Caries Research

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Abstracts

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Caries Research

Session 1
Nathan Cochrane Young Investigator Award

1

The Effect of Different Criteria in the Assessment of Caries Lesions Around Restorations in Children

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This study aimed to evaluate and compare two different visual criteria for evaluation of restorations in primary teeth: World Dental Federation (FDI) and Caries Associated with Restorations and Sealants (CARS). This research is part of a randomized clinical trial (CARies DEtection in Children 3 – CARDEC 3). Restorations in primary teeth (n = 635) of 160 children (3–10 years old) were assessed by one trained and calibrated examiner. Children were randomized to have their restorations evaluated and treated according to the FDI or CARS criteria. After reaching the treatment decision with the allocated group, the same examiner performed another evaluation according to the other criteria. Multilevel linear regression analysis was conducted to compare the time spent during evaluations. Spearman's correlation coefficients (Rho) between the methods and 95% confidence intervals (95% CI) were calculated. Kappa test (95% CI) and Chi-square were conducted to compare the restorations' treatment decision into the categories: (0) no treatment/non-operative treatment, (1) repair and (2) replacement of restorations. Multiple linear regression analysis showed that the evaluations performed with the CARS criteria were less time-consuming (p = 0.005). A strong correlation was observed between CARS and FDI method for marginal adaptation (Rho = 0.792; 95% CI = 0.761 to 0.819) and recurrence of caries (Rho = 0.932; 95% CI = 0.921 to 0.942), while marginal staining (Rho = 0.221; 95% CI = 0.146 to 0.294) showed a weak correlation between them. The Kappa value for the agreement between FDI and CARS was 0.641 (95% CI = 0.591 to 0.692). FDI criteria led to a higher proportion of more invasive treatments. From 635 restorations, 101 (15.9%) were indicated for replacement with FDI criteria. On the other hand, with CARS method, only 31 (4.9%) were indicated for replacement (p < 0.001). In conclusion, both FDI and CARS criteria had a similar performance to assess caries lesions around restorations in primary teeth with FDI being more time-consuming and suggesting a more invasive treatment approach.

The study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; Grant Nº 2017/22897-3), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Grants Nº 141425/2017-2 and 420458/2018-2).

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Children's Acceptability Regarding Two Restorative Treatments in Primary Molars: A Randomized Clinical Trial

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The present study aimed to evaluate the acceptability, represented by self-reported discomfort and cooperation, of children submitted to Conventional Restorative Treatment with Composite Resin under Rubber Dam Isolation and Local Anesthesia (CT) or Atraumatic Restorative Treatment (ART). This study is nested to a cluster-randomized non-inferiority clinical trial whose primary outcome was to compare the longevity of two restorative treatments in primary molars of children aged 3 to 6 years

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(NCT02562456). The treatments were performed in a dental care trailer set up as a regular dental office. Both self-reported discomfort and participants cooperation were measured immediately after the end of each restorative session using the Wong-Baker Facial Scale and a 5-point-scale respectively. To test the association between independent variables and the outcomes discomfort and cooperation, Poisson regression test was applied in multilevel analysis. Both outcomes were dichotomized as absence of discomfort (scores 0 and 1), presence of discomfort (scores 2, 3 and 4), cooperative behavior (0, 1 and 2) and uncooperative behavior (scores 3 and 4). It was performed 509 restorations on 220 participants. The absence of discomfort was reported in 89% of the restorative sessions. There was no statistical difference regarding discomfort (IRR 0.94–95% IC 0.63–1.40) between the treatment groups, which presented similar distribution of the Wong-Baker scores. Children who had already received local anesthesia as well as children with lower caries experience reported less discomfort than their counterparts. Participants cooperated positively in 92% of the restorative sessions. The cooperation was not statistically different between treatments (IRR 0.71–95% IC 0.40–1.28). Girls and older children cooperated better than their counterparts. Discomfort and cooperation outcomes presented significant association. CT and ART present low discomfort and high cooperation rates among children.

The authors would like to thank the Fundação de Amparo à Pesquisa do Estado de São Paulo—FAPESP (grants #2015/11356–6) for funding this trial.

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Is Sealing Moderate Caries Lesions Using Glass Ionomer an Alternative to Restorations? 2-Year Follow-Up

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This study aimed to evaluate, after 2 years, the efficacy of sealing occlusal moderate caries lesions (ICDAS 3–4) in primary teeth using high viscous glass ionomer cement (HVGIC) compared to restore them using the same material. For that, a non-inferiority clinical trial was designed (NCT03005405) and children (3 to 6 years old) were selected. Children were randomly allocated in two groups, according to the interventions: HVGIC restoration (opening using high-speed burs and caries removal using hand excavators) or HVGIC sealant (no opening, no caries removal). Children were evaluated biannually for 24 months and treated teeth were assessed using WHO/ICDAS criteria and the criteria for ART restorations/sealants (Frencken,1998). To estimate the efficacy of treatments, 2 outcomes were chosen: need of restoration/sealant replacement and caries progression (to ICDAS score 5–6). Failed sealants/restorations were replaced, if necessary, during the study. Cox regression analyses were performed using shared frailty models (single first failures) and the conditional risk set models (multiple ordered failures during 24 months) were performed to verify the influence of the intervention on described outcomes and Hazard Ratios (HR) were calculated. Independent variables were used to models adjustment. 105 children (204 surfaces) were included. Approximately 20% of primary restorations/sealants required replacement (restoration: 18%, sealants: 27%, p = 0.10) and a similar mean time until the first single failure was observed between groups (t = 23 months). Including the survival of the replaced restorations/sealants, the failure rates increased to 22% (restorations) and 37% (sealants) (HR = 1.97; p = 0.04). Caries progression was observed in few cases (6%), there were no differences between the treatments (p = 0.55). After a 2-year perspective, sealing moderate caries lesions using HVGIC may be used to arrest the lesions progress in primary teeth, but more treatment failures may require more reinterventions.

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Indirect Pulp Capping in Permanent Teeth: A Randomized Controlled Trial

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This randomized controlled clinical trial evaluated permanent molars and premolars with deep caries lesions, which received indirect pulp protection with calcium hydroxide cement or a universal adhesive system 12 months after the clinical intervention. 88 patients (mean 27 years old [8–55 years]), 86 molars and 30 premolars with deep caries lesions (radiographic involvement ≥50% of dentin depth), pulp sensitivity positive to cold thermal test, absence of spontaneous pain, negative sensitivity to percussion tests, absence of cuspid loss and absence of radiographic image suggestive of periapical lesion. Caries lesions received either conventional treatment or selective carious tissue removal and were randomly allocated to treatment groups: (1) control group which received indirect pulp protection with calcium hydroxide cement (HC) (Dycal, Dentisply, RJ, Brazil) and (2) test group which received universal single bond (UA) (Universal single bond, 3M Espe, Brazil). Treatments and evaluations were carried out by 3 researchers. After 12 months the outcome of success for pulp vitality was evaluated by positive pulpal sensitivity to cold, absence of image suggesting periapical lesion, absence of spontaneous pain and negative sensitivity to vertical percussion. Survival analyses were performed to estimate treatment success rates and to define associated variables using Weibull regression model. Success rates were 92.6% for the control group and 98.3% for the test group (P = 0.21). Five cases of failure were recorded in the HC group (7.8%) and only one in the UA group (1.9%). Results suggest there is no difference using calcium hydroxide cement or universal single

bond for indirect pulp protection in deep caries lesion at 12 months follow-up, with a high success rate with both caries removal technique.

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Diagnosis, Risk Assessment and Treatment Decisions for Tooth Wear: A Case-Based Survey Among Belgian Dentists

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This study validated a case-based survey method and analyzed the extent to which Belgian dentists apply current concepts and strategies for tooth wear diagnosis, risk assessment and treatment decisions. A case-based, pre-coded questionnaire consisting of 10 clinical cases/patients with 20 teeth was developed. The cases were set up in a PowerPoint presentation illustrating patients with different types of tooth wear, levels of severity and risk. Dentists (n = 104), recruited at continued education courses, were from 8% of Belgian municipalities. Content and face validity of the method was established by using a panel of experts (n = 3) to assess the method's reliability using a test-retest procedure (k = 0.88) and establish a benchmark. Measurements of agreement between dentists and benchmark assessments at dentition level showed moderate agreement for overall cases/patients' tooth wear diagnosis (κ = 0.55) and risk assessment (κ = 0.40). At tooth/surface level, assessment of the clinical and radiographic severity of tooth wear was moderate (κ = 0.57) and good (κ = 0.64), respectively. Regarding treatment decisions, Belgian dentists showed poor agreement (κ = 0.38). Multivariate Poisson regression analysis, with the dentists and the cases/patients as cluster variables, showed a significant influence of the overall cases/patients' diagnosis being pathological wear (RR = 1.39; $p < 0.001$) together with the overall case/patients' risk being assessed as moderate or high (RR = 1.39–1.43; $p < 0.001$) for operative treatment. The type of tooth wear being erosion (RR = 1.62; $p < 0.001$) or abrasion (RR = 1.88; $p < 0.00.1$) also influenced significantly the decision for operative treatment. In conclusion, the case-based survey method was validated for content and for face validity. Belgian dentists apply to some extent current concepts on overall patients' tooth wear diagnosis and risk assessment and reasonably well the clinical and radiographic concepts of individual tooth/surface wear severity. Improvement is warranted concerning treatment decisions.

6

Caries Detection Using a 3D Intraoral Scanner Emitting Blue Light. An *in vitro* Assessment

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This study aimed (a) to define optimal cut-off limits for detection of occlusal caries lesions using fluorescence induced by a 3D intraoral scanner and (b) to evaluate the validity of the method. To define optimal cut-offs, 60 freshly extracted permanent posterior teeth were scanned with a 3D intraoral scanner (prototype based on 3Shape TRIOS intraoral scanner, not commercially available) which emits blue light. A texture representing the fluorescent signal from the tissue was mapped onto 3D models using specific software. Red (R) and Green (G) color components from 250 sound or carious sites located on occlusal surfaces of the teeth were used to calculate a function f(R,G). Histological analysis of the teeth was conducted applying the following scale: D0, sound; D1, enamel lesion; D2, lesion into the 1/3 of dentin; D3, lesion into the 2/3 of dentin and D4, lesion into the 3/3 of dentin.

For Sensitivity (SE) – Specificity (SP) sum above 1.6, three optimal cut-offs for the f(R,G) were defined corresponding to histological levels D1, D2 and D3. To assess the method's validity, the defined optimal cut-offs were applied on a new set of 48 teeth (200 sound or carious sites on occlusal surfaces). SE, SP and the area under the ROC curve (Az) were calculated based on histological analysis: D1: SE 0.88, SP 0.9, Az 0.94; D2: SE 1, SP 0.76, Az 0.91; D3: SE 1, SP 0.83, Az 0.87. In conclusion, caries detection using the 3D intraoral scanner emitting blue light showed good *in vitro* performance with high SE, SP and Az values for the three defined optimal cut-offs. Further studies with larger sample size are needed to optimize and validate the defined cut-offs.

This study was supported by Innovation Fund, Denmark and 3Shape TRIOS A/S.

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Is Obesity Associated with Tooth Loss Due to Caries? A Cross Sectional Study with Students

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Individuals with high body mass index have been associated with higher prevalence of dental caries mainly due to unhealthy lifestyle. The purpose of this study was to investigate if there is an association between obesity/overweight and dental loss due to caries among university students in southern Brazil. A cross-sectional study was carried with all entrant students regularly matriculated in the first semester of 2016 in Federal University of Pelotas.

Students were invited to respond a self-administered questionnaire. The body mass index (BMI) was calculated using the self-reported weight and height. Cut-off points were: a) Eutrophic (BMI <25); c) Overweight (BMI between 25 and 30); and d) Obesity (BMI >30.0). The outcome of present study was the self-report of at least one dental loss due to caries (Yes/No). A backward stepwise procedure was used to include/exclude explanatory variables in the model fitting. From 3,237 students eligible to study, 2,089 (64.5%) participated of the present study. Almost 23% of students presented overweight, 8.4% obesity and 362 individuals (17.5%) reported to have at least one tooth extracted due to caries. After control (sex, skin color, age, family income, smoke, depression symptoms, stress, last dental visit and gingival bleeding), the Poisson regression model showed that obese students presented a 32% higher prevalence (PR 1.32 [CI 95% 1.16–1.49]) of dental loss. However, overweight did not show association with dental loss (PR 1.01 [CI 95% 0.91–1.13]). In conclusion, obesity was associated with tooth loss due to caries in this sample.

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Fluoride Concentration in Mouthrinses Marketed in Chile and Brazil

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According to the best scientific available evidence, fluoride mouthrinses for daily use should be formulated with NaF at a concentration of 0.05% (226 ppm F^-). Data about these products marketed in Chile are unknown and in Brazil they are scarce. A convenience sampling of 9 commercial mouthrinses were purchased in Chile and 11 in Brazil. Three bottles of each brand were purchased for most products. Products formulated with NaF were diluted 20x with water. For total fluoride determination (ions F^- +MFP) in the mouthrinse containing MFP, it was diluted 10x and hydrolyzed with HCl; ionic fluoride (F-) was determined directly. For the determinations, the electrode Orion 96–09 coupled to the ion analyzer Orion VersaStar was calibrated with F standards from 1.0 to 32.0 µg F/ml. The determinations were made in duplicates and the variation coefficient was 0.9%. Results were expressed in ppm F (µg F/ml). Total fluoride concentration found in all mouthrinses evaluated ranged from 93.6 to 236.0 ppm F and they were very close to those declared by the manufactures (from 100.0 to 226.2). In Chile and Brazil, a brand of mouthrinse (Listerine) with low fluoride concentration (around 90 ppm F, as NaF) was found. A mouthrinse (Vitis Sensible) formulated with MFP was found only in Chile, in which there was 216.8 ppm F as ion MFP and 4.9 as F^-. In Brazil one mouthrinse (Colgate Mouthgard) was found to contain 180 ppm F (as NaF). The findings show that mouthrinses in the Chilean and Brazilian market, with either MFP or NaF have fluoride concentrations according to the existent scientific evidence that would not be optimally effective for caries control.

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Effectiveness of Toothpastes Based on Calcium, Phosphate and Fluorine in the Remineralization of Initial Caries Lesions

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The objective of this series of studies was to evaluate the effectiveness of four toothpastes based on calcium-phosphate-fluorine on the remineralization in three related studies. Study 1. 104 enamel specimens with artificial caries were divided into six groups: Hydroxyapatite-fluoride (HA-F); Casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF), fluoridated tricalcium phosphate (f-TCP), Calcium sodium phosphosilicate fluoride (CSPS-F), positive control (+C) and negative control (–C). The toothpastes were applied topically with electric brushing. Vickers microhardness (VHN), reconversion (SMHR), change (SMHC) and morphology were measured using SEM. Study 2. 240 specimens were divided into four toothpastes groups (HA-F, CPP-ACPF, f-TCP and CSPS-F). The mineral gain and ultramorphology were measured using SEM-EDS. Study 3. Clinical-trial conducted in 52 children divided into five toothpastes groups. Areas and decalcification of WSL were measured. The appropriate parametric and nonparametric tests were used to analyse the data. Study 1. All dentifrices significantly increased VHN after 30 days after-treatment ($p > 0.05$). HA-F and CSPS-F were also significantly effective at 15-days. A significantly higher value of SMHR/SMHC were observed with HA-F, f-TCP and CSPS-F in the first-remineralization and CPP-ACPF in the second-remineralization compared to the control ($p > 0.05$). SEM images revealed that all groups were able to repair the surfaces morphology (SEM 3 and 4). Study 2. All dentifrices recovered the mineral content significantly after 15-days of application ($p > 0.05$), with the exception of HA-F that recovered the calcium content after 30-days. The mineral gain was positive and was not different between groups ($p < 0.05$). SEM images revealed that all groups were able to reduce permeability by forming a mineral coating (SEM 3 y 4). The treatment of the experimental groups showed a significant decrease in WSL, higher values of RW and a decrease in the severity of the ID, in comparison with the control group. The decrease in DI was significantly associated with dentifrices HA-F, CPP-ACPF and f-TCP (OR < 0.23) unlike the control group (OR > 4.9). Although treatment with CSPS-F was more effective than the control group in decreasing DI (OR = 0.56 vs. OR = 2.1), it was not statistically different. After the analysis of the three studies, it can be concluded that calcium-phosphate-fluoride-based toothpastes are effective for the treatment of initial caries lesions.

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Evaluation of Calcium Ion Diffusion Through Coronal Dentin: An ISE Study

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The success of $Ca(OH)_2$ as a clinical treatment for pulp capping is related to its ionic effect, resulting from the dissociation of calcium and hydroxyl ions that act on the tissue. Liquids mixed with $Ca(OH)_2$ powder play an important role in the overall dissociation because they moderate the ionic transport of the paste constituents. The aim was to test the release of Ca^{2+} from $Ca(OH)_2$ mixed with different carriers in a coronal remaining dentin thickness model (RDT model). Samples were prepared using a coronal RDT model (Gandolfi Iranian Endodontic Journal 7.4 (2012):189). Standardized class 1 cavities were prepared on the crowns. Pulpal dentin was removed using a diamond burr to obtain a standardized pulp chamber. A remaining dentin thickness of 1.0 ± 0.2 mm and 2.0 ± 0.2 mm was prepared. Two aliquots of $Ca(OH)_2$ were prepared using saline or glycerine mixed in 1:1 ratio, placed inside the pulp chamber and restored with composite. External surface was covered with nail varnish. Samples were immersed in 10 mL stirred deionized water. Ca^{2+}-ISEs (Nico2000, UK) were used to continually measure the increase in calcium ion concentration every 20 s for up to 80 h. Transport of free Ca^{2+} from all samples (n = 8) was observed. Two measurements in each group was conducted and average values were calculated. $Ca(OH)_2$/saline in 1 mm thickness showed 00008, 0.0015 and 0.0002 mmol/L per hour, whereas in 2 mm thickness the diffusion was 0.0004, 0.0015 and 0.002. $Ca(OH)_2$/glycerine in 2 mm thickness the diffusion was 0.00003, 0.000008 and 0.000005 mmol/L whereas in 1 mm thickness showed a steady diffusion around 0.0001 mmol/L. In the saline group the Ca^{2+} increased 2 times for 1 mm and 3 times for 2 mm thickness. In conclusion $Ca(OH)_2$ in saline showed a significantly higher and faster release of Ca^{2+} than $Ca(OH)_2$ in glycerine after 80 h.

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Total Daily Fluoride Intake of 1–5-Years-Old Children from the ELEMENT Cohort in Mexico City

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Mexican children are exposed to fluoride in foods, beverages, fluoridated salt and naturally fluoridated water, but the contribution of each source to Total Daily Fluoride Intake (TDFI) is unknown. The aim of this study was to retrospectively estimate daily fluoride intake (DFI) from foods and beverages consumed by 1-to-5-years-old children from the ELEMENT cohort in Mexico City. From 552 eligible children participating in the cohort, those whose caregivers completed a semi-quantitative Food Frequency Questionnaire (FFQ) at each time-point were included in the analytic sample as follows: 1-year-olds (n = 441), 2-year-olds (n = 472), 3-year-olds (n = 443), 4-year-olds (n = 449) and 5-year-olds (n = 375). DFI from foods and beverages was estimated using a fluoride content database of foods and beverages previously developed by our group. DFI estimates from foods and beverages in mg/kg at each age were computed using the software SisNut 5.12. Descriptive statistics and statistical comparisons between groups (foods, beverages, age, sex, socioeconomic level) were performed using SAS® (Kruskal-Wallis and Mann-Whitney tests, significance level 0.05). DFI from foods and beverages in the whole analytical sample of 1–5-year-old children (median, interquartile range) was (0.068, 0.045) mg F-/kg/day. The DFI from foods (0.064, 0.045) was significantly higher than the DFI from beverages (0.004, 0.005), $p < 0.0001$. Differences in DFI were not statistically significant by age or sex ($p > 0.05$), but children from families of higher socioeconomic level had lower DFI ($p < 0.0001$). In conclusion DFI in 1-to-5-year-old children from the ELEMENT cohort in Mexico City from only foods and beverages (not including use of discretionary fluoridated salt) is within the upper limit of the recommended range of daily fluoride intake (0.05–0.07 mg F^-/kg/day). These results constitute a baseline to estimate TDFI in future studies.

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Analysis of Relative Bacterial Activity and ldh-Gene Expression of Caries-Associated-Bacteria in a Site-Specific Natural Biofilm

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The present in vivo study aimed at investigating both the relative bacterial activity and the lactate dehydrogenase (*ldh*) gene expression of caries-associated bacteria in a site-specific natural biofilm. Sixty subjects (age, Mean ± SE: 30.1 ± 1.4) were allocated to two groups: caries-free-subjects (CF) or caries-active-subjects (CA). Mean DMFT of CA was 12.5 ± 8.9. CF presented one sound surface (CFS; n = 30). CA presented two donor sites: a cavitated caries lesion (CAC; n = 30) and a sound reference surface (CAS; n = 30). Real-time quantitative PCR (q-PCR) on species (*Streptococcus mutans*) or genus level (*Lactobacillus* spp., covering all species) and total bacteria was performed targeting the 16S-gene, the 16S-rRNA, the *ldh*-gene, and the *ldh*-mRNA. As the 16S-rRNA-abundance represents the number of ribosomes, while the 16S-gene-abundance represents the number of genomes, the quotient of the relative abundances functions as a measure for the relative bacterial activity (%). Both *lactobacilli* and *S. mutans* showed the highest relative bacterial activity in CAC [(Mean ± SE) 218 ± 60% and 61 ± 16% respectively] and the lowest values for both sound reference surfaces (69 ± 48%; 8 ± 3%). Significant differences were found between CAC and CAS as well as between CAC and CFS for both *lactobacilli* and *S. mutans* ($p < 0.05$). The *ldh*-gene expression of *lactobacilli* and *S. mutans* only showed moderate values in CAC (1.90E+03 ± 2.11E+03; 2.08E+04 ± 4.44E+04 transcripts/µl) and CFS (2.04E+03 ± 2.74E+03; 8.16E+03 ± 6.64E+03 transcripts/µl) consequently no significant differences were detected.

In conclusion caries-associated bacteria (*lactobacilli and S. mutans*) showed highest relative bacterial activity in plaque of cavitated lesions. However, under the conditions chosen, no significant differences in *ldh*-gene expression in the biofilm of caries-active and caries-free subjects could be detected. Future studies should focus on the ability of a sucrose-pulse immediately before biofilm collection in order to activate the *ldh*-gene expression related to sugar metabolism.

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Session 2
Clinical Studies

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Treatment Success of Carious Primary Molars with Marginal Breakdown: Comparison of Three Treatment Techniques

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When cavitated carious lesions are non-cleansable, restorative treatment is indicated. Up to now, there is not a single option to restore occluso-proximal (ICDAS 5) carious primary molars. This study assessed the efficacy of three restorative techniques used to restore carious primary molars with marginal breakdown: Compomer filling (CF), pulpotomy and conventional stainless-steel crown SSC restoration (P+SSC), and performed without pulpotomy (SSC). In this prospective observational study 93 children (2–9 years, mean 6.4 ± 1.8, 53.8% female) with a total of 162 eligible teeth were included (CF = 48, 29%; SSC = 64, 40%; P = 50, 31%). The baseline dmft value was 8.0 ± 3.4. Statistical analyses: Descriptive analysis, paired sample t-test, non-parametric Friedman's Anova test, and decision tree analysis. Mean caries depth from all recorded cavities was 2.85 ± 0.7 mm. CF (n = 22, 57.9%) and SSCs (n = 32, 65.3%) were mostly placed in 3 mm depth cavities while P+SSCs were performed in >3 mm cavities (n = 13, 59%). After a mean of 7.8 ± 2.0 months, 76 (82%) children with 114 (70%) treated teeth were available for assessment. 102 restorations (89.5%; CF = 29, 28%; SSC = 50, 49%; P = 23, 22%) were successful (restoration intact without clinical signs/symptoms of pulpal pathology). Eight teeth (7%) with CF showed at least one 'Minor' failure (reversible pulpitis, caries progression, loss of restoration, secondary caries). Four teeth (3.5%) showed at least one 'Major' failure (irreversible pulpitis, abscess, unrestorable tooth): CF = 3 (7.5%) and SSC = 1 (2%). Survival rate was P = 100%, SSC = 98% and CF = 72.5% (p = 0.0001). Although SSCs without and with pulpotomy (P+SSC) were mostly performed on deeper cavities (≥3 mm), these showed consistently high success rates after 8 months as compared to CF in this high caries-risk cohort.

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TiF_4 Varnish for Treatment of Early Carious Lesions in Children Permanent Dentition: A RCT Study

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This 18-month randomized clinical trial (RTC) compared the effect of TiF_4 varnish with a commercial NaF varnish in the treatment of noncavitated enamel carious lesions in permanent teeth of children. This controlled, parallel and single blind RTC involved 63 children, 6–7 years old, living in Bauru-SP/Brazil. Children were selected according to their caries activity (presence of at least 1 active white spot lesion) and randomly divided into the following treatments: 4% TiF_4 varnish (2.45% F^-, pH 1, FGM); 5% NaF varnish (2.26% F^-, pH 5, Duraphat®-Colgate) and negative control (placebo varnish, pH 5, FGM). The varnishes were applied on all permanent teeth, once a week for 4 consecutive weeks, and also after the 6th and 12th month of study. The clinical examination (ICDAS index) and quantitative fluorescence changes analysis (QLF) were performed. The patient's treatment satisfaction degree was reported after each varnish application. The visual plaque index (VPI) was calculated after 3, 9 and 15 months. The partial results (n = 35) showed slight clinical and fluorescence changes during the period of the study. No significant differences between treatments were found with respect to carious lesions regression or progression by using ICDAS (ANOVA, p > 0.05). With respect to fluorescence changes, only TiF_4 varnish was able to reduce the mean fluorescence loss significantly after 18 months (ΔF: –12.8 ± 1.7%) compared to the baseline values (–16.5 ± 4.1%) (RM ANOVA, p =

0.003). Regardless of the varnishes, the majority of the participants reported being satisfied with the treatments (76.1 ± 9.0, 85.7 ± 6.0 and 75.4 ± 4.7% for TiF_4, NaF and placebo, respectively) and the VPI percentage also reduced significantly after 9 and 15 months compared to 3 months of the study. TiF_4 varnish was the only treatment able to show some carious lesions regression by using QLF.

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Responsiveness of OHRQoL Questionnaires to Dental Caries Interventions: Systematic Review and Meta-Analysis

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The present systematic review and meta-analysis were undertaken to assess the responsiveness of validated Oral Health Related Quality of Life (OHRQoL) questionnaires to dental caries interventions in children, adolescents and young adults. Dental caries interventions included several non-operative and/or operative treatments. Studies eligible for this review were Randomized Clinical Trials (RCTs), Controlled Clinical Trials (CCTs), and Prospective Case Series (PCS) which had OHRQoL questionnaires answered before and after caries intervention(s). The main outcome was change in OHRQoL mean scores following caries intervention. A total of 26 studies were identified for the quality assessment and 14 were selected for the meta-analysis. Eight different instruments were applied for the assessments. The majority of the studies were PCS with a single group pre-test and post-test study design (n = 19). Five studies were CCT and only two were RCT. The number of participants was 3,522 in the control group (baseline = 2002; final = 1520) and 5,917 in the test group (baseline = 3102; final = 2815). The age of the studied subjects ranged from 3 to 19 years. All studies showed significant improvement in OHRQoL following caries intervention. Most of non-randomized studies (n = 15) had low or moderate risk of bias. The meta-analysis showed the effect of caries interventions (standardized weighted mean differences = –1.24; 95% CI: –1.68:–0.81: $p < 0.001$). However, high heterogeneity between the studies was found. The GRADE approach classified the quality of evidence as very low and its strength weak.

In conclusion, there was evidence that the OHRQoL of children and adolescents improved following caries intervention procedures. However, the quality of the evidence was very low. In spite of that, caries interventions are highly recommended as abstaining from treatment is likely to result in a deterioration of OHRQoL.

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Impact of a Community Health Worker on Interprofessional Caries Referrals

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Physicians can play an important role in reducing dental caries disparities and have been encouraged to assess oral health, provide anticipatory guidance, apply fluoride varnish, and establish a dental home. The aim was to evaluate the impact of a community health worker (CHW) on successful medical-dental referrals. Muskegon Family Care implemented a caries prevention program in their medical clinic which included distributing toothpaste/toothbrushes and using a web-based dental registry to facilitate communication and care coordination between medical-dental providers. The registry documented caries risk, dental findings, preventive interventions received, and facilitated electronic dental referrals. Physicians had an option to further involve a CHW to assist with referrals. For this study, medical-dental referral rates for dentate children 0–72 months of age, who had an oral health screening in the medical setting, were monitored for 5 months. Comparisons between subjects with and without a successful dental visit that had received: 1) no referral, 2) a referral without CHW assistance, or 3) a referral with CHW assistance, were performed using chi-square tests. Results: Out of 1,304 well-child medical visits, 489 included an oral health screening and 277 included fluoride varnish. The majority of toothbrush/toothpaste samples were provided by the medical provider (94%), with the remaining 6% by the CHW. There were 111 medical-dental referrals: 77 made without and 34 made with CHW assistance. Referrals involving a CHW resulted in a significantly higher proportion of subjects with a successful dental visit (55%) than referrals without a CHW (28%, $p = 0.005$) and no referral (23%, $p < 0.001$). The latter 2 strategies were not significantly different ($p = 0.410$). Conclusions: In developing and implementing a successful interprofessional caries prevention program, CHW participation increased the success of a dental referral visit. Partially-funded by Colgate.

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Activity Dynamics of Root Caries Lesions Against a Self-Administered Non-Invasive Treatment, a Randomized Controlled Trial

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Although non-invasive therapies with high-fluoride toothpastes with 5,000 ppm F seem an effective approach for preventing and arresting root carious lesions (RCLs), the dynamics of this process over time is mostly unknown. The aim of this study was to assess the inactivation pattern during a 2-year period follow-up of

RCLs, upon a self-administered non-invasive treatment with 5,000-ppm F toothpastes, in independently-living older adults. A population of 276 independently-living older adults participated in this RCT. Subjects were randomly divided in 2 arms; the control group treated with conventional 1,450 ppm F and the experimental arm with a high-fluoride 5,000 ppm dentifrice, used twice/day. A total of 2,071 RCLs were detected at baseline and assessed for activity using Nyvad's criteria and classified within one of the 8 possible patterns of activity or inactivity at 1 and 2 years. A descriptive and a bivariate analysis were carried out. Of all the RCLs, 40.5% remained active after 2 years of treatment with the 1,450-ppm toothpaste, whereas only 3.7% with the 5,000 ppm F. For the 5,000 ppm F arm, there were almost only 2 patterns with all the RCLs. The most prevalent was that in which RCLs were initially active, were inactivated at 1 year and maintained inactive after 2 years of follow-up (A-I-I) (64% of the cases), followed by inactive RCLs at baseline that maintained inactive over time (I-I-I) (30%). No large variations were detected in the conventional toothpaste group, with lesions similarly distributed among all the 8 patterns. In conclusion self-administered non-invasive management with high-fluoride toothpastes appear to be highly effective in inactivating and maintaining inactive RCLs.

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Xylitol Chewing-Gums: Concentration in Saliva and Antibacterial Effect (In-Vivo and In-Vitro Study)

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The salivary concentration of Xylitol released from two chewing gums containing different amount of the polyol, was evaluated in a sample of healthy volunteers. Moreover, the in vitro antibacterial effect of Xylitol was tested. In a cross-over design including 32 subjects, Xylitol concentration using spectrophotometer analysis was determined in saliva after 0.30, 1.30, 2.30, 3.30, 4.30 and 5.30 min after the mastication of two chewing-gums: one sweetened with 550 mg (100%) Xylitol (Group-A) and a second with 110 mg (22%) Xylitol (Group-B). The detection and the estimated level, through checkerboard DNA-DNA hybridisation method of several bacteria associated with caries and periodontal disease were evaluated in subgingival plaque samples before and after 15 and 60 min and 24 h from the contact with the two chewing-gums dissolved in saliva. In Group-A, the highest value (mg/l) of Xylitol concentration in saliva was found after 30 s (mean ± sd 0.31 ± 0.45) with a slow decrement towards the last measurement (mean ± sd 0.24 ± 0.32). In Group-B, the highest Xylitol concentration was observed after 1.5 min (mean ± sd 0.17 ± 0.37). The Xylitol concentrations recorded in the two groups were always statistically significant different in all time points ($p < 0.01$). An antibacterial activity was observed against all bacterial strains after the addition of the two chewing-gums to the plaque samples; the highest bacterial reduction was noted after 60 min for the 22% Xylitol gum and after 24 h for the 100% Xylitol gum. In conclusion the salivary concentration of Xylitol increases after the mastication of both chewing-gums, statistically significant higher with 100% Xylitol gum. Microbial pathogens in subgingival plaque were reduced by both chewing-gums, even if those sweetened with 100% Xylitol produced the highest and most long-lasting antimicrobial effect.

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Impact of Using Different Clinical Criteria in the Assessment of Caries Lesions Around Restorations

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This study aimed to evaluate the effect of the use of 2 visual criteria for the assessment of restored teeth with caries lesions on outcomes related to oral health in adults. This study presents the partial results from a randomized clinical trial (CaCIA – Caries Cognition and Identification in Adults) of two parallel groups. One group of participants received the diagnosis and treatment decision according to the FDI (World Dental Federation) criteria (FDI group), and the other group according to the "Caries Associated with Restorations or Sealants" (CARS) criteria described by the International Caries Classification and Management System (ICCMS group). The restorations were followed for up 20 months. The primary outcome was the restoration failure. A descriptive analysis was performed, followed by a chi-square test. The secondary outcome was the proportions of false-positive results for secondary caries presence (cases in which during the intervention no decayed tissue was found). The sample included 166 restorations (43 patients). 61 restorations were randomized to ICCMS group (86.9% were indicated for monitoring, 9.8% to repair and 3.3% for replacement). 105 restorations were randomized to the FDI group (66.7% indicated for monitoring, 16.2% for repair and 17.1% for replacement). False-positive results were observed on 4 cases on the ICCMS group, and on 23 cases on the FDI group. During the follow-up, 1.9% of the cases from the FDI group failed due to secondary caries. No failure was found on the ICCMS group. There was no statistically significant difference between the groups ($p = 0.532$). In conclusion, the use of the FDI criteria may lead to unnecessary restorative interventions. (NCT03108586).

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Influence of Oral Health Literacy in Preventive Measures Execution Considering Fluoride Use: RCT Preliminary Results

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Oral Health Literacy (OHL) level of parents could interfere on oral health outcomes of their children. Toothpastes are key vehicles for fluorides application, and its correct dosage prevent dental caries and avoid dental fluorosis. The aim was to evaluate if OHL level of parents interfere on execution of preventive measures considering fluoride toothpaste use. The RTC was registered at ReBEC (RBR-93q4gm). Parents of children under 4 year-old attended at UFMG Clinics Hospital, during the period of study data collection were eligible. Parents that were able to read, has Portuguese as mother language and did not received information about fluoride dosage were included (n = 107). OHL was measured through OHL Questionnaire for Adults and classified as inadequate (0–13) or adequate (14–17). Parents were divided randomly into four groups of interventions concerning the rational use of fluoride and stratified by the level of OHL: 1) Oral guidance; 2) Written guidance, 3) Oral guidance and presentation of a photographic model, 4) Written guidance and photographic model. Fluoride toothpaste amount dispensed on toothbrush were measured at baseline and right after intervention, the amount of 0.03 g (according to AAPD Guidelines) were used as reference. Differences between groups were tested using chi-square test. 78 (72.9%) parents were classified with inadequate OHL level and 29 (27.1%) with adequate OHL level. 46.2% of parents with inadequate OHL dispensed the right amount of fluoride toothpaste after intervention, while 62.1% of parents with adequate OHL set the right dosage ($p = 0.023$). A tendency of higher percentage of right dosage was detected on intervention groups that used photographic model. In conclusion parents with adequate OHL level were more prone to perform the right dosage of fluoride toothpaste, despite intervention group.

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Selective Removal of Carious Tissue in Permanent Teeth: A Randomized Controlled Clinical Study

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Selective removal of carious tissue may avoid endodontic treatment in deep caries, however, studies of medium depth lesions of permanent teeth are scarce. A randomized controlled clinical trial in patients with medium or deep active caries lesions compared outcomes from a selective removal to leathery dentin versus the control complete removal (medium lesions-ML) or stepwise two-step complete removal (deep lesions-DL). This was followed by pulp protection with calcium hydroxide if needed, and restoration with total-etch or self-etch adhesive and resin composite. Selectively excavated teeth were not treated further, for stepwise caries removal, a second excavation was made until only hard dentin be achieved. Pulp sensitivity and periapical health of teeth were assessed after 6 months and the restoration evaluation was performed using the functional parameters of clinical criteria approved by the FDI World Dental Federation. Multivariate regression analysis was used to assess the factors associated with failures ($p < 0.05$). Seventy-three teeth were restored, being 44 with medium and 29 with deep caries. For ML, all the evaluated teeth (n = 39; 100%) were considered success in both treatments. For DL, only 3 out of the 23 evaluated treatments failed (one in the selective removal group and two in the complete removal group), resulting in the success of 96.4% for selective removal and 94.1% for complete removal. No differences between the groups were found in the evaluated period regardless of lesion depth ($p = 1.00$). Multisurface restorations (ICDAS 6) showed more failures than single-surface ($p = 0.021$, HR 14.85, CI 95% 1.49;148.16). Although both techniques presented similar results, the selective carious removal may be more beneficial, since it is performed in a single session, preserving a greater amount of dental structure and being more cost-effective (ensaiosclinicos.gov.br U1111-1221-4083).

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Caries Risk Assessment and Quality of Saliva in Diabetes Patients Either With or Without Xerostomia

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The aim was to correlate the physicochemical properties and composition of saliva in diabetic patients with their caries risk profile. Three groups were involved in this study; patients with diabetes and xerostomia (DX, n = 5); patients with diabetes without xerostomia (DN, n = 5); and healthy participants (H, n = 5) without diabetes and xerostomia. Participants were initially screened according to their caries risk using CAMBRA with unstimulated whole saliva (USWS) which was <0.2 ml/min for DX, >0.2 ml/min for DN and H. 15 unstimulated and stimulated whole mouth saliva samples was collected. The matched-age ranged from 30 to 70 years. The saliva samples were analysed for the following; pH with buffer capacity, total protein concentration and osmolality. For each sample, pH and buffer capacity were measured using saliva kit (GC, Japan) and osmolality with Osmometer device (CAMBLAB, Loser Type 2, UK). Results showed that Group DX had extremely high caries risk whereas Group DN was at a high risk, whilst Group H was at lower caries risk (except one being at the moderate category). One-way ANOVA tests showed that participants with xerostomia had lower USWS, SWS, pH however statistically insignificant differences in comparison to the DN and H groups. There were no significant differences for osmolality, total protein concentration, or for MUC7 and amylase between all three groups ($p > 0.05$). There was a significant difference in MUC5b between group DX and group H ($p < 0.05$). In conclusion, saliva physiochemical properties and composition can be used to indicate dental caries risk in diabetes patients. However, salivary parameters need to be assessed further with a larger sample targeting a wide range of age groups and gender in diabetes patients either with xerostomia or without xerostomia.

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Proposing a Utility Scale Related to Dental Caries in Children Based on ECOHIS Scores

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The cost-utility analysis has been extensively used in economic evaluations in health care since it permits the comparison of different treatments for different injuries even when they are measured by different clinical effects. For that, weights for each condition that could be experienced should be established, but there is no consensus about these values for caries in children. We suggested a utility scale based on an oral health related quality of life questionnaire (ECOHIS) scores and to test its ability to discriminate children presenting different clinical conditions related to dental caries. 3-to-6-year-old children who sought dental treatment in a public dental unit and were enrolled in a clinical trial (CARDEC-02/NCT02473107) were selected for this study. Children's dental condition related to caries was registered and ECOHIS questionnaires were answered by parents at baseline. The utility values were obtained after normalizing the ECOHIS scale (0 to 52) in order to obtain utility scores from 1 (perfectly healthy) to 0 (worst health condition). Summary values for each condition of interest were calculated in order to check those situations the scores could discriminate. Utility scores varied from 0.64 to 1.0 (mean = 0.94, median = 0.98, skewness = –1.50). Non-treated frankly cavitated lesions or presence of restored teeth slightly impacted on utility values (median; 95% confidence interval Ą caries-free: 1.0; 0.94–1.0/with caries: 0.96; 0.88–1.00). The early primary tooth loss was the most relevant factor to distinguish utility related to dental caries, especially when associated to frankly cavities and restorations (0.82;0.73–0.91). Although a low variability in utility scores related to dental caries (compatible to the low morbidity of this condition) is observed, the utility scale proposed can discriminate oral conditions known as relevant and should be used and tested in further economic evaluations.

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Dental Treatments Performed After Caries Diagnosis Made by Visual and Radiographic Methods in Primary Molars

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This study aimed to compare caries management approaches chosen for primary molars after two different caries diagnostic strategies: visual inspection alone (VIS) and VIS associated with radiographic method (RAD). This research is part of a randomized clinical trial conducted to compare these two diagnostic strategies, and considering outcomes clinically relevant for the patients (CARies DEtection in Children 1 – CARDEC-1). Children (3 to 6 years-old) were randomly allocated for two groups according the diagnostic strategies (VIS or RAD). Treatment of non-restored surfaces of primary molars at the baseline was conducted according to the decision made by examiners using the allocated method. Comparisons between diagnostic strategies considered some outcomes: number of surfaces that received no treatment, non-operative treatment, and number of surfaces restored. Other outcomes were number of false-positive results (cavities opened but with no caries tissue) and number of teeth endodontically treated or extracted. Comparisons were made by Mann-Whitney test. 216 children who completed the study were analyzed. We did not observe significant differences between the groups considering number of surfaces with no treatment (VIS: mean ± standard deviation – SD = 20.7 ± 10.1; RAD:18.6 ± 9.6; p = 0.095), in surfaces with non-operative treatment (VIS: 10.5 ± 6.1; RAD: 10.5 ± 6.0; p = 0.780); however, there was more surfaces restored in the RAD group (4.2 ± 5.2) than in VIS group (3.2 ± 4.9; p = 0.021). Moreover, children allocated to the RAD group had ten times more surfaces with false-positive results (0.49 ± 1.22) than in VIS group (0.03 ± 0.22; p < 0.001). Number of teeth endodontically treated (VIS: 0.33 ± 0.86; RAD: 0.32 ± 0.78, p = 0.773) or extracted (VIS: 0.22 ± 0.62; RAD: 0.22 ± 0.58, p = 0.809) were similar. In conclusion, more dental surfaces of primary molars are restored and there are more unnecessary restorative treatments when the caries diagnostic strategy is the association of visual and radiographic methods, compared to using visual inspection alone.

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Clinical Performance of Adhesive Restorations Performed in Anterior Primary Teeth – Exploring Recurrent Failures

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Restorative procedures on anterior primary teeth are challenging due to the sensitive restoration technique and the difficulty in controlling moisture in young patients. This study aimed to evaluate the survival rate of adhesive restorations performed on anterior primary teeth, due to caries, without the use of rubber dam, and to identify factors associated with the failures. This retrospective study was based on data from two cohorts of children (3 to 6 years old) previously enrolled in two clinical trials (CARDEC-01/NCT02078453 and CARDEC-02/NCT02473107). In these trials, anterior teeth had been restored, respectively, using resin modified glass ionomer cement (RMGIC) and composite resin (CR). The records of children who received restorations in anterior primary teeth were evaluated. These children were followed for 24 months. Kaplan-Meier test was used to estimate restorations survival and Cox regression analysis were used to identify variables associated with the failures. Analyses considering both single (1st) and multiple failures were performed. Failures were classified as major and minor. 144 children were included in this study. On average, 35% of anterior restorations presented major failures and 10% presented minor failures. Primary restorations (n = 380) performed with CR presented higher failure rate (44%) than restorations performed with RMGIC (23%) (P-value ≤0.001). When including repaired and replaced restorations (n = 456), the CR restorations failed earlier (21.84 months) than RMGIC restorations (30.62 months) in both situations considered. Primary restorations involving proximal surfaces failed more often (HR = 2.05; 95% CI: 1.29–3.28). The failures occurrence was also influenced by children's caries experience. In conclusion, for restorations placed in anterior primary teeth without the use of rubber dam, the performance of RMGIC was superior to CR, but still not clinically acceptable, and restorations involving proximal surfaces of anterior teeth present lower survival rates.

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Radiographic Examination on Diagnosis and Treatment of Caries Lesions in Primary Molars: A Clinical Trial

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The aim of this clinical trial (CARies DEtection in Children 1 – CARDEC 1) was to compare the detection and treatment of caries lesions in primary molars performed with visual inspection associated with radiographic examination (RAD) and with visual inspection alone (VIS), considering clinically relevant outcomes for the patients. Children aged 3 to 6 years were included and randomly assigned to 2 groups according to the method used for caries detection of the primary molars: RAD or VIS. Children were diagnosed and treated according to the allocated group and were followed-up for 2 years. The primary outcome was number of new operative interventions during the follow-up. Other secondary outcomes were considered: surfaces with new restorations, with repair or replacement of the restorations, number of restorations performed since the beginning of the study and others. Comparisons between the groups was made by Mann-Whitney test. Initially, 252 children were included and randomized, and 216 were followed-up for 2 years (follow-up rate = 85.7%, 106 of VIS and 110 of RAD). Regarding the primary outcome, the median (interquartile range – IQ) of number of surfaces requiring operative treatment in the children allocated to the VIS was 0.0 (0.0–4.0), and for RAD was 2.0 (0.0–5.0) (p = 0.112). Regarding the secondary outcomes, children diagnosed and treated with the RAD group had more restorations since the beginning of the study (median = 5.0; IQ = 1.0–10.0) than the VIS group (median = 2.0; IQ = 0.0–7.0) (p = 0.027). The other secondary outcomes did not present significant differences. In conclusion, visual inspection associated with radiographic examination does not have any benefits when compared to the diagnosis performed with the visual inspection alone in primary molars.

The study was supported by FAPESP (Grant #2012/24243-7), CNPQ (Grants # 471817/2012-0 and 471817/2012-0) and CAPES. The trial was registered in clinicaltrials.gov in 4 March 2015 (NCT02078453).

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Effect of Probiotic in Microhardness and Superficial Appearance of Enamel Blocks: In Situ Caries Model

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To establish the differences in microhardness and superficial appearance by Scanning Electron Microscope (SEM) enamel blocks were exposed to sucrose, sucrose and probiotic *Lactobacillus rhamnosus* SP1 and control in an in situ caries model. Healthy volunteers between 18 and 30 years old, with normal salivary flow and without active cavitated caries were recruited for the study. Each of them used an intra-oral acrylic device containing 5 blocks of sterile human enamel for 14 days/24 hours. Six volunteers applied a solution of 20% sucrose every two hours on the enamel blocks and other six volunteers, in addition to applying the same sucrose regimen, applied once a day a drop of lyophilized probiotic reconstituted in water reaching a concentration of 10^8 CFU/ml. A block of enamel in each device was left as a control and varnished with a layer of insulating coat. Blocks were analyzed in the SEM and others were subjected to Vickers microhardness (VH) tests.

SEM evidenced the increase in the porosity of the enamel after the sucrose treatment and a lower porosity in the blocks to which sucrose and probiotic were applied, compared to the control group that does not present porosity. The microhardness mean for the control group was 296.1VH (95% CI: 281.6–310.5), for the sucrose and probiotic group it was 220.1 VH (95% CI: 212.4–227.9) and for the sucrose group it was 156.4 VH (95% CI: 147.4–165.3) finding significant differences between all the groups. In conclusion the use of probiotics in this in situ model suggests changes in the enamel structure observed in SEM and an inhibition of formation of caries like lesions measured by surface microhardness.

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An *In-Vitro* Evaluation of Fluoride Content and Enamel Remineralization Potential of Two Toothpastes Containing Different Bioactive Glasses

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The aim was to compare the discrepancies between declared and real total fluoride (TF) and total soluble fluoride (TSF) concentrations of two bioactive glass based dentifrices and also to analyze their remineralization potential. The TF and TSF concentration of the dentifrices was assessed using fluoride ion selective electrode. For micro-hardness analysis, eighteen human enamel blocks were divided randomly into groups 1 (n = 6; control, artificial saliva group), 2 (n = 6; Novamin® toothpaste group), and 3 (n = 6; BiominF® toothpaste group). The specimens were exposed to 6 wt% citric acid (pH, 2.2) for 5 min to mimic demineralization, and then for 24 h, were kept in AS (group 1) and specimens in groups 2 and 3 were then stored in AS + Novamin® and AS + Biomin® respectively. Data were analyzed statistically. Both Novamin® and BiominF® contained less TF as compared to their label claims with BiominF® containing more TF and TSF (TF: 421.30 ± 12.74, TSF: 414.52 ± 16.54) as compared to Novamin® (TF = 90.35 ± 1.29, TSF: 89.22 ± 1.63) ($p < 0.05$). The BiominF® toothpaste group performed better in terms of remineralizing the surface and improved its micro-hardness post-demineralization (Baseline: 457.03 ± 24.18, Post-demineralization: 390.35 ± 56.38, Post-remineralization: 539.15 ± 31.12) followed by Novamin® group (Baseline: 464.42 ± 17.25, Post-demineralization: 300.84 ± 97.28, Post-remineralization: 544.56 ± 51.42). Both toothpaste groups, showed significant differences ($p < 0.05$) versus the control group, and with their own baseline values after remineralization. In conclusion, BiominF® toothpaste demonstrated more TF and TSF content as compared to Novamin® and a considerable potential to promote remineralization of demineralized human enamel. Future in-vivo studies are suggested to analyze the clinical effectiveness of BiominF® toothpaste.

Session 3
Epidemiology 1

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Perception of Dental Students About Caries Management in the University Clinics and Community Settings

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Colombia achieved a consensus in cariology undergraduates' teaching (2012). Aim: To identify, with a qualitative approach, the perception of last-semester students (El Bosque University) about caries management in university clinics (UC) and community settings (CS).

With IRB, 10th-semester students were invited to participate in focus group (FG). From Consensus document caries diagnosis/management categories and criteria were extracted and FG orientating questions designed. Moderator and relator (8th-semester students to avoid educators' constraint) tape-recorded and transcribed FGs. With leading expert resulting text of each FG was organized into categories in one document. A triangulation process of contrasting written with voice resulting FG aspects and with evidence-based/context related concepts followed. Nine categories were pre-established: risk-assessment, caries-diagnosis, synthesis, prevention/non-operative-care/operative care decision-making, public health, discrepancy and articulation, and 58 criteria derived. Six-FG-orientating questions were designed. Three 45–60 min FG were conducted (5, 8 and 7 students, respectively). After extracting from transcribed text relevant aspects and organized them into categories, two categories emerged: communication, administrative/students'-grading aspects (n = 11 categories). Four triangulation sessions took a 3-month analysis process highlighting that students perceived confidence in their knowledge to address caries lesions, but not caries risk, as teachers don't demand it. In both areas, students perceived adequate caries-lesion teaching; however, without synthesis-process clarity and with teachers' caries-activity assessment disagreement. Decision-making and caries-management activities rendered students' higher autonomy in the CS. Students perceived (CS) the patient as a means of approving requirements increasing the operative-care probability. Patients' follow-up represented a weakness as patients are referred elsewhere (CS) or reassessment is not included in the student's grading (UC). This qualitative analysis from students' point of view adds in a relevant way to understand and overcome caries-current management implementation barriers.

Acknowledgements: Alliance for a Cavity-Free Future – Colombian Chapter.

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Prevalence of MIH and Dental Fluorosis in 12–15 Years Old Schoolchildren from Bogotá, Colombia

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The aim of this study was to describe the prevalence of MIH and dental fluorosis (DF) in a preliminary subsample from a whole sample of 456 12–15 years old schoolchildren from Bogotá, Colombia. Colombia has regulatory whole population salt fluoridation since 1989 (180–220 mg F/salt Kg). Two examiners had been previously calibrated with patients in DF (TFI) by an expert and all four examiners were previously trained in MIH (EAPD index) with photographs by an expert (inter-/intra-examiner reliability Kappa values for both indices ≥0.7). After obtaining IRB agreement and signed consents the clinical examinations were conducted in school settings with portable dental units. Examiners assessed DF on all surfaces with TFI (TFI 0: sound; TFI 1–2: mild fluorosis; TFI 3–4: moderate fluorosis; TFI ≥5: severe fluorosis)

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and MIH including: the severity of the demarcated opacity according to tooth surface involvement (sound; <1/3; 1/3 – <2/3; 2/3) and the presence/absence of associated post-eruptive breakdown (PEB) and atypical restaurations/caries lesions.

A total of 176 children were clinically examined. The prevalence of MIH was of 13.6%. MIH severity distribution was: <1/3 of tooth surface: 5.1%; 1/3 – <2/3 of tooth surface: 5.1% and 2/3 of tooth surface: 3.4%. In total 9.1% of subjects presented the most severe MIH phenotype (demarcated opacity plus PEB); 4% exhibited atypical restorations and 0.6% atypical caries lesions. The prevalence of DF was of 99.3%, distributed in mild DF: 54.5%; moderate DF: 42.6%, and severe DF: 2.2%. The highest TFI score found was TFI 6.

In conclusion, these schoolchildren experienced a high prevalence of DF and a moderate prevalence of MIH, with almost half of children with moderate DF and with most children affected with MIH presenting the most severe phenotypes.

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Predictions and Simulations of DMFT Index Dynamics Among Colombian Adults in the Context of Probability Theory

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This objective of this study was to simulate the trajectories in a predictive nature of decayed, missing, filled teeth DMFT index dynamics among Colombian adults through a methodology based in probability theory, previously implemented to predict dengue, malaria and HIV epidemics. The reported values of dental caries' DMFT obtained from National Oral Health Surveys (ENSAB) ENSAB I, II and III were analyzed, establishing simulations of the dynamics in the interval 1965–1997 that were subsequently assessed setting ranges of amplitude in which these values could vary with the purpose of predicting the DMFT for the year 1998 and contrast with the reported real values for that year by ENSAB III. Simulations were also conducted of the period 1998 to 2015. Four simulations of DMFT index were made. It was predicted that for 1998, with ranges of 2 and 3, obtaining values of 11 and 10.5, achieving percentages of success of 93.64% and 98.10% when comparing the results with the real reported values by ENSAB III. The obtained values of the simulations for 2015 were between 6.07 and 7.21. The methodology based on probability theory to assess the DMFT index dynamics, revealed mathematical orders that allowed to generate predictive simulations designed in an acausal context, providing a method to estimate the future behavior of the variable analyzed, which will facilitate the modelling of promotion and preventions politics and strategies of dental health in Colombia.

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Brazilian Conditional Cash Transfer Program: Five-Year-Old Children Access to Dental Care and Dental Needs

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Dental caries disproportionally affects disadvantaged segments of society placing an additional health burden on vulnerable groups. In Brazil, public policies, such as Bolsa Família (BFP), the Brazilian Conditional Cash Transfer program, have been implemented to reduce the effect of income inequalities, which can impact health and access to care. The objective of the study was to describe access to dental care and dental care needs of BFP five-year-old children. An oral health survey was conducted with five-year old children and their parent/caregiver registered in the BFP in Fortaleza, Northeast of Brazil. Study participants were recruited during visits in randomly selected primary health care facilities in the city. Study participants comprised of children receiving BFP and children waiting to receive the benefit. Demographic, socioeconomic and oral health information were assessed using a questionnaire completed by the parents/caregivers. Prevalence of dental caries was assessed through dental exams. Of the 230 participants, 80% were children's mothers with a mean age of 34.9 ± 0.67 years. More than half of participants had less than high-school schooling, and 71.7% were unemployed. Almost 73% of participants received BF, and around 55% of those were receiving the benefit for more than two years. The majority of participants (65.2%) reported that their children had an unmet dental care need, but had never visited a dentist (63.5%). Dental needs mostly reported were restorative treatment, cleaning and orthodontics. Almost 45% of children presented one or more caries lesion. Although dental care is provided free of charge by the Brazilian Universal Health Care System, our participants have unmet dental needs and still face several barriers to access dental care. Therefore, dental care should be included in the BFP Health Conditionality.

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Burden Disease of Dental Caries in Bogotá, Colombia Assessed Through Conventional DMFT and ICDAS DMFT

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This study aims to estimate the burden disease of caries experience, according to sex and age, using the conventional dmft/DMFT (d/D: ICDAS-merged moderate and severe caries lesions) and the ICDAS dmft/DMFT (d/D: including ICDAS-merged initial caries lesions) system according to the National Oral Health Survey in Bogotá. This ecological study was developed using the 2015 database of the IV National Oral Health Study (ENSAB IV)

for the Colombian population in the city of Bogotá (8,304 subjects). The methodology used is that described by Murray et al. [1996]. Initially, prevalence of caries experience was calculated according to all ages and sex. Subsequently, the synthetic health indicators including: AVADS (years lived with disability), DALY (1 year of life adjusted for disability), and YLD (1 year lost due to disability) were used. The dmft/DMFT burden disease was of 19.18 DALYS/1,000 persons and the ICDAS dmft/DMFT DALYS/1000 persons were of 24.37. According to sex, the female presented the greatest impact by the DALYS in all age groups 18.16 DALYs/1000 persons. The highest burden of the disease was in the group of 30 to 44 years with 30.31 DALYs/1,000. The findings of this study agree with those described in the literature and from the WHO, which have considered caries as the most important global oral health burden. In conclusion, caries is related with a high disability in physical, psychological and social aspects, representing a high cost for the individual and for the Colombian health system.

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Caries Prevention Program Based on Improvement of Oral Health Habits in 7 to 17 Yr-Olds from Moscow, Russia

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The aim was to assess the effectiveness of a caries prevention program based on improvement of oral health habits among 7 to 17-year-old schoolchildren in Moscow.

A total of 300 7–8 (Group-A), 11–12 (Group-B) and 15–17 (Group-C) years old subjects from Central Moscow were included in a program based on children's oral hygiene education and dietary advice focusing on indentified relevant caries risk factors. Children were interviewed and examined for plaque, gingival and caries status at baseline and 4 months later. Chi-Square-test was used to find differences in answers between time points. There was no change in DMFS with simultaneous increase of filled surfaces. After 4 months 61.4% (Group-A), 32.8% (Group-B) and 31.4% (Group-C) were plaque-free. Percentage of children without gingival inflammation increased from 2.8% to 57.1% (Group-A), 14.3% to 74.3% (Group-B), and 11.4% to 38.6% (Group-C) ($p < 0.05$). Significant self-assessed teeth/gum improvements were observed; more children mentioned the reason for visiting dentists was checkups rather than treatment/pain ($p < 0.05$). The number of children brushing their teeth twice-a-day increased from 20% to 91% (Group-A), 64% to 100% (Group-B), 46% to 90% (Group-C) ($p < 0.05$). The number of children using F-toothpaste increased from 24% to 47% (Group-A), 11% to 97% (Group-B), 31% to 89% (Group-C). There was a decrease in consumption frequency of cakes/sweets and tea with sugar in all age groups, and of jam/honey and soft drinks in Group-B and Group-C. The program based on children's intensive education was effective in improving plaque and gingival status; in changing oral health and dietary habits, and in self-assessment of oral health.

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Socioeconomic Inequalities in Early Childhood Caries: Analysis of Four Vulnerable Territories in Colombia

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Colombia is a highly unequal middle-income country recovering from a long internal armed conflict. The aim of this study was to assess whether ECC was a marker of social inequality in four relatively high-vulnerability Colombian territories. We analysed data from a baseline evaluation carried out in 2014, corresponding to before the implementation of an Alliance for a Cavity-Free Future caries prevention/promotion programme. The sample comprised 1,344 children aged 1–6 years. Inequalities in ICDAS-merged moderate/extensive caries lesions and also including ICDAS-merged initial caries lesions were examined by household income and education. We compared age-standardised prevalence/surface-level mean of each outcome by socioeconomic level and derived the relative index of inequality (RII) and slope index of inequality (SII) from regression models adjusted by relevant covariates.

About a third of the children had moderate/extensive caries lesions, while 84% of them had caries lesions of any stage. Social gradients were observed for most outcomes, i.e., a higher prevalence of caries lesions or mean number of surfaces with caries lesions at successively lower income and educational levels. Estimates of the RII and SII revealed the existence of significant relative and absolute inequalities in ECC. Absolute differences indicate a 12.4 percentage points higher prevalence of moderate/extensive caries lesions in children living in households with the lowest education level (SII: 12.4; 95% CI 2.7, 22.1). Also, these children would have 6.7 more tooth surfaces with initial-to-severe carious lesions than those living in households in the highest education category (SII: 6.73 95% CI 4.18, 9.29).

Even in these populations living in vulnerable conditions there were significant socioeconomic inequalities in ECC, posing challenges for designing oral health improvement strategies. Those inequalities were observed when considering two different cut-off points to define caries.

Acknowledgements: Alliance for a Cavity-Free Future – Colombian Chapter.

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First Dental Visit and Caries Experience Among 3-Year-Olds in Moscow: A Retrospective Study

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The aim of the study was to assess age and reason for the first dental visit and caries status among Moscow 3-yr-olds. A retrospective study was performed analysing medical cards of 554 3-year-olds in Moscow public clinic. Children were divided into groups (I-IV) depending on age of first dental visit: 0–12 months (n = 203), 12–24 months (n = 212), 24–36 months (n = 101) and 36–42 months (n = 38). Average age for first appointment was 20 months. In 88.6% the reason for the first visit was check up, 5% – short bridles, 0.4% – dental trauma and 5.4% – pain or visible cavities. The mean visit frequency for prevention was once a year; 6.32% of children came to dentist for professional teethcleaning; 1.81% for orthodontist consultation. Neither information whether children/parents received dental education, nor breastfeeding/dietary habits were available from medical records. The mean dmft was 1.01 (2.54); 14.7% of cavities were complicated caries. Initial caries lesions were recorded only in 9.76% of children. dmft indices were 0.54 (1.53); 1.17 (2.83); 1.41 (2.91) and 2.26 (4.21) in groups I, II, III, IV, respectively. The complicated caries accounted for 21% of cavities in group I; 16.1% – in group II; 10.2% – in group III and 17.9% – in group IV. Among all children 2.64% were sent to be treated under general anaesthesia. In group I, the reason for first visit was: 83.7% for check-up; 13% – short bridles. In group II: 88.4% for check-up; 9.6% pain/cavities. In group III – 52.6% for check-up and 47.4% pain/cavities. In group IV – 50% for check-up and 50% pain/cavities. The most common reason for first appointment was check-up, however further treatment was necessary. Attention to diagnosis of initial caries was insufficient. A first dental visit at the age of 6–8-months old or just at the beginning of the eruption of the first primary tooth is recommended.

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Changes in Iowa Fluoride Study Dental Fluorosis Prevalence and Severity from Age 9 to 23

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Evidence from the few previous studies suggests mild dental fluorosis declines in severity from late childhood to adolescence. This study's aim was to examine changes in fluorosis from ages 9 to 23 in a birth cohort. Fluorosis exams were conducted at 9, 13, 17, and 23 years in the longitudinal Iowa Fluoride Study. Fluorosis severity was defined as the person-level 2nd highest Fluorosis Risk Index (FRI) score for early-erupting, late-erupting, and all-available teeth. Scores were cross-tabulated for each pair of adjacent exam times, and Wilcoxon signed-rank tests used to determine whether median differences in severity scores between adjacent time points were significantly different from 0. Changes in fluorosis were assessed for participants with both 9- and 13-year (n = 523), 13- and 17-year (n = 429), and 17- and 23-year (n = 304) exams. At age 9, the 2nd highest FRI score for early-erupting teeth was fairly evenly distributed across scores of 0 (37.7%), 1 (26.9%), and 2 (34.3%), with 1.1% having FRI scores of 3. By age 23, 65.5% of participants had maximum FRI scores of 0 on the early-erupting teeth, and fewer participants had scores of 1 (24.0%), 2 (9.9%), or 3 (0.6%). Trends were similar for late-erupting and all-available teeth. The median fluorosis score difference was significantly lower than 0, indicating decreased fluorosis severity over time, for all pairs of time points and the three tooth groups, except for the early-erupting teeth from ages 17 to 23. In conclusion fluorosis severity, measured by the person-level 2nd highest FRI score, tended to decline throughout adolescence and early adulthood, and leveled off for early-erupting teeth after adolescence. This trend could be due to wear of the enamel or continued enamel maturation after eruption.

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Dental Caries Prevalence of 10–18 Years Old Students in La Pintana District, Santiago-Chile. 2018

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Dental caries is a chronic non-communicable disease and is one of the more prevalent disease in the world specially in low income populations. The last dental caries prevalence study in Chile (2007) reported 37.5% of 12-year-old individuals were caries free using decay missing filling (DMF) caries detection method. During the last decade Chile has been developing preventive and educational strategies to reduce the number of caries lesions in people under 20 years old, with a focus on the most vulnerable populations. La Pintana district is a low-income population with water fluoridation and the aim of this study was to determine the prevalence of dental caries in school children aged 10–18 years in this district using the International Caries Detection and Assessment System (ICDAS). This was a cross-sectional observational study with all students in school grades 6–12 from two schools invited to participate. A total of 550 students was included. Parents gave informed consent and written assent was received from each participant. The study was approved by the Universidad de los Andes Scientific Ethics Committee. Two examiners, previously trained and calibrated in the ICDAS criteria, examined the students in a dental mobile. The inter-examiner Kappa value was 0.89 and intra-examiner Kappa values were 0.74 and 0.75.

A caries prevalence (ICDAS score >1) of 84.4% was found among those with at least one tooth and with ICDAS ≥3 the prev-

alence was 39.3%. Girls had higher prevalence of ICDAS scores ≥3 than boys (P value <0.00). The mean for D_{3-6}MFT scores for boys was 1.5 and 2.1 for girls. In conclusion, using ICDAS criteria, the 15.6% of individuals were free of dental caries and the constructed DMF index indicated that 60.7% of the scholars were free of dental caries.

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Caries Detection Methods: Comparison of DMF, ICDAS, CAST and Nyvad's Criteria in Italian Schoolchildren

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Methods to measure caries lesions are based on standardized diagnostic thresholds, allowing comparison of caries status in different populations reaching an acceptable level of precision. The most commonly used carious lesion assessment system is still the decayed, missing and filled index (DMFT). Recently, a variety of new methods has been developed to measure caries in a population. This study was a descriptive cross-sectional study, in which four caries measurement methods, DMFT, the International Caries Detection and Assessment System (ICDAS), the Caries Assessment Spectrum and Treatment (CAST), and Nyvad Criteria were applied in the same group. From a sample selected for the Italian National Oral Health Survey, a subsample of 500 12-year-old children (236 males and 264 females) was selected and examined (from January 16th 2017 to February 17th 2017) by four calibrated examiners. Overall Cohen's Kappa exceeded the criterion of K = 0.75 for inter-examiner agreement and K = 0.80 for intra-examiner agreement. All the children were examined four times. The mean time taken to record the DMF was 3.7 ± 1.2 min, for ICDAS 6.3 ± 3.6 min, for CAST, 5.2 ± 4.2 min and for Nyvad 5.1 ± 3.5 min. The variables which showed the greatest differences among systems was the number and percentage of sound teeth ($p < 0.01$): DMFT n = 9942, 77.55%; ICDAS n = 2628, 20.49%; CAST n = 5053, 39.41% and Nyvad Criteria n = 4117, 31.11%. At the level of dentinal Distinct/Active Cavity lesions no statistically significant difference was observed (p = 0.40) between ICDAS (n = 1373, 10.71%), CAST (n = 1371, 0.69%) and Nyvad Criteria (n = 1720, 13.41%). In conclusions the DMF index was the fastest method albeit with a high under-estimation of caries lesions prevalence. ICDAS, CAST and Nyvad Criteria allow collection of comprehensive clinical data at the caries severity level (Nyvad Criteria also records caries activity).

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Candida spp. and Dental Caries: A Systematic Review and Meta-Analysis

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This systematic review and meta-analysis investigated whether the presence of fungi of the genus *Candida* in saliva or dental plaque is associated with dental caries in individuals older than 6-years. Electronic search was carried out in MEDLINE/PUBMED, EMBASE and LILACS databases without restriction of language or date of publication using a pre-defined search strategy following PRISMA guidelines. Information about number of individuals harbouring/not harbouring *Candida* spp. as well as presenting/not presenting dental caries were extracted from the eligible studies by two independent investigators. Quality of evidence was assessed following NIH guidelines. A third investigator solved any disagreement in data extraction and quality assessment procedures. Prevalence ratio (PR) was calculated considering 95% confidence interval (CI). Quantitative meta-analysis was performed using the random effect Mantel-Haenszel model. Twenty-seven out of 129 studies selected for full-reading were included in the meta-analysis (total of 3559 individuals), being 19 and 8 studies related to individuals aged from 6 to 18 years and older than 18 years, respectively. Prevalence of *Candida* spp. in saliva or dental plaque ranged from 7.7% to 78%. Prevalence of dental caries in individuals harbouring *Candida* spp. ranged from 38.9% to 100% and was higher than in individuals not harbouring *Candida* spp. (PR = 1.81 [1.49–2.21]; $p < 0.01$). The subgroup analysis showed that prevalence of dental caries was 96% and 44% higher in individuals harbouring *Candida* spp. at the ages from 6 to 18 years and older than 18 years, respectively (95% CI [1.50–2.56] and [1.20–1.93]; $p < 0.01$). The quality of evidence of most studies (n = 23) was graded as fair. These results suggest that the presence of *Candida* spp. in the oral cavity is associated with dental caries.

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Comparison of Oral Health Knowledge, Attitudes and Practices of Two Cohorts of Young Children's Parents from Anapoima, Cundinamarca

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The Colombian Chapter of the Alliance for a Cavity-Free Future (CC-ACFF) started in 2013 an oral health promotion/prevention project towards young children in the municipality of Anapoima. Despite the fact that it started as a conjunctive programme involving the community, the local government, the health representatives and educators, in the past three years it has shifted more to a programme including in-school settings' dental students' biannual fluoride varnish application, one oral-health talk by the students to parents and a yearly community oral-health promotion ludic activity. This study aims to compare parents' 0–6 years old children-related oral-health knowledge, attitudes and practices (KAPs) of a group who have been in the ACFF Anapoima's programme for ≥1 year with a group who recently joined. It counts with IRB and inform consents signed by parents. A modified unvalidated version from a previously validated parents' KAPs questionnaire of 14 items was applied in 2018 to 160 parents from the two cohorts: ≥1 year (A: n = 85) and new participants (B: n = 75). Regarding knowledge (3 questions) most parents considered as relevant oral hygiene since teeth eruption (>52%), however <45% with fluoridated toothpaste and with no significant improvement in cohort A vs. B ($p > 0.05$). Concerning attitude (1 question), cohort-A considered that dentists share with parents' responsibility for children developing caries ($p > 0.05$). As for practices (10 questions), significantly more cohort-A children received 1–2 yearly varnish-fluoride application (A. 68.3%; B: 45.3%) ($p = 0.003$), but surprisingly more cohort-A children had their teeth brushed at night and at morning (A: 9.4%; B: 5.3% and A: 3.5%; B: 0%, respectively) ($p = 0.005$). In conclusion, parents' KAPs cannot reflect an influence of the ongoing promotion/prevention programme and efforts should be done to redirect it to its principles.

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Association Between Coronal Caries and Partial Dentures in Elderly People of Nursing Homes in Bogotá

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The aim of this study was to identify any association between the use of partial dentures and the presence of coronal caries in institutional elderly (IE) from Bogotá, Colombia. Counting with IRB (012-2016) and informed consents, three examiners calibrated in ICDAS visual criteria (inter-/intra-reproducibility Kappa values ≥0.7) clinically examined the subjects in the elderly institution settings. Assessments included: presence of dental plaque (Silness & Löe modified index), caries risk (Cariogram® software), coronal caries at the surface level (ICDAS-merged criteria), conventional DMFS and ICDAS-merged DMFS, and presence of partial denture in the upper and lower jaws.

Out of 226 partly edentulous IE assessed in 40 nursing homes 66 wore denture. The mean age of this group was of 80.1 ± 9.3 years, mainly 65 years old or older, and most were women (63.7%). The mean number of present teeth was of 11.3 ± 7.5. More than half of the assessed population wore only lower partial dentures (54.5%), followed by partial dentures in both jaws (24.2%) and upper partial dentures (21.2%). The majority had presence of plaque (94.2%) and all (100%) were classified as having high-caries risk. The prevalence of conventional and ICDAS-merged DMF caries experience was 100% with a mean number of ICDAS-merged DMFS of 94.5 ± 26.3 (D: 4.9 ± 9.6; M: 77.8 ± 33.9; F: 11.7 ± 16.4).

A statistically significant association was found between the use of upper partial denture and the ICDAS-merged DMFS ($p = 0.01$). Likewise, there was significant association the use of lower partial denture, with ICDAS-merged DMFS ($p = 0.005$) and with the presence of caries lesions ($p = 0.034$).

This study shows a significant association between the use of partial dentures and ICDAS-merged DMF caries experience in institutionalized elderly in Bogotá.

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Conceptualization of Dental Caries by Dental Students and Its Relationship with Preventive Oral Care Routine

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Considering that cariology concepts learned during dental school may impact how future dentists will control and treat dental caries in their patients and themselves, the aim of this study was to survey dental students on their conceptualization of dental caries, as well as their preventive oral care routine. After ethical approval, dental students of a U.S. dental school (n = 517) were invited via email to voluntarily respond to an anonymous, self-administered electronic survey (REDCap©). Demographic characteristics, caries conceptualization (discursive question), dental care habits and self-caries risk determination were asked. Responses to caries conceptualization were analyzed by the content analysis technique. Data were compared between groups by chi-square test. Overall response rate was 53% (52.5% male). Three researchers independently analyzed discursive answers and classified every answer (n = 274) into one of the 6 caries categories: [1] only signs of the disease (10.2%), [2] bacterial disease – not specifying bacterial metabolism (39.0%), [3] bacteria byproducts – not specifying diet as substrate (13.8%), [4] biological concept or multifactorial disease (24.5%), [5] comprehensive multifactorial disease (8.4%), or [6] other (4.0%). Only 33% completely defined dental caries according to the modern understanding of the disease (categories 4 and 5). Statistical differences were observed between caries conceptualization and diet modification ($p = 0.038$); caries conceptualization and rinsing after brushing ($p = 0.01$); year of school and self-determined caries risk ($p = 0.0005$); year of school and brushing at school ($p = 0.0005$); year of school and rinsing after brushing ($p < 0.0001$); and self-determined caries risk and brushing at school ($p = 0.0065$). Our data suggest that students' understanding of the disease can influence how students manage the disease in terms of diet modification and behavior after brushing. Moreover, self-caries risk determination seems to influence students' brushing behavior.

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Withdrawn

Caries Research

Session 4
De- and Re-Mineralization and Erosion

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Prediction of Resin Infiltration Color Masking Effect on Natural White Spot Lesions

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The aim was to investigate different parameters that could predict the color masking degree of white spot lesions (WSL) after using the resin infiltration technique (RI). Sixty extracted human teeth with natural WSL on smooth surfaces were selected. At the baseline, the laser fluorescence (LF) of WSL and surround sound enamel (SE) was analyzed with DIAGNOdent (KaVo). Standardized photographs were obtained under D65 and UV illuminants. The Lab color coordinates according to the Commission Internationale de l'Eclairage (CIE) and the natural fluorescence (NF) were obtained for SE and WSL. The color difference (Delta E) between the SE and WSL was calculated. The lesions dimensions were determined (area, perimeter, width and height). The resin infiltrant (Icon, DMG) was applied and the assessments repeated. The color masking was evaluated by the reduction of Delta E values. The one-way ANOVA and Tukey's tests were used for LF and NF. The t-test was used for Delta E values before and after the treatments. The correlations between the parameters evaluated at the baseline and Delta E after infiltration were analyzed. The results of LF at the baseline (B) and after infiltration (I) were: SE/B-3.98^{a}; WSL/B-13.28^{c}, SE/I-3.36^{a}, WSL/I-7.95^{b} ($p = 0.001$). The results for NF were: SE/B-7.93^{a}; WSL/B-6.29^{b}, SE/I-7.93^{a}, WSL/I-7.27^{a} ($p = 0.001$). The results for Delta E were: B-13.66^{a}, I-10.45^{b} ($p = 0.001$). A significant reduction of the lesion dimensions was observed after treatment. No correlations were observed between LF (0.00), NF (–0.07), area (0.01), perimeter (–0.12), width (–0.09) and height (0.05) and the Delta E reduction after RI. In conclusion, the WSL has higher LF and smaller NF than the SE, which were respectively reduced and increased after RI. This treatment was suitable to mask most of the natural WSL, but it was not possible to predict the masking effect by the evaluated parameters.

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Enamel Remineralization Potential of Highly Demineralized Lesions with Toothpastes Containing Calcium and Phosphate

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The aim was to evaluate the remineralization potential of toothpastes containing calcium and phosphate on highly demineralized artificial enamel lesions. Enamel blocks 4x4 mm obtained from bovine crowns were embedded in acrylic resin and polished. Artificial caries lesions were created by immersing the blocks in demineralization solution at 37°C for 96 h. Vickers surface microhardness (VMH) and stratification was conducted after lesion formation to ensure no differences at baseline. The enamel lesions were treated with 3M™ Clinpro™ Tooth Crème (CTC) or MI Paste Plus® (MIP). Artificial saliva was used as a negative control for the treatment groups. The enamel blocks were treated with toothpaste slurry 4 times a day for 2 minutes during a 20-days pH cycling model. VMH ($n = 10$), enamel fluoride uptake (EFU) ($n = 7$) and micro-CT ($n = 3$) were taken at baseline, 10-days and 20-days later. VMH recovery (%VHMR) was calculated from VMH baseline values. Volume % mineral recovery (%MR) was calculated by collection of attenuation coefficient profiles from micro-CT analysis. One-way ANOVA and Tukey test were used for statistical analysis.

No differences were found in baseline VHM values ($p > 0.05$). At 10 days of treatment, CTC displayed significant higher values in EFU compared to MIP and saliva control, with 5694.03 (μgF/cm^3) versus 1384.83 (μgF/cm^3) and 224.95 (μgF/cm^3), respectively. At 20 days of treatment CTC presented 91.73% of VHMR compared to 28.8% for MIP ($p < 0.05$). EFU results showed higher values for CTC compared to MIP and control ($p < 0.05$). %MR was higher for CTC with 43% recovery compared to 28.9% for MIP but the difference was not statistically significant. Overall CTC presented higher remineralization potential for highly demineralized enamel lesions compared to MIP and saliva control.

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45S5 Bioglass Doped with Lithium as a Potential Biomaterial for Treatment of Early Dental Caries

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Bioglass® 45S5 (BG) is an alloplastic biomaterial with potential to remineralize early enamel lesion teeth. The incorporation of doping elements has allowed the improvement of therapeutic benefits of bio glasses. From these different therapeutic ions, lithium is stressed due to the ability to improve mechanical properties by generating a more compact silicate network and to increase the cementogenesis process. However, there is no enough information on its contribution in remineralization effect motivating the present study aims to evaluate the effect of doping Bioglass® 45S5 with 5 wt.% of Li (BG-Li) on bio mineralization process. In particular, to analyze the potential use of these materials in remineralization of early enamel lesion produced from *ex vivo* method using *S. mutans* for demineralization. Samples tested were distributed randomly in three groups: artificial saliva (negative control), BG, BG-Li. They were submerged in muds made with products and deionized water for 10 minutes every 12 hours. During that lapse of time, samples were submerged in centrifuge tubes containing artificial saliva in a shaker at 37°C and 80 rpm for 15 days. Micro hardness was evaluated in every stage of the experiment: initial, post demineralization assay and post remineralization assay. Two-sample t test with unequal variances were used for each group, comparing micro hardness post demineralization assay and post remineralization assay. Both experimental groups showed statistically significant differences, with BG-Li being the material that achieved the best results ($p < 0.0001$ versus $p < 0.01$ of BG). In conclusion, *S. mutans* associated with broths rich in dextrose is a good model to produce *ex vivo* demineralization of hard tissues, such as teeth. The present assay confirms the effectiveness of BG and BG-Li in enamel remineralization, with their effect being equivalent to another studies but in shorter periods of time. A better result is observed while using BG-Li when comparing both experimental groups.

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Effect of High-Fluoride Dentifrice and Bracket Bonding Composite Material on Enamel Demineralization In Situ

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The aim of this in situ study was to evaluate the effect of high-fluoride dentifrice (5,000 µg F/g) and fluoride-containing bonding composite resin on enamel demineralization adjacent to orthodontic brackets. Ten volunteers wore palatal appliances containing four bovine enamel blocks (two in each side) with metallic brackets bonded with fluoride-free or fluoride-containing composite resin. During 3 phases of 14 days each, 3 dentifrices with different fluoride concentrations (0, 1, 100, and 5,000 µg F/g) were tested. The cariogenic challenge consisted of 20% sucrose solution dripped 8x/day onto the dental blocks. At the end of each phase, biofilm formed was collected for F analysis. Cross-section hardness was performed in enamel blocks and the lesion area (ΔS) was calculated. Data were analyzed by two-way ANOVA followed by Tukey post-hoc test ($\alpha = 5\%$). The ΔS (±SD) for enamel slabs treated with dentifrices with 5,000, 1,100 and 0 µg F/g and bounded with F-containing or F-free resin were respectively: 3043.00 (761.69); 5443.93 (2001.42); 8823.82 (4361.83) and 3888.75 (832.17); 5931.68 (2119.30); 10527.50 (4693.37). The effect of the bracket-bonding composite resin was not significant for the variables studied ($p > 0.05$), while for dentifrice it was significant ($p < 0.001$). Smaller lesion area and higher F concentration on biofilm were found in 5,000 µg F/g group, irrespective of bonding composite resin. Thus, it could be concluded that high-fluoride dentifrice is effective in reducing demineralization on enamel adjacent to orthodontic brackets, while the fluoride-containing bonding composite resin does not influence it.

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Silver Ions Accelerate Rate of Enamel Demineralisation. An In Vitro Dose-Response SMR Study of SDF

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Topical application of silver diammine fluoride ($Ag[NH_3]2F$) treatments are becoming increasingly popular for prevention and treatment of caries, but its mechanism of actionremains obscure. The modus operandi includes anti-bacteriological properties provided by high concentrations of F– and Ag+. Further, high concentrations of fluoride ions will provide anti-demineralising and remineralising capabilities.

The aim of the study was to use scanning microradiography (SMR) to measure the dose-response efficacy of Ag+ (i.e. without F-) on artificial enamel demineralisation.

Enamel blocks (5 x 5 x 2 mm thickness) were cut from caries free permanent molars, and coated with varnish leaving only the original surface exposed. The blocks were located in SMR environmental cells, and exposed to caries simulating demineralisation solution (pH = 4.0) for 30 h at 23.0 ± 1.0 0C. SMR was used to measure the rate of mineral loss. Subsequently, $AgNO_3$ was added stepwise so that the Ag+ concentration was 0.1, 9.0 and 3565 ppm, and the rate of mineral loss measured. The mineral loss at each Ag+ concentration was linear with time. The percentage changes in rate (from baseline) were +5.1 ± 5.5, –7.8 ± 7.8, and –19.4 ± 10.9% following each Ag+ addition, demonstrating a decreasing log-linear dose-response trend with increasing Ag+ concentration.

This decreasing trend shows that Ag+ increases the rate of enamel demineralisation, unlike the increasing inhibitory trend reported for other ions (including Zn, Cu and Sr). This may be related to the mono-valence of silver ions. In conclusion, Ag+ in solution actually accelerates the rate of enamel demineralisation, suggesting that the cariostatic efficacy of topical fluoride-containing silver agents is related to their F– component, rather than the Ag+. Nevertheless, Ag+ may have a synergistic influence on the efficacy of F– when in combination.

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Effect of Different Concentrations of a Novel Remineralizing Agent on Caries-Like Lesions In Vitro

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The aim was to evaluate the differences of superficial hardness and mineral density in specimens of enamel demineralized in a microbiological model of caries lesion and submitted to a protocol of remineralization with a novel remineralizing agent (BlueRemin) in different concentrations. 24 human enamel surfaces were demineralized in an in vitro mono species cariogenic biofilm (*S. mutans* ATCC 25175) for formation of caries-like lesions. *S. mutans* was cultivated in BHI. Enamel samples (specimens) were exposed to human saliva for the formation of dental pellicle and immersed in BHI. For demineralization we exposed speciemns to 0.1 mM of sucrose four times a day for three days. Specimens were assigned to 3 groups of different concentrations of a novel remineralizing agent, BlueRemin (BR) based on fluoride and graphene, and control. BlueRemin (BR) is protected by Chilean patent application No.201602960 and is not yet available commercially. Microhardness (Vickers microhardness number) and microtomography assessments were used to measure the percentage of recovery of superficial hardness (%RSH) and mineral density (%RMD) as indicators of remineralization. ANOVA test and multiple comparison test were used for statistical analysis. Remineralization occurred in all groups to different degrees; %RSH was 1.32 for control and 30.78, 30.7 and 48.61 for the three different concentrations. All changes from baseline were significant ($P \leq 0.05$) but there were no significant differences between the different concentrations of the novel remineralizing agent. In conclusion, BlueRemin exhibited a remineralization capacity of caries-like lesions in the in vitro model. Further research is required to assess the use of BlueRemin in the dental practice.

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Effect of Blueremin, a New Remineralizing Agent, on Demineralized Enamel and Dentin

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The aim was to establish differences in surface hardness in specimens of hydroxyapatite (HA) discs, enamel and dentin blocks, demineralized by a microbiological caries model and after being subjected to a remineralization protocol with a new fluoride and graphene based agent (BlueRemin). A demineralizing biofilm model using *S. mutans*, ATCC25175, was used to produce artificial caries lesions on HA discs and enamel and dentin blocks that were exposed to sucrose (10%), 4 times a day for 5 min/5 days. Vickers surface microhardness (VH) was determined before and after exposure to biofilm, and after the application of BlueRemin (applied once for 2 min and incubated 16 h with artificial saliva at 37°C). Fluor Silano® and Diamino Silver Fluoride were used as remineralization controls. Using the STATA software, the microhardness of the blocks used was analyzed. Shapiro-Wilk test was applied to evaluate the distribution of the data, the student t-test to compare the VH averages and the (ANOVA) Bonferroni multiple comparison test to compare the VH averages between the remineralizing agents. The initial average of VH for the blocks of HA, enamel and dentin was 558.9, 365.9 and 75.82 VH, respectively. VH after exposure to the microbiological demineralization model was 535.6, 175.0 and 18.40 VH respectively. Finally, after applying BlueRemin®, the VH increased to 552.3, 183.17 and 26.99 in HA discs, enamel blocks and dentin. The difference between the initial values of microhardness and after the demineralization was statistically significant ($p < 0.05$), with lower values in the demineralized areas. The difference in the microhardness values of Vickers after the application of BlueRemin® was also statistically significant ($p < 0.0001$), with higher measurements in the areas exposed to the remineralizing agent. The differences between the measurements of the areas exposed to BlueRemin® and the areas exposed to Diamino Silver Fluoride and Fluorine Silane were not statistically significant. In conclusions, *in vitro* biofilm model of *S. mutans* decreased the surface microhardness of the HA, enamel and dentin blocks. BlueRemin increased the surface microhardness of HA, enamel and dentin. There was no difference in the remineralizing capacity of BlueRemin regarding to Fluor Silane and Diamino Silver Fluoride.

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Characterization of Optical Coherence Tomography (OCT) Intensity Depth Profile of Demineralised Enamel

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The objective of this study was to characterize the depth profile of Optical Coherence Tomography (OCT) backscatter intensity against the histological changes of demineralised enamel. 4x4 mm^2 windows were made on the crowns of nine bovine incisors and immersed in a buffered lactic acid solution (0.1 M, pH 4.5) for 6, 8, 12 and 24 hours. At each time point, one sample was removed for SEM scanning whilst the others were rinsed, air-dried and 3D OCT scans performed of the exposed window. For each sample, a mean backscatter intensity depth-profile (A-scan) was computed from 10 frames extracted from the middle 2x2 mm^2 of the exposed window. Mean A-scans for each time point were then generated. Cross-sectional SEM scanning were performed at the middle of the window at 1, 5 and 20 k x-magnification. All time point demonstrated two distinct layers under 1 k x-magnification, with the depth of the top layer being 13.5 ± 2.2 μm, 19.5 ± 3.0 μm, 48.6 ± 3.9 μm and 53.3 ± 7.7 μm with increasing time interval. 20 k x-magnification shows the top layer to be a combination of inter-prismatic demineralisation and re-deposition of hydroxyapatite crystals. The bottom layer consists predominantly of inter-prismatic demineralisation. The OCT backscatter intensity of the 6 and 8 hours demineralization shows a brief decay from the surface followed by a plateau before continuing to decay. The depth where intensity begins to plateau at 6 and 8 hours were 15.17 ± 2.6 μm and 25.45 ± 9.39 μm. The intensity attenuation of 12 and 24 hours do not exhibit a pronounce plateau but shows changes in attenuation rate at 49.40 ± 8.5 μm and 63.01 ± 20.30 μm respectively. In conclusion, structural changes in enamel demineralization correspond attenuation rate change of OCT backscatter.

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The Impact of Salivary Calcium on the Ability of Fluoride to Reharden Erosive Lesions

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Little emphasis has been placed on how varying saliva calcium concentrations affect the ability of fluoride to reharden erosive lesions. The present laboratory study followed a 3 (calcium concentrations in artificial saliva) x 3 (fluoride concentrations in a topical treatment) factorial design. Early erosive lesions were formed in polished bovine enamel specimens (30 min grapefruit juice; n = 8). Lesions were pH cycled for 5 d with twice-daily fluoride treatments (0/287.5/1250 ppm F), six static acid challenges (0.5% citric acid, pH 2.5) sandwiched between exposures to artificial saliva, varying in calcium concentration (0.66/0.89/1.11 mM). The response variable was percent surface microhardness recovery (%SMHr), calculated using Knoop indentation lengths of the sound enamel, lesioned and pH cycled specimen. Data were analyzed using two-way ANOVA. The two-way ANOVA showed a statistically significant interaction between calcium and fluoride concentrations on %SMHr ($p < 0.0001$). Only two groups exhibited rehardening (positive %SMHr values). Lesions exposed to 1.11 mM Ca/1250 ppm F exhibited the most rehardening (%SMHr = 55), whereas those treated with 0.89 mM Ca/287 ppm F showed the lowest %SMHr (–145%). %SMHr in the 0 ppm F groups were –84; –101; –140 for 0.66; 0.89 and 1.11 M Ca, respectively. In the 287 ppm F groups, %SMHr was –60 and –82 for 0.66 and 1.11 M Ca, respectively. %SMHr in the 1250 ppm F groups were –57 and 14 for 0.66 and 0.89 M Ca, respectively. Only the data in the 1250 ppm F groups followed expected trends. Surface deposition or extensive surface loss were not noted in any of the groups. In conclusion, while higher salivary calcium and topical fluoride concentrations appear to be beneficial in rehardening erosive lesions, the present study was largely inconclusive. Further studies employing additional analytical tools seem warranted.

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Prevalence of Erosive Tooth Wear Among 10–18 Years Old in La-Pintana District, Santiago-Chile

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There are no published data about prevalence of erosive tooth wear in children and adolescents in Chile and there is reported a high consumption of sugar soft drinks. The aim was to determine the prevalence of erosive tooth wear in schoolchildren aged 10–18 years in the La Pintana district, a low income population, in Santiago, Chile. This was a cross-sectional observational study involving 550, 5th grade primary to 3rd grade high school, children. Informed consent of the parents was obtained and the written consent was received from each participants. The study, was approved by the Scientific Ethics Committee of the Universidad de los Andes. The clinical examinations were carried out at the participating schools in a mobile dental clinic. The clinical evaluations were conducted by two examiners, trained and calibrated in the BEWE index with inter-examiner and intra-examiner Weighted Kappa value 0.67 and 0.79–0.75 respectively. A prevalence of 62% was observed for participants having at least one tooth with BEWE value ≥1 and the prevalence was 24.55% when only BEWE 2 and 3 were included. Men presented more severe erosions (BEWE 2/3 and BEWE 3) than women (p value <0.01). In conclusion erosive tooth wear was a prevalent condition in a group of schoolchildren aged between 10 and 18 years of age and living in a low income population. More than half of the individuals have at least one BEWE lesion ≥1 and 10% have at least one lesion with a BEWE score = 3.

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Evaluation of Inhibiting Action of Varnish Containing S-PRG Fillers Against Dentine Loss Through Acid Exposure

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In-vitro studies were conducted to evaluate an inhibiting action of experimental varnishes containing S-PRG fillers which release several ions (Al, B, F, Si, Sr) against dentine loss through acid exposure. Five kinds of vanishes were prepared by adding 0, 10, 20, 30 and 40wt% of S-PRG fillers, respectively. Sixty human dentine-slabs, in which half of the surfaces were treated with the experimental varnishes, were put into warm water for 24 hrs. Afterwards, an exposed window was made within the treated area and another window within the control area. Slabs, each with two windows, were eroded through immersion into 0.83 M acetic acid for 30 min. The slabs were cross-sectioned in the middle of the windows to measure the thickness of dentine loss. Subsequently, three layered samples (15 μm thick) were abraded from the specimen surface using an abrasive micro-sampling technique. The powered samples were dissolved in acid and buffered. Fluoride and the other four minerals, calcium and phosphorus ions were quantified using a fluoride-selective electrode and ICP-atomic emission spectroscopy, respectively. The mineral concentrations were calculated from the assumption that the phosphorus concentration of the dentine was 13.5 wt%. Differences in tissue loss between the treated and control areas were –1.02, 2.04, 1.90, 2.48 and 1.36 μm in the 0, 10, 20, 30 and 40 wt% groups, respectively, indicating that significant reductions were found in the 10 and 30 wt% groups. Levels of fluoride and strontium of the 10 through 40 wt% groups and aluminum only in the 40 wt% group were significantly higher in first layer than those in the 0 wt% group. These results suggest that the varnishes containing S-PRG fillers decrease the influence of acid on dentine loss by penetrating minerals released from the fillers to the underlying dentine.

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Erosive Tooth Wear and Risk Factors in 12–15 Years Old Schoolchildren in Bogota, Colombia

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This study aimed to determine the prevalence of erosive tooth wear (ETW) and describe related factors in a preliminary subsample of 12 to 15 year-old schoolchildren from Bogotá. Counting with IRB and informed consents, two examiners calibrated in the BEWE index, clinically assessed ETW in school settings. All dental surfaces were scored (BEWE index: 0-no wear, 1-initial loss, 2-<50% of, 3-≥50% of surface area affected). For each individual two BEWE measures were calculated: 1) Highest BEWE score (0–3) and 2) Total BEWE score (sum of highest BEWE per sextant: 0–18). An 8-item questionnaire on ETW-related dietary and oral hygiene factors was applied. A total of 176 subjects were assessed (female: 61.9%; mean age: 13.3 ± 1.05 years). Most children were of middle-low social-economic status (53.4%) and most attended private schools (95.4%). The prevalence of ETW was of 73.9%. Children's highest ETW BEWE score distribution was: BEWE-0: 26.1%; BEWE-1: 0.6%; BEWE-2: 14.8%, and BEWE-3: 58.5%, and Total BEWE score distribution was: 0–2: 33.0%; 3–8: 56.8%; 9–13: 9.1%, and ≥14: 1.1% (highest BEWE score was 14). Most relevant questionnaire's aspects disclosed that 13.6% reported gastrointestinal symptoms and only 1.14% experienced frequent vomiting periods; 62.5% reported liking to consume acidic food/drinks; 75.0% consumed fruits (whole/juice) daily and took less than 10 minutes to eat/drink them (76.1%); 48.8% brushed their teeth 2–3 times a day, never before eating (90.3%) and brushed <10 minutes after dining (54.5%). In these schoolchildren the prevalence of ETW was high with over half of children presenting a highest BEWE score 3, a total BEWE score of 3–8 and consuming acidic food/drinks.

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Protective Effect of Fluoride and Stannous Associated to Bioadhesive Polymers on Enamel Erosive Wear

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The anti-erosive effect of polymers is related to the formation of a protective film on the enamel surface, and it has been investigated in dentistry with promising results. The objective of this study was to evaluate if bioadhesive polymers (Carbopol 980 and Propyleneglycol) could potentiate the protective effect of a sodium fluoride plus stannous chloride (NaF+Sn) solution on the control of enamel erosive wear. Polished enamel specimens were divided into three groups (n = 60): NaF+Sn (500 ppm F; 800 ppm Sn -positive control), NaF+Sn+Pol (0.1% Carbopol; 0.3% Propylene glycol), and deionized water (negative control-C). A randomized cross-over in situ model with three phases was used. In each phase, volunteers (n = 15) wore a palatal appliance containing 4 specimens, in which two were submitted to an erosion/remineralization cycle (2 h for pellicle formation, followed by 5 min immersion in 1% citric acid, pH 2.3, 4x/day, with intervals of 1 h in saliva, and 1 min treatment with solutions, 2x/day). The other two specimens were subjected to erosion/abrasion/remineralization cycle, in

which abrasion was performed for 15 s, 2x/day, with active electric toothbrush, before the treatment with the solutions. After 5 days, enamel surface loss (μm) was evaluated by profilometry and data were analyzed by RM-ANOVA and Tukey tests (5%). There were significant differences between both challenges and among the treatment factors. Erosion/abrasion challenge resulted in significantly higher enamel loss than erosion only ($p < 0.05$). The results of Tukey's test for erosion challenge were: C = 14.7 ± 5.8^{b}; NaF+Sn = 9.0 ± 7.5^{ab}; NaF+Sn+Pol = 5.9 ± 3.8^{a}; and for erosion/abrasion: C = 26.6 ± 10.1^{c}; NaF+Sn = 15.0 ± 8.8^{b}; NaF+Sn+Pol = 12.3 ± 7.9^{ab}. It was concluded that the association of the polymers with a NaF+Sn solution protected against enamel erosive wear, but it was not significantly superior to NaF+Sn only.

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Evaluation of Fluoride Mouthrinses with Trimetaphosphate in Reducing Erosive Effects Using an Artificial Mouth

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This in vitro study evaluated the erosion effects of five fluoride mouthrinses with and without micrometric (TMPm) or nanosized sodium trimetaphosphate (TMPn) using an artificial mouth. Each group of mouthrinses had blocks (4x4 mm) bovine enamel specimens (n = 24). The mouthrinses contained 100 ppm F, 225 ppm F, 100 ppm F + 0.2% TMPm, and 100 ppm F + 0.2% TMPn and Placebo (deionized water). Half of each group of blocks underwent an erosion treatment of 1 day or 3 days. The erosion cycles were programmed in the artificial mouth 3x/day with artificial saliva (pH = 7.0) and citric acid 0.05 M (pH = 3.2) by dripping of 1.5 mL/min. The treatment with mouthrinses was performed twice per day for 1 minute. The blocks were subjected to analyzes of % loss of surface hardness (%SH) using a hardness tester (KHN) and the surface erosion wear by profilometry method. The results were subjected to Two Way ANOVA, a Student-Newman-Keuls comparison test and the Spearman correlation Test ($p < 0.05$). The %SH and the erosive wear reported statistically significant interaction between the mouthrinses groups and the erosive treatment ($P \leq 0.001$). The comparison between groups with either TMP and the erosive treatments presented statistical difference ($p < 0.001$) with the other mouthrinses, but did not prevent them for erosive wear 1 day (0.460) and there was no difference between the mouthrinses groups with 1-day treatment. A strong correlation between% SH and erosive wear (rho = 0.843, $p < 0.001$) was found. In conclusion, the effects of loss of hardness and erosive wear were reduced with better effectiveness using mouthrinses with TMP.

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Investigation into the Validity of a New Software to Quantify Erosive Tooth Wear Progression

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The aim was to validate a custom-built software for quantifying erosive tooth wear progression against the current gold standard software. Datasets from 10 natural molar teeth were created with a structured-light model-scanner (Rexcan DS2, Europac 3D, Crewe). A 300 μm depth layer was then digitally removed from the occlusal surface creating a defect of known size. These defects were then measured using volume change, maximum profilometric loss and mean profilometric loss in Geomagic Control (3D Systems, Darmstadt, Germany) and a newly designed custom-built software for measuring wear (WearCompare, Leeds Digital Dentistry, Leeds, UK). The datasets were duplicated, randomly repositioned and re-alignment performed using selective surface alignment. The effect of the re-alignment on conventional measurement metrics was calculated by analysing differences between the known defect size and defect size after re-alignment using the same measurement metrics. Data were normal and analysis performed in SPSS v25 (Pearson correlations, paired t-tests, $p < 0.05$). Excellent correlation was observed between the two softwares when measuring a defect (Volume change: $r = 0.998$, $p < 0.001$; Maximum profilometric loss: $r = 0.971$, $p < 0.001$; Mean profilometric loss: $r = 1.000$, $p < 0.001$). After re-alignment, the mean volume error (SD) was –0.06 mm^3 (0.93) for WearCompare and –0.67 mm^3 (1.14) for Geomagic ($p = 0.217$). The maximum profilometric loss error was –18.3 μm (14.1) for WearCompare and –15.6 μm (33.6) for Geomagic ($p = 0.855$). The mean profilometric loss error was 20.0 μm (9.4) for WearCompare and 24.9 μm (12.4) for Geomagic ($p = 0.059$). Mean measurement errors between softwares were not statistically different. However, the correlation between the two softwares decreased. (Volume change: $r = 0.044$, $p = 0.904$; Maximum profilometric loss: $r = 0.727$, $p = 0.017$; Mean profilometric loss: $r = 0.822$, $p = 0.004$). In conclsion the measurements between WearCompare and Geomagic were highly correlated indicating good agreement when quantifying differences. WearCompare had reduced, but not statistically significant, volume error after alignment.

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Efficacy of Preventive and Non-Operative Approaches for Erosive Tooth Wear. A Systematic Review

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The aim of this study was to assess the efficacy of preventive and non-operative approaches for the prevention and management of erosive tooth wear lesions in enamel. A systematic search of available manuscripts in MEDLINE/PubMed, Web of Science, EMBASE and Scopus was conducted. There was no restriction for language publication. Interventional studies (in-situ, in-vivo and/or clinical), evaluating preventive and non-operative approaches for erosive tooth wear in enamel, and presenting a comparison group, were included. Studies assessing outcomes not related to this review, or conducted in specific groups (such as special needs patients, under medical treatment, or on teeth with developmental defects), were excluded. Two independent reviewers (JSL, TKT) assessed and selected the studies according to the inclusion criteria. A third reviewer (JG) solved any discrepancies. Descriptive analysis of data was performed according to intervention. The search strategy identified 519 potentially relevant articles and 35 were finally included. All included studies were In-situ. No clinical studies met the inclusion criteria. Sodium Fluoride (NaF) was the most studied intervention (49%) followed by Stannous Fluoride (SnF_2) (26%), Titanium tetrafluoride (TiF_4) (14%), and CPP-ACP based products (4%). All studies assessing SnF_2 showed superior results in the prevention and/or remineralization of eroded enamel when compared to other interventions. Although products based on NaF, TiF_4, and CPP-ACP showed potential benefits against demineralization, their effectiveness was lower when compared to SnF_2 approaches. In conclusion, several approaches have been used for the prevention and non-operative management of erosive tooth wear lesions in the enamel. However, Stannous Fluoride seems to be the most effective one for this purpose.

Caries Research

Session 5
Epidemiology 2

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A New Simplified Caries Risk Assessment Model in Children. A Pilot Study

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Pediatricians are the reference stakeholders of child's health. A caries preventive programme was developed with the pediatricians acting as frontline examiners in caries evaluation. A new caries risk assessment (CRA) method was designed and a comparison among this tool and the two most used CRA systems (Cariogram and CAMBRA) was carried out. 71 children (6–14 yrs, mean age 9.5 yrs) were enrolled; caries status (DMFT/dmft) and plaque index (Silness and Loe index) were recorded. Background factors were also collected. The new tool was compared to the risk assessment obtained through the Cariogram and Cambra models. The new tool includes five biological/behavioral/psycho-social factors (socio-economic status, meals' frequency, disability, fluoride exposure, oral hygiene) and six clinical factors (no-cavitated lesions, one cavitated lesion or more, failed restoration, salivary flow, orthodontic appliance) and scores between –1 and +4 were attributed. Three risk levels were measured: low (score <0), medium (score ≥0≤1) and high (score ≥2). Lin's concordance Correlation Coefficient for Agreement (CCA) was calculated to determine how far the observed data deviated from the line of perfect concordance. The Bradley-Blackwood test (BBt) was used for a simultaneous test of their means and variances. The agreement between the different methods ranges from low to medium; CCA = 0.44 (95% CI 0.28–0.51) with a BBt = 16.11 ($p < 0.01$) between the new tool and the Cariogram, CCA was 0.33 (95% CI 0.27–0.48) with a BBt = 16.54 ($p < 0.01$) between the new tool and the CAMBRA were found. In conclusion, the new model does not produce concordant results with those obtained using other standardized models. New simple to use CRA models are needed for clinicians that are involved in health prevention for disadvantaged groups (*i.e.* children, elderly).

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Prevalence of Molar Incisor Hypomineralization in Children Residing in Soc Trang, Viet Nam

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Molar incisor hypomineralization (MIH) frequently occurs in children worldwide. However, MIH condition in Viet Nam has not yet been investigated. The aim of this study was to assess the prevalence of MIH in Vietnamese children residing in Soc Trang city, Viet Nam.

A cross sectional oral health survey was carried out in Soc Trang city, Viet Nam. A stratified sampling was applied to have a sample of 759 children (390 aged 12 years and 369 aged 15 years) from six junior high schools in Soc Trang city. The MIH were evaluated using European Academy of Paediatric Dentistry (EAPD 2003) criteria. The examination was performed by two trained and calibrated dentists. Overall prevalence of MIH was 6.3% (48/759 children). The prevalence was 6.15% and 6.5% in 12 and 15 year-old children respectively. There was no significant difference between male and female. The most prevalent teeth were lower first molars and upper central incisors while the least prevalent were lower incisors. Of the MIH teeth 58% revealed mild defects and 42% revealed moderate (enamel break down) or severe defects. Most severe defects were observed in molars. Among affected children, 23% cases (11/48) had missing first molar which was probably extracted due to MIH.

The prevalence of MIH was 6.3%, similar to other comparable studies. Lower first molars were affected more than upper first molars. The prevalence of teeth probably extracted due to MIH was 23%.

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Using ICDAS in Detecting Caries Around Restorations: A Systematic Review

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This Systematic Review showed that there is a lack of primary research on using ICDAS in the Detection of Caries around Restorations (CARS).

This systematic review was undertaken to assess the use of the International Caries Detection and Assessment System (ICDAS) criteria in research related to detecting Caries Around Restorations (CARS). CARS is a prevalent condition which is poorly diagnosed. Its detection is not straightforward, but it has received little attention in the literature. Specific, detailed, and accurate information about CARS are required by dentists to implement appropriate diagnosis, treatment and preventive approaches for a better prognosis. ICDAS is a visual caries diagnostic tool, which has potential to aid early and accurate CARS detection through systematic examination of clean, dry teeth. The aim of this study was to answer the question "What does the existing literature tell us about whether ICDAS improves the diagnosis of CARS?" Searches linking the terms ICDAS and CARS and its synonyms were undertaken using: Ovid Medline, EMBASE, Cochrane library and Web of Science–Science. Grey literature via Web of Science–Conference Indexes, Dissertations and Theses database, google scholar, research gate and Open Grey database. No restrictions were applied in terms of language or year. Even applying the broadest search strategy and inclusion criteria, three studies were identified. They were all of good quality. However, they discussed the subject from a narrow prospective and offered little to answer the research question posed. The absence of a statistically meaningful study on CARS detection using ICDAS indicates that there is a gap in the literature despite CARS being a widespread problem. These findings suggest further research is necessary in this area.

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Prevalence of Molar Hypomineralization in Indiana, USA

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The aim of this cross-sectional study was to determine the prevalence and severity of hypomineralization in the first permanent molar (molar incisor hypomineralization-MIH) and the second primary molar (hypomineralized second primary molars-HSPM) in a cohort of schoolchildren in the state of Indiana, USA. After obtaining appropriate IRB authorization, all eligible schoolchildren seen as part of a mobile dental program were screened for MIH and HSPM by a single calibrated examiner. A new MIH index consistent with the EAPD judgment criteria (Ghanim et al., 2015) was used. Sociodemographic data were collected from parents' consent forms. Descriptive statistics and Chi-square/Fisher's Exact tests were used for analysis, with a 5% significance level. Two hundred sixty-six subjects (15 public schools/10 of 92 Indiana counties) were examined. The MIH cohort (n = 193, age range 6–15 years, 52% white) had all first permanent molars evaluable for MIH. The HSPM cohort (n = 216, age range 3–14 years, 58% white) had at least one second primary molar evaluable for HSPM. MIH and HSPM had prevalence estimates of 12% and 6%, respectively. The most common defects were demarcated opacities (MIH: n = 71/155, 46%) and post-eruptive breakdown (HSPM: n = 21/69, 30%). Although more Hispanics had MIH and HSPM than Whites, race/ethnicity was not statistically significantly associated with the overall prevalence estimate of MIH or HSPM. Being a resident of a specific Indiana county or living in an urban or rural Indiana were not significantly associated with the overall prevalence estimate of MIH or HSPM but living in urban Indiana was significantly associated with overall prevalence of diffuse opacities. Prevalence estimates of MIH and HSPM in these 10 Indiana counties are comparable to global prevalence statistics.

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Root Caries Experience in Institutionalized Elderly from Bogotá, Colombia

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Being an institutionalized elderly (IE) is associated with high morbidity of oral diseases, including root caries, which adversely affects the quality of life of these people.

The aim of this study was to determine the root caries experience (RCE) through the ICDAS-ICCMS™ classification criteria in older adults living in nursing homes in the city of Bogotá.

This was a cross-sectional study involving institutionalized elderly from Bogotá-Colombia. It counted with IRB and signed informed consents. From 29 nursing homes that were asked to participate, 235 elderly (60–99 years old) living in 23 nursing homes were clinically assessed in the elderly institutions settings and 132 of them fulfilled the inclusion criteria. Clinical assessments included: root caries experience (ICDAS-ICCMA™), Root Caries Index (RCI), and the activity and severity of the root caries lesions. The mean number of remaining teeth was 11.1 (± 7.3). The prevalence of RCE was 55% and the prevalence of root caries was 39%, instead of that, RCE might be underestimated, because it was not possible to stablish if some of the teeth were lost due to root caries. On av-

erage 0.1 (±0.2) teeth presented root caries and 0.04 (±0.1) roots were filled. The RCI was 0.15. Almost all root caries lesions were active (98.7%) and 40.6% of them showed an extensive severity. Most (55%) of IE who participated in the present study showed RCE and there is a high need of treatment evidenced in the fact that almost 100% of the carious lesions were active.

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Oral Health Related Quality of Life and Oral Health Status in Patients with Eating Disorders

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This study aims to show the potential correlations between Eating Disorders (ED), oral health and OHRQoL, evaluated in outpatients of the Unit of Psychiatry and Eating Behavior Disorders of "Sapienza" University of Rome, compared to randomly selected in a territorial Youth Community Center. Two experimental groups (35 for study group and 20 for control group) organized in a coherent way by gender and age were created. The Italian version of the Oral Health Impact Profile 49 (OHIP-49) on OHRQoL was administered. Moreover, subjects were clinically screened and caries (D3MFT), periodontal health (CPI) and erosions (BEWE) were recorded. A statistical comparison of the two groups was performed, significance level was set a 0.05. The outcome of the OHIP-49 questionnaire highlighted that the study group was the one with the significantly higher oral health interference on quality of life, especially regarding psychological and social disability (very often/often respectively for 26% and 42% in study group vs 8% and 2.3% in control group), with repercussions on the sleep-wake rhythm and consequent depression, difficulty in concentration or relaxation and impediments in social relationships. Regarding clinical data, in the study group the average BEWE score was equal to 2.3 vs. 0.5 of the control group; the CPI was found to be 2 in 69.9% of patients with ED (vs. 7.1% of the control group) and the mean D3MFT in the two groups was 7.1 and 5.2 respectively. In conclusion, the present study showed that ED patients have a significant decrease in oral health status and a worsening of the OHRQoL compared to non-affected controls.

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Delays in Translating Evidence into Practice in Cariology: The French Context

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Minimal intervention (MI) in cariology is backed by a substantial volume of publications, recommendations from scientific societies as well as evidence-based professional guidelines. The aim of this analysis is to identify why many French dental practitioners (DPs) do not include MI in their daily practices and provide solutions to facilitate its implementation.

A narrative review of the literature, consensus documents and professional guidelines were cross-compared with the French Dental Health Policies, Public and Private funding of dental procedures, as well as declared frequency of performance data from DPs.

Weak integration of MI into daily clinical practice was identified, which may be explained by various factors such as the lack of recognition in the French classification of medical and dental procedures (CCAM), the absence of funding by the National and Complimentary health insurances or the lack of continuing professional education. Several necessary adaptations to the CCAM, such as the creation of CCAM procedure codes for oral hygiene advice, therapeutic sealants, caries risk assessments are still lacking, preventing DPs from officially performing them. The use of Health Technology assessments (HTAs) as well as resource allocations for treatments that have been recommended by France's own high health authority is also inconsistent, such as the reimbursement for fluoride varnishes and preventive sealants in all age groups.

Although high quality evidence can be found regarding MI in cariology, translation into clinical practice can be impaired by the lack of adaptation of national health policies and proper funding. Translational research into cariology combined with Health Policy can help policymakers identify barriers to implementation and should help improve the quality of health systems.

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Inter-Institutional Agreement for a Consensus on Caries Components in the Oral Health Record in Colombia

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Through an alliance between the Ministry of Health and Social Protection and the Colombian Chapter of the Alliance for a Cavity-Free Future (CC-ACFF) the aim of this study was to achieve an inter-institutional agreement on the caries components of the oral health record in Colombia. Counting with IRB and signed informed consents it included the following phases: 1. Inviting the following institutions: government/international institutions (n = 11); insurers/health providers/companies (n = 16); dental schools (n = 25); academic associations (n = 13). 2. Updating practitioners on caries current understanding. 3. Designing life-cycle evidenced based oral health records with caries components including assessment, diagnosis and management of both caries lesions and caries risk, based on ICCMS™. 4. Assessing sufficiency, coherence, clarity, and syntax aspects for each interview/clinical item on the oral health record. 5. Assessing the participants' perception on using the oral-health record in the practice through a COM-B behaviour model questionnaire.

A total of 47 institutions with inter-institutional and regional representativeness participated. The updated caries course included a 8-h workshop (204 participants), support literature and clinical cases and a 4-h online version (https://drive.google.com/open?id = 14g0vValHwDcoKJ-0Rdd7ET-CnyqJ54WQ). The oral health record included three revisions after: Online inter-institutional/regional representatives work (n = 42); Inter-institutional and regional workshop (n = 47; 87 attendees); Expert peers (n = 6). The interview/clinical items suffered redaction/number modifications. According to the life cycles (early childhood, childhood, adolescence/adults, elderly) the latter varied from 58, 57, 58, 64, respectively to 42, 41, 42, 48, respectively. The questionnaire answers (n = 32) revealed: Capability: confident/very confident (89.6%); Opportunity: considered it very important (89.7%); Motivation: completely satisfied (70.4%); Behaviour: would almost always/always use it (89.2%): ComB model.

An inter-institutional agreement on the caries components in the oral health record was successfully agreed in Colombia. Further steps for its consensus include a pilot study.

Acknowledgements: Alliance for a Cavity-Free Future – Colombian Chapter.

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ICDAS-Merged Visual Coronal Caries Criteria Calibrations: Comparison of Inter-Examiner Reproducibility of Two Training Modalities

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A 2-day training e-learning for ICDAS-merged visual coronal-caries criteria was designed (2016) with an e-learning. The aim was to compare the visual coronal-caries ICDAS calibration trainees' inter-examiner reproducibility of a 4-day (Calibration-A) versus a 2-day training course (Calibration-B). Calibration-A comprised theoretic lectures, pre-clinical workshop on natural teeth and patients, and 1st and 2nd clinical evaluations of patients' tooth surfaces for calculation of inter-/intra-examiner reproducibility with weighted Kappa values. Calibration-B followed the same methodology, but without the workshop on patients and evaluations were conducted on natural-teeth surfaces. ICDAS-merged codes corresponded to Initial (1–2), Moderate (3–4) and Extensive (5–6) caries. The same expert examiner served as the gold standard. Calibration-A was conducted in El Bosque University (2016; n = 37) with a mean of 332.5 ± 25.5 examined surfaces/patients. Most have had previous experience with ICDAS (75.7%). Trainees were from Argentina, Colombia, Chile, Ecuador, El Salvador, México, Dominican Republic, Uruguay, and Venezuela. Calibration-B was conducted in the University of Greifswald (2017; n = 13) and the University of Leeds (2018; n = 11), with 150 natural-teeth surfaces examined per participant. Most haven't had previous experience with ICDAS (70.8%). Trainees were from Egypt, Germany, Greece, Iraq, Jemen, Jordan, Kuwait, Libya, Oman, S. Arabia, and Syria. Intra-examiner weighted Kappa values were >0.65. Inter-examiner weighted Kappa rank values were respectively for Calibration-A and Calibration-B of 0.61–0.88 (mean: 0.74 ± 0.07) and 0.5–0.84 (mean: 0.67 ± 0.09) (t test; p = 0.01). In both training courses the majority (78.4% in Calibration-A and 70.8% Calibration-B) reached a substantial agreement (K = 0.61–0.80), followed in Calibration-A with 21.6% by an almost perfect agreement (K ≥ 0.81), while in Calibration-B with moderate (20.8%) agreement. In conclusion the 2-day calibration course on ICDAS-merged visual

coronal caries criteria is successful in terms of trainees' inter-examiner reproducibility.

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Cross-Cultural Validation of the COM-B ICCMS™ Questionnaire

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The International Caries Classification and Management System (ICCMS™) intends to deliver best-evidence caries management recommendations for clinicians in order to achieve tooth-preserving caries prevention and personalized care plans. Taking into account the COM-B (capability, opportunity and motivation to perform a behaviour) model and the ICCMS™ recommendations for caries diagnosis and management, a 47-item questionnaire was developed and validated for the Colombian population to better understand dentist's current practices. The aim was to conduct a cross-cultural validation of the COM-B ICCMS™ questionnaire for clinicians in the Dominican Republic. After ethical approval the COM-B ICCMS™ questionnaire was answered by a random sample of 276 clinicians from four cities with the largest dentist populations in the Dominican Republic. The participants had ages ranging from 23 to 77 years (M = 40.23, SD = 12.47), had 1 to 53 years of clinical experience (M = 16.60, SD = 12.18), and were predominantly women (80.0%). Dimensionality assessment with parallel analysis and exploratory structural equation modelling supported the theoretical five-factor structure. Measurement invariance analyses with the Colombian sample established configural, scalar, and residual factorial invariance across cultures. According to the categorical omega coefficient, four of the five scales (Capability, Opportunity-Resources, Motivation, and Behaviour) obtained high reliabilities (0.86 to 0.97), while Opportunity-Relevance had a lower reliability of 0.62 due to lack of variability in the item scores. A structural equation model showed that, similar to Colombian clinicians, Opportunity-Resources constituted the most important predictor of Behaviour.

Conclusions: The current findings support the use of the COM-B ICCMS™ questionnaire with the Dominican population and highlight its utility in explaining dentists' caries diagnosis and management behaviours across cultures.

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Parents Perspectives on Oral Health of their Children in the Municipality Amsterdam New-West

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30,000 Amsterdam children, mainly from low socio-economic status (SES) households, do not attend a dentist. The aim was to gain insight into the parent's perspectives on oral health of their children in low SES municipality Amsterdam New-West. A qualitative study was conducted by means of focus group discussions among mothers with low SES in Amsterdam New-West. Topics discussed were: daily routine, diet, exercise and oral care. Interviews were voice recorded, transcribed, coded and analyzed using Atlas.ti.

17 Women of Moroccan (19 children 1–15 yrs; mean 9.4 ± 4 yrs) and 6 of Turkish descend (8 children 5–18 yrs; mean 14 ± 3.6 yrs) participated. In 16 cases children dictated eating, drinking and sleeping habits. 7 children (2–5 yrs) received a bottle with milk or yoghurt to fall asleep. Schools do not allow sweets and soft drinks or juices. This is compensated at home. Four children practiced sports or played outside after school. Oral care was not optimal. Three mothers brushed their children's teeth from the first tooth on, others start at 10–12 months. Parents brushed children's teeth until the age of 5, one child was brushed until age 12. 5 Children brushed twice per day, the rest brushed once like their parents. Two children attended the dentist prior to age 2 due to trauma or caries. Most children attend the school dentist from age 4 or 5. Only few women knew dental care for children up to 18 is free of charge. In conclusion children in low SES families of Amsterdam New-West have an irregular lifestyle with poor dietary choices and oral hygiene habits. Mothers are unaware that regular dental check-ups are preferred from early age and wait until dental care is offered once the child starts school.

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Confidence and Attitude in the Diagnosis of Caries After ICDAS-Training in Denmark, Germany and UK

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ICDAS training was given to dentists in a standardised manner in Denmark (n = 26), Germany (n = 13) and the UK (n = 13). Pre- and post-training COM-B behaviour model questionnaires were used to evaluate whether ICDAS training improved dentists' confidence in, and attitudes towards, their ability to assess and manage dental caries.

Thirty-nine of the 52 participants completed both pre- and post-training questionnaires. Responses originally given in a Likert type scale were converted into a numerical 0–5 scale for analysis. Questions regarding confidence and the importance of specific elements of caries diagnosis or management before and after training were compared using the Wilcoxon signed-rank test (95% CI). After ICDAS training confidence significantly improved in ability to assess both the visual presence ($p = 0.003$) and activity status ($p = 0.039$) of caries lesions. In addition, confidence in the non-operative care of inactive ($p = 0.000$), initial ($p = 0.024$) and micro-cavitated ($p = 0.001$) lesions significantly improved. The rating of the importance of dentists' ability to visually assess caries presence ($p = 0.020$) and non-operative care of inactive ($p = 0.002$) and micro-cavitated lesions ($p = 0.004$) also increased. No significant changes in attitudes were observed toward assessing the activity status of caries ($p = 1.000$) or toward the non-operative care of initial lesions ($p = 0.235$). Professional incentives or resource availability were not reported to significantly influence respondents' caries management and around two-thirds of the areas explored in the questionnaire were not statistically significantly different after training. In conclusion the majority of participants indicated that ICDAS training improved their confidence in the assessment and management of caries, however many attitudes toward caries assessment and management remained largely unchanged though this could be related to factors such as non-operative care of initial lesions being common practice among participants of this study prior to training.

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Dietary Intake and Early Childhood Caries 3- to 4-Year-Old Children in Cali, Colombia

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The aim was to investigate the existence of early childhood caries in relation to the consumption of sugar in food, in a day-care pre-schooler's aged 3–4 years old in Cali, Colombia. A cross-sectional study was conducted in 124 pre-school children attending three public childcare centers. Children attended weekday A menu determined the feeding of the children for breakfast, lunch and two between meals. The clinical evaluation was performed using the International Caries Detection and Assessment System. The overall mean number of decayed, missing or filled teeth (dmft) was obtained and correlated with the dietary exposures. Questionnaire was given to the parents to evaluate dietary habits of children at home. The estimated sugar consumption by children per year was 52.20 kg or 194 grams of sugar per day. On weekends, a pattern of more frequent consumption of unhealthy foods and beverages was observed. The mean $d_{(2-6)}$mft index was 2.95 (SD = 4.27); 2.75 (SD = 3.85) decayed$_{(2-6)}$, 0.10 (SD = 0.39) missing and 0.08 (SD = 0.58) filled. A statistically significant ($p < 0.05$) association was found between the dmft and the drink feeding bottle before bed, consumed of baked goods, confectionery, soft drink, fruit juice, fruit, chocolate milk, and milk/nutritional supplements and wafer of panela. There was no an association between existence of early childhood caries with the consumption of snacks between meals ($p = 0.184$). In conclusion, the differences in the frequency of preschool children's consumption of foods and beverages on weekdays and weekends are additional risk factors in association with early childhood caries.

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Caries Prevalence in Pediatric Patients with Childhood Diabetes

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Dental caries is a multifactorial, slowly progressing disease requiring a careful diagnosis. To date there are few studies on the prevalence of caries diagnosed by the ICDAS system in children suffering from Childhood Diabetes. The aim was to evaluate the presence of caries using the ICDAS system in children aged 6 to 12 years who suffer from childhood diabetes, in order to carry out a protocol of minimally invasive preventive treatment for their dental care. This is a descriptive, crossover observational study, analysed using descriptive statistics. The sample consisted of 56 children treated at the Hospital del Niño y la Mujer in Queretaro, Mexico. After study approval and signed informed consent, children were examined by a calibrated examiner. The data recorded included: caries score, sociodemographic variables and classification

of Diabetes as recorded in the clinical record. 56 children were included, 40 male and 10 female, with an average age of 7.6 years. The prevalence of caries was 87.5%. The severity of caries, according to the highest ICDAS code, was grade 6 (33%). With respect to the least prevalent ICDAS codes, grade 1 and grade 3 together had a frequency of 33.9%. In conclusion, caries prevalence in this group was high and sociodemographic and disease factors should be studied to plan an optimal caries management plan.

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Prevalence of Dental Caries and Risk Assessment Among Children in Tepatitlan De Morelos, Mexico

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The aim of this study was to determine the prevalence of dental caries and associated factors among preschool children (3–5 years-old) and to stablish their risk according to the CARIOGRAM®. A cross-sectional study was conducted including both private/public and rural/urban preschools in the municipality of Tepatitlan de Morelos, Mexico in 2018. Four hundred and nine children between 3 and 5 years of age and their caregivers were included after sample size calculations. A clinical examination for caries and dental plaque was performed by 2 examiners, previously trained and calibrated. Dental caries was determined at 2 thresholds: 1) all lesions (ICDAS 1–6) and 2) cavitated lesions (ICDAS 3–6). A modified version of the CARIOGRAM® was applied to determine the subject's caries risk. Information was obtained through a previously designed questionnaire and a clinical examination. Sociodemographic variables were also included. Fifty-four percent of the sample were females and 46% males. Children were evenly distributed by type of school and location. The prevalence of dental caries was 90% (ICDAS 1–6) and 45% (ICDAS 3–6). Children from rural schools presented the highest caries prevalence at the cavitated threshold with children from rural private schools at 32% and children from rural public schools at 30%. Four-year-old children were the most affected (87%). Regarding risk assessment, 46% of the sample were identified as moderate to high caries risk. Dietary, oral hygiene, fluoride exposure factors and high plaque levels were the major contributors to dental caries in this population. Early childhood caries has been reported as a significant issue for preschool children in Mexico. The presence of caries in the preschoolers in our study was high and significantly associated to dietary habits and oral hygiene practices.

Caries Research

Session 6
Fluoride and Hard Tissues

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Intra-Oral Kinetics of Fluoride-Containing Dentifrices in a Saliva Clearance Study

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This clinical study compared the oral retention of fluoride and concentration of salivary calcium after brushing with two commercially-available toothpastes both containing 1150 ppm declared fluoride as NaF. Toothpaste-A (Sensodyne Pronamel) contained no anionic surfactants or phosphates whilst Toothpaste-B (Colgate Enamel Health Sensitivity Relief) contained anionic surfactant and pyrophosphate. The impact of an acid challenge (Orange Juice [OJ]) on salivary fluoride and calcium concentration was also investigated.

Methods: This was a single-centre, controlled, randomised, cross-over clinical study. 29 healthy subjects were randomised; 28 completed the study. After a 7-Day washout period using a non-fluoride toothpaste, a pre-brushing, unstimulated saliva sample was obtained from subjects. The subjects brushed (2 minutes) with their assigned toothpaste then rinsed with tap water (10 mL, 5 s). Paste and rinse expectorates were collected. Unstimulated saliva was collected at 1, 5, 10, 15, 30 and 60 minutes post-brushing. Subjects then rinsed with either OJ or water and the expectorate collected. Subjects then provided a post-rinse saliva sample. Saliva and expectorates were analysed for fluoride and calcium ion concentration by ion-specific electrode. The concentration of fluoride in saliva collected from the 10 minutes post-brushing time-point onwards was statistically significantly greater for the subjects using Toothpaste-A compared to Toothpaste-B (ratio of Toothpaste A:Toothpaste B from 1.22 [10 min] to 1.52 [60 min pre-rinse]; $p < 0.05$). The concentration of calcium ions in saliva was statistically significantly greater at all time points (difference Toothpaste B-Toothpaste A (ppm) from 2.1 [60 min pre-rinse] to 13.9 [1 min] $p < 0.05$), with the greatest differences see up to 15 minutes post-brushing. Salivary fluoride concentration was statistically significantly higher at the 60 min timepoint following rinsing with OJ compared to water (ratio of Toothpaste A+OJ rinse:Toothpaste A+water rinse of 2.03; $p < 0.0001$). Salivary calcium concentration was statistically significantly lower at the 60 min timepoint following rinsing with OJ compared to water (difference (ppm) of Toothpaste A+OJ rinse:Toothpaste A+water rinse of –12.75; $p < 0.0001$). Toothpastes were generally well-tolerated. In conclusion, this study demonstrates that differences in formulation excipients may impact fluoride retention, possibly through excipients interacting with calcium ions to reduce fluoride deposition in the oral environment.

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A TiF_4/NaF Solution and an NaF/AmF/$SnCl_2$-Containing Product Stain Sound and Eroded Enamel Equally

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Previous *in situ* study has shown protective effect of TiF_4/NaF solution against tooth erosive wear at the same extension as those promoted by NaF/AmF/$SnCl_2$. However, 40% of the participants reported temporary tooth staining by the use of the experimental fluoride solution [Souza et al. J Dent 2018;73:45–49]. Therefore, this study evaluated the staining potential of TiF_4/NaF solution *in vitro*. Ninety bovine enamel samples were polished; half of them remained sound and the other half was eroded (4 x 90 s each, using a 0.1% citric acid). Thereafter, the baseline color reading was performed (T_0). The samples were then subdivided into the following treatments: TiF_4/NaF solution (500 ppm F, 6x1 min), Erosion Protection-*Elmex*® (NaF/AmF/$SnCl_2$, 500 ppm F, 6x1 min) and control (water, 6x1 min). Between the applications, the samples were exposed to artificial saliva for 30 min. The color changes were measured immediately after the applications (T_1-T_6) and after the exposures to artificial saliva (S_1-S_6) by using a spectrophotometer

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(Vita EasyShade®). The ΔE, L*, a* and b* data were compared using 2-way ANOVA/Tukey ($p < 0.05$). For sound enamel, the ΔE values showed that the color changes were perceptible at a glance only. With respect to eroded enamel, the final colors were more similar to the baseline values than opposite. TiF_4/NaF solution induced a lower ΔE values (T1: 2.3 and S6: 12.7) compared to *Elmex*® (T1: 2.8 and S6: 18.4) and control (T1: 2.8 and S6: 17.7) on sound enamel; however, no differences were found between the treatments on eroded enamel (ΔE at S6, TiF_4/NaF: 2.7; *Elmex*®: 2.8 and control: 3.6). Both type of fluoride solutions increased the yellow appearance of enamel. In conclusion, the color changes induced by the experimental TiF_4/NaF solution are not significantly different to those produced by Protection-*Elmex*®.

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Inhibition of Human Salivary MMP-2 and MMP-9 by Different Fluorides

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The emerging literature suggests that, in addition to bacteria, host derived enzymes, specially matrix metalloproteinases (MMPs), play an important role in degradation of the dentin organic matrix. Therefore, some studies have proposed that protease inhibitors may be able to reduce dentin loss under erosive and caries challenges. Thus, the aim of this study was to evaluate the inhibition of the activity of salivary MMPs (MMP-2 and -9) by solutions of titanium tetrafluoride (TiF_4), zinc fluoride (ZnF_2), tin fluoride (SnF_2) and sodium fluoride (NaF). Saliva was collected from 8 healthy individuals. Pooled saliva was centrifuged, the supernatants were incubated for 2 h (26°C) and subjected to gelatin zymography. NaF, SnF_2, ZnF_2 and TiF_4 were added into the incubation buffer in clinically relevant concentrations (from 0.01 to 2 wt %). The eletrophoretic bands were scanned and the transmittance values were analyzed with ImageJ software (NIH). Four bands were detected in the control group, two bands less clear, close to the molecular weight of 70 kDa (corresponding to the pro-active/active forms of MMP-2), and two more evident bands, close to 90 kDa (pro-active/active forms of MMP-9). These enzymes were characterized as MMPs, since their activities were inhibited by EDTA (a known metalloproteinase inhibitor) and not inhibited by NEM (a thiol-proteinase inhibitor). All tested fluorides were able to inhibit at least partially the activity of salivary MMPs. TiF_4 and ZnF_2 had an inhibitory effect at all tested concentrations, whereas at 0.01 and 0.05% NaF and SnF_2 suppressed only partially MMPs activity. In conclusion, TiF_4 and ZnF_2 were able to completely inhibit MMPs expression even at very low concentrations, while the effect of NaF and SnF_2 was dose-dependent.

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Fluoride Concentration in Saliva After Toothbrushing with Electrical and Manual Toothbrush

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Electrical toothbrushes gain increasing acceptance. However, the brush head is generally smaller thus accommodating less toothpaste. This might influence salivary fluoride concentrations. The aim was to assess this in a group of 20 adult volunteers in a 4-legged crossover study. They were instructed to use their habitual quantity of toothpaste (one NaF, one MFP) ad libitum on a manual or electric toothbrush. After 1 minute brushing, salivary samples were taken at baseline and up to 60 minutes after brushing. Salivary samples were electrochemically analyzed for fluoride (ISE) using a method, distinguishing between TF (total fluoride, whole sample with acid digestion), TSF (total soluble fluoride, supernatant with acid digestion) and IF (ionizable fluoride, supernatant without acid digestion) [Cury, Braz.Dent.J,2010; 21:396–400]. For TF, TSF and IF, area under the curve (AUC) was calculated and compared using nonparametric statistical methods. Representative undigested saliva samples were double-checked with gas chromatography (GC) in order to assess method validity. Correlation with GC was high ($r^2 = 0.98$, $p < 0.0001$). It could be shown that the acid digestion/ISE method could be applied for salivary samples. After brushing with MFP toothpaste, there were significant differences between the AUCs for IF and both TF and TSF ($p < 0.01$), but not for the NaF toothpaste. Toothpaste quantity was significantly ($p < 0.05$) higher with manual toothbrushes (manual ranging from 0.3–2.1 g, electric from 0.2–1.2 g). Surprisingly, this difference in quantity was not reflected in AUC in saliva measured with ISE (Spearman correlation, $r^2 < 0.01$, $p > 0.05$). In conclusion, the acid digestion method allows differentiation between total and ionizable fluoride in saliva after brushing with MFP toothpastes. Electrical brushing with ad libitum habitual quantities of toothpaste did not result in lower fluoride availability despite smaller amounts used compared to manual toothbrushing.

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Total, Soluble and Ionic Fluoride of Brazilian Red Propolis Containing-Dentifrice After 1 Year of Ageing

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The aim of this study was to determine the effect of ageing on fluoride concentration in various forms in Brazilian red propolis containing-dentifrice (BRP dentifrice). The toothpaste formulation contained calcium carbonate and sodium monofluorophosphate (MFP). Nominal fluoride content was 1450 µg/g. Batches of 5 tubes each were selected from the 2016 and 2017 production year and analyzed in 2018. Fluoride was assayed according to the method of Cury et al. [Braz. Dent. J 2010; 21: 369–400]. Toothpastes from each tube were analyzed in duplicate using an ion-specific electrode. The concentrations of total F (TF), total soluble F (TSF) and Ionizable Fluoride (IF) were determined (µF/g). Furthermore, insoluble fluoride (TF-TSF) as well as proportion of insoluble fluoride (TF-TSF/TF) was calculated. Data were subject to statistical analysis using ANOVA and post-hoc tests. Total Fluoride was found to be near to the nominal concentration after one year storage (1440 ± 225 µg/g) but decreased significantly after 2 year of ageing,. to 1030 ± 230 µg/g ($p < 0.01$). TSF decreased from 930 ± 100 to 625 ± 165 ($p < 0.01$). IF values decreased within 1 year from 515 ± 260 to 240 ± 55 µg/g ($p > 0.05$). The fraction of insoluble fluoride increased slightly from the 2016 batch to 2017 from 0.33 to 0.36 ($p > 0.05$). The coefficient of variation between individual tubes on TF, TSF and IF was doubled after two years of storage. In conclusion the storage for two years of a calcium carbonate/MFP toothpaste leads to loss of fluoride by about one third for total and total soluble fluoride. Ionizable fluoride is less affected by storage. It is assumed that a part of the MFP may have reacted with propolis.

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Salivary Fluoride Bioavailability as Indicator of Chemical Solubility of MFP/$CaCO_3$-Based Dentifrice

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Dentifrices must be able to release fluoride during the time of tooth brushing or post brushing into the oral cavity to provide anti-caries benefits but there is no standard procedure accepted to measure how much fluoride in a toothpaste may be bioavailable for release (ORCA Workshop, "Methodology for Determination of Potentially Available Fluoride in Toothpastes"). A crossover study with five phases was conducted with 10 participants to evaluate the relationship between the concentration of chemically soluble fluoride in MFP/$CaCO_3$-based dentifrice and fluoride bioavailability in saliva from toothbrushing. The groups were: I – non-F placebo dentifrice; II – Sorriso Dentes Brancos® fresh samples (MFP/$CaCO_3$, 1450 µg F/g; 5% insoluble); Groups III to V – Same F-dentifrice II but with total soluble fluoride (TSF=ions MFP+F) concentrations of 1160, 900 and 597 µg F/g (20, 40 and 60% of insoluble fluoride, respectively). At each phase, the participants brushed their teeth for 1 min with 0.7 g of the respective toothpaste and saliva samples were collected before brushing and from 3 to 120 min after brushing. TSF concentration in saliva supernatant was determined with an ion specific electrode. Fluoride concentration in the dentifrices was determined by Cury's protocol. The areas under curves (AUC= µg F/ml x min) of TSF concentration in saliva versus time (baseline to 15 min) were calculated. Data were analyzed by one-way ANOVA followed by Tukey's and by Pearce correlation. The AUC (mean±SD) was: I. 1.3 ± 0.4^c; II. 30.9 ± 14.5^a; III. 29.5 ± 14.2^a; IV. 24.6 ± 9.8^{ab}; V. 16.1 ± 7.7^b ($p < 0.0001$). A high correlation was found between the TSF concentration in the toothpaste and the TSF AUC in saliva ($r = 0.989$; $p = 0.001$). The concentration of TSF found in MFP/$CaCO_3$-based dentifrice by Cury's protocol is an indicator of fluoride post toothbrushing bioavailability in oral cavity.

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Fluoride Release from Glass Ionomer Cements Mimicking the Caries Process

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Fluoride release from glass ionomer cements (GIC) should be evaluated in a model simulating the process of caries lesion development to have some relevance of the anti-caries potential of these materials. We determined the amount of fluoride released from 15 commercial GIC under pH-cycling regimen simulating the caries process of demineralization and remineralization. Six discs (161.8 mm^2) of each GIC were individually immersed for 6 h in 1.0 ml of demineralizing (De-) and for 18 h in 1.0 ml of remineralizing (Re-) solutions, during 12 days. The solutions were daily changed, in which fluoride concentration was determined with ISE. The results were expressed in fluoride concentration daily found in De- and Re-solutions (µg F/ml), in amount of fluoride daily released in De+Re solutions per area of the specimens (µg F/day/cm^2) and by cumulative release during the 12 days (µg F/cm^2). All GIC showed early burst of fluoride release on the first days followed by a gradual decline but three distinct patterns were observed: Greater fluoride release in De- than Re-solution during the period (A); Initial higher releasing on the De-solution (B) and similar releasing in both solutions during the whole period (C). The GIC differed statistically ($p < 0.05$) either on daily and cumulative fluoride release. Maxxion R released the greatest daily amount of fluoride during

all the period (75.5 ± 16.8 and 22.1 ± 3.8 μg F/cm^2, in the first and the last day respectively) and it showed the greatest cumulative releasing (413.9 μg F/cm^2); Resiglass R released the lowest amount of fluoride (15.0 μg F/cm^2). The GICs evaluated showed distinct qualitative and quantitative patterns of fluoride releasing in conditions simulating the caries process that may reflect their potential anti-caries properties.

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Fluoride Concentration in Bottled, Well and Tap Water in the Tashkent Region, Uzbekistan

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Efficient and safe fluoride administration for children must make the balance between efficacy and avoiding excess quantities which may lead to dental fluorosis. Therefore, fluoride content in oral hygiene products as well as from dietary sources must be known. The aim of the present study was to assess ionizable fluoride content in several samples of tap water, local water wells and bottled water available in the Tashkent area, Uzbekistan.

Water samples were obtained from the water distribution net in the city of Tashkent, four local wells, one in the city and three in the outskirts of Tashkent and from 17 brands of bottled waters bought in local shops. Fluoride was determined electrochemically after 1:1 dilution with TISAB. Fluoride content was compared with the values stated, if available.

Tap water in Tashkent had a concentration of 0.1 mgL^{-1}. This was also the case for most bottled waters with fluoride concentrations ranging from 0.01 to 0.13 mgL^{-1} with one exception having 1.13 mgL^{-1}. Fluoride content was not stated on the bottles. Two of the four local wells had a concentration exceeding 1.2 mgL^{-1}, the other two between 0.3 and 0.6 mgL^{-1}. Consumption of tap or bottled water for infants in children is generally largely below quantities raising concern for dental fluorosis, with the exception of one product which should be avoided for drinking or formula composition for children up to an age of 8 years. Parents should be made aware of this by clear information on the label. The fluoride content of local wells should be examined periodically.

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Concentrations of Fluoride in Infusions of Different Teas

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The aims were to investigate the amount of fluoride in tea infusions of a wide variety of tea products commercially available in the UK and to determine the influence of infusion time, and type and form of tea on the amount of fluoride in tea infusions. Twenty one commercially available tea products of different types (black and green) and forms (bag and loose tea) were investigated using two grams of tea per 100 ml of boiling water based on the International Organisation of Standardisation (ISO) recommendations. The fluoride content was measured at 2, 3, 5, 10, 15 and 30 minutes using a fluoride ion selective electrode.

There was a significant increase in fluoride levels with increasing brewing time (mean ± SD: 5.2 ± 1.90 ppm F at 2 min., 6.33 ± 2.55 ppm F at 5 min). Black tea infusions had significantly higher fluoride levels (5.38 ± 3.12 ppm F) than green tea infusions (3.31 ± 1.18 ppm F) ($p = 0.001$). No significant differences were found between the fluoride levels obtained from the infusions prepared with tea bags or loose tea. In conclusion, the fluoride content of tea infusions increased significantly with brewing time up to ten minutes. Although all the infusions had relatively high fluoride concentrations, this varied widely between tea types and products. However, the black tea types resulted in significantly higher fluoride concentrations than the green tea types. The fluoride content in different types of tea infusions is relatively high and as a result it is important to monitor its consumption especially in young children to avoid any adverse effects.

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Cariogenicity of a Fluoridated Milk-Based Drink, In-Situ Study

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To improve the nutritional status of older adults, the Chilean government provides a nutritional supplement in the form of a milk-based drink (M-BD). Due to the high concentration of sugars in the product, our previous results demonstrated a potential high cariogenicity on root dentin. It has been reported that milk supplemented with low concentrations of fluoride may be effective in controlling caries. The aim was to evaluate cariogenicity of fluoridated M-BD under high cariogenic condition of frequent exposure to sucrose. A randomized, split mouth, cross-over and double-

blind *in situ* study was conducted with 13 adult volunteers who used palatal removable appliances containing 06 bovine dentin slabs during two experimental phases of 14 days each. Sucrose solution (20%) was dripped onto the slabs 8x/day. In 2 of the 8 exposures to sucrose, volunteers had to drip the experimental treatments for 5 additional minutes: 1. deionized distilled water; 2. M-BD; 3.5 ppmF-suplemented M-BD or 4.10-ppmF-suplemented M-BD. Seven-day wash-out period was carried out between the experimental phases. Demineralization of dentin slabs was evaluated by surface Knoop microhardness and integrated mineral loss. Counts of viable cells (total streptococci, *Streptococcus mutans* and Lactobacilli) were quantified on biofilms as well as the concentration of extracellular polysaccharide. Differences between treatments were analyzed by ANOVA followed by Tukey test, $p < 0.05$. No differences were found regarding bacterial counts and extracellular polysaccharide concentration among the experimental groups. Both 5 ppmF- (%SHL: 77.97 ± 4.99) and 10 ppmF- (%SHL: 63.42 ± 7.14) supplemented M-BD (%SHL: 87.93 ± 4.82) were able to reduced up to 11.3% and 27.9% of dentin demineralization, respectively. Regarding mineral loss, supplementation of the M-BD (ΔZ 1183.62 ± 162.52) with 5 ppm and 10 ppm induced a decrease of 50% (ΔZ 592.48 ± 42.44) and 37% (ΔZ 746.72 ± 46.81), respectively. In conclusion, supplementation of M-BD with fluoride decreases its cariogenic potential on root dentin.

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Stability of Fluoride in MFP/$CaCO_3$ Toothpaste Evaluated at Room Temperature and by Accelerated Aging

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Accelerated aging protocols are useful to estimate the stability of drugs and they can be used to estimate the stability of fluoride in toothpaste formulations. Toothpastes should maintain, within their use by date, fluoride chemically soluble in the formulation to be released into the oral cavity during toothbrushing. This concentration should be around 1000 ppm F (μg F/g; w/w) as ion F- (from NaF, SnF_2 or AmF containing toothpastes) or ions MFP+F- (from MFP toothpastes). We evaluated fluoride stability of a MFP/$CaCO_3$-based toothpaste at room temperature (rt) and by accelerated aging. Twelve tubes of toothpaste Sorriso® (MFP/$CaCO_3$, 1450 ppm F, lot 7278BR121J, fab 10/2017 and expiry date 10/2019) were acquired in October 2017. The fresh received samples were analyzed and 6 tubes were maintained at room temperature (mean 22.6ºC) and 6 were placed at 55ºC. The tubes kept at room temperature and 55ºC were re-analyzed after respectively 373, and 98 days. Total fluoride (TF), total soluble fluoride (TSF) and ionic fluoride (F^-) were chemically determined with ISE using standardized protocol of analysis. MFP ion and percentage of insoluble fluoride ($\%F_{ins}$) were further calculated. The data were analyzed by linear regression. The concentrations (mean ± SD; n = 6; μg F/g) of TF, TSF, MFP and F^- in fresh samples were 1447.5 ± 48.9; 1387.0 ± 45.0; 1341.3 ± 43.1 and 45.6 ± 2.2, respectively; 4.2% was as F_{ins}. TSF decreased linearly at rt ($y = -1.0788x + 1400$; $r^2 = 0.94$) and at 55ºC ($y = -7.7602x + 1428.7$; $r^2 = 0.98$). The concentration of TSF found after 365 days at room temperature was also obtained after 54 days at 55ºC. Using this protocol of accelerated aging it is possible to predict how long time after the fabrication a MFP/$CaCO_3$-based toothpaste would be able to maintain the minimum of 1000 ppm of fluoride chemically soluble.

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Total and Soluble Fluoride in the Most Consumed Toothpastes from Lima, Peru

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There is no update information on the quality of fluoride present in the most frequently consumed Peruvian toothpastes. We analyzed in 2018 the three most consumed toothpastes sold in Lima, Peru and, according to the label, they should contain total fluoride of: A = 1,500 ppm F as sodium monofluorophosphate (MFP); B = 1,450 ppm F as NaF (0.1%) + MFP (0.76%) and C = 1450 ppm F as NaF. The samples analyzed were fresh (expiry date 2020) and 19 samples of the same lot of each brand were randomly purchased from wholesale distribution market, by sampling optimization based on the Lot Quality Assurance Sampling Methodology. Total Fluoride (TF) and Total Soluble Fluoride (TSF= ion F + ion MFP) concentrations were determined in the 57 samples with ion specific electrode according to Cury protocol. The results (ppm F=μg F/g; w/w) of TF and TSF in each brand were expressed by mean ± SD; n = 19) and analyzed by ANOVA one-way, followed by Bonferroni for differences between the brands. The results of TF in brands A, B and C were 1471.9 ± 32.4, 1319.0 ± 41.4 and 1479.5 ± 30.4, respectively. For TSF the concentrations found were 1177.2 ± 25.4, 1212.7 ± 31.2 and 1561.3 ± 54.4, and the three brands differed statistically ($p < 0.05$). Only brand B presented insoluble fluoride (16% in terms of TF declared). All brands presented concentration of fluoride chemically soluble (TSF) above 1000 ppm, the minimum concentration that should be bioavailable during toothbrushing. As these analyses were made in the first year after fabrication of these toothpastes, the stability of fluoride should be checked until the expiry date. Although the fresh samples of the three most frequently consumed Peruvian toothpastes have recommended concentrations of chemically soluble fluoride sufficient to interfere with caries the fluoride stability is not known and it should be evaluated.

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Water Ingestion Exposure Doses for Fluoride and Urinary Excretion in Children

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The aim was to evaluate the relationship between exposure dose for fluoride water intake and urinary excretion in children. This study investigated 987 schoolchildren aged between 7 to 11 years, residents in eight municipalities in Colombia. Water for consumption sampling was performed in major aquifers used for daily supply. 185 polyethylene 250 mL containers were collected. On the other hand, 987 urine samples were collected in polyethylene bottles of 25 mL. All the containers were kept under refrigeration and transported at the laboratory until analysis. Fluoride concentrations were obtained by adding TISAB buffer just before analysis with sensitivity for selective ion, using standard reference material. The water ingestion exposure doses (IDag) expressed as risk was calculated using the reference dose (<0.05 mg/kg/day). Dental fluorosis was obtained by visual detection, using TFI. Furthermore, a structured questionnaire was designed for demographic characteristics. For analyses were used correlation coefficient and lineal regression. The municipalities with the highest dental fluorosis prevalence were Margarita-Bolivar (100%) and Manzanares-Caldas (100%), but this last population showed the most severe codes (TFI 5; 23.9%, TFI 6; 8.3%, TFI 7; 5.8%). The highest values of IDag were for people of Algarrobo-Magdalena (0.88 mg/kg/day) and Margarita-Bolivar (0.21 mg/kg/day). The average fluoride concentration in urine samples was 5.1 mg/L. The highest values were for people of Algarrobo-Magdalena (11.8 mg/L). We found correlation coefficients moderate between IDag and urinary excretion of fluoride ($r = 0.40$; $p = 0.000$). But the correlation between IDag and TFI was not statistically significant ($p > 0.05$). In conclusion the relationship between IDag and urinary excretion of fluoride in children with high prevalence of enamel fluorosis was moderate but positive, which suggests that among the different people evaluated may influence diversity of exposures that must be taken into account to validate the urinary excretion of fluoride as a marker of dental fluorosis.

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Toothbrush Bristle Pattern and Brushing Load on Simulated Non-Carious Cervical Lesions Development

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This study investigated the effect of toothbrush bristle pattern and brushing load on the development of non-carious cervical lesions (NCCLs). Human premolars (160) were mounted on acrylic blocks and had their root surfaces partially covered with acrylic resin to simulate gingiva, leaving a 2-mm length area apical to the cemento-enamel junction exposed for brushing. The teeth were brushed under 1- or 3N load with one of the following toothbrushes (n = 16): a) ordinary/flat-trimmed (Oral-B Indicator); b) rippled (Oral-B Contour); c) angled/multileveled/rubber bristles (Oral-B Pro-Health All-in-One); d) angled/multileveled/flex head (Oral-B Pro-Flex); e) feathered (Oral-B Compact Clean). Brushing was performed using a toothpaste slurry (Crest Cavity Protection) for 55,000 double-strokes in back-and-forth motion, to simulate 10 years of brushing. Impressions were taken at baseline and after brushing and scanned by a 3D optical profilometer. The samples were evaluated for dentin loss volume, using image subtraction, lesion shape and lesion angle. Dentin volume loss and lesion angle were assessed through two-way ANOVA and Fisher's LSD test (alpha = 0.05). The ordinary flat-trimmed toothbrush caused higher volume loss to dentin (3.81 mm^3) in comparison to the other toothbrushes that did not differ from each other (2.56–2.92 mm^3). The toothbrush having rubber bristles was associated with NCCLs showing the smallest angle (109.97°) and, along with the rippled toothbrush (53.1%), provoked higher proportion of wedge-shaped lesions (43.8%), whereas teeth brushed with the feathered toothbrush exhibited the lowest prevalence of wedge-shaped lesions (3.1%). The 1- and 3N load applied during brushing affected neither volume loss nor lesion angle. At the loads tested during brushing, the development of NCCLs was dependent on toothbrush bristle pattern, with the ordinary/flat-trimmed version causing the highest abrasion and the feathered toothbrush the least proportion of wedge-shaped lesions.

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Influence of Pit-and-Fissure Sealant Application Protocols on Bond Strength in Caries-Like Lesion and Sound Enamel

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Pit-and-fissure sealants may be applied in sound and in caries enamel lesions, and thus, this study aimed to investigate the influence of varying the sealant application protocol over these substrates. Bovine enamel blocks were prepared and half of the specimens were submitted to a validated cariogenic biofilm model for 7 days to develop caries-like lesions, corresponding to code 2 of the International Caries Detection and Assessment System. A resin-based light-cure sealant with fluoride was used (FluroShield, Dentsply). Sealant application protocol varied in terms of etching time (30 and 60 s) and the use of one etch-and-rinse adhesive (Adper™, Scotchbond™, Multi-Purpose Adhesive, 3M ESPE). Eight groups were prepared: 1) Sound enamel for 30 s, 2) Sound enamel for 60 s, 3) Sound enamel for 30 s and adhesive, 4) Enamel lesion for 30 s, 5) Enamel lesion for 60 s, 6) Enamel lesion for 30 s and adhesive; and negative controls: 7) Sound enamel and 8) Enamel lesion which were sealed without etching or bonding. The specimens were submitted to microshear bond strength test (n = 8) and contact angle evaluation (n = 3). Data were analyzed with two-way ANOVA (enamel condition and protocol) followed by Tukey's test. No significant differences in bond strength values were found for sealants irrespective of the enamel condition ($p = 0.716$) or etching time ($p = 0.360$). The use of the adhesive significantly improved the bond strength of sealants in both enamel conditions compared to all other groups ($p < 0.001$). The enamel condition did not affect the contact angle ($p = 0.270$). Non-bonded groups presented significantly lower contact angles compared to the adhesive groups ($p < 0.05$), whereas control groups presented the higher contact angles ($p < 0.05$). Under the limits of this study, the sealing of enamel caries-like lesions presents similar bond strength when compared to sound enamel, and the use of an adhesive improves the bond strength between the sealant and the enamel tissue.

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Mineral Content in the Mesial and Distal Surfaces of Primary Molar Teeth

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Compared to permanent enamel caries in primary enamel progresses at a faster rate, probably due to their differences in chemical composition and structure (porosity). This in-vitro study aimed to compare the mineralization content of the mesial and distal surfaces of fully erupted sound exfoliated/extracted primary molar teeth using transverse microradiography (TMR). After ethical approval (UEB 129-2011) and parents signed consents, recently exfoliated/extracted teeth were collected. Stereomicoscopic images were taken and one 100 µm thick longitudinal distal-to-mesial section was obtained per teeth. Transverse microradiography (TMR) was used to analyze the proximal surfaces' mineral content. In each proximal surface three zones Zone-a, Zone-b and Zone-c were taken into account. Descriptive statistics and one-way ANOVA were used to identify differences in mineral content between teeth and surfaces. The sample corresponded to 52 first/second primary molar teeth (9 upper first- and 15 upper second-molar teeth; 12 lower first- and 15 lower second-molar teeth). At the macroscopical and stereomicroscopical levels there were no proximal surfaces with initial caries or breakdowns. TMR showed that mean mineral content in the mesial surfaces was of 714 ± 362 vol%min×µm and in the distal surfaces was of 636 ± 386 vol%min×µm ($p > 0.05$). Significantly less mineral content was found in the distal (620 ± 328 vol%min×µm) surface of the upper first primary molar teeth ($p = 0.01$), in comparison to the mesial (892 ± 397 vol%min×µm) surface of the same teeth. In this study there were no statistical differences between first and second molar teeth or upper and lower teeth; there was only a significant difference between mesial and distal surface of the upper first primary molar teeth.

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In Vivo Assessment of the Association of Fluorescence and Dentin Hardness

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The aim of this study was to assess the association between the presence of fluorescence as detected by fluorescence-aided caries excavation (FACE) technology and dentin hardness as determined by the conventional visual-tactile exam in a clinical setting. Eligible patients (N = 15) who were scheduled for restorative procedures at the UNC dental student clinic were consented/assented and enrolled. Calibrated investigators recorded the presence of fluorescence (no/pink/red) as detected by FACE (SIROinspect; Sirona, Salzburg, Austria) and assessed dentin hardness (hard/leathery/soft) with a probe pre- and post-excavation. Association between fluorescence and dentin hardness was evaluated using Kendall's tau-b correlation. Among the 15 enrolled patients, 4 patients were excluded. Pre- and post-excavation data points were combined for the analysis. Kendall's tau-b coefficient indicated a positive association between soft/leathery dentin and fluorescence ($\hat{\tau}b = 0.442$ with 95% confidence interval: 0.129, 0.755); moreover, an exact two-sided test for τb was statistically significant ($p = 0.031$). Coincidentally, leathery or hard dentin fluoresced at a high frequency (15/18 or 83%), although somewhat less than soft dentin (4/4 or 100%). This latter finding agreed with an in-vitro study (Trippe, 2017) which rejected the hypothesis that leathery or hard dentin would not fluoresce. There was a statistically significant association between the presence of fluorescent light detected by the FACE device and dentin hardness.

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Caries Research

Session 7
Pulp Response and Microbiology

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Immunohistochemical Study of Innate Immune Response in the Dental Pulp to Caries

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Innate immunity in human teeth is activated upon the initial invasion of microbes. Antigen-presenting cells are considered important in immunosurveillance as part of the innate response to caries. To characterize the innate immune response we have examined the expression of dendritic cells and macrophages in developing, healthy, and carious human teeth.

In this study we have examined 61 maxillary/mandibular premolars, human teeth under 3 different clinical conditions: developing, healthy, and carious human teeth. Teeth were extracted and immediately cut longitudinally. The specimens were embedded in paraffin, according to standardized laboratory procedure. Sections were cut at 5 μm thicknesses and stained by the streptavidin – biotin complex immunoperoxidase method. To clarify when human pulp acquires an immunologic defense potential and how this reacts to dental caries, antigen-expressing cells were identified with HLA-DR monoclonal antibodies (for dendritic cells) and CD68 monoclonal antibodies (for macrophages).

In the pulp of unerupted developing teeth, HLA-DR-positive cells were distributed mainly in and around the odontoblast layer. A few CD68 positive cells were located more coronary around the blood vessels. In erupted teeth, HLA-DR-positive cells were located, for the most part just beneath the odontoblast layer. CD68 positive cells were also located coronary around the blood vessels. Superficial caries lesions caused an aggregation of HLA-DR-positive cells and macrophages in the dental pulp corresponding to the lesion.

Human teeth are already equipped with an immunological defense potential prior to eruption. In the initial stage of caries infection, an immunoresponse mediated by class-II-expressing cells is initiated in human dental pulp. Thus anti-HLA-DR and anti-CD 68 antibody-positive cells might participate in both an efficient immune system and as a tissue-protective mechanism in the human dental pulp.

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Effect of Herbal/Natural Toothpastes and Mouthrinses on the Prevention of Enamel Demineralization Under Microcosm Biofilm

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This work evaluated the effect of commercial toothpastes and mouthrinses containing natural/herbal agents on the vitality and extracellular polysaccharide (EPS) biovolume of a microcosm biofilm as well as on the prevention of enamel demineralization *in vitro*. Microcosm biofilm was produced on bovine enamel, using inoculum from pooled human saliva mixed with McBain saliva, under 0.2% sucrose exposure for 5 days. The biofilm was daily treated with the toothpastes' slurries or mouthrinses for 1 min: Orgânico natural®-Contente/Uberlândia-Brazil (toothpaste: *Vitis vinifera, Chamomila recutita, Melissa officinalis*, stevioside and xylitol; and mouthrinse: xylitol, *Zingiber officinale* root extract and *Citrus limon* extract); Boni Natural Menta & Malaleuca®-Boni/São Bernardo do Campo-Brazil (toothpaste: *Mentha piperita*, *Calendula officinalis*, *Eucalyptus globulus*, limonene, *Citrus grandis* and *Melaleuca alternifolia*; and mouthrinse: xylitol, *Calendula officinalis*, menthol, *Melaleuca alternifolia*, *Citrus grandis*, aroma, limonene, peppermint and *Eucalyptus globulus*); Propolis & Myrrh®-Tom's Maine/Kennebunk-USA (toothpaste: xylitol, *Commiphora myrrha* and propolis); Colgate Total 12 Clean Mint®-Colgate-Palmolive/São Paulo-Brazil (toothpaste: triclosan and fluoride, positive control); Malvatricin® Plus–Daudt/Rio de Janeiro-Brazil (mouthrinse: *Malva sylvestris*, menthol, sorbitol, triclosan, xylitol, zinc chloride and sodium fluoride); PerioGard®-Palmolive/São

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Paulo-Brazil (mouthrinse: chlorhexidine, positive control) and PBS (negative control). Biofilm vitality and EPS biovolume were measured by fluorescence using confocal microscope and enamel demineralization was quantified by transverse microradiography in biological triplicate. Only Propolis & Myrrh® Tom's (44 ± 19% dead bacteria) and Colgate Total 12® (62 ± 20%) toothpastes as well as Malvatricin® Plus (56 ± 24%) and PerioGard® (56 ± 21%) mouthrinses significantly reduced the biofilm vitality compared to PBS (Toothpaste 22 ± 14%; Mouthrinse 27 ± 16%). With respect to biofilm thickness and EPS biovolume, only PerioGard® (12 ± 2 μm and 7 ± 4%) differed from PBS (16 ± 2 μm; 30 ± 16%) significantly. Despite Propolis & Myrrh® Tom's reduced lesion depth (85 ± 23 μm), only Colgate Total 12® was able to reduce mineral loss (2878 ± 443% vol.μm) significantly. Malvatricin® Plus also decreased both mineral loss (2254 ± 834 %vol.μm) and lesion depth (86 ± 17 μm) ($p < 0.05$), similarly to PerioGard® (3237 ± 781 %vol.μm; 97 ± 18 μm) The commercial herbal products, with exception of Malvatricin® Plus, have limited anticaries effect under this model.

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Effect of Commercial Milk Supplemented with Lactic Acid Bacteria as Probiotics on Children

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Different studies have reported the effect of some probiotics on the decrease of caries but the results with lactic acid bacteria are unclear. The purpose of this study was to determine the effect of a commercial milk supplemented with lactic acid bacteria in the demineralization of carious lesions and in biological factors associated with dental caries in children aged 3 to 5 years. A pilot test of repeated measures was carried out with 121 children distributed in Villavicencio and Pasto, Colombia. The children consumed during 3 months a commercial milk supplemented with probiotic bacteria, *Lactobacillus rhamnosus* GG and *Bifidobacterium longum* as a treatment, and as a control a commercial milk without probiotics. Before and after, the demineralization of the carious lesions (Diagnodent classic), the salivary pH, the variation of the pH after a rinse with sucrose and the quantification of *Streptococcus mutans* in saliva were determined. For results analysis, final sample of 63 children were selected based on meeting the selection criteria throughout the study. The results between the before and the after were analyzed in the paired t-test and the Wilcoxon test according to the normality of the data. It was found that with the treatment there was a non-significant decrease ($p > 0.05$) in the quantification of *S. mutans*, as $\log_{10}$(CFU/ml + 1) (Before: 2.78 ± 0.95 After: 2.70 ± 0.83) and in the pH variation (B: 0.45 (0.15–0.74) A: 0.34 (0–0.80)), a remineralization of 39.4% of the lesions and a significant decrease in the salivary pH ($p < 0.01$) (B: 7.53 (6.98–7.93) A: 7.4 (6.80–7.66)). On the contrary, with the control a non-significant increase in the quantification of *S. mutans* (B: 2.66 ± 0.92 A: 2.78 ± 0.99), pH variation (B: 0.40 (0–0.82) A: 0.47 (0.14–0.78)) and salivary pH was detected ($p > 0.05$) (B: 7.37 (6.80–7.67), A: 7.66 (6.93–8.00)), and a remineralization of 64.2% of the lesions. In conclusion, the commercial milk with probiotics produced a significant decrease in salivary pH and lower degree of remineralization.

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Systemic Evaluation of Rat Model of Simultaneous Dental Caries and Infective Endocarditis

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Infective endocarditis (IE) occurs when bacteria invade the bloodstream and attach to injured heart valves, which can lead to various life-threatening complications. *Streptococcus mutans*, a major pathogen of dental caries and considered to be a causative agent of IE, has been detected in heart valves obtained from IE patients. However, whether severe dental caries is a possible risk factor for that disease and its complications remains unknown. In the present study, we evaluated the virulence of *S. mutans* harbored in severe dental caries for IE development in rats.

Twenty rats (18 days old) were fed a caries-inducing diet containing 56% sucrose until the end of the experiment to induce severe dental caries. Each received a bacterial suspension of *S. mutans* strain SA31 via the oral cavity once a day for 5 days to colonize the oral cavity. At 90 days old, heart valves in the rats were injured using a sterile catheter and wire under general anesthesia. Seven of the rats were euthanized at 1 week, another 7 at 1 month, and the remaining 6 at 3 months after the injury. *S. mutans* was recovered from hearts extirpated from rats at 1 and 3 months after injury. In addition, brains, lungs, kidneys and spleens, known as tissues with major complications of IE, were extirpated. Among the obtained sample tissues, abnormal findings such as inflammatory cell infiltration were observed, except for in the brains, while that was prominent in lungs at 1 month after injury. Our findings suggest that persistent bacteremia induced by *S. mutans* in pulp space causes IE and associated systemic complications.

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In Vitro Evaluation of Antimicrobial Activity of Brazilian Red Propolis Containing-Dentifrice

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The aim of this study was to compare the antimicrobial effect of brazilian red propolis (BRP) extract, BRP containing-dentifrice and one antimicrobial commercial dentifrice (Parodontax) against *S. mutans* in vitro. Strains of *S. mutans* ATCC UA159 were used in the present study. For the evaluation of minimal inhibitory concentration (MIC) the microdilution method was used in culture broth. The strain was activated by incubation at 37°C overnight in Brain Heart Infusion (BHI) culture medium, in an anaerobic jar. To the wells of the microplates were added 100 μL of BHI broth, 20 μL of the substances tested (experimental groups), at concentrations ranging from 100 μL/mL to 0.049 μL/mL, and 80 μL/mL of the standardized microbial suspension (10^6 CFU/mL). An untreated group was used as negative control. The microplates were incubated for 24 hours in a bacteriological oven at 37°C. Visual inspection of the colour changes and reading in BioTek microplate reader at 570 nm was performed to determine the MIC. Mean values and standard deviations were calculated. ANOVA followed by Dunnett's test was performed (against the negative control group); p-value of less than 0.05 was considered significant. The results indicated that the MIC of BRP extract was 0.39 μg/mL ($p = 0.009$) and BRP dentifrice was 0.39 μg/mL ($p = 0.019$). The MIC of Parodontax dentifrice was 0.049 μg/mL ($p = 0.028$). There was no difference between the MIC of the group treated with BRP extract and the group treated with BRP dentifrice. The Parodontax dentifrice obtained the best results. Conclusion: BRP in pure form and processed in a toothpaste shows some antimicrobial activity against *S. mutans* but less than a commercial toothpaste containing herbal extracts and sodium bicarbonate.

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Effect of Fermented Milk Beverages Containing *Lactobacillus* spp. on Dual Biofilm Formation and Enamel Demineralisation

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This study aimed to evaluate the effect of Fermented milk beverages containing spp. (LB) on dual biofilm formation (*C. albicans*-CA and *S. mutans*-SM) and its cariogenic potential in vitro. Dual-species (UA159-SM+ ATCC 90027-CA) biofilms were grown on the surface of sound bovine enamel slabs for 14 days in culture supplemented with 0.5% sucrose, which was replaced daily. The average concentration of both SM and CA on initial culture was approximately 10^2 CFU/mL and 10^4 CFU/mL respectively. The enamel slabs (n = 20) were randomised for the experimental groups: Yakult (G1), Yakult light (G2), Chamyto (G3) and sterile distilled water (G4). During 12 days, the slabs were kept for 5 minutes in the tested solution. The demineralisation of the slabs (visual/tactile criteria) and the microbial composition (total microbial count -TC, concentration of SM, CA and LB) were evaluated. Data were statistically analysed by two-way ANOVA ($p < 0.05$). Microbial composition (Mean±SD $\log_{10}$/mL) were: G1 SM 5.16 ± 0.64; CA 2.69 ± 0.63; LB 6.27 ± 0.51; TC 6.21 ± 0.2; G2 SM 5.01 ± 0.06, CA 2.25 ± 0.15, LB 6.42 ± 0.47, TC 6.02 ± 0.45; G3 SM 4.91 ± 0.57, CA 2.32 ± 0.67, LB 4.21 ± 0.48, TC 5.11 ± 0.65; G4 SM 6.11 ± 0.23, CA 3.94 ± 1.69, LB 0, TC 6.1 ± 0.46. The fermented milk (G1, G2 and G3) significantly decreased the SM counts, but were not able to decrease CA counts significantly. Only G3 was able to reduce TC in biofilm. The demineralisation was similar in all groups ($p > 0.05$). The results suggest that fermented milk beverages had an effect on the biofilm composition, but no signifiacnt effect on demineralisation in the presence of a high cariogenic challenge.

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Cariogenicity of Microcosm Biofilms According to Different Inoculum Conditions

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This study aimed to assess the cariogenicity of microcosm biofilms by varying the inoculum conditions. Biofilms were grown on bovine enamel specimens using saliva from a single donor or pooled saliva from 5 donors (inoculum level), with two distinct levels according to caries activity of the donors (caries activity level): caries active (at least two active caries lesions) and caries free (without any caries lesion). Another condition consisted in pooling saliva from 10 donors (5 caries active and 5 caries free). Biofilms (n = 10 per group) were individually grown over the specimens in 24-well plates, with a defined medium with mucin (DMM), and were submitted to cariogenic challenges (DMM 1% sucrose) for 6 h daily up to 14 days. Outcome variables assessed were mineral loss [surface hardness (SH) change: %SHC = 100 (Final SH – Baseline SH)/Baseline SH)] and microbiological composition of the biofilms (colony forming units counts, $\log_{10}$CFU). Statistical analysis was performed using multivariate general linear model. %SHC values were not affected by the caries activity of the donors ($p = 0.797$), but mineral loss was significantly higher ($p = 0.019$) for the inoculum with 5 donors (87.99% ± 6.41) compared with single-donors (74.62% ± 20.94). Regarding the microbiological counts, some differences were found at the inoculum level, specifically for acid-tolerant bacteria and streptococcus mutans group, showing higher counts for pooled saliva when compared to single-donor saliva. For the caries activity level no significant differences in microbiological counts were found, except for lactoba-

cillus counts, which was significantly higher ($p = 0.017$) in the caries active group (log_{10}CFU 4.13 ± 0.24) compared with caries free group (log_{10}CFU 3.14 ± 0.24). Within the limitations of this study, the present findings indicate that the extent of enamel demineralization was not affected by the caries activity of the salivary donors, but pooled saliva as inoculum source was able to increase the cariogenicity of derived biofilms.

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Bacterial Composition of Bacteriome Associated to Supragingival Biofilm in Non-Cavitated Lesions Related to Caries Activity

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This research compares the bacterial composition from supragingival biofilm (SupGB) collected from subjects with different caries status. Sixteen individuals (13–76; median = 23.5 years-old) recruted at Faculty of Dentistry from the Federal University of Rio Grande do Sul in Brazil participated in the study. Subjects were caries active (CA, n = 7), inactive (CI, n = 3) and caries-free (CF, n = 6). After 12 h without toothbrushing, SupGB pools were collected from different sites from (1) CA group: active non-cavitated lesions (ANCL, n = 6); inactive non-cavitated lesions (INCL, n = 4); sound surfaces (S, n = 6); (2) CI group: INCL lesions (n = 3); CF group: sound surfaces (n = 6). The total RNA was extracted (Lysozyme/UltraClean® Microbial RNA Isolation kit); genomic libraries were prepared (True Seq® Sample Preparation Guide, Low Sample (LS) Protocol Illumina), and sequenced (Illumina HiSeq 3000). All paired-end 2x150 bp sequences were uploaded into MG-RAST (Metagenomics Analysis Server) for bioinformatics analysis. High-quality sequences (3,542,190) was clustered into operational taxonomic units (97% identity; SILVA SSU), representing 915 genera belonging to 29 phyla (higher abundants: Actinobacteria, Firmicutes, Bacteroidetes, Fusobacteria). The presence of a core microbiome was observed (123 shared genus). The α diversity analysis showed less bacterial diversity in disease (CA-S) compared to health (CF-S). The dominant genera included *Actinomyces, Corynebacterium, Capnocytophaga, Leptotrichia, Veillonella, Prevotella, Streptococcus, Eubacterium*, and *Neisseria*. *Veillonella* and *Leptotrichia* were related with disease, and *Prevotella* with health. *Corynebacterium, Capnocytophaga*, and *Actinomyces* clustered together presenting high abundance in health and disease. The Metric Multidimensional Scaling Ordination analysis shows that sites from active subjects (ANCL-CA, INCL-CA and CF-CA) are closer to each other than INCL-CI subjects or CF-S subjects. Supragingival bacterial communities' profile presents an intrapersonal similarity, but interpersonal diversity and different bacterial composition profile reveals that subject's caries activity status matters more than sites.

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Competition or Synergism Between *Candida albicans* and the Commensal *Streptococcus sanguinis*?

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The aim was to test if there was modulation of the viability of *C. albicans* or *S. sanguinis* in co-culture. In addition to examine the survival of the yeast exposed to H_2O_2, a potential virulence factor of the bacteria. Competition tests were carried out in liquid medium: *S. sanguinis* (Ss) SK36 or *S. mutans* (Sm) ATCC 25175 with *C. albicans* (Ca) (ATCC 90029 and clinical isolate from a subject with active caries) in simultaneous and deferred form in microaerophilic at 37ºC during 48 h with shaking; pH of the medium and viable cell count (CFU/mL) of each microorganism was determined under all conditions. The MIC of H_2O_2 for the yeast was determined. Data were expressed as medians. In liquid medium, Ss and both Ca strains increased their counts significantly in co-culture ($p < 0.05$). On the other hand, Sm increased with the reference Ca strain and decreases with the Ca clinical isolate. In presence of Sm, the yeasts decreased their counts relative to the mono-cultures. The pH of the co-culture of both Ca with Ss or Sm was higher than the initial pH of the medium, except that the pH of the Sm mono-culture decreased. The MIC test determined that 0.1 mM H_2O_2 is the concentration that decreases 70% the survival of both Ca strains. In conclusion the growth of *C. albicans* was favored in the presence of *S. sanguinis*. Apparently in the presence of the yeast the pH of the culture medium is alkalized when it is in presence of both bacteria separately. Likewise, both could contribute to maintaining a microenvironment compatible with oral health.

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EPS-Rich Matrix of Biofilms Increases Lactose Acidogenicity

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Lactose is considered less acidogenic than other dietary carbohydrates, but the extracellular polysaccharides (EPS) present in the matrix of biofilm formed under sucrose exposure could contribute to increase its acidogenicity. This study evaluated the lactose acidogenicity of *Streptococcus mutans* biofilms containing EPS (EPS^+), or not (EPS^-). *S. mutans* UA159 biofilms were formed on saliva-coated hydroxyapatite discs (Ø9 mm). After bacterial adhesion, biofilms were grown in UTYEB culture medium containing 1% sucrose for EPS synthesis (EPS^+) or 0.525% glucose + 0.525% fructose (EPS^-). Biofilms were formed for 96 h and the medium was replaced 2x/day. At the beginning of the 5th day, the

biofilms EPS^+ and EPS^- were exposed 5x during 3 min at intervals of 45 min to one of the following treatments (n = 8): 0.9% NaCl, 10% lactose, or 10% sucrose. After 45 min of each treatment the medium pH was measured as indicator of biofilm acidogenicity. Additional biofilms (n = 8) were formed to evaluated viable cells (CFU), biofilm dry weight, EPS (soluble and insoluble) and biofilm architecture by confocal scanning microscopy. pH data was analyzed by two-way ANOVA-Tukey's test and the other analyses by Student's t-test ($\alpha = 5\%$). EPS factor was statistically significant for all time points ($p < 0.05$). Regarding treatment factor, sucrose group differed from NaCl ($p < 0.05$) in all time points, while lactose differed from NaCl only from 180 min. EPS^+ biofilms presented higher dry weight (EPS^+ 4.0 ± 0.5, EPS^- 1.2 ± 1.0 mg; $p < 0.05$), but similar CFU counts (EPS^+ $2.4 \pm 0.5 \times 10^9$, EPS^- $1.5 \pm 0.4 \times 10^9$; $p > 0.05$) than EPS^-. EPS were only found in EPS^+ biofilms (soluble 89.4 ± 6.5, insoluble 613.8 ± 68.9 µg/biofilm), being also visualized by confocal microscopy. In conclusion, EPS-rich matrix of biofilms may contribute to increase the acidogenic potential of lactose.

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Effect of Photodynamic Therapy Using a Non-Coherent Red Light on *Streptococcus mutans* Mature Biofilms

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This study aimed to evaluate de effect of photodynamic therapy-PDT on viability and structure of *Streptococcus mutans* mature biofilms. *S. mutans* UA159 biofilms were formed on saliva-coated hydroxyapatite discs at 37 °C in 5% CO_2 for 5 days. The biofilms were grown in TYB broth containing 1% sucrose and the culture medium was replaced once daily. The non-coherent red light with predominant wavelength of 630 nm, spot size of 113.1 mm^2, fixed output power of 2.24 W was used during 1 min (211.37 J/cm^2) or 2 min (422.74 J/cm^2). Toluidine blue ortho (100 μgmL^{-1}) was used as photosensitizer. After 5 days, the biofilms were treated as follows: biofilms exposed to both TBO and light for 1 or 2 min, biofilms exposed to neither sensitizer nor light, biofilms exposed to the sensitizer only, or biofilms exposed to the light only. After treatments the biofilms were collected and dispersed by sonication. The bacterial viability, the soluble and insoluble extracellular polysaccharides, the variable pressure scanning electron microscopy (VPSEM) and Linkage analysis were performed. The normality distribution was checked using the Kolmogorov–Smirnov test. One-way analysis of variance test followed by a Tukey–Kramer was used ($p < 0.05$). PDT showed a significant reduction in bacterial viability with a 3 logs reduction in the group with 2 min of treatment (counts from $2.84 \times 10^7 \pm 2.05 \times 10^7$ in non-treated group to $2.12 \times 10^4 \pm 1.24 \times 10^4$ after 2 min of PDT treatment). No differences were observed in the results of extracellular polysaccharides among all experimental groups ($p > 0.05$). VPSEM imagens and Linkage results do not revel alterations in treated biofilms after PDT. In conclusion, PDT using a non-coherent red light associated with TBO was effective in reduce viability of *S. mutans* biofilms after 2 min of treatment.

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Streptococcus mutans Gene Expression from Biofilm Associated to Caries Activity in Enamel and Dentin Lesions

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This study aimed to investigate the expression profile of genes of *Streptococcus mutans*: gtfB and gtfC (adhesion); atpD, aguD, nox and fabM (acidogenicity and aciduricity) in in vivo biofilm associated to caries activity in enamel and dentin lesions from children. Site-specific plaque samples were collected from primary teeth and distributed in 5 groups (n = 8): caries-free surfaces (CF), active enamel caries (AEC) and inactive (IEC), active dentin (ADC) and inactive (IDC) caries. Total RNA extraction, purification and cDNA synthesis were performed. RT-qPCR was done for all samples. Data were analyzed by Kruskal-Wallis and Dunn post-test ($\alpha = 5\%$). The results showed that *S. mutans* was detected in all samples. The gtfB gene was more expressed in the ADC, IDC, AEC and IEC groups when compared to the CF group ($p = 0.023$) while the gtfC, atpD and nox genes were expressed at higher levels in the ADC and IDC groups ($p = 0.001$, $p = 0.002$ and $p = 0.005$). The fabM gene was more expressed in the ADC, IDC and IEC than in the AEC and CF groups ($p = 0.004$). No statistically significant differences were found in the expression of the aguD gene ($p = 0.209$). In conclusion, under the evaluated conditions, *S. mutans* is part of the viable microbial community of the biofilm of active and inactive carious lesions. The high expression of the gtfC, atpD, fabM and nox genes in dentin caries groups suggests the relationship of these genes with the progression of caries, and the greater expression of the gtfB gene in the biofilm of all the carious groups suggests the relation of this gene with the presence of biofilm.

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Presence of Archaea in Dental Caries Biofilms: A Pilot Study

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Dental caries biofilms are comprised by a complex microbiota, where the prevalence and functions of bacterial species are well known, but the role of Archaea is not yet established. This study aims to detect archaeal cells in biofilms of coronary and root caries. Carious dentin samples were collected (n = 5), during restorative treatments. Total DNA was extracted and the composition of the microbiota was investigated using two strategies. For the first one, PCR reactions were performed using primers directed to archaeal 16SrRNA gene, amplicons were cloned and sequenced by Sanger methodology (ABI-Prism-3500-Genetic Analyzer). For identification of the closest relatives, sequences of unrecognized inserts were compared to the sequences of the Genbank database. For the second one, samples were submitted to 16SrRNA gene amplification using universal primers and DNA sequencing by a high-throughput method (IonTorrent™). Annotation of sequences was performed using the SILVA database and the relative abundance of OTUs (at genus level) was calculated. Preliminary results detected 15 archaeal clones in a sample of a cervical active caries lesion. From these, 7 were identified as not-yet uncultured archaeon clones MA11-5/Wet60 (88–99% identity); 2 as Arch_J1 (94%); 4 as GJarc2/3 (94–99%); and 2 as Methanocellales (99%). For the same sample, the pair of primers used in the high-throughput sequencing was not sensitive to detect Archaea sequences, showing a relative abundance of *Lactobacillus* (61.4%); Olsenella (16.1%); *Bifidobacteriaceae* (7.5%); and *Streptococcus* (6.4%). In conclusion, members of Archaea were present in dental caries and a group of methanogens was identified. This group closely interact with bacteria, collaboratively metabolizing organic matter under anaerobic conditions. Cloning amplicons of partial archaeal 16SrRNA gene was a good strategy to detect these organisms, although the identification was at the order level.

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Quorum Sensing in *Bifidobacterium dentium* and *Lactobacillus casei*: Perspectives of the Communication in Dental Biofilm

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The aim of this work was determining the quorum sensing system of *L. casei* and *B. dentium* and its role in adhesion and biofilm formation, as well as, in the interaction between both microorganisms. Protocol approved by the Biosafety Committee and the Scientific Ethics Committee, Faculty of Dentistry, University of Chile. *L. casei* ATCC4646 and *B. dentium*ATCC27534, obtained from caries lesions, were used. A bioinformatic, PCR and sequencing analysis were performed to establish that both microorganisms possess a *luxS* gene, predicted to produce autoinducer-2. The functionality was determined measuring *luxS* expression by RT-qPCR and autoinducer-2 production by means of the *Vibrio harveyi* BAA-1117TM reporter strain (it produces luminescence in presence of autoinducer-2). The MTT test was used for adhesion analysis. For biofilm formation, glass coverslips embedded in saliva were used. Results were visualized using Scanning Electron Microscopy. Chemical autoinducer-2 was added to evaluate its effect on adherence and biofilm formation. Also, cell free supernatants (CFS) of both microorganisms (positives for autoinducer-2) were used. *L. casei* ATCC4646 and *B. dentium* ATCC27534 each possess a LuxS quorum sensing system, producing a functional autoinducer-2, capable of generating an increase in the adhesion and biofilm formation of both microorganisms. Similar effect was seen when *B. dentium* CFS were added in adherence assay and biofilm formation of *L. casei*. In conclusion *L. casei* and *B. dentium* were able to establish an intra-species communication through quorum sensing, enhancing its adhesion and biofilm formation. We propose that the effect observed in *L. casei* by *B. dentium* CFS is related to autoinducer-2, suggesting that the presence of *B. dentium* may influence *L. casei* proliferation. These results indicate that cellular communication may generate positive effects among cariogenic microorganisms.

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Relationship between *Streptococcus mutans*, *Lactobacillus* spp with Plaque in 3–4 Year-Old Children in Cali, Colombia

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The aim of this study was to evaluate of the counts of *S. mutans* and *Lactobacillus* spp. (LB) in saliva and their relationships with the Silness-Löe modified plaque index (MPI) in 3 to 4 years old children from Cali, Colombia and to determinate the ability of the MPI to predict caries risk in children. A total of 227 children were included in this study. The MPI was recorded at 0, 3, 6 and 9 months using scores of 0 to 2. Stimulated saliva was collected and the levels of *S. mutans* and LB were determined using selective media. The numbers *S. mutans* and LB were counted, expressed as CFU per millilitre of saliva. The *S. mutans* counts allocated to four categories: (1) very low risk, $< 10^4$; (2) low risk, 10^4–10^5; (3) average risk, 10^5–$5x10^5$; and (4) high risk, $5x10^5$–10^6 colony-forming units per millilitre. For LB counts CFU values <0 and >0 were recorded. On comparing the score of MPI with categories of *S. mutans* at 3 months 49.3% had a score of (1) and MPI = 0 while 7.7% had a *S.mutans* category and MPI = 2. The association between *S. mutans* category and MPI score was significant ($p = 0.013$). However, the associations at baseline, 6 and 9 months were not significant ($p = 0.060$, $p = 0.169$ and $p = 0.409$). The prevalence of LB was very high at between and 99.5 to 97.3% at all sampling times; baseline to 9 months. In conclusion, these results show that the MPI can be an approximation for the evaluation of *S. mutans*, but there was no specific pattern between of salivary *S. mutans* levels and MPI. The relationship between salivary bacteria and caries prediction is complex and requires consideration of additional multiple factors.

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Oral Bacteriome in Children with Dental Caries. A Case Control Study

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Dental caries is a major public health problem affecting 47.11% 5 to 12 years of Colombian children and with the implementation of the ICDAS criteria the national prevalence increased to 86.24%. The oral biofilm is composed of a complex microbial community and changes occur during the process of its transformation to a pathogenic biofilm. Understanding the microbial events that lead to establishment of a stable bacterial ecosystem which may or may not favor the initiation of disease. Molecular-based methods: PCR, microarrays, multiplex pyro- or Illumina-sequencing, have provided insight into cultivable and uncultivable bacterial species in health and disease. The aim of the study was to characterize the bacteriome of supragingival biofilm of children with and without caries. 150 children enrolled in a non-matched case-control study, 75 in each group, 5–9 years of age from Cartagena–Colombia. The biofilm samples were collected from dental surfaces with carious lesions in the case group and in healthy children from supragingival plaque. Next-Generation sequencing based community profiling targeting the V4 region of the 16S RNA genes was used to analyze and quantify the microbial communities. Descriptive analyses and univariate testing of differences and associations, differences between group means were tested using parametric testing (unpaired t-test), Chi-square test was used to test differences in frequency distributions. The contrast between groups of the relative abundance and presence of the identified species and genera was performed by the U-Mann-Whitney test with Benjamini-Hochberg correction for multiple comparisons. Positive reads for 550 probe sequences were found, 439 species and 111 genera identified. The predominant genera detected in both groups: *Streptococcus probe 4, Streptococcus probe 1, Veillonella probe 2 and Fusobacterium probe 4* with a higher prevalence *Neisseria Probe 2* was observed in the case group. *Streptococcus probe 4* was the most abundant in both groups ($p = 0.04$). *Streptococcus sanguinis* was the most frequently identified species in both groups. *Streptococcus mutans and Leptotrichia sp HOT 417* were significantly more abundant in the case group ($p < 0.05$). There was a comparable α diversity in the biofilm samples of the case group (2.98 ± 0.42) and the control group (2.79 ± 0.44). In conclusion, the biofilm microbiome of children with dental caries may contains bacterial biomarker species that may predict the onset of early dental caries.

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