min at 37°C. These three different cycles simulated 1, 3 or 6 months of use. The samples were analyzed through Scanning Electron Microscopy (SEM) to study ultra-structural changes, Infrared Spectrophotometry (micro-FTIR) and Energy-Dispersive X-ray Spectroscopy (EDX) for biochemical and elemental aspects, and colorimetry to analyze color changes. The enamel and restoratives ultra-structure and morphologies did not change with any of the mouthwashes after all treatment cycles. No variations in relative calcium (Ca) and phosphorus (P) concentration for enamel; in Zirconium (Zr), Silicon (Si), Ytterbium (Yb) and Barium (Ba) for composite resin; in Silica (Si), Potassium (K) and Aluminum (Al) for ceramic were observed between pre-post treatments for all the mouthwashes. No changes in biochemical and color properties were detected for all specimens. However for whitening rinse there was a whitening effect on the natural enamel surfaces. Long-term exposure of these mouthwashes that are by design formulated at low pH on human enamel and restoratives caused no ultra-structural and biochemical changes and hence are safe for continuous use.

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Bioconcentration of Organochlorine Pollutants in Deciduous Enamel of Children from a PCB-Polluted Area

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Polychlorinated biphenyls (PCBs) and organochlorine pesticides are lipophilic, persistent and ubiquitous environmental pollutants. We have shown that PCBs cause developmental dental defects and dental caries [Jan, et al., Caries Res 2000;34:469–473]. The aim of this study was to examine the bioconcentration of organochlorine pollutants in deciduous dental enamel of children from areas with different PCB pollution levels. Exfoliated deciduous teeth (n = 46) were collected from 8–14-year-old children who were pre- and post-natally exposed to PCBs in a PCB-polluted area (Semič Slovenia), where an electro-industrial plant used technical PCB mixtures (Pyralene-1.500 and -3000). Control teeth (n = 46) were from children from an unpolluted area (Bršljin). Enamel was cut off and residual levels of PCBs (PCB-28, -66, -74, -99, -138, -153, and -180) and organochlorine pesticides [hexachlorobenzene (HCB) and 1,1-bis (4-chlorophenyl)-2,2-dichloroethene (DDE)] were determined by high-resolution gas chromatography with electron capture detection. The levels of PCB-28, -66, -74, -99, -138, -153, and -180 in deciduous dental

enamel from Semič; were 2.8 ± 0.19 , 1.4 ± 0.13 , 0.91 ± 0.10 , 0.70 ± 0.12 , 4.8 ± 0.09 , 4.2 ± 0.65 , and 2.3 ± 0.13 µg/g orig. basis, respectively. PCB levels in enamel from Bršljin were under the limit of detection. Levels of HCB and DDE in enamel from Semič were 0.50 ± 0.12 and 3.2 ± 0.47 µg/g orig. basis, respectively, and from Bršljin 0.41 ± 0.16 and 2.9 ± 0.37 µg/g orig. basis, respectively. The difference was statistically significant for PCB congeners (p = 0.000), but not for DDE (p = 0.46) and HCB (p = 0.50). This was the first study to show that organochlorine pollutants bioconcentrate in human dental enamel. PCB levels in deciduous enamel from the PCB-polluted area were higher than from the unpolluted area. It is suggested that dental deciduous enamel could be used as an environmental pollution marker.

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Mastication Resistance of an RMGI Coating Material in an Artificial Oral Environment

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Aim: Sealants are critical in the prevention of tooth decay; however, they must endure the severe stress of chewing in order to perform this function. The aim was to determine the wear and adherence of sealants under simulated non-contact chewing conditions with a food bolus. Sealants studied were 3M™ ESPE™ Vanish™ XT Extended Contact Varnish (VXT: resin-modified glass ionomer); 3M ESPE Clinpro™ Sealant (CP: resin); GC™ Fuji™ Triage Capsule (FT: glass ionomer). Experimental Approach: Human maxillary third molars were scanned with a contact digitizer before and after sealant placement per manufacturer's instructions. Mastication with opposing human palatal cusp and food bolus (millet & rice) was done in an Artificial Oral Environment [DeLong, et al., 1991]: 37°C, 150 k cycles, 10–12 N load, 1 mm lateral excursion, n = 5. Teeth were scanned after mastication; digital scans were analyzed with Ansur® software (DeLong, 2007) to calculate volume and depth differences. Data were analyzed via one-way ANOVA and compared with Tukey's T-test (p < 0.05). **Results:** Volume change (mm³, mean (StDev) and depth change (mm, mean (StDev): VXT 0.778 (0.606) A, 0.001 (0.013) a; FT 1.620 (1.074) A, 0.003 (0.024) a, CP 1.226 (1.276) A, 0.004 (0.029) a. Superscript letters denote groups that are not statistically different. **Conclusions:** The total volumetric and depth wear of all three materials were not statistically different. Both FT and CP showed evidence of material flaking away in the regions around the two-body contact; VXT did not show this effect. Thus, it is concluded that VXT resists chewing wear as well as CP and FT in this in vitro model.

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Evaluation of Treatment Decision for Carious Lesions by Undergraduate Dental Students

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In Brazil, dental caries is still the most prevalent disease in the oral cavity. Therefore, dental students need to receive a systematic education in cariology based upon current best evidence. The object of this study was to evaluate the therapeutic decision making for dental caries, of the undergraduate dental students in all universities of the Federal District, Brazil. After approval of Ethics Committee of the Catholic University of Brasilia, all students in the last semester of the dental course (160 students) were invited to participate to the investigation. In the federal district

there are 4 universities of dentistry and all of them were included in this research. After signed the informed consent, a questionnaire previously validated in a pilot study was completed by students. The questionnaire containing eight multiple choice questions based on a diagram representing five different stages of carious lesions on interproximal sites from deciduous and permanent teeth. The data obtained were statistically analyzed by the chi-square test at a 5% level of significance. A total of 132 questionnaires was obtained. 30.3% of respondents opted for immediate restorative treatment for the injuries to deciduous teeth in the dentin-enamel junction. Statistical analysis showed a significant difference in the philosophy of universities concerning the removal of dental caries in permanent teeth ($p = 0,001$). In conclusion, it was observed that there was no consensus in the teaching of dental caries decision making in universities of the Federal District. Therefore, there is a need to make a document with the knowledge base of cariology in order to implement the teaching strategies for training the future professional accordingly to the best evidence in the study of dental caries.

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