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## Abstracts

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## Clinical Studies

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### Impact of a Gameful Learning Approach on Cariology Teaching and Learning

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Gameful learning involves pedagogical systems designed to motivate, activate and engage adult learners, to enhance the sense of competence and autonomy in their learning and increase academic achievement. A gameful learning approach was started in 2018 in the first year cariology courses at the University of Michigan. The objective was to compare the perceived value of this pedagogy and the impact on learning of cariology between 2 cohorts of students (2017 pre-gameful and 2018 post-gameful). A software program, GradeCraft, was employed to facilitate implementation of a gameful learning environment in the cariology courses. Students' grades in the cariology winter 2017 and 2018 course were compared using the Mann-Whitney Rank Sum Test. A questionnaire used at the University of Michigan to assess students' values and behaviors associated with learning was adapted and used to assess differences between the 2 cohorts. The post-gameful cohort had significantly higher ( $p < 0.001$ ) overall course grade and clinical test case grade (mean+sd = 96.9%+4.4%; 96.9%+5.0%, respectively) than the pre-gameful cohort (89.1%+5.5%; 96.4%+3.8%, respectively). In 2018, 48% of students stated employing gameful learning strategies helped improve their learning experience, while 36% felt this did not affect their learning and 16% stated this strategy was not helpful. Also, 73% felt confident in their ability to learn material in the course (vs. 61% in 2017); and 63% felt free to choose which assignments to complete (vs. 29% in 2017). Students valued the use of a gameful learning environment, and use of this pedagogical strategy helped improve their performance in the cariology course.

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### Caries Risk and Lesion Assessments for Chilean Oral Health Program for Older Persons, e-Delphi Study

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The present study was undertaken to define an expert e-Delphi consensus regarding suitable caries risk assessment and caries lesion detection/assessment for a Preventive Oral Health Exam for Older Persons (EDePAM) in Chile. A three-round Delphi study via e-mail was conducted. A total of 11 Chilean cariology experts agreed to participate as part of the panel. All experts completed Round 1 questionnaire, 90% completed round 2 and 81% responded to round 3. Round 1 comprised open-ended questions regarding suitable caries risk assessment and caries lesion detection/assessment to be incorporated in the EDePAM. In rounds 2 and 3, the panel of experts rated the degree of agreement with the statements, which were built with answers from the Round 1 and 2, respectively. An agreement percentage >70% (totally agree or agree answers) was used as the cut-off for consensus among experts. Several factors for caries risk assessment in older persons in the Chilean context were agreed upon by the participants. The factors considered as more relevant were: fluoride access, diet, polypharmacy related with hyposalivation or xerostomia, exposed root surfaces, salivary flow, level of dependency/autonomy, head and neck

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cancer, systemic conditions, socioeconomic level, use of dental prosthesis and past caries experience. To detect and assess caries lesions, consensus was reached on using the ICDAS classification, along with the Nyvad criteria for lesion activity assessment, for coronal and root caries lesions. These results will contribute to the development of the EDePAM, to be proposed for its use in a preventive program for oral health for older persons.

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### **Radiographic Yield for Clinical Caries Diagnosis in Young Adults: Indicators for Radiographic Examination**

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This prospective cohort study investigated the distribution pattern of carious lesions diagnosed by visual-tactile and radiographic examinations, assessed the radiographic yield for clinical caries diagnosis and estimated how accurately commonly used indicators for caries identified young adults who would benefit from radiographs. Overall 576 patients aged 16-32 years seeking a first consultation were included. Patients were examined for caries and answered a validated questionnaire on socio-demographics and oral health behavior. Almost 10% of clinically sound approximal surfaces presented radiolucency in enamel/dentine. Of the clinically diagnosed non-cavitated approximal (22.5%) and occlusal lesions (17.7%) presented radiolucency reaching dentine. Non-cavitated/enamel lesions detected radiographically were mainly at approximal surfaces (73.2%) while rarely at occlusal surfaces (0.7%). More than half of approximal dentine lesions were only detected radiographically (61.3%) whereas more than half of occlusal dentine lesions were only clinically diagnosed (57.1%). The hierarchical logistic regression analysis showed that patient's caries activity, D1MFS scores  $\geq 17$  and frequent consumption of soft drinks were significantly associated with detection of approximal lesions. Also, patient's caries activity and frequent consumption of soft drinks were significantly associated with occlusal dentine caries ( $p \leq 0.05$ ). The indicator power of grouping these indicators as a predictor for radiographically detected lesions showed high sensitivity (0.84-0.91) and moderate specificity (0.64-0.73) for all surfaces and thresholds tested. In conclusion, radiographs increased significantly the number of approximal enamel/

dentine and occlusal dentine lesions diagnosed. The ability of the predictor to identify patients with approximal lesions was satisfactory. Since an essential contribution of bitewing radiographs is the detection of approximal non-cavitated/enamel lesions that can be inactivated, our results support the prescription of radiographs in young adults seeking a first consultation. Updating of current guidelines' recommendation of radiographs is warranted.

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### **Resin Sealing of Sound and Carious Occlusal Surfaces – Rct: 5.6 Year Results**

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Aim was to investigate longevity and effectiveness of resin fissure sealings of sound and carious occlusal surfaces in permanent molars. Prospective, split-mouth, RCT study on pairs of molar teeth with at least one active occlusal enamel or dentin caries lesion in each pair. 636 sealings in 6-16-year-old patients were performed by 22 dentists and 21 dental hygienists from 12 PDHS in Denmark. After stratification and randomization, each clinician used two of six resin sealants. Treatments were annually followed clinically and radiographically. Data were analysed using Chi-square and Cox regression. Sixty-six sealings (10%) were excluded because of lacking control examinations. Data analyses were performed on 570 molars sealed with Clinpro Sealant<sup>®</sup> (17%), Control Seal<sup>®</sup> (17%), Delton FS+<sup>®</sup> (18%), Grandio Seal<sup>®</sup> (15%), UltraSeal XT hydro<sup>®</sup> (17%), and UltraSeal XT+<sup>®</sup> (16%). 26% of the sealings were of sound surfaces, 44% of enamel and 30% of dentin caries lesions. After 5.6 years, 76% of the sealings of sound surfaces, 75% of enamel caries, and 60% of dentin caries were well-functioning ( $p < 0.01$ ). Further, 22% of the sealings of sound surfaces, 23% of enamel lesions, and 30% of dentin lesions were repaired/replaced by new sealings. The remaining 2% of sealings of sound surfaces, 3% of enamel lesions, and 10% of dentin lesions were replaced by restorations ( $p < 0.01$ ). The sealings prevented caries progression in 96% sound surfaces, 96%, enamel and 88% dentin caries lesions ( $p < 0.01$ ). Overall, annual failure rates for the six sealants varied between 3.9%-6.4%. Neither brand of the sealants nor profession of clinicians influenced the longevity and effectiveness of the sealings ( $p > 0.05$ ). Longevity and effectiveness of resin sealings of sound occlusal surfaces and enamel lesions were statistically superior compared with sealed dentin lesions.

## Undergraduate Students' Knowledge Retention After Theoretical-Laboratory Training in Caries Lesion Detection: Assessments After 1.5 Year

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This study aimed to evaluate undergraduate students' medium-term knowledge retention after such learning activity related to caries lesions detection. This study is part of a multicenter study (IuSTC studies). Dental students from the last years were invited to participate. They had been exposed to the activity in the beginning of Pediatric Dentistry Course and were assessed regarding knowledge retention after 1.5 year. Theoretical and practical evaluations were prepared to evaluate knowledge, abilities and attitudes. Each evaluation was scored from 0 to 10. These scores were set as the outcomes. Multilevel regression analyses were used to assess the association between students' performance and his/her perception about the importance of such learning activity in curriculum. Particularities related to students' performance in specific topics and types of evaluations were also explored. 320 students participated in the evaluations. On average, students achieved 6.68 (SD = 1.88) in theoretical and 5.5 (SD = 2.27) in practical assessment. Student's perception about the learning activities were associated with their performance on tests. In theoretical test, students performed worse (21% of correct answers) in linking clinical and histological condition or justifying clinical decision-making for inactive caries (36% of correct answers). Other theoretical or attitudinal questions achieved from 64-75% of correct answers. In the practical test, 70-90% demonstrated ability to detect, assess activity and make the decision about management of initial or severe lesions, while 50% of students could assess adequately moderate caries lesions and only 32% demonstrated correct attitude related to their management. Undergraduate students' knowledge retention related to the detection of caries lesions is moderate in a medium-term analysis. It is noted that for some topics (e.g. not related to practice/justification of attitudes), the students' performance tends to be worse.

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## Caries Management of 6- to 18-Year-Olds in a Japanese Private Dental Clinic

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The aim of this study is to assess caries management of 6- to 18-year-olds in Sugiyama Dental Clinic (SDC). Eighty-six subjects (32 males and 36 females) were selected from the patient database

of SDC on December 15, 2019 (n = 14,855) based on the following criteria: had received preventive treatments between 5 and 7 years old (age 6: 5.5±0.68 years) and between 17 and 19 years old (age 18: 18.3±0.85 years). The subjects' status at age 18 was: university student n = 39 (57.4%), high-school student n = 13 (19.1%), technical school student n = 12 (17.6%), working after high-school graduation n = 4 (5.9%). The treatment period was 12.9±1.1 years, the number of preventive treatments was 23.2±9.3, the DMFT increase was 1.31±2.29, the DMFS increase was 1.68±2.99. DMFS = 0 in 42 subjects (61.8%), sealant applied to 50 subjects (73.5%) and 252 surfaces (3.71±2.73), and Icon applied to 5 subjects (7.4%) and 6 surfaces. The number of filled caries lesions was 49 on molar occlusal surfaces, 29 on molar approximal surfaces, 13 on molar buccal and lingual surfaces, and 13 on anterior proximal surfaces. Comparing regular visits (RV: at least once a year or with an interruption of one year, n = 41) and irregular visits (IRV: more than one-year interruption, n = 27), the DMFS increase was no statistically significant difference (RV: 1.22±2.99 and IRV: 2.37±2.91, p > 0.05). The highest filling subject (DMFS = 16) was in RV, two teeth were extracted due to caries in IRV. In conclusion, DMFT increase was lower than Japanese surveys (Survey of Dental Disease in JAPAN 2015; 17-19 years = 3.0, First Visiting Survey in JHCDA 2014-2017; 17-19 years = 5.6), however, we need to improve the caries management system for IRV and extreme high-risk patients in RV.

## In Vivo Caries Detection Using a 3D Intraoral Scanner and Visual Inspection

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This study aimed to investigate occlusal caries detection by an intraoral scanner (IOS) combining fluorescence with 3D imaging, and to compare this method with visual inspection (ICDAS). Twenty-three 18-year-old participants were monitored for 6 months. The occlusal surfaces of 364 teeth at baseline and 360 after 6 months were examined using ICDAS criteria. Additionally, the upper and lower jaw of the participants were scanned using the IOS (Trios 4, 3Shape Trios A/S, Denmark) which emitted both white and blue light (415 nm). Aided by specific software (TRIOS – Dental Desktop, 3Shape A/S, Denmark), the IOS allowed creating 3D models of the teeth with the fluorescence signal as overlay. The 3D models obtained were analyzed with a custom-made software employing an automated caries scoring system (ACSS) based on the difference in fluorescence between lesions and sound surfaces. Presence or absence of caries lesions in the pits and fissures of all posterior teeth was registered independently for each method, both at baseline and after 6 months. Only the most severe lesion per tooth was registered. At both timepoints, ACSS and ICDAS agreed in classifying ~50% of all examined surfaces as carious and ~30% as sound. However, on ~10% of the surfaces, a caries lesion

was detected by ICDAS only and on another ~10% by ACSS only. McNemar's test showed no significant difference between the two methods (p<sub>baseline</sub> = .817, p<sub>6-month</sub> = .488). In conclusion, the IOS employing fluorescence and the automated scoring system showed promising results: agreement between ACSS and ICDAS was observed in ~80% of the examined occlusal surfaces both at the baseline and the 6-month follow-up examination.

This study was supported by Innovation Fund, Denmark (grant no.8053-00005B) and 3Shape TRIOS A/S.

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### 3-D Imaging with Intra-Oral Scanners is a Valid Tool for Recording Dental Plaque

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A disadvantage of recording plaque clinically is momentariness, and taking conventional images of all tooth areas throughout the dentition is difficult. Thus, 3-D imaging using intra-oral scanners could be an ideal tool for time-efficient, comprehensive and repeatable plaque recording. The present study therefore investigates whether plaque levels can be validly detected and monitored on such 3-D images; gold standard for comparison were digital 2-D digital images. Seven volunteers (27.3±6.2 years) were included. Plaque was disclosed with Mira-2-Tone, and 2-D and 3-D images (intraoral-camera CS1500, intraoral-scanner CS3600; Carestream Dental, Stuttgart, Germany) were taken at T1 (habitual oral hygiene), after 72 hours without oral hygiene (T2), and after following toothbrushing (T3). The oral and vestibular surfaces of the Ramfjord-teeth (16, 21, 24, 36, 41, 44) were investigated using the Rustogi-modified-Navy-plaque-index (RMNPI). Kappa values (mean±sd) for inter-rater agreement were 0.89±0.09 for 2-D and 0.87±0.12 for 3-D images. Statistics: Kolmogorov-Smirnov-test, Wilcoxon-test, Chi-square-test. At T1, T2 and T3, the RMNPI (median (min;max)) from 2-D images was 5.0 (0.0;8.0), 7.0 (0.0;9.0) and 4.0 (0.0;8.0) and from 3-D images 5.5 (0.0;8.0), 6.0 (0.0;9.0) and 4.5 (0.0;9.0). RMNPI increased significantly from T1 to T2 (2-D: p≤0.001; 3-D: p≤0.05) and decreased significantly from T2 to T3 (2-D: p≤0.001; 3-D: p≤0.001). Except T1, there was no significant difference between 2-D and 3-D images. Chi-square tests of the nine different areas of the RMNPI revealed no significant differences between 2-D and 3-D images except two areas at T3, where more plaque was detected on 3-D images. The phi-coefficient (mean±sd) was somewhat lower for oral than for vestibular surfaces (0.52±0.23 versus 0.64±0.13 resp.; p≤0.05). Dental plaque can be validly detected and monitored on 3-D images from intra-oral scanning.

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### Does Parents Oral Health Literacy Level Influence the Choice of Adequate Toothpaste for their Children?

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There are many options of toothpastes for children available for purchase. Toothpastes are key vehicles for fluorides application, and its correct choice and use are important on caries prevention and control. Oral Health Literacy (OHL) level of parents could interfere on oral health outcomes of their children. The objective of this study was to evaluate if OHL level of parents interfere on execution of preventive measures considering fluoride toothpaste use. 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 use were included (n = 161). OHL was measured through OHL Questionnaire for Adults and classified as inadequate (0-13) or adequate (14-17). Parents received information about adequate fluoride concentration on toothpaste, at least 1000 ppm, according to AAPD Guidelines. Four different toothpastes, with different fluoride concentration, indicated for children and available at Brazilian market were given to parents: GalinhaPintadinha<sup>®</sup> - Oralgift (0 ppm F), Oral-B Stages<sup>®</sup> - GSK (500 ppm F), Tandy<sup>®</sup> - Colgate Palmolive (1100 ppm F), MalvatrikidsJunior<sup>®</sup> - Daudt (1450 ppm F). Parents answer was classified as "correct" when choosing one or two adequate toothpastes or "incorrect" when choosing at least one inappropriate toothpaste. Differences between groups were tested using chi-square test. Concerning OHL level, 116 (72%) parents were classified with inadequate and 45 (28%) with adequate OHL level. When choosing toothpaste, 104 (64.6%) parents were correct and 57 (35.4%) were incorrect. No significant difference were observed on OHL scores between parents who choose adequate toothpastes or not (p = 0.463). Better description of fluoride concentration on toothpastes labels could help parents to choose the adequate toothpaste for their children more efficiently.

### Atraumatic Restorative Treatment (ART) and Hall Technique (HT) for Occluso-Proximal Lesions in Primary Molars- RCT

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This study aims to evaluate the survival rate of Atraumatic Restorative Treatment (ART) and the Hall Technique (HT) for managing occluso-proximal lesions in primary molars after 36 months. After securing approval from local Ethical Board (#1.293.935) and registration in ClinicalTrials.gov (NCT02569047), children aged 5 to 10 years old (n = 131) presenting at least one occluso-proximal cavitated dentine carious lesion in a primary molar were selected and treated in schools of Tietê, Brazil. One tooth per child, fitting inclusion criteria, was randomly allocated to receive an ART restoration using high viscosity glass ionomer or a stainless-steel crown placed using the HT. One trained and calibrated outcome assessor evaluated the restorations after 1, 6, 12, 24 and 36 months. The primary outcome is the restoration survival, a composite measure of the absence of Minor (restoration/crown defect) or Major failures (signs/symptoms of pulp involvement, e.g. dental fistula/abscess, tooth fracture or irreparable failures). Kaplan-Meier survival analysis and log rank tests were carried out and Cox regression test investigated associations between survival and other variables ( $\alpha = 5\%$ ). After 36 months, 112 (85.5%) children had the tooth included in the study evaluated. Restoration survival rates were: ART = 32.7% (SE = 0.08; 95%CI = 0.17-0.47) and HT = 93.4% (SE = 0.05; 95%CI = 0.72-0.99),  $p < 0.001$  calculated by log rank test. Occluso-proximal cavities restored using crowns placed with the HT reveal better survival rates after 3 years.

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São Paulo – FAPESP (#2015/18098-2 and #2018/12143-4), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). FMM and DPR also receive scholarships as awards for Research Productivity in Brazil from the CNPq.

### Sugary-Snack Consumption and its Association with Caries Prevalence and Severity in Chilean Schoolchildren: Pilot Study

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The aim of this study was to describe frequent snacks consumed by Chilean schoolchildren and to assess whether there was an association between caries prevalence, severity and the amount of total sugars in them. Methods: A cross-sectional pilot study was carried out in a convenience sample (104 students) in two public schools in Santiago. We determined: 1) snacks regularly consumed, 2) the amount of total sugars in them 3) caries prevalence and severity and 4) whether there was an association between the amount of sugars and caries prevalence and severity. Interviewers registered the snacks at schools. Cases in which children carried money were also recorded. Clinical examinations were performed at the school setting using ICDAS criteria. Spearman correlation and logistic regression were performed,  $p < 0.05$  was considered statistically significant. Results: 499 observations/snacks were recorded and grouped into 39 categories. 10 out of the 11 more consumed categories were processed foods with at least one warning sign. The most frequent warnings were high in calories and sugars. The most commonly consumed snacks were sugar juice boxes, fruit, cookies, sugar milk box, and sweet biscuits. Caries prevalence was 84.61% (dicdas2-6mft>0) and 75% (Dicdas2-6MFT>0). Severity was dicdas5-6mft  $2.07 \pm 2.56$  and dicdas2-6mft  $6.97 \pm 5.69$ ; and Dicdas5-6MFT  $0.32 \pm 0.72$  and Dicdas2-6MFT  $2.09 \pm 1.92$ . We found no statistically significant correlation between the amount of sugars in snacks and caries prevalence in deciduous OR = 0.97, ( $p > 0.05$ ), nor permanent dentition OR = 0.97, ( $p > 0.05$ ) nor with caries severity (dicdas2-6mft) Spearman's rho = -0.0348, ( $p > 0.05$ ), (Dicdas2-6MFT) Spearman's rho = 0.0869, ( $p > 0.05$ ). In conclusion, caries prevalence, severity and amount of sugars in children's snacks were independent variables for this sample.

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## Quality of Reporting Methods in Caries Histological Gold Standard - A Critical Review

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Research in caries detection generally uses histology as gold standard. Reproducibility and clarity make it imperative to explain the methodology used. The aim of this review was to assess methods to establish the histological gold standard. Methods: Studies published between 2000-2018 were retrieved using PubMed database resulting in 2645 hits. Titles and abstracts resulted in 324, full text reading in 237 selected papers. Inclusion criteria were: detection of natural, primary carious lesions and original method description. Papers were analyzed via a validated score list regarding number of teeth, applied technique, number of observers, reproducibility, scoring (categories, central depth), sample size and lesion distribution considerations. Eventually, reproducibility of each study was assessed on a 5-point Likert scale. Results: most studies reported occlusal caries lesions (52%), followed by approximal (39%). Median number of teeth employed was 70 (IQR 44-102). Histology was mainly performed by serial sections (61%) with microscopic evaluation. In most studies, sections were observed by 1 (23%) 2 (62%) or more (15%) observers. Criteria by which a section was considered to be carious was clearly stated in 37% of the studies only. In 40% of the studies, caries was scored in consensus. Reproducibility as given by Kappa statistics, correlation or % agreement was presented in 16% of the studies resulting in values from 0.61 to 0.95. Distribution of lesion type was given in 86% of the studies, however a sample size calculation only in 4%. General assessment of the reported method yielded a score of >3 in 30% of the papers. In studies on in vitro carious lesion detection, methodology is not always clearly reported, making reproduction and appreciation of possible bias difficult for the reader.

## Caries Experience in German Adults with Intellectual Disabilities Working in Sheltered Workshops

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In Germany, only very few studies to determine the caries experience of persons with intellectual disability (ID) have been conducted. Aim of this study was to assess the caries experience of adults with ID living in a region of the German Federal State of Nordrhein-Westfalen. In 2017, the legal guardians of 847 persons being employed and cared for in sheltered workshops for adults

with ID in the administrative district Unna were asked to give informed consent to the dental examination of the persons they are responsible for. In this region, no specific caries prevention program for persons with ID is offered. The dental examinations were carried out between September 2017 and July 2018 by one person in the workshops mentioned above according to WHO criteria (WHO 2013). Artificial light and dental mirrors were used and D<sub>3</sub>MFT values were calculated. In total, 137 persons aged between 18 and 65 years could be examined. Their mean age was 35.5 years (SD 12.8) and their caries prevalence rate (D<sub>3</sub>MFT>0) was 90.5%. The mean D<sub>3</sub>T, FT and MT values were 0.5, 4.4 and 4.8. A mean D<sub>3</sub>MFT value of 9.7 (95% confidence interval 8.2 – 11.1) was calculated. This value was significantly lower than the one which been determined 10 years before in adults with ID working in sheltered workshops from another German region (Schulte et al. 2014). For these persons a mean age of 35.5 years and a mean DMFT of 12.3 (95% confidence interval 11.6 – 12.9) had been calculated. It seems that in Germany persons with intellectual disability have benefited from multiple caries preventive measures that, since 1989, have been introduced in Germany for the general population.

## Testing an In-Situ Enamel Gap Model to Evaluate the Bactericidal Activity of a New Composite

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To prove that a newly developed in-situ enamel gap model can demonstrate the bactericidal activity of a dental composite with quaternary ammonium silica dioxide (QASi) nanoparticles incorporated with other fillers into the resin type restorative material. Seven subjects, wearing lower removable partial dentures with acrylic flanges on both sides of the mouth, were recruited into the 4-weeks in-situ study (IRB #18-26344). The gap model consisted of an enamel slab placed next to a composite, separated by a small, controlled gap (38 µm). In the split mouth design on one side of the denture the composite was the Nobio Infinix composite, on the other side a widely used composite, serving as control. For each subject, enamel slabs originated from one tooth. The gap model with the enamel slab and composite was recessed into the flange, allowing microbial plaque to accumulate on top of it and in the gap. After 4 weeks of day and night wearing decalcification - ΔZ mineral loss - of the enamel slabs adjacent to the gap was determined by cross-sectional microhardness testing in the laboratory. Results: The mineral loss (ΔZ) for the Nobio test side was with 213 ± 257 (mean ± standard deviation [SD]) significantly lower compared to ΔZ of the control side (611 ± 574; mean ± SD) (paired t-test, p = 0.046; mean of Nobio minus control -397.43, 95% confidence interval of difference: -785.98 to -8.87). The in-situ enamel gap model was able to demonstrate the bactericidal effects of a dental composite with quaternary ammonium silica dioxide by significantly reducing the ΔZ mineral loss in enamel adjacent to the gap over a 4-week period.

### Caries State on Mesial Surface of the Permanent First Molar in Children with Mixed Dentition

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Immediately after the removal of the second primary molar, a white spot can be often observed on the mesial surface of the PFM. After the PFM erupt and make contact with the proximal surface of the second primary molars, it takes approximately three years in average for the second primary molars to exfoliate. Considering the proximal caries lesion of the PFM tends to worsen over time and is very likely to lead to pulpal lesion. Because early proximal caries lesion have potential to progress gradually, early diagnosis and management of caries in mesial surface of the PFM is important. The purpose of this study is to investigate the caries state on mesial surface of the PFM and its relationship with adjacent to the second primary molar. For this retrospective study, 211 children who visited the pediatric dentistry of Pusan National University Dental Hospital in the last five years were examined for ICDAS classification through direct visual examination of the mesial surface of the PFM after 30 minutes of extraction. The condition of the second primary molars is also recorded using the ICDAS code. The average age of extraction of the second primary molar was 10.6 years. The mesial surface of PFM evaluated by ICDAS was 20.9% (code 0), 10.9% (code1), 61.1% (code 2), 6.6% (code 3), 0.5% (code 4). The correlation values of mesial and distal surface of the second primary molar with the mesial surface of the PFM were 0.24, 0.38, respectively ( $p < 0.01$ ). Meanwhile, the white spot lesion on mesial surface of the PFM was treated with resin infiltration, and the cavity lesion with composite resin. The mesial surface of the PFM is undergoing periodic check-up by radiographic examination.

### Defective Restorations Management: Knowledge, Opinions and Practices of Members of a New French Dpbrn (ReCOL)

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Reducing the rate of restoration replacement is one of the cornerstones of Minimal intervention (MI) in Cariology first described early 1990's. More recently (fall 2019), the Fédération Dentaire Internationale edicted its first statements on the topic to encourage practitioners to implement less invasive strategies than systematic replacement. Several surveys have been undertaken in France to describe MI implementation into practice in terms of restorative management, risk assessment, sealants. The next topic to be studied was thus the Management of Defective Restorations (MDR). The aim of the study was to explore, using a questionnaire, knowledge, opinions and practices toward MDR among private practitioners who are members of the newly launched French dental practice-based research network "ReCOL". ReCOL members were solicited to reply to an online structured questionnaire assessing attitudes toward MDR. Univariate and bivariate (Fisher test) statistical analyses were performed. Practitioners working exclusively in academic settings were excluded from the analysis. A total of 378 private practitioners participated to the study (100%). If 61% of the respondents thought their undergraduate education on MDR was insufficient, 82% declared opting for repair DRs, from rarely (39%) to often (8%); the main reasons for choosing this strategy were 'reduction of toxic effects on the pulp' (58%) and 'dental hard tissues preservation' (47%). About 34% declared that repaired restoration must be considered as temporary. When asked about treatment attitudes for four different clinical cases of DRs, wide disparities were shown among respondents. Demographic characteristics (age, gender) seemed to influence some MDR patterns ( $p < 0.05$ ). This questionnaire study, the first of its nature in France and the first among ReCOL members, shows the need to further communication/education and harmonization about MDR.

## A Retrospective Study on the Survival of Dental Sealants in Disable Children: A Long-Term Follow-Up

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The most caries susceptible surface in permanent teeth are the occlusal surfaces and sealants are highly effective in preventing and treating incipient lesions. A retrospective study on the survival of resin-based dental sealants in a group of disable children was carried out. The dental charts of 321 children (8-22yy at the moment of the study) with neurodevelopmental disorders were scrutinized; children with at least one resin-based sealant on first molars and grade 1 or 2 of the Frankel scale of collaboration were enrolled. A hundred-fifty-two patients were included with 551 sealed teeth. Data on the date of sealant first application, any subsequent re-application and any restorative treatment needed for failure of the sealant and presence of dentinal caries, check-ups dates were extracted. Associations were assessed using Fisher's exact test at a 5% level of significance. Kaplan-Meier survival analysis was used to evaluate the survival of the sealants. The follow-up period ranged from 2 to 15 years. Overall 369 (66.97% of the total) sealants showed total retention and survived, with no statistically significant differences among the four first molars, ranging from 62.12% of the total of survived sealants for first right lower jaw molar to 70.42% for first right upper jaw molar. Partial retention of sealant was observed in 102 (18.51%) teeth, 59 in the upper jaw and 43 in the lower jaw ( $p = 0.04$ ). Total failure was observed in 80 teeth (14.52%) and for 52 (9.44%) a caries lesion developed, needing so a restoration. Resin-based sealants are effective to prevent dental caries in first molars even if applied without the rubber dam in low-collaborative children.

## Milk Supplemented with Egg Ovalbumin Inhibits Sucrose-Induced Salivary and Biofilm pH Drop, In Vivo

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Our previous studies have shown a potential anticaries effect of egg ovalbumin, in vitro and in vivo, but its combined effect with other foods is unknown. We aimed to assess the effect of ovalbumin-supplemented milk, whole or skim and with or without sucrose, on oral pH dynamics. Thirteen healthy volunteers, aged between 18-30 years, enrolled this double-blind crossing-over randomized study, with 6 experimental phases. After refraining from oral hygiene for 48 h and a 8 h fasting period, participants rinsed with a 10% sucrose solution for 1 minute followed by a rinse with

one of the 6 randomly-assigned treatments for 1 additional min: 1. whole milk, 2. whole milk supplemented with 2% ovalbumin, 3. whole milk with sucrose supplemented with 2% ovalbumin, 4. skim milk, 5. skim milk supplemented with 2% ovalbumin and 6. skim milk with sucrose supplemented with 2% ovalbumin. Saliva and biofilm pH were monitored at baseline, 2, 5, 10, 15, 20 and 30 min after rinsing, using a pH-meter and pH-sensitive strips located in the proximal area of upper molars, respectively. Data were analyzed by Shapiro Wilk and Kruskal Wallis tests. All treatments induced a decrease in salivary pH after rinsing with sucrose at 2 min. Although no statistically significant differences were detected ( $p > 0.05$ ), there were clear trends among the experimental groups. At min 5, whole milk supplemented with ovalbumin showed the highest pH: 6.99 (6.5–7.3). Conversely, the lowest pH values were detected with the both milk types with sucrose added; whole: 6.70 (6.1–7.1) and skim 6.71 (5.2–7.4). In all groups, pH increasingly recovered up to baseline values from min 10 onwards, up to min 30. Compared with saliva, a similar trend was observed with the biofilm pH, albeit without statistical differences ( $p > 0.05$ ). Whole milk supplemented with 2% ovalbumin seems to effectively counteract pH drop induced by sucrose. The addition of ovalbumin to dairy products could help in caries prevention.

## Effect of Irradiation of Aluminium Gallium Arsenide Laser on Incipient Pit and Fissure Caries: A Clinical Study

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Aim was to clinically evaluate the caries inhibitory potential of Aluminium Gallium Arsenide (AlGaAs) LASER irradiation on the incipient pit and fissure caries. 279 patients aged 18-25 years, were screened for bilateral Site-1 Size-0 (Mount and Hume classification), non-cavitated carious lesion on mandibular first and/or second molar. Those scoring V0, V1, V2 and R0 & R1 by Ekstrand clinical and radiological criteria respectively, were shortlisted. The final selection of 104 patients, was done by occlusal scanning with LASER fluorescence (LF) device (DIAGNOdent), and teeth recording values between 20-35 were included in the study (Lussi criteria) and these values served as the baseline. By random allocation, the tooth was assigned to Test (Group A) and the contralateral tooth type served as control (Group B). Group A cases were irradiated with 3.5 watts of 810 nm AlGaAs LASER for 30 seconds followed by application of CPP-ACP-F remineralizing paste. In Group B only remineralization paste CPP-ACP-F was applied. The LF values were recorded after 7 days and protocol repeated. Follow up done at 1, 3, 6, 9, 12 months by LF scanning. Fall in values from baseline values indicated remineralization. The data were statistically analysed by parametric tests i.e. student unpaired 't' test and paired 't' test, Chi-square test of significance for proportion analysis. Life survival was assessed by Kaplan Meier analysis. Control group had 53 (51%) patients who required operative intervention whereas Test group had 3 (2.9%) patients. In the control group, 38 (36.5%) patients required repeating remineralizing protocol

whereas it was 5 (4.8%) patients in the test group. The optimal wattage and time of irradiation were 3.5 watts and 30 secs respectively (in-vitro studies were done to standardize protocol). There is a significant difference in mean between test and control at all the time intervals with  $p$ -value  $<0.001$  at a 99% confidence level. Preoperatively mean + sd of LF values in control was  $25.58 + 2.92$  and in the test, it was  $26.76 + 2.42$ , i.e. significantly higher difference in the test group. However, postoperatively after 12 months mean + sd LF in control increases to  $29.73+7.04$  and in test group it decreases to  $12.44+5.80$ , which is very significantly lower in the test group. Based on the clinical results it was concluded that Irradiation with 3.5 watts of 810 nm (AlGaAs) LASER has a caries inhibitory role in non-cavitated lesions.

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### The Clinical Effect of Dental Caries Detection in Primary Teeth Using QLF Technology

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The early detection of dental caries in primary teeth is important for children because they could be more active or destructive. This study aimed to identify the clinical detection ability of QLF (Quantitative Light-induced Fluorescence) technology for dental caries in primary teeth compare to visual inspections and radiographic examinations. Ninehundred-thirty-seven surfaces of primary teeth from 3 to 12-year-old children were investigated in this study included normal and caries surface of occlusal caries and proximal caries respectively. They were assessed by the scoring system of visual inspections (modified ICDAS-II) and radiographic examinations (ICCMS). A quantitative analysis about fluorescence loss ( $\Delta F$ ) and red fluorescence ( $\Delta R$ ) were produced using QLF images obtained by Qraypen C, which is the pen-type portable QLF device. The sensitivity, specificity, AUROC (area under the receiver operating characteristic curve) and cut-off point for dental caries of both quantitative parameters were calculated and compared to the results of visual inspections and radiographic examinations.  $\Delta F$  showed an excellent AUROC value (OC = 0.976, PC = 0.824), whereas  $\Delta R$  was a relatively lower (OC = 0.758, PC = 0.672). Cut-off point of  $\Delta F$  to detect dental caries could be estimated as  $-6.30$  (OC) and  $-6.35$  (PC). The sensitivity and specificity of QLF (OC = 0.969 and 0.849, PC = 0.701 and 0.806) were similar to radiographic examinations (OC = 0.828 and 0.875, PC = 0.738 and 0.866) and visual inspections (OC = 0.946 and 0.656, PC = 0.750 and 0.843). \*\*Occlusal caries = OC, Proximal caries = PC,  $p$  value  $\leq 0.05$  for all data. In clinical conditions, QLF technology for dental caries detection in primary teeth would be reliable as other existing methods.

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### Development of a Root Caries Risk Model in a Population of Primary Care Dental Attenders

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Root caries is a preventable disease where the majority of the disease burden affects approximately one third of the population. However, there is no accepted practice to identify high risk individuals. The aim of this study was to develop a diagnostic model for root caries in a population of UK adult regular dental attenders. 2372 patient participants were examined clinically at study baseline and made dentally fit by the recruiting dentist. Data on participant demographics, oral health indicators and oral health attitudes was collected through baseline patient questionnaires. Participants attended clinical assessments by study researchers four years after recruitment to determine tooth level root caries incidence. A regression analysis at two outcome thresholds was conducted: root caries (i)  $> 0$  teeth, and (ii)  $\geq 3$  teeth. Root caries data was collected for 1432 participants. 324 (22.6%) had at least one root caries lesion, and 48 (7.8%)  $\geq 3$  lesions. The results from the multivariate regression analysis indicated that individuals who smoked regularly (OR 6.3, 95% CI 3.60-10.80,  $p < 0.001$ ), had more than eight restored teeth at baseline (OR 2.0, 95% CI 1.80-2.30,  $p < 0.001$ ), had worse oral health knowledge (OR 1.8, 95% CI 1.20-2.60,  $p = 0.006$ ), were older (OR 1.07, 95% CI 1.06-1.09,  $p < 0.001$ ) and attended for dental check-ups more frequently (OR 1.10, 95% CI 1.00-1.20,  $p = 0.002$ ) were more likely to develop root caries over a four year period. Use of an electric toothbrush reduced the risk of developing root caries compared to manual toothbrush users (OR 0.72, 95% CI 0.53-0.98,  $p = 0.034$ ), over the study period. The model sensitivity was 76.0, specificity 72.6, and AUC 0.82. This study highlights variables that accurately predict development of root caries in adult regular dental attenders and may aid dental practitioners identify those who would benefit most from root caries preventive interventions.

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### Oral Health Self-Efficacy and its Association with Oral Health Status in Chilean Pregnant Women

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Discussions regarding psychosocial factors have gained relevance on caries research in recent years. This study aimed to explore self-efficacy and its association with oral health status in

pregnant women from an urban primary healthcare centre in Santiago, Chile. A cross-sectional study was performed in a convenience sample of 49 pregnant women attending an oral health primary care program. Self-efficacy was measured through Syrjala's (modified) questionnaire and oral health status was determined after a clinical examination. DMFT, the simplified Greene and Vermillion plaque index and the simplified Löe and Silness gingival index were recorded. Descriptive analysis and Pearson's correlation were performed,  $p < 0.05$  was considered statistically significant. The mean age of the sample was 28 years old. General oral health self-efficacy was 31.57 points  $\pm$  6.89 (score range 15–48 points  $\pm$  standard deviation), tooth brushing self-efficacy subscale was 14.42 points  $\pm$  3.73 and 17.14  $\pm$  4.75 to visit the dentist self-efficacy subscale. Mean DMFT was 7.5  $\pm$  5.14, the gingival index was 0.64  $\pm$  0.74 and the plaque index was 0.60  $\pm$  0.61. A statistically significant correlation was found between "visit the dentist self-efficacy" and "decay component" from DMFT ( $r = -0.296$ ;  $p = 0.039$ ), between "general oral health self-efficacy" and "decay component" ( $r = -0.338$ ;  $p = 0.017$ ) and between "tooth brushing self-efficacy" and plaque index ( $r = -0.334$ ;  $p = 0.022$ ). In conclusion, a significant correlation was found between oral health self-efficacy and oral health status regarding plaque index and decay component from DMFT.

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### Myth Busted – Breastfeeding Does Not Cause Early Childhood Caries in Serbia

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Considering that there are interprofessional opposing opinions regarding association between breastfeeding practices and occurrence of early childhood caries (ECC), there is strong need to clarify this issue. A cross-sectional epidemiological survey was conducted involving children aged 12 to 91 months of age attending public kindergartens in Serbia. Sample involved 3 age groups: toddler group (12 to 36 months of age), kindergarten group (36–71 months of age), and preschool group (72–91 months of age). The final study sample was calculated according to total number of inhabitants in targeted age groups, using the official data from Statistical Office of the Republic of Serbia. Stratified cluster sampling was used in order to obtain a nationally representative sample; additionally, it was stratified according to urban, peri-urban and rural types of residency involving most important population subgroups. Results were presented as frequencies (percentages) or mean  $\pm$  standard deviation (SD) depending on data type. A total of 3676 preschool children participated in the study (52.2% males; 47.8% females). The results showed that 13.4% of toddlers, 50.9% kindergarten and 80% older preschoolers had caries experience. The majority of ECC was untreated – 98.9% in toddlers, 93.5% in kindergarten group and 90.6% in preschoolers. No statistically significant difference ( $p = 0.185$ ) was observed between duration of breastfeeding in children with ECC who were breastfed for 11.8 ( $\pm$ 7.4) months vs caries free children who were breastfed for 10.9

( $\pm$ 6.8) months. ECC was statistically significantly ( $p < 0.05$ ) less prevalent in children who were exclusively breastfed (17.1%) vs. children who were predominantly/partially breastfed (38.7%). Conclusions: The results from the present national survey suggested that both parents and health professionals need additional education about healthy lifestyle habits that could tackle both oral and general health.

The data were collected within Program for Oral Health Improvement in Children and Youth in Serbia, approved by the Government of Serbia, Ministry of Health, Program number 1802, Project activity number 4015.

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### Detection Methods for Early Caries Diagnosis: A Systematic Review and Meta-Analysis

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Aim was to appraise existing evidence on the diagnostic performance of a range of means for the detection of incipient caries in vivo. Five databases of published and unpublished research were electronically searched for eligible studies from January 2000 to October 2019. Search terms included "early caries", "caries detection". Inclusion criteria involved in vivo diagnostic test accuracy studies for early caries detection of both permanent and primary teeth. Risk of bias assessment was performed using the QUADAS-2 tool. Study selection, data extraction and risk of bias assessment were done in duplicate. The review protocol was a priori registered in the Open Science Framework. Of the initially 22964 search results, 50 articles were included. For permanent teeth, when histologic examination was considered as reference for occlusal surfaces, the sensitivity (Se) range appeared high for DIAGNOdent Pen (DD Pen: 0.81–0.89), followed by ICDAS-II (0.62–1), DIAGNOdent (DD: 0.48–1) and bitewing radiography (BW: 0–0.29). Corresponding specificity (Sp) range was: DD Pen (0.71–0.8), ICDAS-II (0.5–0.84), DD (0.54–1), BW (0.96–1). No data were available for approximal surfaces. When operative intervention served as reference for occlusal surfaces, again DD means valued the most promising results on Se: DD (0.7–0.96), DD Pen (0.89), followed by ICDAS-II (0.54–0.93) and BW (0–0.83). Sp range was formed as follows: DD (0.54–1), DD Pen (0.54), ICDAS-II (0.44–1), BW (0.6–1). For approximal surfaces, the sensitivity range (Se) was: BW (0.75–0.83), DD (0.6), ICDAS (0.54), while the specificity range was: BW (0.6–0.9), DD (0.2), ICDAS (1).

For primary teeth, under the reference of histologic assessment, the Se range for occlusal surfaces was: DD (0.545–1), DD Pen (0.63–1), ICDAS-II (0.42–1), BW (0.31–0.96), while the respective Sp: DD (0.5–1), DD Pen (0.44–1), ICDAS-II (0.61–1) and BW (0.79–0.98). For approximal surfaces, the sensitivity range (Se) was: DD pen (0.58–0.63), ICDAS-II (0.42–0.55), BW (0.14–0.71). Corresponding specificity (Sp) range was: DD pen (0.85–0.87),

ICDAS-II (0.73–0.93), BW (0.79–0.98). There was considerable variation in Se and Sp values detected, due to apparent heterogeneity in the experimental settings of individual studies. Evidently, robust conclusions cannot be drawn and different diagnostic means should be used as adjuncts to clinical examination.

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### Oral-Health and -Behavior of Adolescents with Mild/Borderline Intellectual- and Developmental Disabilities in the Netherlands

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Like in general health, less favorable outcomes concerning oral health are reported for people with intellectual disabilities (ID). Concerning the sub-group of adolescents and young adults with Borderline Intellectual Functioning (BIF; IQ 70–85) and mild ID (IQ 55–70), no data are available for The Netherlands. Having their own household and autonomy this group is considered even more vulnerable for not adhering to healthy behavior than their peers without ID. The aim of this study is to assess to what extent adolescents (16–18 years of age) with mild intellectual disabilities or BIF differ from their peers in a national representative sample in clinical and non-clinical outcomes of oral health. Ninetyseven adolescents with mild ID/BIF were invited to fill out a questionnaire and to undergo a comprehensive oral health screening. Outcomes were compared to data of 17-year old Dutch adolescents (n = 581) from a large national epidemiological study on oral health and oral health behavior of the Dutch population. A higher number of decayed, missing and filled carious teeth (3.08 vs 2.23 DMFT), a higher number of nearly cavitated carious lesions (1.6 vs 0.75 ICDAS 3 lesions), a higher periodontal treatment need (37% vs 32% bleeding of the gums and calculus) and a poorer oral hygiene (mean OHI-s 1.28 vs 0.69) was found in people with mild ID compared to their peers in the general population. Furthermore, people with mild ID were found to have less desirable non-clinical outcomes as well. The results of this study confirm the presumption that adolescents with BIF and mild ID show less desirable oral health and dietary behavior and have a poorer oral health than their peers without mild ID. Development of targeted interventions to reach this group are necessary.

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### Dental Caries Experience in a Japanese Adult Outpatients in Japan. A Preliminary Report of the Multicenter Study

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The purpose of the present multi-center study was to investigate the caries experience of the outpatients in Tokyo and the rural city. All the adult outpatients who visited the dental clinic and received comprehensive carious examination and full mouth radiographs were consecutively included and assessed in 2019. The evaluated items included were age, gender, the total number of teeth present, number of decayed, missing, filled tooth, caries status, and type of restoration. Caries status was evaluated using both clinical and radiographic data and assessed as D1/D2 as enamel lesion and D3/D4 as dentin lesion. The oral status assessment was done by 2 evaluators for each case. There was a calibration session but when evaluated results were different, they were discussed until the consensus was reached. Caries experience of each patient is calculated as of D (untreated D3/D4), M (missing), and F (filled) components (DMFT). Two hundred fifty-eight adults (160 female 98 male) aged 16 to 84 (Mean±SD: 41.8±12.2) from 2 centers were analyzed. People lost teeth (Mean±SD: 4.1±3.0) gradually by increasing age. Almost half of the remaining teeth were restored (Mean±SD: 13.4±6.4). There was great variability among people. Although the mean number of untreated caries (Mean±SD: 3.6±3.5) is low for all age groups, DMFT score was high (Mean±SD: 18.5±7.3). Oneway-ANOVA revealed DMFT has a statistically significant difference among age groups (p<0.001). The results also showed DMFT score underestimates the real carious status in our materials. On average, mean (±SD) number of initial caries lesion (D1/D2) was 2.5 (±2.8). Furthermore, it should be realized that the restored teeth have significantly (p<0.01) more carious problems (19.5%) than unrestored teeth (6.9%). We need prompt action for caries preventive strategies in Japan.

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### The Role of Vitamins in Dental Health. A Systematic Review and Meta-Analysis

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The present review aimed to systematically evaluate the most recently papers investigating the role of vitamins in the prevention and treatment of dental caries, enamel defects and erosion.

Randomized controlled trials, cross-sectional studies, cohort studies, comparative studies, validation studies and evaluation studies, following the PRISMA guideline, reporting associations between vitamins and hard dental tissues diseases or the use of vitamins to prevent or treat dental diseases in patients of any age were included. PubMed, Embase and Scopus were searched to November 2019 using an ad hoc prepared search string. All the papers meeting the inclusion criteria were subjected to a quality assessment. The search identified 889 papers; 328 were selected after removing duplicates. A total of 182 articles were excluded after title and abstract evaluation; 146 papers were full-text assessed and 126 were discharged after full text evaluation. Finally, 20 papers were included, 13 on caries, 5 on enamel defects/erosion and 2 on both diseases. Fifteen papers were ranked of as being of good quality, three were classified of fair quality and only two of poor quality. Funnel plot analysis showed that no study was trimmed for caries and enamel defects, and the overall effect sizes observed were 1.04, (95%CI 0.92, 1.18;  $p = 0.52$ ) and 0.27 (95%CI -0.04, 0.57;  $p = 0.09$ ), respectively. No significant publication bias existed based on the Egger regression analysis ( $p = 0.79$  and  $0.19$ , respectively). Heterogeneity analysis for caries showed the highest value for Vit. C (95.50%), while a substantial I2 value was observed for Vit. D (70.06%). Considering enamel defects, there were not enough data for performing this analysis. No scientific evidence for hard dental pathological processes and vitamins was found.

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### ICCMS™ Root Caries Lesions Stages and their Underlining Depth towards the Pulp

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The aim was to examine the relationship between the visual features of root caries lesions and the underlining depth of the lesion towards the pulp. In order to determine the best way of performing the cut, 21 extracted teeth with root caries lesions were hemi-sectioned (HS) through the lesion and data showed that the outline of the pulp assessed by the stereomicroscope (SM) was much clearer on the horizontally cut compared to the vertically cut lesions (SIGN-test  $p = 0.03$ ). Also, the underlining lesion depth, assessed by the SM, towards the pulp was not influenced by the cutting direction (horizontally/vertically, unweighted Kappa 0.86;  $p < 0.001$ ). Later, 100 extracted teeth were visually and by a peri-probe classified with ICCMS™ as: 0 = sound; 1 = initial-lesion (non-cavitated); 2 = moderate-lesion (cavity depth  $\leq 2$ mm) and 3 = extensive-lesion (cavity depth  $> 2$ mm). Then, lesions were cut

horizontally through them. After HS the depth of the underlining lesion, one of the section faces was stereomicroscope assessed independently by two of the authors with the following criteria: 0 = no lesion; 1 = lesion in outer 1/3-; 2 = middle 1/3- and 3 = inner 1/3 of the dentine towards the pulp. Kappa, Specificity and Sensitivity, and Spearman Correlation analyses were performed. Weighted Kappa-values for Intra- and inter-reproducibility were  $\geq 0.85$ ; the accuracies (Spearman's rho values) were = 0.94 and Specificity- and Sensitivity values at three different thresholds were  $\geq 0.92$  and  $\geq 0.91$  respectively. The reproducibility, accuracy and, the specificity and sensitivity values of the ICCMS™ root caries scoring system were high.

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### Accuracy of Different Approaches for Detecting Proximal Root Caries Lesions In Vitro

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Objective was to compare the diagnostic accuracy of radiographic evaluation (XR), visual-tactile assessment (VT) and laser-fluorescence (LF) (DIAGNOdent Pen/KaVo) to detect proximal root caries lesions in vitro. Two-hundred extracted permanent posterior human teeth with and without proximal root caries lesions were allocated to 50 diagnostic models (two premolars and two molars/model) simulating the proximal contacts between teeth (6 proximal tooth surfaces/model), and mounted in a phantom dummy head. The different caries detection methods were independently applied by two examiners. The accuracy to detect any (ICDAS W1 and W2) or advanced (only ICDAS W2) root caries lesions was evaluated, with histologic evaluation of the lesions serving as reference. Receiver operating characteristic (ROC) curves were employed, and sensitivity, specificity and the area under the ROC curve (AUC) calculated. Significant differences in mean AUCs between approaches were assumed if  $p < 0.05$  (two-sample t-test). The sensitivity/specificity to detect any proximal root caries lesion was 0.81/0.63 for XR, 0.76/0.88 for VT and 0.81/0.95 for LF, and the sensitivity/specificity to detect advanced lesions was 0.43/0.94 for XR, 0.66/0.99 for VT and 0.83/0.78 for LF, respectively. Mean AUCs for LF and VT were significantly higher compared to XR for both any and advanced root caries lesions ( $p < 0.05$ ). LF was also significantly more accurate than VT to detect any root caries lesions ( $p = 0.01$ ), while there was no significant difference for advanced root caries lesions ( $p = 0.59$ ). LF and VT were more accurate than XR to detect proximal root caries lesions, with LF being particularly useful for initial lesion stages.

### Usability and Satisfaction of a Digital Learning Tool for ICDAS: A Practice-Based Research

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This practice-based research aimed to evaluate the usability and satisfaction of a digital learning tool (DLT; [www.ufrgs.br/icdas](http://www.ufrgs.br/icdas)) [Luz et al., 2015 and Alves et al., 2018] available online for teaching, training and calibration of examiners for the visual detection of caries lesions using ICDAS. The DLT is an interactive website quiz with 60 questions based on pictures of both sound and carious deciduous and permanent teeth. The DLT was online spread through social media in order to achieve dental students and dentists. In a non-controlled environment, users were instructed to fill out a profile questionnaire and a satisfaction questionnaire after completing the DLT. Data on number of sessions used to complete the DLT and its average time were collected through Google analytics. The satisfaction questionnaire reported the following characteristics: content, interface, personalization and user learning. A total of 343 users started the DLT, however 175 completed it and filled out both questionnaires. The DLT was most popular among dental students (79.6% of users). Users spent 1.7 sessions to respond the DLT in an average time of 16 minutes and 30 seconds each. Satisfaction questionnaire showed that over 81.1% of users found in the DLT exactly what they expected and 89.7% found the DLT useful. Around 87% found that the DLT helps discussing ICDAS with colleagues (87.4%) and superiors (86.9%), 84% found that the DLT provides immediate results and 84.6% reported the higher level of satisfaction with the DLT. The DLT was most useful to dental students and overall satisfaction with the DLT was 84.6%. This study showed that the DLT is indicated for teaching, training and calibration of examiners for the visual detection of caries lesions using ICDAS.

### Maternal Exposure to an Oral Health Program and Teeth Affected by Caries in 2-Years-Old Children

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It has been shown that pregnancy is a useful time for intervention to prevent Early Childhood Caries (ECC). In Chile, there is a program called Integral Oral Health for Pregnant Women (IOHPW), which is an explicit health warrantee (GES) by the State of Chile. It aims, among other goals, to protect the child's oral health in the future. The aim of this study was to evaluate the relationship between maternal exposure to the IOHPW program and

the number of teeth affected by caries in 2-year-old children. A cross-sectional study was conducted. 911 children were examined in their kindergarten to find out the number of teeth affected by caries (including cavitated and non-cavitated lesions) or restorations, for each child, as the outcome variable. Exposure to the IOHPW program was assessed by determining if the mother had received services from the program during pregnancy. We determined whether the mothers received IOHPW program services using health services databases. This information was available for the mothers of 696 children. Children were nested in 30 kindergartens. Sex and age were also variables measured for each child. A multilevel negative binomial regression was done. Rate ratios and their 95% confidence intervals were estimated. The Incident Rate Ratio (IRR) obtained was 1.13 (95% CI 0.77 - 1.67). In conclusion, a relationship was not found between maternal exposure to the IOHPW program and the number of teeth affected by caries in 2-year-old children.

### Detection of Early Dental Caries: Cochrane Diagnostic Test Accuracy Reviews

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Objectives were to produce a comparison of available tests for the detection and diagnosis of early dental caries. Cochrane Diagnostic Test Accuracy (DTA) Reviews have been completed for five groups of tests: visual examination, radiography, fluorescence devices, electrical conductance and transillumination. These employed the QUADAS-2 method for risk of bias and applicability. Meta-analyses were completed using hierarchical bivariate and SROC methods. Further statistical analyses used the extracted data from all eligible studies that evaluated more than one method to arrive at a comparative accuracy for the five test areas. The series of reviews included 262 studies which generated 318 datasets. Summary sensitivity and specificity results were: visual (0.86 (95% CI 0.81 to 0.90), 0.77 (95% CI 0.71 to 0.82)); radiography (0.48 (95% CI 0.43 to 0.54), 0.88 (0.85 to 0.91)), fluorescence (0.78 (95% CI 0.71 to 0.86), 0.81 (95% CI 0.74 to 0.88)), electrical conductance and transillumination (0.76 (95% CI 0.62 to 0.86), 0.87 (0.81 to 0.91)). The diagnostic odds ratios were: visual examination 20.8, radiography 7.1, fluorescence 14.0, electrical conductance 15.7 and transillumination 20.8. A substantial amount of heterogeneity existed in the design of the included studies and the quality of included studies was low, therefore these results need to be interpreted with caution. There is potential for transillumination devices to be beneficial during the detection of early/enamel caries and the results suggest that visual examinations are also successful. Radiographs are not able to detect early lesions. The fluorescence based tests are limited due to the heterogeneity of devices used and variety of thresholds applied, while the number of included studies was low for electrical conductance.

### Reasons Why Radiographs bring More Harm than Benefits in the Caries Diagnosis of Preschool Children

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We aimed to evaluate the management and clinical course of surfaces from primary molars diagnosed (and consequently treated) by two different strategies: visual inspection alone or associated with radiographs. Secondary analyses from a randomized clinical trial on diagnostic strategies for caries detection in preschool children were conducted. All dental surfaces of 216 children (3-6 years-old) who sought oral health care were diagnosed by visual inspection and subsequently, through radiographic assessment. Treatments were conducted in accordance with the allocated diagnostic strategy. Dental surfaces (unit of analysis) with no restoration needs, or those restored in the beginning of the study were followed-up for 2 years. The outcome was the failure occurrence (new operative intervention or restoration replacement) during follow-up. Thus, 4,383 proximal and occlusal surfaces of primary molars were diagnosed and treated according to the diagnostic strategies abovementioned. When we considered all types of treatment (non-operative or operative treatments), decision made by the radiographic assessment was different from that made by visual inspection in about 30% of the surfaces. However, most disagreements occurred for initial lesions; radiographs tended to underestimate these type of lesions. Contrariwise, discordances for operative-needing lesions occurred in less than 5% of all surfaces. Moreover, treatments performed accordingly to radiographs were not more successful than those made after visual inspection alone. In fact, we observed that radiographs brought more harms than benefits due to false-positives occurrence, overdiagnosis or lead-time bias. Therefore, we concluded that simultaneous association of visual and radiographic method for caries detection in preschool children causes more harms than benefits. Visual inspection conducted alone is more beneficial for children at the pediatric dental office setting and therefore, should be indicated for daily clinical practice.

### A School-Based Report of Factors Associated with High and Extreme Caries Risk

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The identification of factors driving the caries disease process aids on assessing caries risk and developing individualized

management plans. This retrospective study evaluated the outcomes of the caries risk assessment tool used at the University of Florida College of Dentistry (UFCD) with emphasis on patients assigned at high and extreme caries risk. Electronic dental records from 2,000 patients were previously used to assess data on demographics, and the frequency of caries disease indicators, and risk and protective factors. Chi-square statistics and multivariate logistic regression were used for statistical analysis. Of the population studied, 743 patients were included in this study and 85.6% (n = 636) of these were assigned at high (mean age: 52.4 years) and 14.4% (n = 107) at extreme risk (mean age: 51.5 years). When compared to high-risk, extreme-risk patients presented higher frequency of: caries lesions radiographically into enamel (p = 0.005) and those into dentin (p = 0.003), clinically active enamel lesions (p = 0.022), coronal cavitated lesions (p = 0.006), and root lesions (p < 0.0001). With regards to risk/protective factors, extreme-risk patients presented with less frequent flossing habits (p = 0.019) and dental visits (p = 0.008), less exposure to fluoride (p < 0.0001), higher sugar intake (p = 0.010), increased medical risk factors (p < 0.0001) and self-reported dry mouth (p < 0.0001), and less visible salivary flow (p < 0.0001) as compared to high-risk patients. Logistic regression model showed that medical risk factors, dry mouth and fluoride exposure were significantly associated with extreme risk (p < 0.05). The combination of individual factors such as medical risk factors, reported dry mouth and daily exposure to fluoride were the main variables used to assigned patients at extreme risk. The need for increasing daily fluoride exposure to offset medical risk factors must be emphasized to patients for continue success of the caries management program.

### Systemic and Systemic/Topic Effect of Probiotic in Surface Microhardness in an In Situ Caries Model

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Aim was to establish the differences in microhardness of enamel blocks exposed to sucrose, control and two different way of probiotic administration in an in situ model of caries. Healthy volunteers between 18 and 30 years old, free of periodontal and gingival disease, with at least 22 permanent teeth, 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 d and 24 h and were distributed in 3 groups. Group 1 applied a solution of 20% sucrose every 2 h on the enamel blocks (positive control group); group 2 in addition to applying the same sucrose regimen, drank 100 ml of water with lyophilized probiotic strain reaching a concentration of 108 CFU/ml, without the device in the mouth (systemic group) and group 3 similar to group 2 but drank the probiotic with the device in mouth (systemic / topic group). A block of enamel in each device was left as a control and varnished with a layer of insulating coat (negative control group). All blocks after the experiment were removed from the devices and were subjected to Vickers microhardness tests. The microhardness mean for the

sucrose group was 156 HV (95% CI: 147-165), for the systemic group was 254 HV (95% CI: 246-262), for the systemic/topic group was 285 HV (95% CI: 281-289) and for the control group was 298HV (95% CI: 287-310).

There were significant differences between all groups. The use of systemic/topic probiotic in this in situ model suggests a greater inhibition of formation as caries like lesions measured by surface microhardness.

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### Scientific Evidence for the Cavitated Caries Lesion Treatment in Anterior Primary Teeth: A Systematic Review

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The aim of this systematic review was to evaluate the current scientific evidence about the management of cavitated caries lesion in anterior primary teeth. Literature searching was carried out in MEDLINE/Pubmed, Scopus and Web of Science databases until December 2019. Grey literature was also checked as well as the list of references of eligible studies. Studies evaluating the cavitated caries lesion treatment in anterior primary teeth with at least 12 months of follow-up were included. Manuscripts that fulfilled the inclusion criteria were fully assessed, being excluded those which didn't report the success of treatment as outcome or separate data for anterior primary teeth. Qualitative analysis of data was performed according to intervention. Search strategy identified 2092 potentially relevant studies and 26 of them were included in the review. The majority of studies were designed as retrospective (65.4%) and focusing in treatment of early childhood caries (46.2%). Overall, three approaches to treat anterior caries lesion were evaluated: direct restoration (resin composite - RC: 50%; resin-modified glass ionomer cement - RMGIC: 7.7%), RC strip crowns (26.9%) and indirect crowns (zirconia - 7.7%, pre-veneered stainless steel crowns (SSC) - 7.7%, resin-veneered SSC - 3.8%, copolyester jackets - 3.8%). Nine studies considered recurrent caries as outcome and almost all approaches presented high success rate, ranged from 87.2% (RC strip crowns) to 100% (RC, RMGIC and zirconia crowns). All studies evaluated retention rate. Among them, results that come up were from 61% for resin-veneered SSC in 17.3 months to 100% for RC strip crowns/RC in 12 months and RMGIC in 50 months of follow-up. In conclusion, the several approaches available for the management of cavitated anterior caries lesion seems to be effective for this purpose.

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### Use of ICDAS II and Fluorescence-Based Intraoral Camera in Early Caries Detection: A Clinical Study

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Aim of this in vivo study was to compare two performance of ICDAS II and Intraoral camera for the detection of early caries lesions. Ninehundred fifty-one permanent teeth (259 premolars and 691 molars) in 13-20 years old patients were evaluated in this in vivo study. Two blind operators inspected the occlusal surfaces of each tooth and the first one assigned an ICDAS II code, while the second one assessed the VistaCam score by using VistaCam ix Proof, Durr Dental. Level of agreement between the two procedures was expressed by using Cohen's/Fleiss' kappa statistic. Two-hundred sixty-six (28%) of the assessed teeth had ICDAS II code 0, 316 (33.3%) code 1, 178 (18.7%) code 2, 164 (17.2%) code 3, and 27 (2.8%) code 4. VistaCam assessed in 482 (50.7%) sound enamel [0-1.2], 274 (28.9%) initial enamel decay [1.2-1.5], 195 (20.4%) deep enamel decay [1.5-3]. ICDAS II code 0 (n.266) was classified by fluorescence camera as 244 sound, 12 enamel decay, 10 deep enamel decay. ICDAS II code 1 and 2 were diagnosed in 22 and 41 cases respectively, as deep enamel decay. McNemar's chi-squared value was 142.15, when df = 1 with p-value of < 2.2e-16. The weighted kappa test showed coefficients ranging from 0.553 to 0.641 with an estimate of 0.61. Our data demonstrate a moderate agreement between the two diagnostic methods, especially in ICDAS II Code 1 and 2 assessment. Future randomized study will better define the role of the operator's visual inspection and the fluorescence-based camera. Until then, usage of ICDAS II and fluorescence-based cameras should continue to be considered the standard of care and begin to be widely used in epidemiological examinations.

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### Caries Intensity Among Teenagers Treated with Fixed Appliances

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The aim of this study is to investigate the caries situation in a group of Polish adolescents, who were undergoing the orthodontic treatment with fixed appliances. An age- and gender-matched orthodontic and control group was selected from 144 patients aged 12-18 years from the same city (Lublin, Poland). The study was

divided into three stages: before treatment (I), at 1 month (II), and at 6 months (III) follow-up. Caries and fillings presence on teeth were diagnosed clinically in both groups with full permanent dentition. The DMFT index was assessed and statistically analysed withing groups during the period of observation. There were no significant differences regarding DMFT index between groups although it was higher in the orthodontic group than in the control group, both during the first study (4.35 vs 4.10) and after 6 months of treatment/observation (4.48 vs 4.15). However, a significant differentiation ( $p < 0.05$ ) was revealed in the orthodontically treated group during the six-month observation period. The DT index in the orthodontic treated group, as opposed to the control group, decreased significantly ( $p < 0.05$ ) after 6 months of observation. At the same time, the FT index in the group with fixed braces increased significantly ( $p < 0.05$ ) during each of the three studies, while in the control group only a slight increase in number of FT was observed between the first and third study. No teeth removed due to caries ( $MT = 0$ ) were recorded among all teenagers. Orthodontic treatment does not significantly affect the increase in the DMFT index in the presented group treated with orthodontic braces compared to the control group in the first six months of therapy.

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### Effect of Lactobacillus Brevis CD2 on Plaque-pH and Cariogenic Bacteria: A RCT in Diabetic Children

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The short-terms effect (60 days) of probiotic lozenges vs placebo on variables related to caries and gingivitis in type-1 diabetic children were evaluated. Material and Methods: 68 diabetics (4-14 yy) were assigned to probiotic or placebo group, including 34 subjects per group. The probiotic lozenges contained 2,000,000,000 colonies of Lactobacillus brevis CD2. Saliva, plaque pH samples and gingival status were assessed at baseline (t0), 30 days from baseline (t1), at the end of treatment (60 d from baseline, t2) and in the follow-up period (90 d from baseline, t3). Primary and not primary cariogenic bacteria using the checkerboard DNA-DNA hybridisation method were assessed. The signals were coded as 0 = no signal; 1 = signal lower <105 bacteria; 2 = 105 bacteria; 3 = >105 but <106 bacteria; 4 = 106 bacteria and 5 = higher than >106 bacteria. Differences between groups were evaluated by two-way ANOVA. Results: In the probiotic group, all cariogenic bacteria considered, except Lactobacillus salivarius, decreased: Streptococcus mutans dropped from  $3.11 \pm 1.13$  at baseline to  $1.82 \pm 0.72$  and to  $2.06 \pm 0.56$  at t2 and t3 respectively ( $p \leq 0.01$ ). The lowest pH and the maximum pH fall increased significantly in the probiotic group changing from  $5.37 \pm 0.41$  at baseline to  $5.49 \pm 0.24$  at t3 ( $p \leq 0.01$ ) and from  $1.20 \pm 0.46$  to  $0.98 \pm 0.29$  ( $p \leq 0.05$ ) respectively. No inter-groups differences regarding pH were detected except for the lowest pH at t2. Bleeding score decreased significantly in both groups,

but children using the probiotic showed a lower bleeding score at t2 compared to placebo group (25.6%, 95%CI 21.5–32.7 vs 29.5%, 95%CI 25.2–34.9,  $p \leq 0.05$ ). In conclusion, 60 d administration of Lactobacillus brevis CD2 has shown to improve variables related to caries and gingivitis in diabetic children.

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### Are Glass Ionomer Sealants an Efficient Option for Treating Moderate Caries Lesions?

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Sealing may be a non-invasive alternative to restore moderate lesions (ICDAS 3-4) on occlusal surface of primary molars. However, the remaining question is if sealing with high viscous glass ionomer cement (HVGIC) is an efficient way to allocate resources considering possible uncertainties around the estimates. This study performed an economic evaluation based on a non-inferiority clinical trial (NCT03005405) considering the treatment success (no caries progression) and patient-reported acceptability. A time horizon of 2 years and a societal perspective were chosen. Children were randomly allocated in two groups: HVGIC restoration (opening, removal using excavators) or HVGIC sealant (no opening/ no caries removal). Child's acceptability was evaluated using a facial scale. Caries lesions progression to ICDAS 5-6 was recorded, the proportion of children without discomfort at baseline and without caries progression were considered as effects for two years. Costs were valued per child, the incremental cost and effect of performing sealants as alternative to restoration were used in two cost-effectiveness analyses. A cost-effectiveness plane was drawn using 10,000 simulated situations. Probabilistic analyses and acceptability curves were used to explore uncertainties around incremental values. 101 children were included (mean  $\pm$  SD) (2 teeth  $\pm$  1.03 and 5 years old  $\pm$  0.8). There were no statistically significant differences between treatments for effects and costs. Despite not statistically significant a  $\Delta$ cost = \$10.87 (90% CI = -0.604 – 0.197) was observed. When treatment efficacy was considered, 30% of simulations represent HVGIC sealants as a cost-effective option compared to restorations. As the perspective was the patient-centered outcome, this probability increased to 50%. HVGIC sealants may be a cost-effective alternative to restorations for 30-50% of children depending if success or discomfort are used as expected effects. If uncertainties were ignored, sealing option might be erroneously forgone for the entire children population and its possible benefits ignored.

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### Clinical Criteria for the Assessment of Caries Adjacent to Posterior Restorations Impacts Treatment Decisions

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This cross-sectional study nested in a clinical trial aimed to evaluate the influence of two different visual criteria for the assessment of caries around restorations on the treatment decision for restored permanent teeth. The two visual criteria used for the evaluations of restored teeth were the FDI (World Dental Federation); and the CARS (Caries Associated with Restorations or Sealants – International Caries Classification and Management System) criteria. One trained and calibrated examiner assessed the restorations in adults (18 to 60 years old), which were randomized according to one of the criteria. After the diagnosis and establishment of the treatment decision according to the sorted criteria, the same restoration was examined with the other system for comparison. Spearman's correlation coefficients and 95% confidence intervals (95%CI) between the scores obtained with both criteria and respective treatment decisions were calculated. Poisson multilevel regression analyses were performed including exploratory variables related to patients (caries status, gender, age), restored tooth (tooth type, position in the mouth) and restorations (type of restorative material, number of restored surfaces); the outcome variables were decisions related to restoration replacement, any operative intervention and presence of secondary caries. A number of 717 restorations on posterior teeth from a total of 185 patients were included. The strongest correlation observed between the visual criteria was for secondary caries ( $Rho = 0.829$ ). CARS criteria indicated replacement for 16 restorations (2.2%), while FDI criteria indicated replacement for 83 restorations (11.6%). Association was found among the outcomes and the explanatory variables, where patients with higher DMF-T, higher caries activity and teeth with more than 3 restored surfaces had more indications for restorative interventions ( $p < 0.05$ ). Using FDI as a diagnostic method promoted an increase in the prevalence ratio of 5.2 for restoration's replacement, 4.2 for the indication of any treatment, and 2.7 for the detection of secondary caries ( $p < 0.001$ ). The patient risk factors, and the method of visual assessment used to evaluate the restored tooth influences the decision to replace or not the restoration.

### Evaluation of Brazilian Red Propolis Toothpaste against Salivary Lactobacillus spp.: A Randomized Clinical Trial

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The objective of this study was to evaluate the clinical efficacy of a toothpaste containing 1% Brazilian Red Propolis (BRP, INPI Patent Number BR1020170110974) against salivary Lactobacillus spp and plaque formation in orthodontic patients. This was a randomized and double-blind study. Fourty participants, caries-free, aged 12-18 years, from both genders were selected and randomized into two groups. The first group (G1) received BRP toothpaste (1450 ppm F) and the second group (G2) received fluoridated common toothpaste (1450 ppm F). The participants brushed their teeth for 2 min thrice a day for 4 weeks. Saliva was then collected at the baseline (D0) and 4 weeks after day 0 (D28) to verify the effectiveness of BRP toothpaste treatment in the reduction of Lactobacillus spp. The microbiological analysis was repeated twice, establishing dilutions of 1:10 mL and 1:100 mL on Rogosa agar medium. Lactobacillus spp. isolates were identified by its characteristic colony morphology and the values were expressed as log (CFU/ml). Data (mean  $\pm$  SD) on visible plaque index (VPI) was recorded on D0 and D28. Results: Comparisons between different times within the same dilution were carried out by repeated measures analysis of variance associated with Tukey's multiple comparisons test. Results: We found the VPI as follows: G1: (D0:  $38.10 \pm 17.95$ ; D28:  $20.60 \pm 16.44$ ;  $p < 0.0001$ ); G2: (D0:  $38.38 \pm 19.65$ ; D28:  $27.40 \pm 14.63$ ;  $p < 0.01$ ). The CFU found in G1 was: (D0:  $1.15 \pm 0.41$ ; D28:  $0.68 \pm 0.15$ ;  $p < 0.0001$ ) and G2: (D0:  $1.33 \pm 0.52$ ; D28:  $1.84 \pm 0.39$ ;  $p < 0.0001$ ). Conclusions: These data indicate that the BRP toothpaste showed antimicrobial activity against Lactobacillus spp and decrease VPI for up 4 weeks in orthodontic patients. Further studies to identify the anticaries effect of this BRP toothpaste are required to establish its use in caries prevention.

### Evaluation of Salivary pH and Plaque Index After Consumption of Mineral Water Following Meal Intake

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Dietary habits are acknowledged as a major cause of dental caries. Mineral water due to mineral ingredients affects re-mineralization process and caries development. This clinical study aimed at evaluating changes in dental plaque formation and salivary pH level after consumption of natural mineral water following meal intake. A sample of 51 participants in the age of 15 and 25 years were divided in two groups: experimental (36 participants) and control (15 participants). Experimental group consumed natural mineral water (Jamnica) as opposed to control group (tap water) immediately after meal intake. All participants followed the same dietary protocol (meal) and were instructed on oral hygiene. Informed consent was signed as required by the ethical board and prior to commencing the study.

pH value, salivation and dental plaque were evaluated at a single visit prior to and 30 min after meal intake and drink consumption. Each drink was swallowed following meal intake and 1 min gurgling. Salivary pH was determined by using standard CRT tests for caries risk assessment (Ivoclar / Vivadent / Lichtenstein). Salivation was defined by the quantity of stimulated saliva (ml) collected in a probe over 5 min. Dental plaque was estimated based on the API index (Approximal Plaque Index). As opposed to controls, the results in the experimental group after consuming natural mineral water showed a significant increase in the number of participants with higher pH values (61.1% before vs. 88.9% after;  $p = 0.036$ ) and a significant decrease of API index ( $\chi = 75.3$  before vs.  $\chi = 35.2$  after;  $p = 0.000$ ).

### Caries-Preventive Efficacy of Magnolia Bark Extract and Xylitol Chewing Gums in Adults: A 2-Year RCT

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The caries preventive effect of a sugar-free chewing-gum containing magnolia bark extracts and xylitol in a high-risk adult population was investigated (NCT02310308). Two-hundred seventy-one high-caries-risk subjects were assigned to three chewing-gum groups: polyols (Pols), xylitol (Xyls) and xylitol plus

magnolia (XylMags). ICDAS, gingival bleeding, salivary mutans streptococci (MS), and plaque pH were re-evaluated after 2 years from baseline in 64 Pols, 66 Xyls and 64 XylMag subjects. Net caries increment for initial, moderate, and severe lesions were evaluated using the nonparametric Mann–Whitney U test. After one-year of chewing-gum use (t2), caries increment for initial ( $0.09 \pm 0.15$  Pols,  $0.05 \pm 0.12$  Xyls and  $0.04 \pm 0.14$  XylMags) and severe ( $0.18 \pm 0.38$  Pols,  $0.11 \pm 0.12$  Xyls and  $0.09 \pm 0.44$  XylMags) lesions was significantly different among groups ( $p \leq 0.05$ ) and this difference increased one-year after the end of the chewing-gum use (t3) for initial ( $0.20 \pm 0.67$  Pols,  $0.14 \pm 0.37$  Xyls and  $0.10 \pm 0.34$  XylMags  $p \leq 0.01$ ) and severe lesions ( $0.44 \pm 0.73$  for Pols group,  $0.30 \pm 0.52$  Xyls and  $0.25 \pm 0.38$  XylMags  $p \leq 0.01$ ). MS concentration differed among groups at t2 ( $p \leq 0.05$ ) and t3 ( $p \leq 0.05$ ). Plaque-pH as mean Areas Under the Curves (AUC) were statistically different among groups (AUC5.7  $p \leq 0.05$  and AUC6.2  $p \leq 0.05$  at t2 and  $p \leq 0.05$  and 0.05 respectively at t3). XylMags subjects had a Reduction of Risk rate (RRR) of 39% respect to those treated with Pols with a Number Needed to Treat (NNT) of 32; Xyls subjects had an RRR of 23% respect to those treated with polyols with NNT of 55. Significantly lower gingival scores were observed in Xyl+Mag and Xyl groups ( $p \leq 0.01$ ). The present study provides robust but still non-conclusive evidence of the higher efficacy of a chewing-gum containing xylitol plus magnolia compared to xylitol alone in caries prevention.

### The Effect of Chlorhexidine Mouthwashes on Incidence of White Spots and Oral Hygiene in Orthodontic Patients

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The purpose of this study was to determine the effect of chlorhexidine mouthwashes 0.2% (CHX) on oral hygiene status and incidence of white spot lesions (WSLs) in adolescents wearing fixed orthodontic appliance. This clinical trial included 40 patients (20 females and 20 males), who were treated in the Orthodontic Clinic at the University Dental Clinic Center in Skopje. The parameters evaluated in this study were dental oral hygiene status (oral hygiene index-simplified OHI-S) and white spot lesion (WSLs-index). For all the patients, each index was scored at the beginning of orthodontic treatment, then 3 months, 6 months, and 9 months afterwards. Data were analyzed using Kruskal–Wallis and Mann–Whitney tests. The level of significance was set at 0.05. The results of Kruskal–Wallis test showed significant changes in indices after the 9-month period ( $p < 0.05$ ). After using CHX for 9 months, OHI-S decreases significantly ( $p < 0.05$ ). Based on the results of Mann–Whitney test, a significant decrease in OHI-S was observed when comparing CHX with control ( $p < 0.01$ ). CHX effectiveness in WSL reduction was also significant compared at the beginning

of orthodontic treatment ( $p < 0.001$ ). Adding chlorhexidine mouthwashes to daily oral hygiene program reduces bacterial plaque accumulation and improves OHI-index. Orthodontists are recommended to enhance their patients' oral hygiene by requiring the use of chlorhexidine mouthwashes in addition to daily brushing. The usage of CHX resulted with better oral hygiene status and reduced the incidence of white spot lesions.

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### Ozone Therapy For Early Childhood Caries (ECC) Treatment: A Prospective Study

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The primary aim was to investigate the effectiveness of ozone therapy in increasing the consistency of decayed dentin in ECC-affected teeth in uncooperative patients. Secondary aims were to evaluate any changes in salivary bacterial count, tooth hypersensitivity and patients' quality of life. Seventeen subjects (3-5 years of age) uncooperative for conventional restorative treatments were enrolled and treated with a 60 s ozone application/week for 4 weeks (OzoneDTA, Sweden&Martina). Patients were evaluated at baseline (T0), after 4 ozone applications (T1) and 1, 2 and 3 months after treatment (T2, T3, T4). At T0 and T1, dentin was classified according to the Affected Dentine Scale (ADS), salivary bacteria measured using the Saliva Check Mutans Test (GC), hypersensitivity registered with the Visual Analogue Scale (VAS) and patients' quality of life evaluated through the Early Childhood Oral Health Impact Scale (ECOHIS) questionnaire submitted to patients' parents. ADS and VAS were registered at T2, T3, T4. ADS and salivary tests results were analyzed using non-parametric tests. Statistically significant differences were detected for both ADS score and salivary bacteria count between T0 and T1 ( $p < 0.01$ ). ADS increased from  $3.23 \pm 0.34$  at T0 to  $1.82 \pm 0.63$  at T1. Bacteria count decreased from positive results in 82% cases at T0 to 9% at T1. At T1 tooth hypersensitivity was completely solved and did not reappear over time. A reduction of ECOHIS scores was recorded at T1 compared to T0. The ozone therapy was a valid aid for ECC treatment. By reducing salivary bacteria and improving dentin's consistency it may arrest caries progression, decrease hypersensitivity and improve patients' quality of life.

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### The Impact of Metabolic Control of Diabetes Type2 in the Saliva Characteristics and Caries Status

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The aim of this study was to investigate the association between factors such as metabolic control and duration of type 2 diabetes with quantitative characteristics of saliva and caries in adults. Twentythree adults with diagnosed type 2 diabetes (DM) and 18 healthy adults (control group) participated in the clinical trial. Patients with type 2 DM divided into two groups depending on their metabolic control (well- controlled-w.c: HbA1c <7%, poorly-controlled-p.c: HbA1c > 7%). The clinical parameters which were recorded and evaluated in each participant were: 1) number of natural teeth, 2) the clinical index of coronal caries ICDAS II – DMFT, 3) saliva pH 4) salivary flow and buffering capacity of saliva-based on GC Saliva Check Buffer. 5) check of the subjective feeling of xerostomia with a questionnaire completed by patients. DMFT was significantly higher in the p.c. and w.c group compared to the control group. Saliva flow rate was significantly lower ( $5,45 \pm 2,91$ ) in the w.c. and p.c ( $4,87 \pm 1,70$ ) compared to the control ( $6,5 \pm 2,44$ ). Significant differences were also recorded in the saliva pH and buffering capacity between the control and p.c. patients. The duration of the DM was significantly related to the pH, while the HbA1C was significantly related to the saliva flow rate and the buffering capacity. Xerostomia was significantly affected by the duration of the DM. Differences were found between the control and the p.c. groups in the saliva flow rate, the buffering capacity and the DMFT. The control group and the w.c. patients differed in the saliva flow rate, buffering capacity, pH and DMFT. Finally, W.c. and p.c. patients differed in the saliva flow rate and DMFT.

### Comparative Efficiency of Caries Infiltration and Amelogenin Peptide Application Methods for Incipient Caries Treatment

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Aim was to evaluate the comparative efficiency of incipient caries treatment using caries infiltration and amelogenin peptide application methods in children aged 10–16 years.

This prospective study was approved by the Volgograd Regional Research Ethic Committee and signed informed consents of adolescents and parents were obtained. Split-mouth design was used in 8 children aged 10–16 years in 110 permanent teeth with incipient caries lesions on vestibular surfaces (ICDAS-II, codes 1–2). Two methods of treatment were used: caries infiltration (Icon, DMG, Germany) – 52 teeth, CI group, and application of amelogenin peptide (InnoDentm Repair, BACHEM, Switzerland) – 58 teeth, AP group. The results were assessed with QLF-digital system (Qraycam, AIOBIO, Seoul) after 12 months. The mean value of fluorescence loss (FL) before and after the treatment,  $\Delta$ FL and standard error ( $M \pm m$ ) were calculated in each group, the differences were assessed by Student-test at  $p$ -value  $< 0.05$ .

Clinical success was found in all the cases in CI and AP groups. The mean FL values before the treatment were  $-9.079 \pm 0.523$  in CI group and  $-8.395 \pm 0.373$  in AP group ( $p > 0.05$ ), mean FL values after the treatment in 12 months were  $-6.127 \pm 0.591$  and  $-7.806 \pm 0.383$  respectively ( $p < 0.05$ ). Mean  $\Delta$ FL values were  $2.583 \pm 0.539$  in the CI group and  $0.587 \pm 0.218$  in the AP group, the demineralization decreased by 28.45% and 6.99% respectively ( $p < 0.01$ ). After 12 months, according to QLF, no fluorescence loss (FL = 0) was revealed in 8 teeth in the CI group and in 1 tooth in the AP group (15.38% and 1.72% respectively,  $p < 0.05$ ). Clinical efficiency of incipient caries treatment was revealed in all treated teeth after 12 months. However, according to QLF-assessment, demineralization value decreased significantly in caries infiltration group comparing with amelogenin peptide application group.

### Efficiency of Fluoride Varnish and Highly Fluoridated Toothpaste for White Spot Treatment Lesions in Children

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Aim was to compare the efficiency of fluoride varnish and highly fluoridated toothpaste for treatment of white spot lesions (WSLs). The study was conducted after the approval of the Regional Ethics Committee. The parents' informed consents were obtained. Thirty 10-16 year old children with WSLs on vestibular surfaces of 293 permanent teeth were randomized into two groups. In the first group (G1) 155 WSLs were treated with fluoride varnish (5% NaF, Duraphat, Colgate) every 3 months. In the second group (G2), 138 WSLs were treated with highly fluoridated toothpaste (2800 ppm F<sup>-</sup>, Duraphat, Colgate) which was used for tooth brushing twice a day during 1 month, 4 times per year. The laser fluorescence (LF) device (DIAGNOdent Pen, KaVo) was applied for WSLs evaluation before and after the treatment. The results of WSLs treatment were assessed after 12 months according to LF-score. LF-score mean-value and standard error ( $M \pm m$ ) were calculated. Student's t-test was used to determine differences between G1 and G2 at  $p$ -value  $< 0.05$ . At baseline, the mean LF-scores were similar in G1 and G2 ( $20.21 \pm 0.43$  and  $20.99 \pm 0.41$ ,  $p > 0.05$ ). After 12 months the mean LF-score decreased to  $18.47 \pm 0.39$  in G1 and to  $19.96 \pm 0.40$  in G2 ( $p < 0.01$ ). WSLs evaluation revealed that individual LF-scores decreased more frequently in G1 than in G2 (69.0% and 37.9%,  $p < 0.001$ ); LF-score increase was found less frequently in G1 than in G2 (23.9% and 55.6%,  $p < 0.001$ ). Clinical examination after 12 months did not show WSLs which had turned into cavities in both groups. Similar clinical outcomes of WSLs treatment were demonstrated after 12 months in both groups; according to LF-score assessment, more favorable results of WSLs treatment with fluoride varnish compared to highly fluoridated toothpaste were found.

## Dental Hard Tissues

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### Study Regarding the Efficiency of Some Nano-Hydroxyapatite Toothpastes On Dentine Tubules Closure and Dentine Remineralization

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The aims of the study were to evaluate the capacity of three commercial nano-hydroxyapatite toothpastes to close the dentine tubules and to remineralize the dentine. Forty dentine disks were obtained from sound human extracted teeth. In order to open the dentine tubules and to demineralize the dentine the disks were submersed in citric acid 40% for 30 s. The disks were equally and randomly included in four groups: three study groups where three nano-hydroxyapatite toothpastes (Karex, Dr Kurt Wolff GmbH & Co. KG, Bielefeld, Germany- group 1, Biorepair Plus Sensitive, Coswell SpA, Bologna, Italy- group 2 and Dr.Wolff's Biorepair, Dr. Kurt Wolff GmbH & Co KG, Bielefeld, Germany- group 3) were applied by brushing in a mechanical device simulating the movements during toothbrushing and a control group where there was no product application- group 4. The dentine samples were then evaluated by scanning electron microscopy at 2000x magnification to determine the dentine tubules closure according to a 5 grades scale. For each sample the mean score value resulted by nine evaluations. Energy dispersive X-ray spectroscopy (EDX) was used to determine the calcium and phosphorous ions concentration. The lowest mean score value was recorded in group 1 ( $2.20 \pm .632$ ), followed by group 2 ( $2.30 \pm 1.059$ ), and group 3 ( $3.00 \pm 1.155$ ). Statistically significant increase of calcium and phosphorous ions concentration was recorded in groups 1-3 when comparing to control and in groups 1 and 2 when comparing to group 3 (ANOVA and LSD post-hoc statistical tests). Karex and Biorepair Plus Sensitive toothpastes were more efficient in dentine remineralization when comparing to Dr.Wolff's Biorepair toothpaste, but there was no difference between the toothpastes in dentine tubules closure.

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### The Use of 3D Optical Coherence Tomograph on Artificial Root Caries Using Different Resin-Based Materials

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The aim of this laboratory-based study was to evaluate progression of artificial root caries using different resin materials. Forty-eight freshly extracted sound teeth were collected from Emergency Clinics. Each tooth was cleaned using prophylactic brush with non-fluoridated paste and embedded in acrylic resin leaving 4x4 mm square-window on the root surfaces. These root samples were immediately immersed in 60 ml of demineralisation solution (pH 4.8), and kept in a shaker incubator for five days (37°C). Subsequently, samples were rinsed in distilled water for 30 s and randomly allocated into three test and one control groups (n = 12). Group 1: Resin infiltration material (ICON, DMG, Germany), Group 2: Acetone-based universal adhesive agent (G-Premio Bond, GC, Japan), Group 3: Experimental flake glass with light-cured UDMA-TEGDMA resin (resin from Sigma Aldrich, UK), Group 4: Control group had no treatment. Each root was then placed in a sample holder and imaged using OCT imaging system, developed in-house specifically for dental research (QM, London). All resin-based materials were then applied according to manufacturer's instructions. Subsequently, second demineralisation was carried out. Surface changes with sub-surface lesion severity were estimated from OCT images by measuring the depth at which most of OCT imaging light is attenuated. Results showed that all groups exhibited significant caries progression after second demineralisation ( $p < 0.001$ ). The universal adhesive group demonstrated lowest  $\Delta CD$  [ $69.80 \mu m$ ] followed by glass flake+resin group [ $71.75 \mu m$ ] ( $p > 0.05$ ). Resin infiltration [ $100.48 \mu m$ ] and control groups [ $132.83 \mu m$ ] showed similar  $\Delta CD$ , whilst control group presented significantly higher  $\Delta CD$ , compared to universal adhesive agent and glass flake with resin groups ( $p < 0.05$ ). Universal adhesive and glass

flake materials have potential in protecting initial root carious lesions compared to resin infiltration and control groups.

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### The Correlation of Fluorescein Penetration with Artificial De- and Remineralized Caries Lesions in Bovine Enamel

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The aim of this study was to evaluate the dye-enhanced light-induced fluorescence (DELFT) method to detect fluorescein penetration in different types of artificial caries lesions in bovine enamel. On 126 flattened bovine enamel surfaces a 1×1 mm window was made with transparent nail varnish. The specimens were demineralized for 3 d (D3), 5 d (D5), and 10 d (D10) using a 2% carbopol demineralizing solution. Then half of the specimens from each group were remineralized for 10 d (RD3, RD5, and RD10, respectively) using a 2% sodium fluoride solution and kept in refrigerator under 100% humidity conditions for storage. The specimens were dried with compressed air for 15 s. Then the specimens were dyed for 10 s from the top surface with a wet cotton pellet containing 100 µM sodium fluorescein. A fluorescence image of the dyed specimens was captured with QLF-D (Inspektor Research Systems BV, The Netherlands). The green value intensity of the caries lesion with respect to sound enamel was measured in the fluorescence images using Image Pro (Media Cybernetics, USA). The G value was calculated as follows.  $G \text{ value} = \frac{\text{Green intensity}_{\text{caries lesion}}}{\text{Green intensity}_{\text{sound enamel}}}$ . G values (mean ± s.d.) were D3 (1.19 ± 0.10) and RD3 (0.86 ± 0.08), D5 (1.28 ± 0.10) and RD5 (0.86 ± 0.06), D10 (1.34 ± 0.17) and RD10 (0.82 ± 0.11). For all three groups G value was statistically significantly different between demineralized and remineralized caries lesions ( $P < 0.001$ ). It was found that the fluorescein penetration into demineralization lesions was significantly higher than in remineralized caries lesions.

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### Effect of Silver Ions and Fluoride Ions on Remineralisation of Hydroxyapatite Discs: An ISE Study

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Ag<sup>+</sup>, either on their own, or in combination with F<sup>-</sup> ions (SDF for example), are often incorporated in professional cariostatic products for early caries treatment, and as a prophylactic. However,

the use of Ag<sup>+</sup> for remineralisation is less well explored. We have previously shown that Ag<sup>+</sup> and F<sup>-</sup> ions in combination are effective inhibitors of demineralisation, using ion selective electrodes (ISEs) to measure Ca<sup>2+</sup> loss as a proxy for mineral loss. The objective of this study was to compare the remineralisation efficacy of previously demineralised hydroxyapatite discs, treated at similar concentrations, with Ag<sup>+</sup> ions, and Ag<sup>+</sup> in combination with F<sup>-</sup> ions, using ISEs for real-time measure of Ca<sup>2+</sup> uptake, as a proxy for remineralisation. Windowed (3x3mm) hydroxyapatite discs (Plasma Biotol, UK, 20% porosity) were immersed into acetic acid (pH = 4.0) at 37°C for 4 h. Treatments were either: 3.16 M AgNO<sub>3</sub>, Riva Star silver capsule (SDI, Australia), 3.16 M AgF, 3.16M KF, and deionised water (control), with each treatment applied by microbrush for 10 s. All discs were then immersed in remineralising solution (2 mM CaCl<sub>2</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 150 mM NaCl at 37°C) for a further 10 h. ISEs were used to measure the decrease in Ca<sup>2+</sup> concentration with time, as a proxy for remineralisation rates following each treatment. In descending sequence, the rate of Ca<sup>2+</sup> concentration uptake (remineralisation) was: 3.16 M AgF, 0.052±0.011 mM/h; Riva Star silver capsule 0.044±0.014; 3.16 M KF, 0.027±0.005 mM/h; 0.017±0.001 mmol/h; 3.16 M AgNO<sub>3</sub>, 0.005±0.004 mM/h; deionised water 0.007±0.003 mM/h. The simple combination of Ag<sup>+</sup> and F<sup>-</sup> ions (as AgF) was the most effective treatment for promoting remineralisation, suggesting synergistic remineralisation capability of Ag<sup>+</sup> and F<sup>-</sup> ions. Ag<sup>+</sup> ion treatment alone has little effect on remineralisation.

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### Radiopacities Assessment of Novel Radiopaque Infiltration Resins in Artificial Carious Lesions

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To assess the changes of radiopacities within artificial carious lesions after infiltration with novel radiopaque resins contrasting Bromine-Methacrylate (BrM). Six caries-free premolars were coated with nail varnish leaving a 2x2 mm<sup>2</sup> window on each tooth surface. The teeth were immersed in a demineralising solution (pH 4.5) for 72 hours. Each tooth was scanned using an X-ray Microtomography (XMT) scanner (MuCAT, QMUL). An infiltrant (20%UDMA, 80% TEGDMA), based on the ICON<sup>®</sup> formula was prepared as the control. Three radiopaque resins incorporating 30%, 50% and 70% BrM (2,3-dibromopropyl methacrylate, by weight) in the control infiltrant were prepared. For each tooth, the demineralised lesion was infiltrated by one of these 4 resins using the ICON<sup>®</sup> manufacturer instruction. The teeth were re-scanned. For each tooth, on 5 XMT slices (250 µm apart), 3 line-profiles were used to measure and compare the Linear Attenuation Coefficients (LACs) of the lesions before and after infiltrations. The mean lesion LAC was the mean LAC along the line and the bulk LAC was the sum of the LAC along the line within the lesion. The demineralised depth was the distance from the lesion surface to normal enamel. The mean demineralised lesion depth was 231±41.5 µm. After control resin infiltration, the mean lesion LAC for all the slices was decreased by 0.12±0.06 cm<sup>-1</sup> to 1.74 cm<sup>-1</sup> and mean bulk lesion was decreased by 1.58±0.76 cm<sup>-1</sup>. After 30%BrM,

50%BrM and 70%BrM infiltration, the mean lesion LAC was increased by  $0.97\pm 0.20$  cm<sup>-1</sup>,  $1.50\pm 0.33$  cm<sup>-1</sup>, and  $1.63\pm 0.38$  cm<sup>-1</sup> to have a mean LAC of 2.46, 2.97, and 3.00 cm<sup>-1</sup> respectively; and the bulk lesion LAC was increased by  $12.15\pm 2.85$  cm<sup>-1</sup>,  $16.44\pm 3.29$  cm<sup>-1</sup>, and  $20.75\pm 4.20$  cm<sup>-1</sup> respectively. The changes in LACs between pre- and post- infiltration were significant ( $p\leq 0.005$ ). In the post-infiltrated lesions with the control resin, there was a mean surface loss of  $50\pm 46.7$   $\mu\text{m}$ .

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### Effect of an Experimental Dentifrice with Hydroxyapatite Nanoparticles and High-Fluoride Concentration on Root Dentin Demineralization

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An in vitro study was carried out to evaluate the effect of experimental dentifrice with high fluoride concentration and nanohydroxyapatite (nano-HA) on root dentin demineralization. After formulation of dentifrices, 60 specimens of bovine root dentin with pre-determined initial hardness were randomly assigned to six groups using different treatments: (1) placebo dentifrice; (2) dentifrice with nano-HA and without F; (3) dentifrice with 1100  $\mu\text{g F/g}$ ; (4) dentifrice with 1100  $\mu\text{g F/g}$  + nano-HA; (5) dentifrice with 5000  $\mu\text{g F/g}$ ; and (6) dentifrice with 5000  $\mu\text{g F/g}$  + nano-HA. The pH cycling used consisted of 10 cycles. The blocks were kept for 6 h in demineralizing solution (1.4 mM Ca, 0.91 mM P, 0.06  $\mu\text{g F/mL}$ , 0.05 M acetate buffer, pH 5.0) and 18 h in remineralizing solution (1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05  $\mu\text{g F/mL}$ , 0.1 M Tris buffer, pH 7.0) each day. The treatments were performed twice a day and consisted in the immersion of the blocks in dentifrices slurries under agitation for 5 min. After that period, the longitudinal hardness was evaluated and the area of demineralization ( $\Delta S$ ) was calculated. The formulated dentifrices were evaluated regarding primary stability, cytotoxicity, and other parameters. Statistical analysis was performed using two-way ANOVA and Tukey test, with  $p$  set at 5%. The experimental dentifrices were stable and had no cytotoxicity. The values of  $\Delta S$  ( $\pm$ SD) of groups 1 to 6 were respectively: 2436.33 (396.82), 595.66 (168.73), 872.22 (202.23), 619.49 (158.13), 253.26 (74.45) and 123.09 (48.11). There was a statistically significant difference between the placebo and all other treatment groups ( $p<0.001$ ). The dentifrices containing 5000  $\mu\text{g F/g}$ , regardless of the presence of nano-HA, led to a smaller lesion area in relation to the other treatments ( $p<0.001$ ). Furthermore, there was a strong negative correlation ( $r = -0.85$ ,  $p<0.001$ ) between F concentration in dentifrices and lesion area, regardless of the presence of nano-HA, showing that increased F concentration leads to decreased lesion area. The findings of this study suggest that nano-HA has an effect on dentin demineralization, and dentifrice with 5000  $\mu\text{g F/g}$  + nano-HA promotes greater reduction of demineralization.

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### Hydroxyapatite vs Fluoride Gel for Remineralization of Caries Lesion: An In Vitro Comparison

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The present in vitro study compared the effectiveness of a hydroxyapatite (HAP)-based oral care gel and a commercially available fluoride-based oral care gel in remineralization of early caries lesions. Three tooth blocks were produced from each of 20 bovine teeth. Caries-like lesion was created on each block by 4 d demineralization in acidified gel. Subsequently, the blocks were randomly assigned to 3 remineralizing groups (20 blocks/group); (A) hydroxyapatite-based oral gel (Karex gelée, 15% HAP), (B) fluoride-based oral gel (Elmex gelée, 12,500 ppm F), and (C) artificial saliva (AS). Remineralization was conducted using pH cycling model for 28 d with storage in AS. The cyclic treatment regimen consisted of daily 2-h acid challenge in demineralization solution (pH 4.5), while the remineralization treatment consisted of daily 3 min HAP gel application (Group A), weekly 3 min fluoride gel application (Group B), or daily 3 min fresh artificial saliva treatment (Group C), respectively. Baseline and post-test mineral loss were quantified using transverse microradiography. Using one-sided t-tests ( $p<0.05$ ), pairwise comparison (baseline vs. post-test; 95% confidence interval (CI)) indicated significant ( $p<0.0001$ ) remineralization by HAP ( $1713\pm 130$ ; CI:1652–1774 vs  $1017\pm 153$ ; CI:945–1088) [mean diff (MD) = 697; CI:640–753], fluoride ( $1726\pm 105$ ; CI:1677–1775 vs  $1030\pm 232$ ; CI:921–1139) [MD = 696; CI:622–770] gels and AS ( $1759\pm 131$ ; CI:1697–1820 vs  $1652\pm 125$ ; CI:1593–1710) [MD = 106.8; CI:89.07–124.5]. When compared against each other using post-hoc multistep comparisons (Tukey), there was no statistically significant difference in remineralization between the two gels but both gel exhibited significantly ( $p<0.001$ ) higher mineral gain (HAP:39 $\pm$ 7%; fluoride:41 $\pm$ 11%) relative to AS (6 $\pm$ 2%). In conclusion, oral gel containing HAP was as effective in remineralizing early caries lesion as fluoride-based oral gel containing 12,500 ppm F. Thus this study confirmed that HAP oral gel can serve as an effective alternative to fluoride gel for caries management.

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### The Effect of Fluoridated Milk on Surface Loss of Enamel Under Erosive/Abrasive Conditions In Vitro

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Aim was to assess and compare the effect on surface loss in enamel of fluoridated milk 2.5 and 5.0ppm under erosive and erosive/abrasive conditions in vitro, using Surface Profilometry. A total of 90 bovine enamel slabs were subjected to a 28 d pH

cycling model with erosive, and erosive/abrasive challenges. They were randomly assigned to 6 treatment groups with fluoridated milk: Erosive challenge: 0 ppm F, 2.5 ppm F, 5.0 ppm F. Erosive/abrasive challenge: 0 ppm F, 2.5 ppm F, 5.0 ppm F. During pH-cycling the slabs were exposed to five times for 2 min periods of erosive challenge with 0.3%- 2.6 pH citric acid, twice for 5 min periods in one of the concentrations of fluoridated milk, followed by 10 min in a milk /saliva slurry twice daily. The abrasive challenge was provided by an automated tooth brushing machine (200 g load for 2 min/twice daily). Throughout the cycling period the slabs were stored in artificial saliva at 37°C. The slabs were analysed for surface loss at days 7, 14, 21 and 28 of the pH cycling period. After 28 days, data analysis was carried out using one way ANOVA with Bonferroni multiple comparisons to compare between groups 0, 2.5 and 5.0 ppm F milk. There was a statistical significant reduction in enamel surface loss both without brushing (Mean Diff  $\pm$  SE) (3.26 $\pm$ 0.5  $\mu$ m); (7.24 $\pm$ 0.5  $\mu$ m); (3.98 $\pm$ 0.5  $\mu$ m) (p<0.001) and with brushing (3.67 $\pm$ 0.9  $\mu$ m; 6.41 $\pm$ 0.9  $\mu$ m) (p<0.001), (2.73 $\pm$ 0.9  $\mu$ m) (p<0.01). In addition, using an independent t-test to compare non-brushed with brushed groups showed significantly more surface loss (-5.48 $\pm$ 0.8  $\mu$ m), (-5.07 $\pm$ 0.6  $\mu$ m), (-6.31 $\pm$ 0.7  $\mu$ m) (p<0.001) in the groups with brushing. Treatment with fluoridated milk resulted in a statistically significant reduction in enamel surface loss after 28 days when subjected to an erosive or an erosive/abrasive challenge.

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### Decision Making Flow-Chart in Root Caries Management

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Root caries, by definition, is the degeneration of tooth root tissues, due to an imbalance of the demin/remin process and to a collagen degradation. The aim of the study is to conduct a literature review to develop a flow-chart in order to facilitate practitioners in clinical decision making. Studies from 2013 to 2019 were included in the review. PICO and PRISMA methods were used in the formulation of the research question and selection of the articles. The final search string obtained is as follows: "primary prevention" AND "root caries", "secondary prevention" AND "root caries", "risk factor" AND "root caries". After processing the final search string and selecting the various filters, a final number of 24 articles out of 266 total references were selected. On the review's result, a decision making flow-chart, sorted by risk factors analysis was developed, highlighting the most evidence based preventive, non-operative and operative best practices to be performed, along with the best effective materials to be applied in root caries clinical management. Given the CAL loss related to the prolongation of life expectancy, an increase in root caries is likely. In this perspective, knowing how to manage the disease becomes of primary importance in order to guarantee the increase of untreated caries, pain, discomfort and tooth-loss, compromising the OHRQoL in adulthood and older age.

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### Management and Prevention of Age-Relevant Caries Risk

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Dental caries still represents one of the most common pathologies globally. Early diagnosis and timely and targeted intervention can avoid the unbalance of the stomatognathic system. The aim of the study was to conduct a literature review to identify the best performing procedures in intercepting the age-relevant caries risk factors. Studies of the last five years were included in the review, from 2013 to 2018, relating to professional treatments in primary and secondary caries prevention. PICO and PRISMA methods were used in the formulation of the research question and selection of the articles. The final search string obtained is as follows: "early enamel lesion" OR "caries non cavitated" AND "treatment" OR "minimal intervention" OR "remineralization". Seven hundred total articles were selected. Following the abstracts and full texts reading, a final number of 64 articles was reached. Type of patient included age ranges from childhood to over 70 years. To improve readability and usability of the review results, we developed a table sorted by age ranges, topography of carious lesion, risk factors, highlighting the most effective treatments to be performed. The final diagram categorizes the scientific papers according to the age range and type of preventive tool and material: 5 articles (8%) relate to remineralizing pastes; 29 articles (45%) to sealants; 20 articles (31%) to fluoride; 10 articles (16%) to possible associations and comparisons between abovementioned products. Minimally invasive treatment turns out to be the most effective tool capable of supporting the "teeth for life" motto, with selective interventions capable of reducing the clinical impact and economic burden of carious pathology.

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### Tooth Structure Loss Prevention with White-Opaque Flow Composite Depth Marking in Class II Cavities

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Composites are frequently used as filling materials to restore class II cavities. The removal of aesthetic filling materials during a revision results in loss of hard tooth structure, as the extension of the filling material cannot always be estimated due to a superior shade matching. This in vitro study investigated the influence of a white-opaque flow composite depth mark as an optical revision aid. That should contribute to tooth structure preservation and

shorten the excavation time. Cavities in 50 human caries-free extracted molars were prepared, filled and removed. The fillings weight and volume were measured in before and after the filling revision. The revision time was measured and the remaining filling remnants were optically evaluated. A flow composite matched with the tooth shade was used as the control group. The loss of hard tooth substance was significantly lower with previous underfilling with the opaque flow-baseliner. The weight and volume mean values were  $-0.037 \pm 0.021$  g and  $-0.016 \pm 0.013$  cm<sup>3</sup> ( $p < 0.001$ ), respectively. In the tooth-colored flow composite, the weight and volume mean values were  $-0.067 \pm 0.043$  g and  $-0.028 \pm 0.019$  cm<sup>3</sup> ( $p < 0.001$ ), respectively. The revision time measurements did not show any differences ( $p = 0.800$ ) between the investigated groups. The revision time was  $4.43 \pm 0.86$  min for the tooth-colored flow composite and  $4.47 \pm 0.89$  min for the opaque flow baseliner. The application of an opaque flow baseliner helps to preserve hard tooth substance during a filling revision. The filling material has no influence on the revision time. Cavity deep marking with an opaque flow-baseliner showed a significant tooth structure protection during a filling revision.

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### The Performance of Three Different Methods in Detecting Caries and Predict Severity around Composite Restorations

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This in vitro study aimed to compare the performance of three different methods in detecting caries and predict its severity around composite restorations in permanent extracted teeth. A total of 116 restored teeth was included, and selected sites were classified according to the FDI-criteria (FDI) and the CARS-system (CARS). In addition, gap sizes were visually assessed as 0 = no gap, 1 = gap ≤ 1mm and 2 = gap > 1mm and further by Trios 3D intraoral scanner, expressed in mm. Treatment decisions (no treatment, non-operative treatment, repair and replacement) were expressed with FDI and CARS. One examiner assessed the teeth with both methods twice, another used the 3D scanner and yet another did the histological examination (reference standard) on hemi-sections through the site using a stereomicroscope (x5). Intra-examiner agreements were assessed using Weighted Kappa statistics: FDI presence of caries = 0.92; [95%CI = 0.80–1.00]; FDI adaptation = 0.96; [95%CI = 0.92–1.00]; FDI staining = 0.86; [95%CI = 0.76–0.95]; CARS scores = 0.98; [95%CI = 0.97–1.00]; FDI/CARS treatment decisions, respectively = 0.79; [95%CI = 0.57–1.00] and = 0.89; [95%CI = 0.76–1.00]; Histological

scores = 0.94; [95%CI = 0.89–0.99]. Spearman's correlations between the following variables versus histology were: FDI presence of caries (Rho = 0.64,  $p < 0.001$ ); CARS scores (Rho = 0.64,  $p < 0.001$ ); Gaps for visual inspection (Rho = 0.48,  $p < 0.001$ ); Gaps measured by scanner (Rho = 0.37,  $p < 0.001$ ); FDI treatment decision (Rho = 0.46,  $p < 0.001$ ); and CARS treatment decision (Rho = 0.62,  $p < 0.001$ ). The correlation between Gap sizes assessed with the two methods was (Rho = 0.49,  $p < 0.001$ ). The reproducibility of the score systems used to assess caries around restorations in the present study was excellent. The FDI and CARS criteria were associated moderately to lesion depth and similar in performance. The 3D scanner did not add further information to gap size than the visual accessed gap size.

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### Antimicrobial Effect of Antimicrobial Photodynamic Therapy in Artificial Carious Lesions in Human Teeth In Vitro

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The aim of the present study was to evaluate the antimicrobial effect of antimicrobial photodynamic therapy in artificially created and infected cavities of human wisdom teeth. Defined cavities were prepared into the lateral surfaces of extracted human wisdom teeth using a round diamond bur. The teeth were incubated in a saliva solution, pooled from collected human saliva from four volunteers. The cavities were incubated in the saliva solution for 6 d and then sampled using a cut sterile paper point. The teeth were randomly distributed into three groups with 20 cavities in each group. In the first group, antimicrobial treatment was performed using antimicrobial photodynamic therapy (aPDT). The photosensitizer, a toloum chloride solution, was brought into the cavity and incubated for 60 s. The photosensitizer was activated using the light source at 635–645 nm for 60 s. In the second group, antimicrobial treatment was performed using 0.2% chlorhexidine (CHX) solution. In the third group, no antimicrobial treatment was performed. After the treatments, a final sample was taken using a cut sterile paper point to determine the remaining contamination of the cavities. The samples were collected in tryptic soy broth solution and cultured on Columbia blood agar. Survival fractions of the samples were calculated by culturing on blood agar and counting colony-forming units. The highest contamination was found in the untreated cavities. Statistical analysis showed a significant difference between the groups. Treatment using CHX killed 86 % of the bacteria, whereas in the aPDT group a reduction of bacterial contamination of only 74 % was observed. APDT significantly reduced bacterial contamination in artificial dental cavities. However, CHX was significantly more effective in reducing bacteria viability.

### Correlation Between QLF and ICDAS Modified Visual Criteria for the Detection and Assessment of Root Caries

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Aim was to describe the correlation between quantitative light-induced fluorescence (QLF) and the ICDAS visual criteria (modified) in the detection and assessment of root caries. After receiving IRB and obtaining consent, human teeth were collected from dental clinics and human teeth banks and stored in a 0.02% thymol solution diluted with phosphate-buffered saline at 4°C. A trained examiner classified the surfaces with the root-ICDAS modified caries visual criteria - as a clearly demarcated discoloured area and, based on loss of anatomical contour- into four categories: RS- Root sound; RI- Root initial lesion (<0.5 mm); RM- Root moderate lesion (>0.5 – ≤2 mm); RE- Root extensive lesion (>2 mm). Corresponding QLF images (v-3.0.0.35-Inspektor) were obtained (variables: A, ΔF, and ΔQ at threshold 5). The correlation between visual diagnosis (root-ICDAS modified) and QLF variables was evaluated with Kendall's tau with the range-relation scale interpretation. Out of 230 collected teeth, 130 were included. Corresponding QLF ΔF mean±SD (range) values according to root-ICDAS categories were: RS (n = 20): -6.5±3.05 (-19.2 to -5); RI (n = 50): -30.2±12.67 (-60 to -6.9); RM (n = 40): -36.4±12.03 (-58.6 to -10.2); RE (n = 20): -43.1±8.07 (-57.8 to -26.4). Statistically significant positive moderate Kendall τ correlations were observed between visual diagnosis and all three QLF variables (A: 0.59, ΔF: 0.51, and ΔQ: 0.58; p < 0.001). QLF showed a moderate correlation with modified root-ICDAS caries visual criteria. Histological validation of both methods will follow.

### OCT Intensity Profile Characterization of ICDAS Code 0-2 Stages

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Aim was to characterize and differentiate the backscattered depth-resolved intensity profile (A-scan) of ICDAS code 0-2 using Optical Coherence Tomography (OCT). Fifty-four extracted human premolars with sound or non-cavitated fissure caries, amounting to 107 investigation sites (ISs) were staged using ICDAS code 0-2. 3-dimensional scans of 3mm in x-y-z axis were

performed using a Swept-source OCT. The ISs were sectioned and imaged using Polarized Light Microscopy (PLM). Final categorization of ISs were done on PLM images using the Ekstrand (Ek) histology criteria with final sample size of Ek0 = 17, Ek1 = 30 and Ek2 = 60. The matched OCT B-scan was identified based on fissure anatomy and processed using bespoke software developed in MATLABM. 50 A-scans at each IS were collated to generate a mean A-scan and an overall mean A-scan for each EK code was computed. The A-scans were divided into two phases. Phase I involves the increase of intensity at the tooth-air interface until it reaches a peak (Imax) and its subsequent exponential attenuation followed by Phase II where attenuation assumes a more linear pattern. Imax was the highest in Ek0 while lowest in Ek1. Ek0 demonstrated steep increase in intensity due to an absence of backscattering and Phase II begins at about 30 μm subsurface. Compared to Ek0, Ek1 & Ek2 both exhibited slower increase in intensity, lower Imax and slower attenuation with Phase II starting at approximately 130 μm subsurface. Ek2 showed higher backscattering than Ek1 and attenuated faster. Kruskal Wallis analysis followed by post hoc test showed that attenuation rate of Ek0 in Phase I was significantly different from Ek1 and Ek2 (p < 0.001) respectively. OCT was able to characterize and differentiate ICDAS codes 0-2.

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### Correlation Between VistaCam, ICDAS-II and Cavity Extent After Lesion Excavation: An In Vivo Pilot Study

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Aim of this in vivo study was to correlate fluorescence based camera (FC) and visual inspection (ICDAS-II) to the extent of caries after excavation. The occlusal sites of 50 permanent first and second molars with ICDAS-II 3 and 4 score in 36 young adolescents (12±2 years) were examined at 1st Observation Unit of Department of Oral and Maxillofacial Sciences, "Sapienza" University of Rome. The assessment of ICDAS-II and FC was performed by a trained operator. Then a second blinded operator removed the carious lesion. To access the cavities, a #557 diamond bur in a high-speed handpiece (Star Dental, Lancaster, PA, USA; NSK Pana-Max2 M4, NSK Tokio, Japan) under water-cooling was used and caries was removed using stainless steel #2 and #4 round burs in a slow-speed handpiece (W&H WD-74 M, W&H Dentalwerk, Bürmoos, Austria). Then the extension (depth, width, length) was evaluated with a scaled probe (CP-15/CP-11.5B, Hu-Friedy Inc, Chicago, IL, USA) with 0,5 mm accuracy. VistaCam assessments ranged from 1.7 to 2 (deep enamel lesion with possible extent to dentine) with a mean of 1.7. ICDAS II detected score was

of 3 and 4. The extent of the cavities was determined by three consecutive measurements: depth, width and length, with a mean of 3.5±1.11, 2.8±1.15 and 3.2±1.78 mm respectively. This in vivo comparative diagnostic pilot study showed that ICDAS-II and VistaCam detected all the occlusal caries lesions. VistaCam mean measurement of 1.7 was correlated to a cavity with 3.5, 2.8 and 3.2 mm of depth, width and length. ICDAS-II and FC is proposed as caries detection method of choice on occlusal surfaces in young adolescent population which may help to avoid multiple diagnostic testing, overdiagnosis, overtreatment and exposure to x-rays.

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### **Novel Non-Destructive Analysis of Carious Lesions with CLSM and Image Processing Validated by Transverse Microradiography**

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The aim was to assess the ability of confocal laser scanning microscopy (CLSM), coupled with a novel image processing technique, to measure mineral loss as a function of depth ( $\Delta Z$ ) in artificial carious lesions. Ground/polished areas (4x5 mm) were created on bovine enamel samples (n = 14) using acid-resistant nail

varnish. Artificial carious lesions were created (8% methylcellulose gel, 0.1 M lactic acid, pH 4.5, 38°C, 10 d). Samples were removed, rinsed with deionised water, the varnish removed, and were submerged in 0.1 mM Rhodamine-B (24 h). CLSM captured fluorescent images (depth = 115  $\mu\text{m}$ ) using a 100x oil immersion lens. ImageJ was used to process the images and to provide fluorescent volume data as a function of depth (FV). Samples were sectioned for TMR encompassing the sound and carious enamel lesion to provide changes in  $\Delta Z$  and relating this to  $\Delta FV$ . CLSM showed the distinct zones of a carious lesion with a total depth of 82.83±12.08  $\mu\text{m}$ . Changes to pore morphology and space were observed between the characteristic zones of enamel carious lesions (mineralised, lesion body, dark and translucent zones). The mineralised surface zone was observed as a dark area beneath the surface 3.28±1.03  $\mu\text{m}$  thick. The lesion body below the mineralised zone was distinct with a bright fluorescent band originating from the prism cores and inter-prismatic space with a thickness of 50.05±9.05  $\mu\text{m}$ . A translucent and dark zone beneath the lesion body was 29.7±7.20  $\mu\text{m}$  thick with fluorescence originating from the inter-prismatic space only. The FV data mirrored that visualized by CLSM producing distinct fluorescent volume depth profiles of multiple carious lesions. In conclusion CLSM combined with a novel image processing method allowed non-destructive cross-sectional analysis of  $\Delta Z$  by fluorescence.

## Epidemiology

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### Acceptance and Feasibility of the Current Early Childhood Caries Control Strategies in Germany: Pilot Project

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Data from the last German National Oral Health Survey (2016/17) in children showed for 3-year-olds a substantial caries prevalence (14%) with low care index (26%). In Germany, recently implemented strategies for early childhood caries (ECC) control at the individual level include early referral from paediatricians to dentists, early oral examination (FU), training of parents in oral hygiene by their children (FUPr), and fluoride varnishes application (TF), which were implemented before in a study region (Pirmasens-Zweibrücken, Rhineland-Palatinate) as a pilot project. This questionnaire-based study assessed the acceptance and feasibility of current ECC control strategies by parents of children (6-33 months), dentists and paediatricians in the German region of Pirmasens-Zweibrücken. The study sample included 27 dentists (56% of all practices), 6 paediatricians, and 68 caregivers who agreed to participate. Nearly all dentists provided parents with counselling on oral hygiene (96.3%), nutrition (92.6%), caries etiology (81.5%) and fluoride use (88.9%). However, oral hygiene demonstration (63%) or training (40.7%) was performed less frequently. Fluoride varnish was applied for initial (77.8%) and cavitated carious lesions (66.7%) in the majority of dental practices. 54.4% of children had been referred to the dentist by the paediatrician. Paediatricians reported that they provide information on caries prevention (100%), nutrition (83.3%) and oral hygiene (66.7%). However, only one third (33.3%) recommended the use of fluoride tooth-paste from the first tooth onwards. All parents were satisfied that their child was referred to a dentist by the paediatrician and do not consider dental diseases in children to be normal. There was a

general respondent's acceptance on the implementation of early strategies for ECC control. The recent extension to the whole Germany within the health system framework appears to be accepted and feasible.

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### Dental Caries Prevalence in 3–6 Years Old Children

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The aim of this study was to investigate the prevalence of dental caries in 3-6 years old children. A retrospective analysis was performed, using medical cards of 300 children from 3 to 6-years-old. Children were divided into 4 groups depending on age of first visit: 3-year-olds (n = 50), 4-year-olds (n = 50), 5-year-olds (n = 100), 6-year-olds (n = 100). Registration of all dental caries lesions was performed in clinical conditions using the International Caries Detection and Assessment System (ICDAS) and DIAGNOdent Pen. In addition, the dmft/s index was calculated. The study was carried out in the period 2015-2017, with the permission of the University Scientific Research Committee. The results were obtained by processing the data with a STATISTICA Manual, Version 10.0, 2010.

Parallel to the increase of age, the number of lesions increased and the average value of carious lesions into the total group of participants from 3 to 6 years old was  $4.40 \pm 0.21$  (dmft) and  $6.35 \pm 0.65$  (dmfs). Patients in group 1 had a dmft of  $2.80 \pm 0.25$ , in group 2 of  $3.00 \pm 0.21$ , in group 3 of  $4.30 \pm 0.29$ , and in group 4 children had the highest caries prevalence ( $5.10 \pm 0.32$ ). There was a significant difference in the intensity of caries for children with different number of primary teeth in their dentition. There is a strong proportional relationship between the number of carious primary teeth and surfaces.

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### Oral Health Survey in Ladakh. Caries Prevalence and Related Factors in Children

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Ladakh is a region administered by India as a union territory and constituting a part of the larger region of Kashmir, which has been the subject of dispute between India, Pakistan, and China since 1947. This study presents the result on the Oral Health Survey conducted in Ladakh, reporting the severity and prevalence of caries in children (age range 3–16 years). Clinical examinations were carried from July 2019 to August 2019 recording the DMFT/dmft following the WHO criteria and the gingival bleeding score. Several background variables as gender, BMI, living with the family or at school/ monasteries, geographical area of living (rural or urban area), oral hygiene habits were considered. One thousand four hundred seventy-four children (41.18% males and 58.82% females) were investigated by ad hoc calibrated examiners. Caries free subjects ranged from 4.37% in children aged 7–10 years to 17.05% in adolescents aged more than 14 years; caries experience in primary dentition was  $6.58 \pm 4.35$  in children aged 3–6 years and  $5.44 \pm 3.66$  in children aged 7–10 years. DMFT index was  $1.11 \pm 1.35$  in children aged 7–10 years,  $2.27 \pm 2.35$  in children aged 11–14 years and  $3.31 \pm 2.67$  in adolescents. Gingival bleeding was observed in 33.92% of the sample. Mean BMI and dmft/DMFT were statistically significant different (One-Way-Anova  $p < 0.01$ ). Children living at school/ monasteries had statistically significant lower DMFT index (One-Way-Anova  $p < 0.01$ ) than children living in family settings. In conclusion, high caries levels were observed in all the age groups. There is an extreme need for oral health services switch from urban areas to remote and rural areas.

### Change in Knowledge of Parents of Children Involved in Punctually Applied Caries Preventive Program in Moscow

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Punctually applied caries preventive program to children aged 6–8 months, until their age was 3, was initiated in one Moscow district. Aim was to describe young children's parents' knowledge change during the 2½ years of the program. Methods: in 2015, 100

6–8-month-olds were involved in a program based on intensive parental education of their child's oral health, diet advice, toothbrush training with fluoride toothpaste related to dental status. Parents were interviewed about their child's oral health and feeding habits at baseline, after 1- and 2½ years. Chi Square-test was used to discover differences in answers. The number of parents brushing their child's teeth every day increased from 15% at baseline to 100% after 1- and 2½ years; 86% of parents brushed their child's teeth twice a day after 1 year and 91% after 2½ years ( $p < 0.02$ ). The number of parents choosing toothbrush and toothpaste according to dentist's advice increased from 72% to 89% ( $p < 0.005$ ), and from 57% to 89% ( $p < 0.0001$ ), after 1 year after 2½ years, respectively. Toothpaste use increased from 9% at baseline to 100% after 1- and 2½ years. At baseline 8% used non-fluoride toothpaste, after 1 year all parents used non-fluoride toothpaste, after 2½ years 73% used fluoride toothpaste ( $p < 0.0001$ ). The bottle feeding decreased from 67% at baseline to 14% after 1 year ( $p < 0.05$ ), none used bottle feeding after 2½ years; night feeding decreased from 88% to 69% after 1 year and to 6% after 2½ years ( $p < 0.0001$ ). The number of children fed more than 8 times per day decreased from 48% to 14% and 11% after 1- and 2½ years, respectively ( $p < 0.0001$ ). The use of pacifier decreased from 61% at baseline to 57% and 12% after 1- and 2½ years, respectively ( $p < 0.0001$ ). Conclusion: The study showed significant improvement in parents' oral health knowledge after 1 year of the program, remaining or even further improving after 2½ years of program.

### Punctually Applied Caries Prevention Strategy Among 6–8-Month-Olds in Moscow: 2 ½ -Year Follow-Up Results

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The aim of the study was to assess the effectiveness of the punctually applied caries prevention strategy in one Moscow district initiated among 6–8-month-olds until they become 3-year-olds. In 2015, 220 6–8-month-olds were enrolled and randomly allocated to a study group ( $n = 100$ ) and a control ( $n = 100$ ) group. Study group: at every appointment, parents received training in toothbrushing relating to the child's tooth status, intensive dental education and diet recommendations focusing on fewer intakes and reduced amount of sugar consumption. At first tooth eruption, 1000 ppm fluoridated toothpaste was recommended, followed with 1450 ppm fluoridated toothpaste from 29 months onwards. At the first appointment, the mothers' caries status was used to allocate the child into the "high-risk" or "low-risk" group. During follow-up appointments, visible plaque accumulation on incisors, signs of caries and breast feeding over 16 months were used as risk factors. The intervals between visits, from 4 (high-risk) to 8 months (low-risk), were identified. Fluoride varnish was applied to the "high-risk" children. Control group: the participants followed the

traditional dental care protocol. Plaque indexes, caries recorded by ICDAS, number of visits in the two groups were the outcome variables. At the end of the study plaque indexes were 0.15(0.36) and 0.64(0.75) in the study and control groups, respectively ( $p < 0.005$ ). The intra- and inter-examiner reliability of the ICDAS assessed by Kappa was 0.75 and 0.8, respectively. Combining D1-D2 scores, the means were 0.72(SD = 1.41) in the study and 1.41 (SD = 1.74) in the control group ( $p < 0.05$ ); D3-D4 were 0.19(SD = 0.49) and 1.42(SD = 1.62), respectively ( $p < 0.05$ ). The mean D5-D6 were 0.72(1.60) observed only in the control group. During the 2½ years of the program, 1030 visits were done in the study group and 350 in the control group. The punctually applied preventive program was effective in reducing plaque accumulations and preventing caries particularly to stages where operative intervention is necessary, but requires more short visits than in the control group.

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### Completion of the Inter-Institutional Agreed Caries-Care Oral Health Records within Colombian Practice

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Caries risk/lesions' assessment/management life-cycle oral health records (OHR), based on CariesCare International (CCI), were nationally agreed (2018). This study aimed at testing the OHR's completion within NHS/private/dental-school practice in terms of time and patients/dentists' satisfaction. Having secured IRB approval and signed informed consents, we invited dentists to conduct: 1. A COM-B behaviour-model caries-management questionnaire (Global-Collaboratory-for-Caries-Management); 2. The completion of life-cycle OHRs (Early-childhood-EC, Childhood-C, Adolescents-Adults-AA, Elderly-E); 3. Patient/parent and dentist-satisfaction related questionnaires. 1. Questionnaire answers: 77 dentists (66 females; 16 cities; 47 institutions); graduated: 1982-2019 (before 1991: 43%); practicing for 1-34 years (mean: 16.7±8.5); postgraduate studies: 58%; 31% teach; 97% know about ICDAS/ICCMS/CCI. Most considered a comprehensive caries risk/lesions diagnosis/management very important (99%); feeling very-moderately confident using it (94%); always-almost always conducting it (83%), having available needed resources (78%; with only 57% for risk assessment), and feeling motivated remuneration-wise (76%; 69% for risk). 2. In total 352 OHR (EC-50, C-35, AA-218, E-49) were conducted by 34 dentists (28 females; 6 cities; 22 institutions). Mean time spent: 41.7±15.9 minutes (EC-47.1±13.4, C-42.2±9.7, AA-40.1±17.1,

E-41.0±16.0;  $\chi^2$ ;  $p > 0.05$ ). 3. Most patients/parents were overall very satisfied (EC-72.7%, C-95.9%, AA-95.1%, E-91.1%;  $\chi^2$ ;  $p > 0.05$ ). Dentists felt very satisfied-satisfied with: caries-risk assessment (80%), caries-severity/activity assessment (96%), caries-risk/lesions synthesis (80%) and correspondent care plan (92%), while fewer (56%) with instrument's language and application related to its extension (34%), complexity (34%), design (9%), lack of training (6%), and inconsistency with NHS guidelines (16%). NHS-guideline inconsistency and/or training needs were confirmed by internal analysis regarding correspondence of risk classification (low-caries risk: EC-50%, C-47.1%, AA-55.3%, E-48.9%) with a non-indicated consequent fluoride-application decision (EC-60%, C-68.8%, AA-21.1%, E-47.8%). Modern-concept caries-care OHR completion showed high satisfaction among patients and dentists with opportunities for a friendlier/shorter/simpler version, dentists' training and NHS-guideline adjustment.

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### Oral Health Behaviors and Caries in Low-Income Children with Special Health Care Needs

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Our goal was to evaluate the associations between oral health behaviors and dental caries in low-income children with special health care needs (CSHCN). We ran the 3M Clinical Risk Grouping Software on a cohort of Medicaid enrollees in Washington State, U.S.A. to identify CSHCN and recruited 116 CSHCN ages 7-20 years for a single visit observational study. We administered a survey to caregivers and conducted a tooth-level dental screening on child participants. The main outcome was caries prevalence, defined as the total number of pre-cavitated, decayed, missing, and filled tooth surfaces. Predictor variables included toothbrushing, caregiver concerns about fluoride, having a personal dentist, receipt of topical fluoride, and diet. We ran univariate and multiple variable log-linear regression models and calculated prevalence rate ratios (PRR). The mean age of participants was 12.4±3.1 years, 41.4% were female, and 38.8% were white. About 69% had an episodic chronic condition, 23.3% a life-long chronic condition, 0.9% a catastrophic chronic condition, and 4.3% a malignancy. Only two variables were significantly associated with caries prevalence in the univariate models: caregiver concerns about fluoride and sugar-sweetened beverage intake. CSHCN of caregivers who were "not at all" or "not too concerned" about fluoride had significantly higher caries prevalence than CSHCN whose caregivers were "somewhat" or "very concerned" (PRR: 2.06; 95% confidence interval [CI]: 1.03, 4.09;  $P = 0.04$ ). CSHCN who consumed >4 sugar-sweetened beverages per week had significantly higher caries prevalence than CSHCN who consumed <4 per week (PRR: 2.40; 95% CI: 1.30, 4.44;  $p < 0.01$ ). Only sugar-sweetened beverage consumption was significant in the final model. Our findings suggest sugar-sweetened beverages are a key target of future behavioral interventions aimed at preventing dental caries in low-income CSHCN in Medicaid.

## Untreated Carious Lesions in Schoolchildren of Talca City, Chile

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The PUFA/pufa index relates to various pulp and associated surrounding tissue complications of untreated carious lesions. The objective was to determine the PUFA/pufa index in schoolchildren from Talca city and to associate the index values with socio-economic variables. The sample was derived from primary schools. Socio-economic information (i.e. sex, age, type of school, place of residency, type of health cover and reason for dental visit) was collected and a single calibrated examiner performed a full mouth clinical evaluation to participants. The PUFA/pufa experience was calculated in the same way as DMFT/dmft index. Code P/p: pulpal chamber visible or root fragments left. Code U/u: ulceration. Code F/f: fistula. Code A/a: abscess. The PUFA + pufa prevalence was calculated as the proportion of the population reporting one or more teeth scored with a PUFA/pufa greater than zero. The PUFA/pufa ratio is the proportion of cavitated carious lesions that have progressed to a pulpal complication, calculated as [(PUFA+pufa)/(D+d)] x 100. Analyses included descriptive statistics, non-parametric bivariate analyses and binary regression models. Results: 577 children were examined. The PUFA and pufa for the sample population were 0.06 (s.d. 0.30) and 0.26 (s.d. 0.76), respectively. PUFA/pufa, PUFA/pufa ratio and PUFA/pufa prevalence were statistically different according to socio-economic variables. Children from public schools (OR = 5.81, 95%CI 2.10-16.04, p<0.001) and those who visited the dentist only for emergencies (OR = 4.81, 95%CI 2.79-8.29, p<0.001) had increased likelihood for PUFA/pufa prevalence. Despite the high caries experience reported in this region, the sequelae of untreated carious lesions seem moderate. However, the PUFA/pufa expressed marked inequalities in this sample. This evidence should be considered by health policy makers when developing oral health care programs, aiming at the relief of pain and infection amongst children.

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## Pre-Schoolers' Oral Health Availing New Examinations in German Health System in a Specialized Dental Clinic.

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The caries prevalence in German 3-year-olds is quite high with 13.7% and a mean deft of 3.6 in the affected group. This led to the introduction of new free of charge preventive dental visits for 6-33 month old children in the German healthcare system in July 2019.

The aim of this study was to characterize the participants seeking those services in a specialized pediatric facility. The oral health data (age, deft, initial caries lesion) were collected from all pre-schoolers (age: 6-33 months) attending these new preventive services at the Department of Preventive & Pediatric Dentistry of the University of Greifswald, Germany from July to November 2019 and compared to national data. The mean deft of the participants (n = 84, mean age 22.1 ± 6.4 months) was 1.7 (± 3.3) and higher than in German 3-year-olds (0.5 deft). 71.4% of the children were caries-free on defect level leaving 18.6% with a mean deft of 5.96 ± 3.51. The d-component comprised 91.6% of the index (ft = 5.6%, mt = 2.8%, German 3-year-olds: dt = 73.9%, ft = 17.3%, mt = 8.7%, resp.). By including initial lesions the proportion of caries-free children was reduced to 63.1% (mean ideft 2.4 ± 4.2; German 3-year-olds: 81.3% caries-free, mean ideft 0.68, resp.). Compared to the representative data in German 3-year-olds, the study population presents higher caries values. Thus, at least in specialized practices small children with high caries experience are over-represented using the new services for controlling early childhood caries. Still, the majority of participating children attend caries-free at the defect level.

The study is funded by the regular budget at University Medicine/Greifswald and was approved by the Ethics committee of the University of Greifswald (Vote Nr. BB 028/16).

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## Caries Development in Germany: Children and Patients in Specialized Pediatric Practice

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Aim was to compare the caries levels in children treated in a specialized paedodontic services with representative national surveys in Germany. This analysis reviewed the caries experience levels of children recruited for three different clinical studies [a) Santamaria et al. 2017; b) Mourad 2019; c) Lauenstein-Krogbeumker 2016] performed in the Department of Preventive and Pediatric Dentistry at the University of Greifswald/Germany compared to the according age groups of representative epidemiological surveys in Germany [DAJ 2017]. In the first study (a) using a randomised clinical design, 169 children (mean age 5.56 years ± 1.5) received chairside dental treatment (mean 5.6 dmft), the second, retrospective study (b) on dental treatment under nitrous oxide sedation included 456 children (mean age 6 years ± 2.1) with a mean of 5.81 ± 0.23 dmft for preschoolers (3-6-year-olds; n = 277), 4.02 ± 1.81 dmft in schoolchildren (7-12-year-olds; n = 179) and 1.77 DMFT for 12-year-olds. The third study (c) on 139 patients (mean age 4.35 years ± 2.0) treated under general anesthesia presented with a mean dmft of 8.76 ± 4.27. All these values are 3-4 times higher than recent German epidemiological data [DAJ 2017] in 6-7-year-olds of 1.7 dmft and for 12-year-olds of 0.5 DMFT and match the national caries data from about 25 years ago. The results of this study clearly show that large groups of children treated in specialized pediatric practice analysis still present with caries levels

of more than two decades ago. Therefore, effective prevention strategies are required to reach this subgroup of children earlier in life.

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### Impact of Molar Incisor Hypomineralization on DMF-T Index in 8-Year-Old Children

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The aim of this cross-sectional study was to evaluate the impact of Molar Incisor Hypomineralization (MIH) in the occurrence of dental caries in permanent dentition in 8-year-old children. Participation was voluntary and the study was approved by the ethics committee. The sample consisted of 450 students enrolled in public schools in Petrópolis, Rio de Janeiro, Brazil. DMF-T and def-t indices were used to assess caries in permanent and primary dentition respectively. The criteria set by the European Academy of Paediatric Dentistry was used to diagnose MIH. To investigate socioeconomic factors, a questionnaire was answered by the mothers. SPSS software was used for descriptive and association analyses. Kruskal-Wallis, Mann-Whitney, chi-square tests were used and regression analysis was performed. MIH prevalence was 28.7%, def-t was 3.04 ( $\pm 2.90$ ) and DMF-T was 0,60 ( $\pm 1.18$ ). There was a significant correlation between the family income and caries in the deciduous teeth (OR = 1.86; CI = 1.12–3.10). Caries experience in deciduous teeth (def-t  $\geq 1$ ) and MIH were associated to higher chance of caries in permanent teeth (OR = 4.02; CI = 2.09–7.70 and OR = 1.77; CI = 1.02–3.07, respectively). DMF-T index was significantly higher in children who experienced caries in deciduous dentition and in those with MIH. Children who presented both conditions were even more susceptible to caries in permanent teeth.

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### Proportion and Manifestation of Molar Incisor Hypomineralization and Enamel Hypomineralization in Permanent Dentition

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The aim of this study was to assess the proportion and manifestation of molar incisor hypomineralization (MIH) and enamel hypomineralization in children. A cross sectional oral health survey was carried out. A total of 755 children (8–10 year olds) were selected from primary schools in Tra Vinh city, Viet Nam. Enamel hypomineralization was scored on all teeth in the permanent den-

tion. A surface and tooth related level were scored based on the criteria of the European Academy of Paediatric Dentistry (EAPD) by three calibrated dentists. Children were grouped according to their distribution pattern of enamel: children with hypomineralization on first permanent molar only (MH); with hypomineralization on permanent incisor only (IH); with hypomineralization on at least one first permanent molar and permanent incisor (MIH); with hypomineralization on the other permanent teeth (canine/premolar/second molar) and at least one first permanent molar (MH+); and with hypomineralization on the other permanent teeth only (H). The overall proportion of affected children was 16,2 percent. Among affected children, the proportion and mean number of teeth were: 18.9% and  $2.09 \pm 1.20$  (MH), 33.6% and  $1.39 \pm 0,59$  (IH), 20.5% and  $3.6 \pm 1.76$  (MIH), 12.3% and  $4.2 \pm 2.54$  (MH+), 14.8% and  $1.72 \pm 1.67$  (H). Out of affected teeth, 90.2% of permanent incisors and 45.5% of permanent molars was scored as demarcated opacities; 54.5% of molars was considered severe (PEB/ atypical caries/ atypical restoration/ missing). Enamel hypomineralization can manifest in any tooth in different phenotypes in permanent dentition with varying extent and severity.

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### Factors Related to Inequalities of Dental Caries Experience Divided by Significant Caries Index (SiC)

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This study aimed to determine the factors related to inequalities of dental caries experience in children divided by Significant Caries Index (SiC). The stratified cluster-sampled data from Korea National Children's Oral Health Survey in 2018 were analysed. The study included 22,371 children aged 12 years who underwent oral health examinations and answered questionnaires regarding demographic and socioeconomic status (SES) and oral health behaviours related to dental caries. Three calibrated dentists examined dental caries by the diagnostic criteria of WHO protocol and mean number of decayed, missing and filled teeth (DMFT) in permanent dentition was obtained. The SiC index group was assigned to one-third of population with the highest DMFT scores. The complex samples multivariable logistic regression analysis was employed to determine the factors related to inequalities of dental caries experience. The dependent variable was SiC index group or not. The independent variables were SES and oral health behaviours. DMFT of total population was 1.83; DMFT of SiC index group, 4.62. The SiC/DMFT ratio calculated as DMFT of SiC index group divided by DMFT of total population was 2.52. The study findings showed that factors related to SiC index group or not were sex, household income, pocket money and daily frequency of cariogenic snacks. Odds ratio (OR) belonging to SiC index group was higher in females [adjusted OR: 1.32, 95% CI(1.19–1.47)], in low household income [adjusted OR: 1.07, 95% CI(1.19–1.47)], in high pocket money [adjusted OR: 1.06, 95% CI(1.03–1.09)] and in high daily frequency of cariogenic snacks [adjusted OR: 1.04, 95%

CI(1.00–1.08)]. Interventions for caries prevention should be developed such as community oral health programmes using fissure sealant and fluoride, and oral health education considering children's SES and oral health behaviours.

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### The Impact of Pediatricians on Prevention and Early Diagnostic of Early Childhood Caries

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Aim was to investigate the caries-preventive services by pediatricians in the German national health care system. In this interview-based investigation, 677 of 5821 practitioner pediatricians in Germany were randomly chosen and called by phone. The structured interviews focused on demographics, knowledge and attitudes towards caries prevention with specific respect to ECC. The answers were collected in Google Forms then transferred to Excel for further analysis. Eighty pediatricians accepted to be interviewed and most of them (60.0%) routinely advise a dental visit for small children, 36.3% only with treatment needs. Almost all pediatricians (98.8%) instructed the parents in oral hygiene and most recommended toothbrushing twice a day (77.5%) with a fluoride containing age appropriate toothpaste (73.8%) with parents brushing their children's teeth (67.5%). Still, most pediatricians prescribe fluoride tablets, often in combination with vitamin D3 (41.3%), 22.5% refrain from tablets or give them needs-based (10%). Most of the pediatricians (68.8%) would be willing to stop fluoride tablets in favor of fluoride toothpaste. All the pediatricians stated to screen the oral cavity routinely, but most of the pediatricians consider brown spots as earliest sign of caries (70%). Only few recognize white spots as initial caries (28.7%) or comprehend initial caries as a reversible process (22%). In case of caries, almost all pediatricians recommend seeing a dentist (97.5%), but few refer specifically to pediatric dentists (6.3%). Many pediatricians see caries as a hereditary disease (71.3%), few believe that prolonged breastfeeding is a cause of early childhood caries (10%) and a minority thinks that they have enough knowledge on this topic (30%). Although pediatricians provide parents with caries-preventive services, there is a clear need of more interaction with the dental professionals.

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### Added Sugar Behind Chronic Oral Diseases: Higher Caries and Periodontal Diseases Rates

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The World Health Organization (WHO) recommends reducing the consumption of added sugar to less than 10% of total daily energy intake to prevent non-communicable diseases. This study

evaluated the association of high sugar consumption to caries and also to periodontal disease in adolescents (18-19 years old). The sample included data from the RPS Cohorts Consortium, São Luís, Brazil (n = 2,515). The exposure was high daily added sugar intake ( $\geq 10\%$  of total daily energy intake), obtained from food frequency questionnaire. The outcomes were dental caries (number of decayed teeth) and periodontal disease (number of teeth affected by bleeding on probing, periodontal probing depth  $\geq 4$  mm, and clinical attachment level  $\geq 4$  mm). The regression models were adjusted for household income, adolescent's educational level, sex, alcohol use and smoking, using multinomial logistic regression categorized into: 0 (<2 teeth affected), 1 (2 to 3 teeth affected), and 2 ( $\geq 4$  teeth affected). To test for consistency, a zero-inflated Poisson (ZIP) regression model was used to estimate the prevalence ratios. High sugar intake was associated with higher categories:  $\geq 4$  teeth decayed teeth (OR = 1.63 95%; confidence interval (CI) = 1.27–2.09;  $p < 0.001$ ) and also  $\geq 4$  teeth affected by periodontal disease (PR = 1.42; CI = 1.03–1.94;  $p = 0.030$ ). Consistency ZIP analysis confirmed that high sugar intake was associated to a higher number of teeth affected by caries (MR = 1.22; CI = 1.14–1.30;  $p < 0.001$ ) as also as periodontal disease in adolescents (MR = 1.15; 95% CI = 1.03–1.29;  $p = 0.011$ ). Preventive strategies focused on reducing sugar intake as recommended by WHO may have an effect not only on dental caries, but also on periodontal disease.

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### Caries Experience of 6-Years Old Children in Romania – The National Romanian Oral Health Survey

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There have been few comprehensive oral health surveys in Romania. Objectives: To evaluate caries experience of 6-years old children from rural and urban areas in Romania. Material and Methods Within the National Romanian Oral Health Survey for children, an estimation of the required sample size, assuming a sampling error of plus or minus 3% at 95% confidence level was conducted. The number of children enrolled, as a stratified, random sample, in the first grade, in each Romanian school were centralised. The stratification has been done on the administrative units and 45 schools distributed in rural and urban areas have been selected. A total of 718 children (mean age 6.48 years) were examined. Dental caries (ICDAS criteria), and fillings were recorded on surface basis of all present deciduous and permanent teeth, teeth missing due to caries have been recorded according to ICDAS

criteria. Examinations have been performed by 10 previously calibrated examiners. Results: A percentage of 18.3% (130 children) don't have any surfaces with dentinal caries (ICDAS codes 4–6). A total of 59.9% (427 children) have between 1 and 16 surfaces affected by carious lesions and 11.79% (84 children) have between 16–24 carious surfaces with a mean of 10.56 (95% CI 9.74–11.4). Out of the total sample size, 12% (86 children) have missing teeth due to caries. At least one ICDAS 97 code has been recorded in 7.8% (56 children) of children, and 1.8% (13 children) have more than 2 extracted teeth with a mean of 0.21, (95% CI 0.208–0.212). Conclusions: The level of caries experience and missing teeth is high in the selected sample for the specific age group.

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### **Pufa is Related to Higher Impact on Quality of Life Due to Caries in Children**

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The aim of this cross-sectional study was to evaluate the impact of caries prevalence and severity on the quality of life in preschool children. Participation was voluntary and the study was approved by the ethics committee. The sample comprised 277 5-year-old children. Def-t and pufa indices were used to assess caries and pulpal involvement, respectively. Impact on quality of life was assessed using the questionnaire used in the national caries prevalence survey conducted by the Health Ministry in Brazil. The questionnaire has 10 questions related to pain, discomfort when eating, brushing, sleeping, and other daily activities. Impact on quality of life could range from zero (no impact) to 10 (maximum negative impact). SPSS software was used for descriptive and association analyses. Kruskal-Wallis test and Spearman correlation were used. Caries prevalence was 59.6% and def-t was 2.94 ( $\pm 3.62$ ). Among children with caries, 21.2% had at least one tooth with pulpal involvement (pufa = 0.38;  $\pm 0.86$ ). The questionnaire score was: a) 0.79 ( $\pm 1.53$ ) for the whole sample; b) 0.36 ( $\pm 0.95$ ) when def-t = 0; c) 0.99 ( $\pm 1.73$ ) when def-t  $\geq 1$  and pufa = 0; and 1.46 when def-t  $\geq 1$  and pufa  $\geq 1$  ( $p = 0.000$ ). Correlation between the questionnaire score and def-t score ( $p = 0.000$ ) and pufa score ( $p = 0.001$ ) was significant. The main complaints were related to tooth pain and discomfort when eating, brushing, and sleeping. The presence of cavitated caries impacted the quality of life of the children negatively and the impact increased significantly when there was pulpal

involvement. PUFA index is a valuable tool to identify children whose quality of life is significantly more impacted due to caries.

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### **The Co-Occurrence of the Two Main Diseases: Dental Caries and Periodontitis**

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Having clear information on the epidemiological relationship between periodontitis and dental caries would result of uttermost importance in order to set individual and population-based prevention strategies, as well as to correctly plan comprehensive oral treatments. Therefore, this cross-sectional population-based study aimed to verify whether dental caries and periodontitis co-occur in a representative sample of the South Korean population. Experimental approach: A total of 23,405 subjects representative of 36.2 million adults were examined, following the WHO recommendations. Univariate and multivariate regression analyses using 5 different models were applied, controlling for age, gender, smoking status, frequency of toothbrushing, use of interproximal toothbrushes and flossing, educational level, income, gum diseases treatment and tooth filling in the previous year, BMI, Vitamin D serum levels, alcoholism, diabetes status, stress, and carbohydrates dietary intake. Information regarding missing teeth was intentionally excluded from the data analyses as edentulism could be there caused either by periodontitis or caries. Results: In the fully adjusted model, more untreated caries surfaces and teeth (mean 0.82 95%CI: 0.41–1.23 and 0.36 95%CI: 0.22–0.50, respectively) was observed in participants with periodontitis than participants without periodontitis, with an OR to have at least one untreated caries surface of 1.96 (95%CI: 1.66–2.32). However, cumulative caries experience (DF scores) and periodontitis were not associated. In this large nationally-representative population, periodontitis and untreated dental caries co-occur, independently from common risk factors. However, when considering cumulative caries experience (DF scores), the two diseases appear not to be independently related.

### Dental Caries Prevalence and Dental Health Behavior Among Pre-School and School-Children in Madagascar

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Madagascar is still considered one of the ten poorest countries in the world. This epidemiological investigation aimed to examine the prevalence of dental caries among children in Noyse Be Island, Madagascar. A calibrated examiner registered clinical condition using International Caries Detection Assessment System (ICDAS) scores on a sample of 568 children, 332 pre-school (58.45%) and 236 school-children (41.55%). Children were previously directly interviewed about dietary and hygienic habits. Hands hygiene was also evaluated and used as a proxy for personal hygienic standards. ICDAS scores were merged as follows: 1/2 enamel lesions, 3/4 pre-cavitated lesions and 5/6 cavitated lesions. Difference among ICDAS scores and background variables were analyzed using One-way Anova. All the enrolled subjects (100%) reported to consume sweets both at school and at home; moreover 74% of the sample has never brushed their teeth. Only 94 children (21.08% pre-school and 10.17% school-children) were caries-free. In pre-school children mean number and standard deviation of ICDAS scores were  $1.10 \pm 1.32$  for enamel lesions,  $1.53 \pm 1.62$  for pre-cavitated lesions and  $1.88 \pm 2.66$  for cavitated lesions (one-way ANOVA  $p < 0.01$ ). In school-children, ICDAS values were  $1.68 \pm 1.92$ ,  $1.52 \pm 1.50$  and  $2.22 \pm 2.56$ , respectively ( $p < 0.01$ ). When sorting the sample by gender only cavitated lesions were statistically significant higher in male ( $2.16 \pm 2.85$  vs  $1.55 \pm 1.63$  in female  $p = 0.01$ ). Cavitated lesions were statistically significant lower in subjects with clean hands ( $2.02 \pm 2.74$  vs  $2.43 \pm 2.95$   $p = 0.03$ ). This preliminary study should be considered a first step to design larger-scale population-based investigations aiming to raise awareness of this community issue and to empower initial preventive strategies as sugar intake reduction and standardized tooth brushing time at school. Health promotion strategies are extremely needed in this population.

### Epigenetic Differences in Dental Caries: A Study of Twin Children

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Aim was to assess associations between DNA methylation in cord blood leucocytes at birth and caries experience at six years-of-age. This study was nested within a birth cohort study of 500 twin children. Genome-wide analysis of DNA methylation was performed on a subset of 27 twin pairs (14 dizygotic and 13 monozygotic) using the Illumina Infinium MethylationEPIC BeadChip array with DNA from cord blood. The presence/absence of (i) 'any' caries, (ICDAS codes 2 to 6 and/or past treatment) and (ii) 'advanced' caries' (ICDAS codes 4 to 6 and/or past treatment) were determined from dental examinations conducted at six years-of-age. Using linear regression, methylation values were adjusted for birth-weight, sex, maternal smoking, cell counts and batch effects. Residuals from this model were then inverse-normalized and used as the dependent variable in linear regression models in which each dental outcome was the independent variable and which accounted for twin correlation. 'Any' caries and 'advanced' caries affected 19 and 15 individuals respectively. For 'any' caries, there were 15 pairs with neither twin affected, seven concordant pairs with both twins affected and five discordant pairs with only one affected twin. For 'advanced' caries, there were 16 pairs with neither twin affected, four concordant pairs and seven discordant pairs. Applying a false detection rate (FDR) of 0.05, 'any' and 'advanced' dental caries were associated with 39 and five differentially methylated probes (DMPs) respectively. Genes implicated by these probes included BCL2A1 and SHANK2 for any caries and MMP28 and PRDM16 for advanced caries. This exploratory study has identified several differentially methylated genes at birth that are associated with dental caries in childhood, and which warrant further investigation in larger studies.

### Dental Health in an Early Modern Swedish Population

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Examination of ancient skull materials can give important information about our ancestors. The prevalence and distribution of dental caries and tooth wear was determined in complete and partial human dentitions from an early modern time city

graveyard (1500–1620) excavated in Gamlestaden, Gothenburg, Sweden. In addition, other clinically detectable dental pathology was noted. The study population consisted of 308 individuals; 205 adults and 103 children. Altogether 4951 teeth in adults and 1660 teeth in children were examined through visual inspection and use of a dental probe under strong light source. Age and gender of the individuals had previously been assessed. Caries prevalence in the total studied population was 55%. The highest caries prevalence was found for the adults, where 68% of the individuals showed at least one carious lesion. The corresponding number among the children was 28%. Caries experience (DMT>0) in the entire population was 60%. In the adult group caries experience was 76%. Women had significantly higher caries experience compared to men ( $p < 0.05$ ). Caries was most prevalent in the molars and least prevalent in the incisors and canines. Tooth wear increased significantly with age ( $p < 0.0001$ ). In the adult group, molars were most affected by tooth wear, while incisors were mostly affected in the child group. Other findings were clinical signs of apical lesions, crowding of teeth, aplasia, non-erupted canines and calculus. These findings show that both caries, tooth wear and dental infections affected a high number of the individuals in this early modern population and indicates that women were more predisposed to dental disease than their male counterparts.

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### Added Sugars Intake and Obesity in Mother-Child Dyads with Oral Disease Burden in Early Childhood

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The association between of added sugars intake and obesity in mother-child dyads with burden of chronic oral disease (BCOD) in early childhood was investigated. Population-based study with a representative sample of preschoolers (3-6 years) from public day care centers, São Luis, Maranhão, Brazil ( $n = 679$ ) and their mothers. In the theoretical model were analyzed socioeconomic factors, obesity and sugar consumption in the dyads, associated with the outcome burden of chronic oral disease, using structural equation modeling. The BCOD in children was a latent variable deduced from the shared variance between visible plaque index, gingivitis and number of teeth with active caries lesions in children. The BCOD was a good latent variable because all indicators had factorial load above than 0.4 ( $p < 0.001$ ). The higher intake of added sugar by the child (standardized coefficient [SC = 0.208];  $p = 0.021$ ) explained the burden of chronic oral disease. Higher sugar consumption by the mother had an indirect effect increasing the burden of chronic oral disease via higher sugar consumption by the child (SC = 0.047;  $p = 0.031$ ). Maternal obesity was associated with the highest z-score of the child's BMI (SC = 0.136;  $p = 0.048$ ). A transgenerational effect of obesity and higher sugar intake in mother-child dyads was identified. In addition, indicators of caries and the onset of periodontal disease (gingivitis) were correlated with each other in early childhood, explained by higher sugar intake. Together, our data support the World Health Organization's

position that the approach to prevention of oral noncommunicable chronic diseases should be directed to common risk factors.

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### Access to Professional Dental Care for Young Children in Low SES Municipality Amsterdam New-West

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Aim was to investigate the provided care to young children in dental practices of Amsterdam New-West, a low SES municipality. Practices in Amsterdam new-West were approached with a brief questionnaire regarding the intake of children as patient and a request to interview the dentist. Interview questions related to age of children at first intake and oral exam; age and/or reasons for first radiographs; description of a routine exam or treatment. There are 41 dental practices in Amsterdam New-West, of which 36 practices participated in the questionnaire and 23 also had one dentist interviewed. All practices accept children, 4 practices only accept children if their parents are enrolled patients. Ages at which children can be enrolled varies from up to age 2 (12); from age 2-3 (12); from age 3 (8) to from age 4 (3). The first intraoral exam is reported to take place from the first tooth (4), from age 2 (6), age 3 (12) or from age 4 and up (9). Only 10 practices stimulate pregnant women to bring their child along from the eruption of the first tooth. Nineteen practices inform parents that dental care for children is free of charge. Standard control intervals are 6 months and a shortened interval in case of high caries risk. Radiographs are taken according to the ALARA principle in 6 practices, while 10 practices start with x-rays once the first permanent molars are erupted. One practice makes x-rays from age 9, three from age 12 and one from age 15.

While most practices are open to the treatment of young children, only 12 out of 36 participating practices were considered child-friendly.

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### Geriatric Oral Health Related Quality of life in North Macedonia Associated with Socio-Demographic Factors and Dental Status

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Aging of population in North Macedonia increase interests in health-related quality of life. The aim of the study was to evaluate oral health-related quality of life OHRQoL associated with socio-demographic factors and dental status. A cross-sectional study was conducted in 2 geriatric institutions and data from 160 elders, aged

65 to 84 years (mean age 78, 8, 51.3% female) were evaluated after clinical examination and questionnaires for socio-demographic characteristics, health-related behaviors, dental status and General Oral Health Assessment Index (GOHAI) score. Results showed average number of remaining teeth  $5,81 \pm 7,34$ , average number of remaining roots with caries  $1,37 \pm 1,66$ , only 4, 11% had minimum of 20 remaining teeth while total anodontia was found in 47, 95%. Men had significantly higher number of remaining teeth than women  $9,07 \pm 7,34$ . Over half of participants 53, 43% obtained primary school education, 38, 56% finished high school. DMFT index (decayed, missed, filled tooth) was very high with average value  $28,84 \pm 4,71$  with highest value of extracted teeth  $25,41 \pm 8,03$ . The mean GOHAI score of the participants was  $48.23$  (SD 7.62), range 19 to 60, meaning moderate self-perception about their oral health. Higher values of DMFT score, number of natural teeth, teeth with root caries and functional occluding pairs are significantly associated with the GOHAI score ( $p < 0.001$ ). For elders living in North Macedonia, variables for OHRQoL were gender, DMFT score, self-rated general and dental health, number of natural teeth and roots. Educational level, smoking, drinking, brushing habits, number of functional occluding pairs were not the explanatory variables. Poor oral health is very important public health issue and high prevalence rates of caries and oral diseases must be reduced.

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### Dental Caries in 12-Year Old Children Residing in Four Administrative Regions of Republic of Serbia

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The aim of the study was to examine dental caries profile of 12-year old children in Serbia, and to assess differences between the four administrative regions of Serbia. The survey was undertaken between March and July 2019. Basic Methods for Oral Health Surveys of the WHO were implemented. Stratified cluster sampling was used in order to obtain a nationally representative sample. Republic of Serbia is divided into 4 administrative territorial units: 1) Central and Western Serbia, 2) Southern and Eastern Serbia, 3) Vojvodina (northern region), and 4) the City of Belgrade (the capital). At least 50 12-year olds were included at each site: 4 sites in capital (2 urban, 2 periurban), 2 sites in 3 large towns (1 urban, 1 periurban in each town), and 1 site in 4 rural areas, aiming to have a minimum of 700 participants. A total of 1300 children were included. The mean DMFT for 12-year olds in Serbia was  $2.40 \pm 2.76$ . Thirty-six percent was caries-free. Significantly worse caries-status was recorded in Southern and Eastern Serbia (mean DMFT =  $3.10 \pm 2.6$ ) compared to the capital city of Belgrade

(mean DMFT =  $2.02 \pm 2.72$ ), Central and Western Serbia (mean DMFT =  $2.23 \pm 2.52$ ), and Vojvodina (mean DMFT =  $2.29 \pm 2.72$ ) ( $p < 0.05$ , Kruskal-Wallis test). In addition, a tendency to have more decayed teeth has been observed in children residing in periurban and rural areas. The level of caries experience in 12-year old children in Serbia may be considered moderate. Additional strategies must be implemented to further improve oral health in Serbian children, and to reduce territorial discrepancies.

Funding: the data were collected within the project "Improvement of oral health of children and youth in Republic of Serbia" funded by the Ministry of health, Government of Serbia.

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### Caries Incidence and Oral Hygiene Status in Autistic Spectrum Disorder Children in Central Italy

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Autism spectrum disorder (ASD) is a pathology with a strong social impact and important public health implications. In this respect, oral health problems represent a key component. These children have significantly more untreated caries and a higher prevalence of periodontitis and gingivitis compared to the average population. No study of this type is available for Italy, this study is aimed to create an epidemiological oral care profile for children with ASD, by investigating their oral health status. The study was conducted using a cross-sectional design. Children with ASD attending the Special Needs Paediatric Dentistry Unit, Policlinic Umberto I in Rome (Italy) were included in the study. Each patient received a complete intra-oral examination to investigate Plaque Index ((LOE-SILNESS, 1963), decayed, missing and filled primary and permanent teeth (dmft/DMFT), for each patient D3 threshold was used. Overall caries experience was calculated by dmft/DMFT. Clinical examinations were performed between June 2013 and November 2018. A total of 285 children, aged between 3 to 18 years, were included in this study. The overall average caries experience (dmft/DMFT) was  $2.87$  (SD 3.08). One third (33.68%) of the sample had no obvious decay experience, and approximately 25% has a high level of caries experience scoring dmft/DMFT > 5. For the PI, only 38% has an acceptable level of oral hygiene, while in 62% the level is poor. Our study, in line with others, shows a worsening of caries experience associated with ASD. Promoting a personalized path for the treatment of these patients, based on the interception and prevention of oral pathologies, can limit the most invasive interventions and the use of general anesthesia.

### Validity of CARIOGRAM® for Early Childhood Caries Prediction. A One-Year Follow-Up Study

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Objective was to evaluate the validity of Cariogram® in relation to the early childhood caries increment (natural development/progression of caries lesions) over a 12-month follow-up period. In 2018, a cohort of 409 Mexican preschool children (3-5 years old) received an oral examination to determine caries prevalence at different thresholds and associated factors. Additionally, the caries risk profile was also assessed using the Cariogram® software (excluding *S. mutans* count, buffer capacity and salivary flow items). Caregivers were informed about children's oral status and encouraged to seek dental care. One year later, re-examination using the same criteria was performed on 381 subjects from the original sample (dropout 7.09%). Possible correlated variables were analyzed using the principal component analysis (PCA). The performance of Cariogram® in predicting caries incidence/increment was evaluated by the Receiver Operating Characteristic (ROC) analysis. After one year, 36 (9.47%) subjects had developed new ICDAS 3-6 caries lesions. The first two eigenvalues of PCA analysis account for 71.68% of the total variance at baseline and 69.56%, at follow-up. In both D-plots of the first two principal coordinates, gingival status, diet content, and clinical judgment tend to form a separate cluster. Goodness of fit was high for these variables. Sensitivity measured by ROC analysis were 0.77 at baseline and 0.83 at follow-up, while specificity was 0.76 and 0.81, respectively, and the area under the ROC curve was 0.50 and 0.59, respectively. A strong association between caries risk profiles at baseline and dentinal caries incidence after one year was found. Conclusions: This study's results confirm the Cariogram® predictive validity for early childhood caries being a suitable, precise, accurate and easy-to-use model for caries risk assessment in young children.

### It's Not Just Brushing – Social and Economic Performance Influences Children Oral Health in Serbia

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Having in mind how much social determinants affect general health, the influence of macro-level or country level factors on children oral health is underinvestigated area. This epidemiological study was a cross-sectional epidemiological survey including a representative sample of children aged 36 to 71 months of age attending public kindergartens in Serbia. The final study sample was selected according to WHO's Basic Methods for Oral Health Surveys using stratified cluster sampling. This subdivision of the country was made in accordance with European Union method – NUTS level 3 (districts of the country). Country-level variables that were analyzed involved: social-economic performance of districts – participation of district in country gross value added (GVA), unemployment rate, and type of employment. A probability value of <0.05 was considered significant. The final sample involved 864 preschool children (51% males; 49% females). The results showed that 41.1% of preschoolers had early childhood caries (ECC) experience. The majority of ECC was untreated (91.8%). Occurrence of ECC ranged from 18.6% to 54.8% in districts ( $p < 0.01$ ). Children with ECC were living in districts with 1% lower participation in country GVA ( $p < 0.05$ ), 0.4% higher unemployment rate, and 1% higher employment in private sector ( $p < 0.05$ ). The results from the present survey suggested strong influence of social-economic macro-level factors on children oral health.

The data were collected within Program for Oral Health Improvement in Children and Youth in Serbia, approved by the Government of Serbia, Ministry of Health, Program number 1802, Project activity number 4015.

### Prevalence of Molar-Incisor Hypomineralisation Among Schoolchildren in Tepatitlan de Morelos, Mexico

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The aim of this cross-sectional study was to evaluate the prevalence and severity of Molar-Incisor Hypomineralisation (MIH) in Mexican schoolchildren and evaluate the association with socio-economic factors. After local ethical approval (CUA/CEI/110/2018), a total of 441 6–13 year-old children (8.85±1.70) were examined in three different public schools in Tepatitlan de Morelos, Mexico. MIH was evaluated using the EAPD diagnostic criteria and dental caries according to the WHO criteria (DMFT/dmft) by trained and calibrated examiners (Kappa values = 0.82–0.94). A questionnaire containing questions of socio-economic factors and household situation was given to the parents. The association between MIH, dental caries and gender was tested using chi-square test. Adjusted logistic regression was performed to evaluate the association between the prevalence of MIH and socio-economic factors ( $\alpha = 5\%$ ). The overall prevalence of MIH was 39.4%, with a total of 483 teeth affected. Regarding the severity of the lesions, 81.3% of the teeth ( $n = 393$ ) showed demarcated opacities, 9.9% ( $n = 48$ ) showed post-eruptive breakdown, while 8.6% ( $n = 42$ ) showed atypical restorations. No differences between female and male patients were found ( $p = 0.10$ ). The prevalence of caries was 51.92% ( $n = 229$  DMFT/dmft > 0) with no association with the prevalence of MIH ( $p = 0.478$ ). No association was found between MIH and socio-economic variables such as marital status and education level of the parents, family income, household crowding and number of siblings ( $p > 0.05$ ). Although the prevalence of MIH and dental caries in this study population was high, no association was found between the variables. Socio-economic factors were not associated with MIH.

### National Pathfinder Among Children's Oral Health in Italy. Survey on the 12-Year Old Children

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In Italy, nationally representative caries data are rare, as oral health is private based. In 2016, the second National Survey conducted in Italy on children's oral health was promoted. This study presents the result of that National Survey on children's oral health, reporting the prevalence and severity of caries in 12-year old children. Clinical examinations were carried out in school settings from October 2016 to April 2017, according to ICDAS (International Caries Detection and Assessment System) criteria and gingival bleeding score. To facilitate the reporting of the data, ICADS codes 1 and 2 and codes 5 and 6 were merged, and each subject was coded at the maximum ICDAS score recorded. Seven thousand sixty-four children (51.03% females and 48.97% males) were examined by ad hoc calibrated examiners. No clinical sign of caries (ICDAS 0) was observed in 30.45% (95%CI: 27.41%–33.27%) of the study population, enamel lesions (ICDAS 1/2) was recorded in 20.37% (95%CI: 17.84%–25.02%) of the sample, localized enamel breakdown without clinical visual signs of dentinal involvement (ICDAS 3) in 3.08% (95%CI: 1.16%–5.31%), underlying dark shadow from dentine (ICDAS 4) in 6.16% (95%CI: 5.04%–7.26%) and frank cavitation in dentine (ICDAS 5/6) was found in 28.73% (95%CI: 26.14%–30.44%) of the population. Gingival bleeding was observed in 24.41% of the sample. An inverse linearly proportional regression was found between ICDAS scores, caries recorded at ICDAS levels 5/6 and more than 2 lesions and Gross National Product per capita ( $p < 0.001$ ). Despite the overall caries decline in Italy, 12-year-old children still present high caries levels with statistically significant differences in caries values among children from different socio-economic backgrounds, which calls for more effective preventive and, possibly, restorative efforts.

## Dental Erosion and Health Issues

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### Effect of Different Citric Candies on the Development of Enamel Erosion In Vitro

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This in vitro study evaluated the erosive potential of citric candies on bovine enamel samples with respect to the quantification of loss. Ninety bovine crowns were prepared and randomly distributed in 6 groups (n = 15): Group 1 – 0.1% citric acid solution (standard control, pH 2.5); Group 2 – Coca-Cola<sup>®</sup> Soft Drink (pH 2.6); Group 3 – Fini<sup>®</sup> Diet (Lactic acid and citric acid, pH 3.3); Group 4 – Fini<sup>®</sup> Gelatinas Kisses (citric acid and lactic acid, pH 3.5); Group 5 – Fini<sup>®</sup> Chicle Fruit Salad (maleic acid, pH 2.6); Group 6 – Fini<sup>®</sup> Regaliz Acid Tubes (maleic acid and citric acid, pH 3.1). The candies were dissolved in water in proportion 40 g/250 mL deionized water. The samples were submitted to pH cycling for 7 days, consisting of 4 daily immersion cycles in the acid solutions for 90 s each. Between the erosive challenges, the samples were immersed in artificial saliva. Enamel loss was measured using contact profilometry ( $\mu\text{m}$ ) and the data (median value [interquartil interval]) were submitted to Kruskal-Wallis/Dunn test ( $p < 0.0001$ ). All citric candies had high erosive potential. Fini Diet (2.40 [1.18]) and Fini Regaliz Tubs (2.15 [0.49]) presented the highest erosive potential, not differing from 0.1% citric acid (2.30 [0.74]) significantly. Fini<sup>®</sup> Regaliz Tubs was even more erosive than Coca-Cola<sup>®</sup> (1.36 [0.45]). Citric candies have relevant erosive potential and their frequent consume may represent an important factor in the development of erosive tooth wear.

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### The Effect of Theobromine on the In Vitro De- and Remineralization of Enamel Carious Lesions

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The aim of this mechanistic in vitro study was to investigate the effect of theobromine on the de- and remineralization of enamel carious lesions under plaque fluid-like conditions. Early carious lesions were created in 272 bovine enamel specimens and assigned to 16 groups (n = 17) based on Knoop surface microhardness (SMH). Lesions were demineralized again under plaque fluid-like conditions in the presence of fluoride (0.2 or 1.0 ppm) and theobromine (0; 10; 100 or 200 ppm) at different pH values (5.5 and 7.0) in a factorial design. SMH was determined again and percent SMH recovery (%SMHr) calculated. Three-way ANOVA was used for the fixed effects of fluoride, theobromine and pH levels to compare the differences between each level. The three-way ANOVA was not significant ( $p = 0.71$ ). The two-way ANOVA between fluoride and pH was significant ( $p = 0.03$ ), whereas those between fluoride and theobromine as well as that for pH and theobromine were not ( $p = 0.48$  and  $p = 1.0$ , respectively). Theobromine did not affect %SMHr at any of the tested concentrations (e.g. %SMHr; mean  $\pm$  standard deviation: pH 5.5/0.2 ppm F/0 ppm theobromine –  $-3.5 \pm 18.0$ ; pH 5.5/0.2 ppm F/200 ppm theobromine –  $2.0 \pm 21.2$ ). There were trends for the higher fluoride concentration and the higher pH resulting in more rehardening with the lesions exposed to 0.2 ppm fluoride at pH 5.5 ( $2.3 \pm 16.4$ ) displaying significantly less rehardening than those exposed to 0.2 ppm fluoride at pH of 7.0 ( $11.1 \pm 25.2$ ;  $p = 0.018$ ) and lesions exposed to 1 ppm fluoride at pH of 5.5 ( $12.1 \pm 16.2$ ;  $p = 0.008$ ). In conclusion, theobromine does

not affect de- or remineralization of enamel carious lesions under the presently studied conditions.

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### Proteomic Analysis of Unstimulated Saliva of Head-And-Neck Cancer Patients Treated with Radiotherapy

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Saliva is essential to maintain the homeostasis of the oral cavity. Salivary glands are highly affected during radiotherapy in the head and neck region, leading to a dramatic reduction in salivary flow and changes in salivary composition. Besides negatively affecting the oral soft tissues, this can also affect the teeth, since saliva is the most important biological factor protecting against dental caries. Thus, the present study evaluated the effect of radiotherapy in the proteomic profile of unstimulated saliva in patients with head-and-neck cancer (HNC). Nine HNC patients had their saliva collected before (BRT), during (2–5 weeks; DRT) and after (3–4 months; ART) radiotherapy. Saliva was also collected from healthy volunteers (control; C). The proteins were extracted in biological triplicate, the samples were then concentrated, reduced, alkylated and digested with trypsin. Samples were purified and processed for nLC-ESI-MS/MS analysis. In total, 1,055 proteins were identified, among which 47 were common to all groups, while 86, 86, 286 and 395 were exclusively found in C, BRT, DRT and ART, respectively. Remarkably, alpha-enolase was increased 35-fold DRT compared with BRT, while proline-rich proteins were decreased. After radiotherapy, there was a 16-fold increase in scaffold attachment factor B1 and a 3-fold decrease in alpha-enolase and several cystatins. When compared with Control, salivary proteins of BRT patients showed 25-fold increase of vitamin D 25-hydroxylase, besides increases in lipocalin, cystatin C, lysozyme C, histatin-1 and proline-rich proteins. Both HNC and radiotherapy remarkably changed the salivary protein composition. These results provide important insights for designing more effective dental products for these patients and contribute to a better understanding of the progressive changes in salivary proteins induced by radiotherapy.

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### In Silico Modelling of the Effect of Hyposalivation on Biofilm Dysbiosis and Dentin Demineralization

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Hyposalivation significantly increases the risk for caries development, but detailed investigations on the effect of hyposalivation

on plaque biofilm dynamics are not available. We have previously shown (Head et al.: Sci Rep 2017;7: 17413) that increasing the frequency of acid challenges (i.e. sugar exposures) can drive the biofilm composition from symbiotic to dysbiotic. Here we used the same in silico methodology to determine the effect of time for sugar clearance (used as a proxy for hyposalivation) on biofilm dysbiosis and expected dentin demineralization. Sugar clearance half-times of 2, 4 and 6 min were used. The longer the clearance half-time, the longer it took for the pH versus time curve to reach values that were above those required for dentin demineralization; increasing clearance half-times from 2 to 6 min doubled the amount of time the biofilm pH was below 6.2 (from about 19 min to about 38 min). The effect on the biofilm dysbiosis, assessed at 50 d, showed the conversion from a symbiotic to a dysbiotic biofilm to happen at a frequency of 6 sugar intakes/d when the clearance half-time was 2 min, and between 3 and 3.4 sugar intakes/d when the clearance half-time was 6 min. Taken together, the results confirm the drastic effect that a prolonged sugar clearance has on the dynamics of dental biofilm and caries, and suggests that this in silico model can be employed to predict the effect of anticaries agents that reduce biofilm metabolism on dental caries in hyposalivation patients.

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### Resin Infiltration in Naturally Occurring Non-Cavitated Enamel Lesions Using Optical Coherence Tomography (OCT)

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This study aims to investigate using OCT, the degree and depth of resin infiltration (ICON<sup>®</sup>) in non-cavitated enamel caries lesion (NCECL), and the association between these parameters and the post-infiltration colour change. Twenty extracted human teeth with ICDAS 1 or 2 lesions (n = 10) were infiltrated with ICON<sup>®</sup> following the manufacturer's instruction. Pre- and post-infiltration 3D scans and colour values (CIE L\*a\*b\*) were obtained using OCT and a spectrophotometer respectively. Mean OCT intensity depth profiles of the regions-of-interests, pre- and post-infiltration, were extracted. Ratio of infiltration depth (Dr) and percentage of integrated reflectivity reduction ( $\Delta$ IR) were computed from the mean A-scans. Independent T-test were used to compare Dr,  $\Delta$ IR and colour change pre- and post-infiltration ( $\Delta$ E) between the two ICDAS groups. Pearson's correlation test was used to correlate  $\Delta$ E with Dr and  $\Delta$ IR respectively. Dr for ICDAS 1 and 2 lesions were  $0.61 \pm 0.39$  mm and  $0.72 \pm 0.37$  mm respectively and were not significantly different.  $\Delta$ IR for ICDAS 1 and 2 lesions were  $3.60 \pm 0.88\%$  and  $6.12 \pm 2.08\%$  respectively and were significantly different.  $\Delta$ E for ICDAS 1 and 2 lesions was  $3.96 \pm 6.28$  and  $3.1 \pm 3.27$  and not significantly different. In both ICDAS lesions, three patterns of infiltration were observed; A) full lesion depth and complete infiltration, B) full lesion depth but incomplete infiltration C) partial lesion depth infiltration. Significant correlation ( $p < 0.05$ , Pearson Correlation coefficient = 0.924) was observed between  $\Delta$ E and Dr in infiltration pattern C, with linear regression

R2 of 0.854. It can be concluded that colour changes between pre- and post- resin infiltrated NCECL is more influenced by the depth of infiltration than the degree of infiltration and has a linear relationship with the ratio of depth of infiltration.

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### Effect of Sodium Lauryl Sulphate on Developing Zebrafish Embryos

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Toothpastes are indispensable products to provide personal oral hygiene. Besides their comprehensive benefits, they contain potentially harmful ingredients, including some that they may lead to localized and systemic adverse effects. Some of these effects have been associated with sodium lauryl sulphate (SLS). The most common detergent used in toothpaste is the anionic compound SLS. Being genetically similar to humans zebrafish is used in toxicity potential testing. Zebrafish embryotoxicity test is fast and simple method to study chemical toxicity during embryogenesis. Externally fertilized zebrafish embryos grow and develop quickly, both embryo and larvae are transparent, making them accessible to observation and manipulation at all stages of their development facilitating experimental techniques. Aim of this study was to evaluate any toxic effect of SLS on zebrafish embryos. Adult AB strain zebrafish were used according to Institutional Animal Care and Use Committee protocols. Fish were kept in an aquarium rack system (Zebtec, Tecniplast, Italy) at  $27 \pm 1$  °C under a light/dark cycle of 14/10 h. Normally dividing, spherical embryos were exposed to low and high dose of SLS (0,01 mg/10 ml and 0,04 mg/10 ml) in well plates containing 20 embryos, having 3 replicates. Mortality rate and hatching rate were evaluated for 72 h. One-Way ANOVA and Tukey Multiple Comparison Tests were used for statistical analysis. Our findings showed that zebrafish embryos which were exposed to the high dose of SLS had higher rates of mortality and hatched slower when compared to the embryos exposed to low dose SLS and the control group ( $p < 0,05$ ). No significant difference was observed in the low dose SLS exposed embryos when compared with the control group ( $p > 0,05$ ).

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### Caries Detection Using Targeted Fluorescent Starch

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Fluorescent, cationic submicron starch (FCSS) particles can label the subsurface of carious lesions as demonstrated in proof-of-concept experiments. Aims of this study: to evaluate the ability of FCSS to detect carious lesions over a range of severities and with different light sources using histology as the reference standard and compared to visual exam. A clinical examiner selected 100 extracted human teeth with sound (50), non-cavitated (25), or cavitated (25) carious lesions in pits and fissures. Teeth were immersed in FCSS dispersion, rinsed in deionized water and evaluated for the presence of fluorescence by two independent “blinded” examiners, using orange UV-protective glasses with two different lights: a standard dental curing light and an LED penlight. Teeth were then sectioned and evaluated by histology (Downer criteria) as a gold standard for presence of caries. Cohen’s Kappa for inter-examiner and intra-examiner agreement was  $0.74 \pm 0.07$  and  $0.80 \pm 0.06 / 0.94 \pm 0.03$  for the curing light, and  $0.74 \pm 0.07$ ,  $0.88 \pm 0.05 / 0.82 \pm 0.06$  for the pen light, indicating high degrees of repeatability and reproducibility. Sensitivity, specificity and ROC-AUC were: 88.9%, 94.6%, 0.92 (curing light); 79.3%, 91.9%, 0.85 (penlight); 77.8%, 97.3%, 0.88 (visual exam). For early lesions (limited to enamel), sensitivity, specificity and ROC-AUC were: 76.9%, 94.6%, 0.86 (curing light); 61.5%, 91.9%, 0.75 (penlight); 46.2%, 97.3%, 0.72 (visual exam). In conclusion, FCSS is a promising novel technology for caries detection with superior sensitivity and comparable specificity to visual exam, particularly for early carious lesions (comparison of ROC-AUC,  $p < 10^{-4}$ ). Higher irradiance light sources improve visibility of FCSS fluorescence. Detection of early non-cavitated caries will allow for improved implementation of non-restorative treatments which can subsequently be monitored for efficacy, shifting treatment towards non-invasive medical approaches.

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### Development of Slow-Release Muco-Adhesive Anti-Caries Tablets: In Vitro Cytotoxicity Evaluation and In Situ Adhesion

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Aim was to develop a new formulation of slow-release muco-adhesive tablets, containing casein phosphopeptides (CPPs) (rem-ineralizing agent), pomegranate peel extract (antibacterial agent), Stevia rebaudiana Bertoni (non cariogenic sweetener), designed for being settled on the inner-mouth mucosal surface and to assess the in vitro cytotoxic effects as well as to evaluate the in situ adhesion. Tablet composition : 1 mg of CPPs, 1 mg of pomegranate peel extract, 3 mg of carbopol 71G, 3 mg of HPMC (HydroxyPropyl-MethylCellulosa), 1.5 mg of guar, 0.25 mg of Stevia rebaudiana and 0.25 mg of magnesium stearate. The in vitro cytotoxicity of slow-release muco-adhesive tablets was assessed on human gingival fibroblasts. In situ trial: 40 volunteers were instructed to press the tablets against gums, above upper second molar. The in situ adhesion was evaluated at the application (t0), after 6 h (t1) and after 12 h (t2). At the end of the procedures data were processed with the Statistical Package for Social Sciences (SPSS). A regression binary logistic analysis was made. Statistical significance level was set at  $p < 0.05$ . Slow-release muco-adhesive tablets did not interfere with physiologic cell growth in vitro. In situ trial: at t0 tablets adhered in 97.5% of cases. At t1 tablets were still attached in 94.9 % of cases. At t2 all the remaining tablets were still attached. The differences in the in situ adhesion between t0 and t1, t0 and t2, t1 and t2 were not statistically significant, respectively. Slow-release muco-adhesive drug delivery system could be a novel route of anti-caries bio-active molecules administration by offering prolonged contact at the site of application, thanks to long term adhesiveness.

### Substitution of Sn Ions, Applied as Sn<sup>2+</sup>, Into Hydroxyapatite In Vitro

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The aims were to (i) assess the practicability of substitution of Sn ions, applied as Sn<sup>2+</sup>, into hydroxyapatite (HA), and

(ii) investigate any subsequent physico-chemical modifications. Sn<sup>2+</sup>-doped HA was prepared by wet precipitation. 1mol/l (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> was added dropwise (vigorous stirring) to 1.75 mol/l Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O (150 ml.l<sup>-1</sup>, pH10.5, room temperature). HA and Sn<sup>2+</sup>-doped HA, precipitated from solutions containing 10/50/100/500/1000/5000ppm Sn<sup>2+</sup>, as SnCl<sub>2</sub> ([Sndoping]), were prepared. All samples were rinsed (1 mol/l NaCH<sub>3</sub>COO·3H<sub>2</sub>O, pH8.8, agitated) to extract weakly adsorbed Sn<sup>2+</sup>, immersed in CH<sub>3</sub>COOH (37%, pH 5.00), and freeze-dried. Finally, the solid residue was digested in 2 N HCl. The resultant solutions were analysed for Sn using ICP-OES. XRD (Rietveld-refinement as appropriate)/FTIR were used to measure crystallite size, a- and c-axis dimensions and investigate substitution site (Ca(I) or Ca(II)). Linear relationships were assessed (Pearson's), treatment groups compared (ANOVA) (sig at  $p < 0.05$ ). Sn<sup>2+</sup> substitution increased significantly with increasing [Sndoping] ( $p = 0.999$ ), with a mean Sn<sup>2+</sup>-substituted:[Sndoping] of 0.558. Any Sn present in HA was below ICP-OES limits of detection. With increasing [Sndoping] Sn<sup>2+</sup> initially substituted preferentially into the Ca(1) site, but preferentiality ceased as [Sndoping] increased, with a suggested 'critical concentration' of ca. 4% of stoichiometric Ca<sup>2+</sup> concentration. Samples were apatitic below 1000 ppm [Sndoping], but traces of SnO<sub>2</sub> and SnP<sub>2</sub>O<sub>7</sub> appeared at and above 1000 ppm. C-axis dimensions in doped samples decreased significantly ( $p = -0.85$ ) with increasing log<sub>10</sub>[Sndoping] (range 30.8-26.2 nm); 10 ppm (30.8 nm) was significantly larger than HA (28.6 nm), 1000 and 5000 ppm [Sndoping] significantly lower (27.2/26.2 nm respectively). A-axis dimensions in doped samples were generally significantly larger (range 11.4-10.7 nm) than HA (9.27 nm), but there was not a clear trend. In conclusion, incorporation of Sn ions, into the HA lattice, applied as Sn<sup>2+</sup>, has been demonstrated, modifying physical parameters in a dose-dependent fashion.

### Predictive Factors for Erosive Tooth Wear in Patients with Gastroesophageal Reflux Symptoms

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Patients with gastroesophageal reflux (GOR) are at high risk of developing erosive tooth wear (ETW). This cross-sectional case-control study used two techniques which have not been used together previously aiming to assess the predictive factors for ETW in patients with symptoms of GORD. A total of 261 consecutive patients participated, underwent High resolution manometry (HRM) test for 10 min followed by the insertion of the 24 h pH-impedance reflux monitoring probe, and clinical assessment of ETW using Basic Erosive Tooth Wear Examination (BEWE). Those with a cumulative score of <sup>3</sup>12 and at least 1 area scoring 3 were included in the ETW-group (ETW n = 150) and the rest were included in the No-ETW-group (NETW n = 111). This study evaluated the following collected data: GORD diagnosis, the total acid reflux episodes, percentage of reflux episodes in both upright and recumbent position, DeMeester score and symptoms reported

during the test. Patients who were off Proton pump inhibitors (PPI) for seven days prior to the monitoring were included. Data were analysed using STATA software,  $p < 0.05$  was considered significant. Parameters with correlation to ETW were: pathological GORD diagnosis (NETW = 34.5%, ETW = 65.4%;  $p = 0.007$ ), abnormal acid exposure time (NETW = 29.9%, ETW = 70.01%;  $p = 0.001$ ), abnormal acid exposure in upright position (NETW = 26.09%, ETW = 73.91%;  $p = 0.002$ ), DeMeester score  $> 14.7$  (NETW = 34.69%, ETW = 65.31%;  $p = 0.05$ ). Symptoms reported by patients during the monitoring test with relevance to ETW were: heartburn (NETW = 33.33%, ETW = 66.66%;  $p = 0.001$ ) and regurgitation (NETW = 36.36%, ETW = 63.63%;  $P = 0.02$ ). In conclusion, results of this study suggests that the likelihood of developing ETW is increased with: diagnosis of GORD (2 times), having heartburn (2.33 times), and regurgitation (1.75 times).

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### Effect of $\text{TiF}_4/\text{NaF}$ /Chitosan Solution at Different Viscosities on the Protection of Enamel Erosion

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This in vitro study evaluated the protective effect of experimental solutions containing  $\text{TiF}_4/\text{NaF}$  and chitosan at different viscosities on enamel erosion. Bovine enamel samples were distributed to the following groups ( $n = 15$ ): (1) commercial solution with  $\text{SnCl}_2/\text{NaF}$  (800 ppm  $\text{Sn}^{2+}$ , 500 ppm  $\text{F}^-$ , pH 4.5, elmex<sup>®</sup>, GABA Int. AG, positive control); (2) experimental  $\text{NaF}/\text{TiF}_4$  (190 ppm  $\text{Ti}^{4+}$ , 490 ppm  $\text{F}^-$ , pH 3.8); (3) similar to 2 plus 0.5% chitosan (Ch 500 mPas, pH 4.5), (4) similar to 2 plus 0.5% chitosan (Ch 2000 mPas, pH 4.5), (5) no treatment (negative control, pH 6.1), (6) 0.5% chitosan (Ch 500 mPas, pH 4.0) and (7) 0.5% chitosan (Ch 2000 mPas, pH 4.0). The tested chitosan (Heppe Medical Chitosan GmbH) presented 75% deacetylation. The samples were submitted to pH cycling (0.1% citric acid, 4x90s/d, interposed by artificial saliva) and daily treatment application (after the last erosive challenge, 1x30s/d) for seven days. The enamel loss was quantified by contact profilometer ( $\mu\text{m}$ ). The data were compared using Kruskal-Wallis/Dunn ( $p < 0.05$ ). Enamel samples treated with elmex<sup>®</sup> presented the lowest loss (0.72/0.18  $\mu\text{m}$ ) followed by those from both  $\text{TiF}_4 + \text{Chitosan}$  (500 mPas: 1.24/0.49  $\mu\text{m}$  / 2000 mPas: 1.28/0.25  $\mu\text{m}$ ), which did significantly differ from negative control (1.70/0.27  $\mu\text{m}$ ).  $\text{TiF}_4/\text{NaF}$  and Chitosan alone did differ from elmex<sup>®</sup>, but not from the other groups, including the negative control. The experimental solution containing  $\text{TiF}_4/\text{NaF}$  and chitosan 2000 mPas presents promising effect on the protection against enamel erosion when compared to elmex<sup>®</sup>.

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### Maximum BEWE Score on Index Surfaces and Associated Erosive-Tooth Wear Risk Factors in Colombian Schoolchildren

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Aim was to assess common ETW (Erosive-Tooth-Wear) risk factors in schoolchildren from Bogotá, Colombia using index surfaces. A risk factors' questionnaire was applied on 454 schoolchildren of 12 to 15-years old. Two calibrated examiners assessed ETW by BEWE (Basic Erosive Wear Examination) on index surfaces (buccal of upper-central incisor- and occlusal of lower-first molar-teeth). Maximum-BEWE score categorised patients into with (BEWE:2-3) and without wear (BEWE:0-1). These were compared in terms of demographic, clinical, dietary and other factors with bivariate analysis and logistic regression models. Schoolchildren's mean age was  $13.5 \pm 1.1$  years (61.7% females). Most were middle-low social-economic status (71.1%) and attended private schools (68.7%). The time used to assess ETW on index surfaces was  $39.3 \pm 3.1$  s in average. The prevalence of ETW was of 34.6%. The maximum-BEWE score distribution among the population was: 0 = 59.5%; 1 = 5.9%; 2 = 8.8%; 3 = 25.8%. Questionnaire highlights included: 33.2% with gastroesophageal symptoms; 61.4% liking acidic food/drinks; 38.9% consuming fruit daily; 53.3% conducting 2-3 daily toothbrushing; 74.7% never brushing teeth before eating; 22.0% chewing gum 1-3 daily. Bivariate analysis displayed association between ETW and hypersensitivity ( $p = 0.038$ ), acid mango consumption ( $p = 0.04$ ), and chewing gum frequency ( $p = 0.03$ ). Logistic regression model showed that those who chew gum once daily were 2.23 times more likely to have wear (95% CI: 1.27–3.91,  $p = 0.005$ ) compared to those who did not chew gum daily, and 1.88 times more likely to have wear (95% CI: 1.14–3.12,  $p = 0.013$ ) compared to those who did not consume fruit daily (multivariate logistic regression). In conclusion, 12–15 years-old Colombian schoolchildren showed a high prevalence of ETW reporting associated factors. The use of index surfaces was time saving and useful to assess ETW associated risk factors.

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### Does Cannabis Protect against Dental Erosion?

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Nowadays, due to its numerous health benefits, there is an increasing interest in products containing Cannabis. Cannabis tea is an accessible mode for consumption. In this study, we aimed to investigate the effect of Cannabis (hemp) teas on the salivary pellicle regarding its protection against dental erosion. Enamel specimens were submitted to five cycles, each consisting of salivary

pellicle formation (30 min, 37°C) with whole human saliva, incubation in the different teas (2 min, 25°C, 70 U/min), another salivary pellicle formation (60 min, 37°C), and finally immersion in citric acid (1 min, 1%, pH 3.6, 25°C). The investigated groups/teas (n = 15/group) were: Control (Ctrl; deionized water, pH 6.4), commercial Cannabis plant tea (CT; 100% Cannabis plant parts, pH 8.4), commercial Cannabis flower tea (CFT; 100% Cannabis plant parts, from which 77% were flowers, pH 8.5), and commercial green tea (GT; 100% Camellia sinensis, pH 5.3). Relative surface microhardness (%SMH) and total amount of calcium released to the citric acid (CaR, in nmol/mm<sup>2</sup>) were evaluated and data were analyzed with Kruskal-Wallis and post-hoc Dunn-Bonferroni tests. The results are presented as median, interquartile range. None of the Cannabis teas improved the protection of enamel against dental erosion compared to Ctrl (54.5, 52.6–58.6). GT presented the highest %SMH values (59.6, 55.6–63.5), and significantly differed from CFT (54.3, 50.8–56.1), which had the lowest values. The significantly lower CaR (3.28, 2.87–4.24) observed in GT comparing to all other groups (Ctrl: 5.65, 3.65–7.37; CFT: 5.43, 4.27–7.59; and CT: 4.76, 3.94–6.28) suggests that green tea has promising protective properties. However, both Cannabis teas did not protect the enamel against dental erosion, but neither did they cause further demineralization.

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### Pellicle Engineering to Protect against Dental Erosion on Native Enamel

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This study evaluated the effect of pellicle engineering with two proteins (a new Cystatin derived from sugarcane – CaneCPI-5 – and human Hemoglobin), on the prevention of dental erosion in vitro. Sixty native human enamel specimens (4x4 mm) were prepared from molars and divided into three groups (n = 20) according to the treatment: 1) Deionized water (control), 2) 0.1 mg/ml CaneCPI-5 solution; 3) 1.0 mg/ml Hemoglobin solution. The specimens were exposed to the treatment solutions for 2 hours at 37°C under agitation. Then, for the formation of the acquired pellicle, they were incubated in stimulated saliva for 2 hours at 37°C under agitation. For the erosive process, the specimens were immersed in 1% citric acid (pH 3.6) for 2 min at 37°C. Each specimen was treated once/day for 5 days. Calcium released into the citric acid was determined using an Atomic Absorption Spectrometer and normalized to the surface area of each specimen (CaR, nmol/mm<sup>2</sup>), and relative surface reflection intensity (%SRI) was assessed using an optical reflectometer. Data were analyzed with Kruskal-Wallis and post-hoc Dunn's tests (p<0.05). The results are pre-

sented as median, interquartile range. Significant protection for enamel was observed with treatments with CaneCPI-5 (%SRI: 85, 70–106, p = 0.003; CaR: 11, 9–12, p = 0.030) and Hemoglobin (%SRI: 83, 67–98, p = 0.015; CaR: 9, 8–11 p = 0.000) when compared to the control (%SRI: 66, 57–75; CaR: 13, 11–17), but no differences were observed between CaneCPI-5 and Hemoglobin (p = 1.00 for %SRI; p = 0.251 for CaR). In conclusion, pellicle engineering with CaneCPI-5 or Hemoglobin significantly protected native enamel against dental erosion.

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### 3D-Surface Texture Characterization of In Situ Simulated Erosive Tooth Wear Lesions

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Detection and differentiation of erosive tooth wear (ETW) by enamel 3D-surface texture analysis has shown promising laboratory results. This study tested this approach in clinically relevant conditions, using a 14-day in situ ETW model simulating different dental erosion severities. This study consisted of a 3-arm cross-over intra-oral ETW simulation, where 20 participants were enrolled and had to wear their own partial denture holding two human enamel specimens (per arm). In each study arm they were assigned to one of three different erosion protocols: severe (lemon juice, pH 2.5); moderate (grapefruit juice, pH 3.5); and no erosion (drinking water, control). Enamel specimens were evaluated by white-light scanning confocal profilometry for 3D-surface texture study. Individual point clouds were analyzed using standard texture characterization protocols, and fractal complexity (Asfc), anisotropy (Str) and roughness (Sa) values were generated at baseline, 7 and 14 days. Data were analyzed by ANOVA models suitable for the cross-over design with repeated measurements, and correlation coefficient used to examine the relationship between outcomes. Asfc and Sa increased with ETW severity (no-erosion<moderate<severe, p<0.001) at days 7 and 14. Asfc and Sa were lower at baseline compared to days 7 and 14 (p<0.001) for moderate and severe challenges. Asfc was lower at day 7 compared to day 14 (p = 0.042), for the severe challenge. For Str, ETW did not have a significant effect overall (p = 0.15). Asfc and Sa were highly positively correlated (r = 0.89, p<0.001), while Asfc and Sa were not correlated overall with Str (r<0.1, p>0.25). 3D-surface texture parameters of complexity (Asfc) and roughness (Sa) were able to detect and differentiate ETW levels, with Asfc also being able to monitor severe lesions. No clear characterization of ETW lesions could be provided by the anisotropy (Str) parameter.

### Effect of Salivary Pellicle from Adults and Children on the Protective Effect of Fluoride

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Fluoride can interact with the salivary pellicle and modulate enamel demineralization. Since salivary pellicles from children and adults differ, fluoride could also have different effects depending on the kind of pellicle. We, therefore, investigated the effect of a sodium fluoride (NaF) rinse (250 ppm F<sup>-</sup>; pH 5.8) on enamel covered with salivary pellicle from children or adults. Enamel specimens from permanent molars (n = 40) were submitted to five cycles, consisting of salivary pellicle formation (30 min, 37°C) with saliva either from adults (AP; n = 20) or children (CP; n = 20), then incubating half of each group in deionized water (DW) and the other half in NaF for 2 min (25°C), later another period of pellicle formation (60 min, 37°C), and finally immersion in citric acid (1 min, 1%, pH 3.6, 25°C). Relative surface microhardness (%SMH) and total amount of calcium released to the acid (CaR, in nmol/mm<sup>2</sup>) were evaluated and analyzed with Mann-Whitney test (p < 0.05). The results are presented as median, interquartile range. No differences were observed in the specimens treated with DW either covered with AP (%SMH 62.4, 59.5–64.5; CaR 20.0, 17.1–21.9) or CP (%SMH 64.1, 60.8–70.7; CaR 19.1, 16.9–20.0). Treatment with NaF resulted in significantly less (p = 0.005) surface microhardness loss in enamel covered with AP (%SMH 80.5, 72.4–82.7) than that covered with CP (%SMH 71.2, 68.7–75.6), but treatment with NaF also resulted in significantly increased CaR: AP 31.7, 25.4–33.8 and CP 32.2, 29.8–43.7, with no differences between the two kinds of pellicle (p = 0.353). Different kinds of salivary pellicles have different impacts on the protective effects of NaF, where specimens with salivary pellicle from adults will probably have better protection against demineralization than those with salivary pellicle from children.

### Does Using Mouthrinse Before/After Toothbrushing Impact on Enamel/Dentine Erosive Tooth Wear? An In Situ Study

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This study aimed to evaluate the impact of using a mouthrinse before/after toothbrushing on the progression of erosive tooth

wear. The treatment groups were: Control – toothbrushing without dentifrice; B-only – toothbrushing with a 1400 ppm F<sup>-</sup> and 3500 ppm Sn<sup>2+</sup> dentifrice; B+R – toothbrushing with F<sup>-</sup>/Sn<sup>2+</sup> dentifrice and then rinsing with a 500 ppm F<sup>-</sup> and 800 ppm Sn<sup>2+</sup> mouthrinse; R+B – rinsing (F<sup>-</sup>/Sn<sup>2+</sup>) and then toothbrushing (F<sup>-</sup>/Sn<sup>2+</sup>); and R-only – rinsing (F<sup>-</sup>/Sn<sup>2+</sup>). In each of the 5 phases, the volunteers (n = 15) used removable mandible devices containing 3 enamel and 3 dentin specimens, which were subjected to an erosion-abrasion cycling model (5 min immersion in 0.3% citric acid, followed by 60 min saliva exposure in situ; 4x/d for 5 d). Treatment, according to the groups, was performed in situ twice a day after the first and last erosive challenges; toothbrushing was performed with an electric toothbrush placed for 5 s on each specimen (total of 2 min exposure to the slurry) and rinsing was performed with 10 ml of solution for 30 s. Surface loss (µm) was evaluated with optical profilometry (presented as mean±SD). Data were analyzed with two-way repeated measures ANOVA and Tukey tests (α = 0.05). Dentin specimens presented significantly greater surface loss than enamel (p < 0.001). Control showed the greatest surface loss for both enamel (24.58±11.32) and dentin (32.32±10.10). The least surface loss was observed for R-only on both enamel (8.30±4.96) and dentin (16.15±8.29), but it did not significantly differ from B+R. None of the groups involving toothbrushing (B-only, B+R or R+B) differed from each other (p > 0.05). In conclusion, when toothbrushing with a F<sup>-</sup>/Sn<sup>2+</sup> dentifrice, no additional protection was observed with the F<sup>-</sup>/Sn<sup>2+</sup> mouthrinse, whether used before or after the brushing.

### Pellicle Modification with Casein and Mucin does not Affect Surface Loss from Erosion and Abrasion

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A combination of casein and mucin is known to improve the salivary pellicle's protection of the underlying enamel. This in vitro study aimed at investigating whether this protection is confined to erosion, or if it extends to abrasion. We prepared 72 human enamel specimens and randomly assigned them to four groups. They underwent five cycles, consisting of a pellicle/treatment part, an erosion (3 min, 1% citric acid, pH 3.6, 25°C, 70 rpm), and an abrasion (50 toothbrush strokes within 25 s in toothpaste slurry, 200 g load). The pellicle/treatment part consisted of 3 groups: 2 h incubation (25 °C, 70 rpm) in whole human saliva followed by 2 h incubation in a 1% casein and 0.27% mucin mixture (group PCM); 2 h incubation in saliva only (group P); 2 h incubation in the casein/mucin mixture only (group CM). The fourth group (Ctrl) served as control without pellicle/treatment. Enamel surface loss was analysed by optical profilometry, and relative surface microhardness (%SMH) was measured initially and after each experimental step. The results were analysed with Kruskal-Wallis and Wilcoxon tests with Bonferroni corrections.

The treatments did not show differences in surface loss (p > 0.05) and therefore did not protect enamel from surface loss by abrasion

(median, IQR across all four groups: 1.88  $\mu\text{m}$ , 1.55–2.06). However, differences were seen in the %SMH values, Ctrl (48.9%, 45.2–55.4) being significantly softer than P, CM or PCM, with no differences between the latter three (P: 64.5%, 61.0–68.4; CM: 61.6%, 53.1–68.5; PCM: 60.5%, 56.7–65.6). This suggests that different abrasion protocols would lead to differences in surface loss, and further investigations on whether pellicle modifications can lead to increased resistance to abrasion remains worthwhile.

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### Potential of Hydroxyapatite for the Prevention of Erosive Tooth Wear

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Hydroxyapatite-containing products claim to prevent erosive wear and to provide repair of eroded tooth surfaces. Aim of the present study was to investigate, whether such formulations are able to impact tissue loss in enamel in an in vitro erosion/abrasion model. Five test formulations were examined: two hydroxyapatite toothpastes (HAP-TP1/2), two hydroxyapatite toothpastes with fluoride (HAP-F-TP1, 1450 ppm F<sup>-</sup>; HAP-F-TP2, 1490 ppm F<sup>-</sup>) and a hydroxyapatite mouthrinse (HAP-MR). Positive controls were a Sn/F toothpaste and a Sn/F mouthrinse (Sn-TP, 1000 ppm F<sup>-</sup>; Sn-MR, 500 ppm F<sup>-</sup>). The placebo toothpaste (P-TP) had no active ingredients. Human enamel samples (16 groups; n = 16 each) were cycled for 10 days through demineralization (0.5% citric acid; 6x2 min/day) and remineralization. After the first and the last daily acid exposure, samples were immersed in toothpaste slurries (1:3 by weight; 2 min). Half of the samples were brushed during slurry immersion (15 s, brushing machine). The mouthrinses were used for 2 min after each P-TP slurry/-brushing procedure. Substance loss was measured profilometrically (mean $\pm$ SD;  $\mu\text{m}$ ). Statistics: Kolmogorov-Smirnov-test, ANOVA. Compared to P-TP (7.8 $\pm$ 2.0), substance loss increased significantly after slurry immersion in HAP-TP2 (11.7 $\pm$ 3.1; p $\leq$ 0.01) and HAP-F-TP2 (13.0 $\pm$ 2.2; p $\leq$ 0.001) but not after immersion in HAP-TP1 (6.9 $\pm$ 1.9; n.s.), HAP-F-TP1 (6.6 $\pm$ 1.4; n.s.) and HAP-MR (5.99 $\pm$ 1.2; n.s.). Sn-TP (4.1 $\pm$ 1.2; p $\leq$ 0.001) and Sn-MR (1.3 $\pm$ 0.5; p $\leq$ 0.001) inhibited substance loss distinctly. After additional brushing and compared to P-TP (11.4 $\pm$ 2.9), substance loss increased significantly after treatment with HAP-TP1 (15.3 $\pm$ 3.4; p $\leq$ 0.05), HAP-TP2 (20.6 $\pm$ 3.2; p $\leq$ 0.001) and HAP-F-TP2 (16.7 $\pm$ 2.6; p $\leq$ 0.001) but not after treatment with HAP-F-TP1 (10.4 $\pm$ 2.9; n.s.), HAP-MR (10.5 $\pm$ 3.9; n.s.) and Sn-TP (9.5 $\pm$ 2.9; n.s.). Sn-MR (1.3 $\pm$ 0.5; p $\leq$ 0.001) inhibited substance loss nearly completely. Hydroxyapatite-containing formulations had no preventive effects on erosive demineralization and abrasion.

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### Can Proanthocyanidin Enhance the Protective Effect of Acquired Enamel Pellicle Against Initial Erosion?

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The aim of this study was to evaluate the effect of Proanthocyanidin gel applied over acquired pellicle on the inhibition of enamel demineralization, in a short-term erosive challenge. For that purpose, 64 enamel blocks were equally divided and treated according to the following groups: G1- acquired pellicle formed in situ and 6.5% Proanthocyanidin gel application, G2- only 6.5% Proanthocyanidin gel, G3- only acquired pellicle formed in situ and G4- without treatment (without gel and without acquired pellicle). The acquired pellicle was formed in situ by the use of intra-oral palatal appliance for 2 h (G1, G3). Gels were applied for 1 min (G1, G2). After treatment, enamel blocks were immersed in 0.5% citric acid (pH 2.5) for 30 s to promote a short erosive challenge. Surface microhardness analysis was performed before and after all procedures and the response variable was the percentage of surface microhardness change (%SMC). Data were analyzed by Kruskal-Wallis and Dunn's test (p $<$ 0.05). Results: [%SMC Median/1st Quartile/3rd Quartile]G1: 7.7a/4.7/10.3; G2: 17.7b/10.8/23.4; G3: 19.1b/11.1/21.1 and G4: 24.9b/19.4/31.9]. The statistical analysis showed that Group 1 (Proanthocyanidin gel and acquired pellicle) presented the lowest value of %SMC compared to the other groups (p  $<$ 0.001). Based on these results, Proanthocyanidin gel could be considered as a promising therapy for preventing enamel erosion because it is able to enhance the protective effect of the acquired enamel pellicle.

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### Quercetin is Able to Reduce Erosive Dentin Wear

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The aim of the present study was to evaluate the effect of quercetin on the acid resistance of human dentin through both in vitro and in situ investigations. Two hundred and twelve dentin blocks (2 mm x 2 mm x 2 mm) were prepared and used. For the in vitro investigation, dentin specimens were randomly divided into 8 groups (n = 12): deionized water, ethanol, 1.23  $\times$  10<sup>4</sup>  $\mu\text{g}/\text{ml}$  sodium fluoride (NaF), 120  $\mu\text{g}/\text{ml}$  chlorhexidine, 183.2  $\mu\text{g}/\text{ml}$  epigallocatechin gallate (EGCG), and 75  $\mu\text{g}/\text{ml}$ , 150  $\mu\text{g}/\text{ml}$ , and 300  $\mu\text{g}/\text{ml}$  quercetin (Q75, Q150, and Q300). The specimens were treated with respective solutions for 2 min and then subjected to in vitro erosion (4 cycles/d for 7 d). The surface microhardness loss (%SMHL), erosive dentin wear, and surface morphology were evaluated and compared. For impact on MMP inhibition, the release of crosslinked carboxyterminal telopeptide of type I collagen

(ICTP) and the thickness of the demineralized organic matrix (DOM) were measured using additional dentin specimens. For the in situ investigation, the specimens were intraorally treated with NaF or Q300 for 2 min and then exposed to in vivo erosion cycles for 7 d. The %SMHL and erosive dentin wear of the specimens were measured to determine whether quercetin similarly inhibits of erosion in situ. The quercetin-treated group had a significantly lower %SMHL (Q300:  $8.75 \pm 4.95$ ) and erosive dentin wear (Q300:  $2.26 \pm 1.16 \mu\text{m}$ ) than any other group, and the effect was concentration-dependent in vitro ( $p < 0.05$ ). Dentin treated with quercetin produced significantly less ICTP (Q300:  $5.97 \pm 0.88 \mu\text{g/L}$ ) and had a thicker DOM (Q300:  $15.45 \pm 2.59 \mu\text{m}$ ) than the control dentin ( $p < 0.05$ ). After in vivo erosion, the %SMHL and erosive dentin wear of the Q300 group were significantly lower than those of the control group ( $5.22 \pm 2.94$  vs.  $19.23 \pm 5.78$ ,  $0.77 \pm 0.34 \mu\text{m}$  vs.  $2.61 \pm 0.97 \mu\text{m}$ , respectively, all  $p < 0.05$ ). The application of quercetin was shown, for the first time, to increase the acid resistance of human dentin, possibly through MMP inhibition and DOM preservation.

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### Factors Influencing the Inhibiting Action of Varnish Containing S-PRG Fillers Against Substance Loss of Eroded Dentine

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In-vitro studies were conducted to evaluate factors influencing the inhibiting action of varnishes containing surface pre-reacted glass-ionomer (S-PRG) fillers which release mineral ions against dentine loss through acid exposure. Sixty-six human dentine slabs were randomly divided into 6 groups. Half of each slab's surface was treated with varnishes containing 20 or 40wt% of S-PRG fillers and then put into water at 37°C for 24, 48 or 72 h. Afterwards, an exposed window was made within the treated area and another within the control area. Slabs, each with two windows, were eroded 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  $\mu\text{m}$  thick) were abraded from the specimen surface apart from the windows, using an abrasive micro-sampling technique. The powdered samples were dissolved in acid and buffered. Fluoride and the other minerals (Al, B, Si, Sr), 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 (mean $\pm$ SEM) in tissue loss between the varnished and control areas soaked for 24, 48 or 72 h were  $0.20 \pm 0.65$ ,  $2.01 \pm 0.74$  and  $1.26 \pm 0.59 \mu\text{m}$  in the 20 wt% and  $1.43 \pm 0.48$ ,  $1.87 \pm 0.67$  and  $0.90 \pm 0.78 \mu\text{m}$  in the 40 wt% groups, respectively, indicating that significant reductions were found except for the 20%-24 h and 40%-72 h treatments. Although fluoride and strontium profiles within treated areas rose significantly with a negative gradient, no significant relationship was found between eroded dentine loss and both mineral concentrations,

suggesting that differences in acid erosion rate among dentine samples could influence the effect of erosion inhibiting action of varnish.

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### Environmental Scanning Electronic Microscope Analysis of Enamel Surface Morphology Following Demineralization and Remineralization Processes

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The enamel erosion induced by chemical exogenous acids causes dissolution of hard tissues of teeth. The aim of this study was to analyse the erosive effect of three different soft drinks on enamel surface and remineralizing potential effect after applying a paste containing Biomimetic Hydroxyapatite crystals (Zinc Substituted Carbonate-Hydroxyapatite Microrepairs<sup>®</sup>, Biorepair desensitizing Paste) using the ESEM (Environmental Scanning Electron Microscope) technique. Fourteen human third molar teeth specimens were analysed in order to assess the effects of Zinc-substituted Carbonate-Hydroxyapatite on enamel tooth, after demineralisation because of the soft drinks acid attack. Morphological characterization of teeth was performed by means of Environmental Scanning Electron Microscope. The surface of specimens was examined before and after immersion into three recipients containing Coca Cola, RED BULL and orange juice, and after remineralization process for the purpose of comparison. Environmental Scanning Electron Microscopy analysis showed enamel morphology alterations after acidic soft drink exposure. Furthermore, in solutions with pH values of 2.53 and 3.41, "lesions" were formed with well-defined surface layers, whereas, in solutions with pH, "lesions" were produced with no apparent surface layers. It is concluded that high concentration of exogen acids with the prolonged exposure time change the enamel composition.

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### Palm Oil Component Responsible for its Preventive Effect Against Enamel Erosion

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Palm oil has demonstrated preventive potential against erosive demineralization in vitro and in situ, but its mechanism is not known. The aim of this study was to analyze the in vitro effect of the different components of palm oil in order to understand their mechanism of action against a single initial erosive challenge.

Eighty four bovine enamel blocks were randomly assigned into the following groups (n = 14 per group): PO- 100% Palm Oil; TO- 85% Tocotrienol solution; VE - 100% Oily Vitamin E; VA - 100% Oily Vitamin A; DW - Deionized water (negative control); SN- Sn containing commercial fluoride solution (Elmex® Erosion Protection Dental Rinse /positive control). Acquired enamel pellicle was formed in situ for 2 h. After application of the oils, water and fluoride solution (5 drops, 30 s) the blocks were immersed in artificial saliva for 2 min and subjected to short-term acid exposure in 0.5% citric acid, pH 2.4, for 30 s, to promote enamel surface softening. The response variable was the percentage of surface hardness loss  $[(SHi - SHf) / SHf] \times 100$ . Data were analyzed by one-way ANOVA and Fisher's test ( $p < 0.05$ ). Enamel blocks of the positive control, palm oil and vitamin E groups (SN- $25.11 \pm 6.49\%$ , PO- $23.70 \pm 11.64\%$ , VE-  $24.73 \pm 11.86\%$ ) presented significantly less percentage of enamel hardness loss compared to the other groups under study (VA- $32.03 \pm 7.59\%$ , TO- $36.99 \pm 9.15\%$ ), including the negative control (DW- $34.98 \pm 7.78\%$ ) ( $p < 0.001$ ). Vitamin E seems to be the component of palm oil responsible for its protective effect against enamel erosion.

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### In Vivo Effects of a Hydroxyapatite Gel on Calcium and Phosphorus Levels of Dental Plaque

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The aim of this in vivo pilot study was to analyze the 3-day effects of a newly developed hydroxyapatite-based oral care gel (HAP;  $Ca_5(PO_4)_3(OH)$ ) on the calcium and phosphorus levels within the dental plaque of children. This study was conducted in Kebon Padang, Bali, Indonesia. 34 children (mean age 8.9; mean DMF-T 0.6; mean dmft-t 4.5) were included in the study. The gel was applied 3 times for 3 d by an experienced dentist. Dental plaque was collected at baseline (control) and after the study. The plaque-collection after the study was performed 1 h after application of the gel. The plaque-samples were dried onto silicon single crystal substrates at 60° C for 1 h. Levels of calcium and phosphorus of plaque samples were analyzed by energy-dispersive X-ray spectroscopy (EDX). 3 EDX-analyses were conducted for each sample. The EDX-analyses were performed at 10 kV at a probe current of 0.9 nA. Quantitative analysis was carried out by calculating the relative concentrations of chemical elements in the sample from the relative X-ray counts and applying matrix corrections. The calcium level increased after 3 d of application of the HAP-gel from 0.25 wt% (median) to 0.40 wt% (median;  $p = 0.5$ ) while the phosphorus level increased from 1.17 wt% (median) to 1.41 wt% (median;  $p = 0.3$ ). However, variations of both calcium and phosphorus levels measured in the pooled dental plaque samples were high.

Within the limitations of the study, the 3 d application of the oral HAP-gel in children increased the median of both calcium and

phosphorus levels in plaque. A positive influence on the remineralization/demineralization-process is very likely (ClinicalTrials.gov: NCT03956992).

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### Effects of Dentifrices Differing in Fluoride Content on Remineralization Characteristics of Demineralized Dentin In Vitro

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In vitro models should be capable to demonstrate a (significant) fluoride dose-response similar to the anticipated clinical effect. However, until now pH-cycling models were only capable to demonstrate a fluoride dose-response for enamel but not for dentin. Thus, the aim of this study was to compare the caries-preventive effect of highly fluoridated dentifrices and gels on artificial dentin caries-like lesions. Bovine dentin specimens (n = 240) each with one caries lesion were prepared and randomly allocated to one highly (6x120min demineralization/day[H]) and one lowly (6x60 min demineralization/d[L]) cariogenic pH-cycling model. Treatments during pH-cycling (28 d) were: brushing 2x/d with: 0 ppm F [H0/L0], 1450 ppm [H1450/L1450], 2800 ppm [H2800/L2800], 5000 ppm [H5000/L5000], 5000 ppm+tricalcium phosphate (TCP) [H5000+TCP/L5000+TCP] and 12500 ppm [H12500/L12500] containing dentifrices/gels. Dentifrice/gel slurries were prepared with deionized water (1:2wt/wt). Differences in integrated mineral loss ( $\Delta\Delta Z$ ) and lesion depth ( $\Delta LD$ ) were calculated between values before and after pH-cycling using TMR. For mineral loss, significantly increased values between before and after pH-cycling were observed for H0/L0, H1450/L1450, H2800/L2800, and H5000+TCP/L5000+TCP, indicating further demineralization, whereas specimens of H12500/L12500 showed significantly decreased values, indicating remineralization ( $p \leq 0.022$ ; two tailed paired t-test). For  $\Delta\Delta Z$  significant differences could be found between H0, H1450, H5000 & H12500 ( $p \leq 0.028$ ; ANCOVA). Contrastingly, in the lowly cariogenic model significant differences could only be found for L5000 and L12500 when compared with L0 ( $p \leq 0.028$ ; ANCOVA). A significant and strong/moderate correlation between  $\Delta\Delta Z$  & F was observed for both the highly ( $r = 0.691$ ) and the lowly ( $r = 0.500$ ) cariogenic model ( $p < 0.001$ ), indicating a fluoride dose-response. For both pH-cycling conditions a dose-response for fluoride could be revealed. For elderly people with exposed root surfaces the use of gels containing 12500 ppm F instead of standard fluoride dentifrices should be further investigated, as it offered higher caries-preventive effects in vitro.

### Efficacy of S-PRG Filler Varnishes on Enamel Caries Remineralization

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The objective was to evaluate the efficacy of different concentrations of bioactive S-PRG fillers versus fluoride in experimental varnishes on enamel remineralization. One hundred twenty enamel specimens were obtained and polished. Half of the surface was protected with light-curing adhesive. Artificial caries lesions were created on the unprotected area. The specimens were assigned into 6 groups (n = 20): four varnishes containing different concentrations S-PRG (10%, 20%, 30% and 40%) and one containing 5% sodium fluoride (SF) as positive control (Shofu, Japan). A non-treated group was used as negative control (NC). The varnishes were applied on specimens' surface, followed by immersion in artificial saliva for 24 h. After that the varnishes were removed with acetone and the specimens submitted to a pH-cycling (2 h of demineralization and 22 h of remineralization) for 8 days. Then, varnish application and pH cycling were repeated. The specimens were cross-sectioned and the transversal Knoop microhardness (KHN) was evaluated each 10 µm, from the surface up to 100 µm depth. The percentage of microhardness of the caries affected/ treated area in relation to the sound enamel (100%) was calculated for each depth and used for statistical analysis. The groups were compared for each depth using one-way ANOVA and Tukey's test. Results: Significant differences among the groups were observed on each depth, for 10-80 µm (p<0.05). The varnishes containing 40%S-PRG and 5%SF produced a significantly higher KNH than the NC for the depths of 10-50 µm, without differences between them. For the depths of 60, 70 and 80 µm, only the 40%S-PRG varnish showed significantly higher KHN than the NC. The means (SD) were: for 60 µm: NC-79.8(12.2)a, 10%-82.1(17.0)a, 20%-88.4(17.7)ab, 30%-89.2(11.6)ab, SF-91.8(13.3)ab, 40%-96.0(12.4) b; for 70 µm: NC-85.2(11.8)a, 10%-87.5(10.8)ab, 20%-92.2(13.1) ab, 30%-94.8(11.2)ab, SF-95.1(12.8)ab, 40%-97.7(11.5)b; for 80 µm : NC-89.6(12.1)a, 10%-95.1(10.3)ab, SF-95.8(12.3)ab, 20%-97.1(9.4)ab, 30%-98.2(8.0)ab, 40%-99.9(9.1)b. The varnishes containing 40%S-PRG and 5%SF were effective and similar on the remineralization up to the depth of 50 µm. On deeper areas, only the 40%S-PRG had a significant effect.

### Monitoring Lesion Activity After Silver Diamine Fluoride (SDF) Application Using Nondestructive Near Infrared Modalities

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Aim was to investigate the lesion activity after the application of SDF by measuring the dehydration rate using thermal imaging and optical coherence tomography. Twelve extracted teeth with cavitated enamel and dentin lesions were used for this in vitro experiment. Optical coherence tomography (1300-nm) and thermal imaging (6-10-µm) were used to measure the lesion structure and activity before and after application of SDF according to UCSF protocol. Each sample was imaged before application using both methods to determine its activity by the presence of the hypermineralised layer and acquire time-resolved thermal images during dehydration with air. After application of SDF the measurements were repeated at: 1 h, 24 h, 5 days and 2 weeks to determine the changes in lesion activity. Changes in the time-resolved thermal dehydration curves were monitored. Samples were sectioned (100–200 µm thick) and images using high resolution digital microscopy (Keyence) and three-dimensional micro computed tomography (µCT) were acquired to examine the penetration of the product into enamel and dentin lesions. Analysis showed that the area enclosed by the time-intensity curve, ΔQ, measured with thermal imaging decreased significantly (p < 0.05) after application of SDF. The mean ΔQ dropped from 2297 (SD 501.1) to 985 (SD 647.8) after 1h, 319 (SD 84.3) after 24 h. After 4–5 d the mean ΔQ went slightly up to 473 (SD150.7) and then down to 312 (SD 42.9) after 2 weeks. OCT showed the formation of a superficial layer of silver precipitation that faded with time to reveal a hypermineralised transparent layer underneath in some samples. The new technologies used in this study provide for the first time a method to evaluate the lesion activity after SDF application. These results demonstrate that thermal imaging and OCT may be ideally suited for non-destructive lesion activity assessment and for monitoring the effectiveness of the application and the need for re-application.

## The Association Between Long-Term Corticosteroids Therapy in Transplant Recipients and Dental Erosion and Tooth Decay

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Corticosteroids are part of the management of many pathological conditions and are widely used for their immunosuppressive properties in conjunction with immunosuppressant drugs in transplant recipients, to prevent the rejection. For transplant patients, taking medications is a regular part of the daily routine, with variable dosage. The long-term corticosteroids therapy side effects on the dentition, such as tooth decay and erosion, have been

a subject of debate in the research. The present study aimed to evaluate the association between the corticosteroid administration and dental erosion manifestation and to record the potential correlation between the severity of dental erosion and different drugs dosage. Furthermore, the prevalence of tooth decay was calculated. Eighty transplant patients were included. The degree of dental erosion was calculated using the Basic Erosive wear Examination (BEWE) index, recording the most severely affected surfaces of each sextant. In addition, the decayed-missing-filled teeth (DMFT) index was used to assess dental caries prevalence. The corticosteroid daily dosage was recorded. To establish the correlation between the variables, the Pearson's correlation was conducted, and descriptive statistical analysis of results was performed. There was a statistically significant correlation between corticosteroid administration and dental erosion, with a p-value <0.01. Transplant patients taking corticosteroids may be at risk to develop dental erosion, with major exposure to dental caries and enamel fracture.

## Fluoride

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### Dose-Response Cariostatic Efficacy of Combined Stannous and Fluoride Ions: An In-Vitro Ise Study

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Stannous ions, individually and in combination with fluoride ions, are used in oral health care products to reduce demineralization in enamel and dentine. Computer interfaced ion selective electrodes (ISEs) are used to continuously monitor calcium ( $\text{Ca}^{2+}$ ) release rate as a proxy for demineralization rate in artificial demineralization studies. The aim was to use ISEs to investigate the dose-response cariostatic efficacy of stannous ions as a function of concentration, with and without fluoride ions present, from enamel and dentine surfaces. Three dentine and three enamel blocks were coated with nail varnish leaving windows 2mm x 2mm exposed. Caries-simulating acetic acid solutions (pH = 4.0) were prepared and the blocks immersed. ISEs were used to measure  $\text{Ca}^{2+}$  concentrations every 10 s for 1 h throughout demineralization. Demineralization solutions containing tin fluoride ( $\text{SnF}_2$ , at concentrations: 0, 3.22, 6.25, 9.10 ppm) and tin acetate ( $\text{SnAcetate}$ , at concentrations: 0, 0.32, 0.62, 0.91 ppm) were also prepared. Each block was then immersed sequentially into each of these demineralization solutions for 1 h each. ISE calcium release measurements continued throughout. The percentage demineralization inhibition was calculated following each treatment on each substrate. The percentage demineralization inhibition ( $y$ ) was plotted as a function of log of  $\text{Sn}^{2+}$  concentration ( $x$ ). All experiments were carried out at  $25.0 \pm 2.0^\circ\text{C}$ . For  $\text{SnF}_2$  the dose-response plots had gradient: for enamel  $y = 1.87\ln(x) + 74.9$  ( $r^2 = 0.8$ ), for dentine  $y = 22.6\ln(x) + 16.8$  ( $r^2 = 0.99$ ). For  $\text{SnAcetate}$ , the dose-response plots had gradient: for enamel:  $24.56\ln(x) + 69.7$  ( $r^2 = 0.99$ ), for dentine,  $y = 39.7\ln(x) + 99.1$  ( $r^2 = 0.92$ ). Stannous ions are effective inhibitors of enamel and dentine demineralization, and this follows a

log-linear dose response similar to that seen for fluoride ions. The demineralization inhibition efficacy of stannous ions is further enhanced by fluoride ions, suggesting different cariostatic mechanisms of each ion.

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### Reactivity of Commercial Fluoride Mouthrinses with Carious Enamel and Dentine

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The formation of reaction products on enamel and dentine is one of the mechanisms of action of fluoride mouthrinses on caries control. This chemical reaction is dependent on the fluoride concentration, pH, and type of fluoride salt. Given that commercial fluoride mouthrinses differ in fluoride concentration, pH and fluoride type, we evaluated the ability of nine mouthrinses; eight containing NaF (96.5-233.5 ppm F) and one with  $\text{Na}_2\text{FPO}_3$  (221.7 ppm F), and pH ranging between 4.26-7.66, to form loosely (" $\text{CaF}_2$ ") and firmly (FAp) bound fluoride in carious enamel and root dentine. Enamel and dentine slabs with chemically-induced caries lesions ( $n = 15/\text{group}$ ) were sectioned in halves. One half was used as control and the other was treated for 10 min with the assigned mouthrinse. " $\text{CaF}_2$ " and FAp were extracted with alkali and acid, respectively, and fluoride in the extracts was determined with F-ISE by the direct technique. The amount of fluoride found in the treated half was subtracted from the control half and the results for " $\text{CaF}_2$ " and FAp formed in each dental substrate were expressed as  $\mu\text{g F}/\text{cm}^2$ . Data for enamel and dentine were separately analyzed by ANOVA followed by Tukey's test ( $p < 0.05$ ). The concentration of " $\text{CaF}_2$ " formed ranged from 3.2-36.2 and 5.1-55.0  $\mu\text{g F}/\text{cm}^2$ ,

respectively, for enamel and dentine. Greater reactivity ( $p < 0.05$ ) was found for mouthrinses presenting higher NaF concentration and lower pH, for enamel and dentine. The  $\text{Na}_2\text{FPO}_3$  product presented the lowest reactivity ( $p < 0.05$ ). The concentration of FAP formed ranged from 0.36–1.66 and 0.90–3.28  $\mu\text{g F/cm}^2$ , respectively, for enamel and dentine. In conclusion, caries control by mouthrinses may be dependent on fluoride concentration, pH and fluoride type.

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### Effect of Different Toothbrushing Routines on Fluoride Concentration in Saliva

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This study aimed to evaluate the effect of different toothbrushing routines and different kinds of toothpaste on the interproximal fluoride concentration after toothbrushing and its clinical relevance to the recommendations given to patients regarding the process of toothbrushing. Eight adults participated totally 8 times in order to test different toothbrushing routines with different amount of toothpaste (1 or 2 cm), duration (1 or 2 min) and amount of water after toothbrushing (10 or 20 ml). Another 8 adults participated 6 times in total to test different forms of administration (paste, gel, foam) of toothpaste with different amount of water after toothbrushing (no rinsing or 10 ml). Interdental saliva samples were collected from the proximal sites 25/26 and 46/45 using small paper points, before and up to 60 min after toothbrushing. The fluoride concentration was measured by an ion-specific electrode. The area under the curve, saliva fluoride concentration versus time, was calculated. Differences between the groups were tested by ANOVA with Tukey's multiple comparisons test. An increase of fluoride concentration with 47.2% was observed when the amount of toothpaste increased ( $p < 0.01$ ), with 41.2% when reducing the amount of water rinsing ( $p < 0.01$ ) and with 26.8% when increasing the duration ( $p < 0.01$ ). The paste and gel resulted in higher fluoride concentration ( $p < 0.01$ ) compared to foam. These findings suggest that the amount of toothpaste, duration and amount of water have a significant effect on fluoride concentration after toothbrushing. Furthermore, despite the lower amount of fluoride, the gel gives almost the same fluoride concentration after toothbrushing as the toothpaste. The results indicate the importance of giving clear advice to patients regarding the process of toothbrushing.

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### A randomized Clinical Trial on the Anti-Plaque and Anti-Gingivitis Effect of 3 Dentifrices

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Plaque-induced gingivitis is the second most common oral disease after dental caries. Gingivitis begins in the childhood and its prevalence increases with age. Self-performed mechanical plaque removal is a proven method of controlling plaque and gingival disease. However, tooth brushing and flossing are difficult tasks and many patients might not be able to completely remove plaque on all dental surfaces. For this reason, antimicrobial agents have been added to dentifrices in order to enhance the efficacy of mechanical tooth-cleaning procedures. The aim of the study was to evaluate antiplaque and antigingivitis properties of 3 different commercially available dentifrices. Twenty-five subjects, aged between 18 and 55 years, were selected for a randomized, double-blind, three-treatment crossover design study performed in 3 experimental phases of 14 d, each followed by a 5 d wash-out interval. Patients were allocated in three groups and instructed to use different dentifrices: 1) an herbal dentifrice (HD); 2) a calcium sodium phosphosilicate bioactive glass dentifrice (CSPS) and 3) a Zn-CHA nanocrystal-based dentifrice (CHA). Measurements of plaque (FMPS), bleeding index (FMBS), antibacterial activity (salivary sampling) and patient's appreciation (VAS) were recorded at the different time intervals. All subjected showed an improvement in FMPS and FMBS. Groups using HD and CSPS, however, demonstrated a more pronounced antibacterial activity compared to group using CHA ( $p < 0.05$ ). Moreover, HD group showed higher VAS than the others ( $p < 0.05$ ). Thus, the herbal dentifrice, even if do not contain antimicrobial agents, can be recommended for improving bleeding and controlling bacterial plaque, being also highly appreciated by the patients.

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### Bias Risk Assessment in References of the 2013 German Guidelines for Caries Prevention Using Fluoride

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Risk of bias is a serious concern in evidence-based science. High risk of bias tends to falsely enlarge the effect size of interventions, which can make the results unreliable. However, risk of bias assessment has almost completely been ignored in the development of guidelines and recommendations until recently. As there is still a debate about the most appropriate form of fluoride administration for caries prevention in early childhood in Germany due

to different interpretation of the underlying studies: Analyzing these studies under the scope of risk of bias might aid in solving some controversies regarding the best method for fluoride use to prevent dental caries in children. Aim was the assessment of risk of bias in the cited papers of the latest German guidelines for caries prevention using fluoride in children. Cited papers were divided into categories according to study design and assessed using appropriate tools: RoB 2 for randomized clinical trials, ROBINS-I for non-randomized trials, and ROBIS for systematic reviews. 58 out of 80 studies remained for assessment after exclusion of double, non-accessible and non-assessable papers. "High" risk of bias was found in 14 out of 29 randomized clinical trials, all the included 13 non-randomized trials, and 3 out of 16 systematic reviews. None of the 7 studies regarding fluoride tablets was assessed with "Low" risk of bias, while the 6 included Cochrane reviews had a "Low" risk of bias. The high risk of bias in the assessed papers shows that researchers and authors of guidelines or recommendations need to have intensified understanding of "risk of bias" and its sources, as well as train to use the assessment tools to raise the reliability of studies' outcomes and clinical recommendations.

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### Evaluation of Fluoride in Saliva After Brushing with Brazilian Red Propolis Toothpaste

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This study aimed to evaluate the availability of fluoride after use of a toothpaste incorporated with brazilian red propolis (BRP, INPI Patent Number BR1020170110974) and to compare it to a fluoride toothpaste in healthy participants. This study was conducted in a double-blind, randomized, controlled, and crossover design. Following a washout period with non-fluoridated toothpaste for three days, participants brushed with the common fluoride toothpaste, followed by three more washout days and a new brush with the propolis toothpaste. Saliva samples of participants were collected at the times: 0 - baseline and 5, 15, 30, 45 and 60 min after brushing with each toothpaste. The samples were electrochemically analyzed for fluoride (ISE) using the method described by Cury et al. [Braz.Dent.J,2010; 21:396-400]. Salivary fluoride concentrations per sampling time point were submitted to the Kolmogorov-Smirnov normality test and compared by Wilcoxon and Mann-Whitney tests between both toothpastes. The value of  $\alpha$  was set at 0.05 in all cases. Salivary fluoride concentrations showed no statistically significant difference when comparing the two treatments ( $p > 0.05$ ). After 1 h, all available fluoride concentrations in

saliva decreased, with no significant difference between BRP and common fluoride dentifrice treatment samples ( $p > 0.05$ ; two-way ANOVA). The results showed that, after 1 h of brushing with the different toothpastes, there was no difference between the fluoride concentrations analyzed, suggesting that the propolis in the toothpaste did not interfere with the kinetics and bioavailability of Fluoride ion in saliva samples.

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### Fluoride and Carbohydrates in Diet and Dental Caries in Adolescents from the ELEMENT Cohort Study

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A positive association between intake of carbohydrates and dental caries has been well established; this association is modified by fluoride. This cross-sectional study aimed to examine whether dietary intake of carbohydrates and fluoride were associated with dental caries in 402 adolescents from the ELEMENT birth cohort in Mexico City. An oral examination was performed evaluating dental caries using the International Caries Detection and Assessment System (ICDAS). Usual dietary intake of daily fluoride and total carbohydrates were estimated with a semi-quantitative Food Frequency Questionnaire (FFQ). Negative binomial models were run to estimate associations between the number of caries lesions, dietary fluoride and carbohydrate intake, adjusted for age, sex, Body Mass Index Z-score, SES, age of tooth brushing initiation, and energy intake. The participants' mean age was 14.5 (SD 1.7) years; 80% presented dental caries. Mean scores for D1MFT and D4MFT were 6.2 (SD 5.2) and 0.67 (SD 1.32), respectively. The median intake of fluoride reported in the FFQ was 0.015 mg/kg/d (SD 0.007); the intake was statistically higher in those participants with a D4MFT = 0 than those with a D4MFT > 0 (0.90 vs 0.82 mg/d,  $p = 0.02$ ; 0.016 mg/kg/d vs 0.014 mg/kg/d,  $p = 0.04$ ). For D1MFT, D1MFS, D4MFT and D4MFS scores, there was a statistically significant reduction in the number of lesions with higher fluoride consumption (mg/d) from foods and beverages (fluoridated table-salt was not taken into account). Total carbohydrate intake 332 g/d (SD 145) was positively associated to the number of lesions. In conclusion, higher fluoride intake through foods and beverages was associated to lower ICDAS scores; this effect was seen even when the dietary intake of fluoride was 0.015mg/kg/d, which is lower than the average intake recommendation. In contrast, a higher amount of total carbohydrates intake was associated with a larger number of caries lesions.

### Fluoride Varnish Containing Nano-Sized Sodium Trimetaphosphate Reduces Enamel Demineralization In Vitro

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The aim of the present study was to assess the effect of fluoride (F) varnishes supplemented with nano-sized sodium trimetaphosphate (TMP) on enamel demineralization in vitro. Bovine dental enamel blocks (n = 48) were selected by surface hardness (SH) and randomly assigned into 4 experimental groups, according to the varnishes to be tested: Placebo (without F or TMP - negative control); 5% NaF (positive control); 5% NaF + 5% micrometric TMP; and 5% NaF + 5% nano-sized TMP, hereafter abbreviated as PLA, 5NaF, 5TMPmicro and 5TMPnano, respectively. Blocks received a single application of the varnishes and were immersed in a remineralizing solution for 6 h. Varnishes were then removed, and the blocks were transferred to a demineralizing solution (for 6 h) and to a remineralizing solution (18 h). This cycle was repeated for 5 d. After the experimental phase, SH was determined again, allowing the calculation of the percentage of SH loss (%SHL). Following, blocks were longitudinally sectioned for the determination of sub-surface hardness loss ( $\Delta$ KHN). Data presented normal and homogeneous distribution, and were submitted to one-way ANOVA, followed by Student-Newman-Keuls' test ( $p < 0.05$ ). Significant differences were observed among all test groups, for both variables analyzed. The lowest %SHL values were observed for 5TMPnano (-22.5%), followed by 5TMPmicro (-28.3%), 5NaF (-44.0%) and PLA (66.2%) groups. The same trend was observed for  $\Delta$ KHN values, with the corresponding mean values of 1,821.9, 3,186.4, 3,671.4 and 4,084.4, respectively for 5TMPnano, 5TMPmicro, 5NaF and PLA. It was concluded that the addition of TMP to a conventional, colophony-based F varnish significantly enhanced its protective effects against enamel demineralization in vitro, and that the use of nanoparticles further increased such effects.

### Effect of Different Dentifrices Containing Either Bioglass with Low Fluoride or High Fluoride on Artificial Root Caries

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The objective of this laboratory-based study was to compare the efficacy of different fluoride concentration dentifrices either with fluoride+bioglass or fluoride alone on artificial root caries. The crowns of two extracted teeth without any dental caries were cut by leaving root surfaces and divided into four pieces (n = 8). One sample used for baseline, seven were stored in demineralisation

solution (pH = 4.8) at 37°C for five days to develop artificial root caries. Each sample received standardised toothbrushing twice daily with assigned dentifrices: Group 1. Bioglass+540 ppmF, Group 2. 5,000 ppmF, Group 3. 1,450 ppmF, and Group 4. No treatment. Subsequently, all samples were immersed in pH-cycling model by alternating between demineralisation (pH = 4.8) and remineralisation solutions (pH = 7) at 37°C for 13 d. Knoop micro-hardness measurements (HK) and non-destructive sequential 3D X-ray microtomography (XMT) were performed at baseline, after the development of artificial caries, and following the 13 d pH cycling. The HK results showed that there were significant changes in the HK for each group ( $p < 0.001$ ). Mean  $\pm$  SD values of all samples were  $47.90 \pm 11.70$  at baseline and after the development of artificial caries was  $13.3 \pm 1.61$ . Following the 13-day of pH cycling, bioglass with 540 ppm F group had HK of  $47.41 \pm 10.04$ , whilst HK in 5,000 ppm fluoride group was  $46.80 \pm 7.28$ , 1,450 ppm fluoride group had  $46.04 \pm 11.50$  and the HK in control group was  $42.13 \pm 5.79$ . XMT showed an increase in mineral concentrations within artificial root carious lesions in both bioglass with 540 ppm fluoride and 1,450 ppm fluoride ( $p < 0.001$ ). In conclusion, bioglass with low fluoride dentifrice rehardens root dentine on artificial initial root carious lesions in the HK test, and it is confirmed by XMT, however, these dentifrices need to be assessed with a large sample size on natural root carious lesions.

### Effect of pH on Fluoride Formation in Enamel and Dentin Sound and Carious

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Total fluoride (TF) formed on enamel or dentine could be used instead of CaF<sub>2</sub>-like sub-product of reaction as indicator of the anticaries potential of professional fluoride application, but this topic has not been explored. We evaluated the formation of TF, CaF<sub>2</sub>-like and FAp formed in slabs of enamel (E) and dentin (D), sound (S) and carious (C). Enamel and dentin bovine slabs (4x4x2 mm) were obtained. Sound slabs were selected by surface hardness. For carious slabs, firstly caries-like lesions were induced by immersion in demineralizing solution for 16 h and the carious slabs obtained were selected by %SHL. Therefore, 16 slabs of ES, EC, DS and DC were randomized to neutral (NF, pH = 6.8) or acidulated phosphate fluoride (APF, pH = 4.5) solutions containing 10,000  $\mu$ gF/ml (as NaF). Slabs were divided in half for paired test; 16 hemi-slabs were subjected to the treatments and the other 16 were control. Treatments were made during 4 min. TF was determined in 8 hemi-slabs control and 8 treated. CaF<sub>2</sub>-like and FAp were determined in the others 8 hemi-slabs. For TF determination, hemi-slabs were subjected to sequential six acid-etching extractions in 0.5 M HCl and subsequent addition of TISAB II (50% v/v in 0.5 M NaOH). For CaF<sub>2</sub>-like and FAp, hemi-slabs were firstly immersed in 1 M KOH during 24 h for CaF<sub>2</sub>-like extraction, followed by addition of TISAB II (50% v/v in 1 M HCl). Further, FAp was extracted as described for TF. The depth ( $\mu$ m) of fluoride reaction was calculated based on phosphate concentration found

in the extracts. Fluoride extracted was determined with F-ISE. Results found for the treatments were subtracted from controls. Data were analyzed by two-way ANOVA and Tukey test ( $\alpha = 5\%$ ). Fluoride reacted on up to 30  $\mu\text{m}$  from the surface for each substrate. The highest ( $p < 0.05$ ) TF,  $\text{CaF}_2$ -like, and FAp concentrations (mean  $\pm$  SD;  $n = 8$ ) were found for DC treated with APF ( $0.71 \pm 0.34$ ,  $493.5 \pm 318.9$ , and  $0.10 \pm 0.06$ , respectively). A positive linear correlation was observed between FT and  $\text{CaF}_2$ -like for DS ( $r = 0.711$ ,  $p = 0.002$ ) and DC ( $r = 0.815$ ,  $p < 0.01$ ). TF determination may be able either to differentiate the reactivity of vehicles for professional fluoride application with E and D, and to differentiate these vehicles by the depth of fluoride reaction.

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### TSF Concentration but not TF is an Indicator of Fluoride Bioavailability from MFP/ $\text{CaCO}_3$ Toothpaste

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Analytical methods of fluoride determination in toothpaste should be able not only to indicate how much of the total fluoride (TF) is released into the oral cavity during the time of tooth brushing or post brushing [Martinez-Mier et al.: *Caries Res* 2019;53:119–136], but should also estimate the systemic effect of fluoride if ingestion occurs. Therefore, we evaluated if the chemical determination of TF and total soluble fluoride (TSF) in  $\text{Na}_2\text{FPO}_3/\text{CaCO}_3$  toothpaste by Cury's protocol [Cury et al.: *Braz Dent J* 2010;21:396–400] is valid for these purposes. Data of two clinical studies ( $n = 10$  volunteers) done with fresh and aged toothpaste Sorriso Dentes Brancos<sup>®</sup> ( $\text{Na}_2\text{FPO}_3/\text{CaCO}_3$ , 1450  $\mu\text{g}$  F as TF/g) were analyzed. The first study evaluated fluoride bioavailability in saliva during toothbrushing (local effect) and the second determined fluoride in blood after toothpaste ingestion (systemic effect). The TSF concentrations in the fresh toothpaste and in the three aged samples were respectively: I = 1,378.2; II = 1,160.9; III = 900.0 and IV = 597.2  $\mu\text{g}$  F/g for the first study and I = 1,334.0; II = 1127.5; III = 807.9 and IV = 686.6  $\mu\text{g}$  F/g for the second study. TF and TSF concentrations ( $\mu\text{g}$  F/ml) in the toothbrushing residues (TR = toothpaste slurry+saliva) and area under the curve of fluoride concentration in blood ( $\text{AUC} = \text{ng F/ml} \times \text{min}$ ), respectively for the 1st and 2nd study, were correlated with TF and TSF found in the toothpaste. The TR (mean  $\pm$  SD) values were: I = 196.6  $\pm$  47.5; II = 199.0  $\pm$  35.2; III = 188.3  $\pm$  22.7; IV = 178.0  $\pm$  13.5 and I = 206.1  $\pm$  59.0; II = 168.7  $\pm$  31.9; III = 113.6  $\pm$  24.8; IV = 59.1  $\pm$  13.1 for TF and TSF, respectively. The AUC values were: I = 17,725.6  $\pm$  4,622.2; II = 15,517.9  $\pm$  2,288.5; III = 10,720.3  $\pm$  3,169.7 and IV = 6,682.9  $\pm$  4,189.0. Statistically significant correlations were found between TSF in the toothpaste with TR ( $r = 0.85$ ;  $p = 0.0001$ ) and with AUC ( $r = 0.69$ ;  $p < 0.0001$ ), but not for TF ( $p > 0.05$ ). The TSF concentration found in  $\text{Na}_2\text{FPO}_3/\text{CaCO}_3$ -based toothpaste by Cury's protocol is an indicator of local and systemic fluoride bioavailability.

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### Fluoride Soluble in HCl 0.01 N Found in $\text{Na}_2\text{FPO}_3/\text{CaCO}_3$ -Based Toothpaste Indicates Fluoride Systemically Bioavailable

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Regarding fluorosis risks, the chemical determination of fluoride concentration in toothpastes should indicate how much of the total fluoride (TF) would be systemically bioavailable. We hypothesized that fluoride soluble in 0.01 N HCl (TSF), found in  $\text{Na}_2\text{FPO}_3/\text{CaCO}_3$ -based toothpaste, but not TF fits this assumption. An in vivo crossover study of four phases was conducted, during which 10 participants ingested an amount of toothpaste equivalent to 70.0  $\mu\text{g}$  of TF/kg body weight from the toothpaste Sorriso Dentes Brancos<sup>®</sup> ( $\text{Na}_2\text{FPO}_3/\text{CaCO}_3$ , 1450  $\mu\text{g}$  F of TF/g), fresh and aged. The treatment groups were: I- Fresh sample (1332.5  $\mu\text{g}$  F/g as TSF); II to IV: Aged samples containing 1113.5, 796.6 and 667.4  $\mu\text{g}$  F/g as TSF, respectively. Blood was collected before (baseline) and up to 180 min after toothpaste ingestion. Total urine (24 h before and 24 h after ingestion) was collected. Fluoride in plasma and urine was determined with F-ISE, using inverted-electrode or conventional one, respectively. TF and TSF concentration in the toothpastes were determined by Cury's protocol, but the samples were prepared with 0.01 N HCl. The areas under the curves of absorption ( $\text{AUC} = \text{ng F/ml} \times \text{min}$ ) and the peak of maximum fluoride ( $\text{ng F/ml}$ ) concentration ( $C_{\text{max}}$ ) were calculated. Data were analyzed by regression analysis ( $p < 0.05$ ). The mean ( $\pm$  SD) values for AUC were: 17,725.6  $\pm$  4,622.2; 15,517.9  $\pm$  2,288.5; 10,720.3  $\pm$  3,169.7 and 5,430.9  $\pm$  2,511.1, respectively for groups I to IV. For  $C_{\text{max}}$  were, respectively: 176.8  $\pm$  51.2; 147.1  $\pm$  27.0; 100.5  $\pm$  29.2 and 71.8  $\pm$  13.6. The correlations between AUC vs. amount (mg) of TSF ingested, and  $C_{\text{max}}$  vs. TSF were:  $r = 0.6838$ ;  $p < 0.0001$  and  $r = 0.7200$ ;  $p < 0.0001$ , respectively. For TF, the correlations were not statistically significant ( $p > 0.05$ ). AUC and  $C_{\text{max}}$  data were supported by urine fluoride analysis. The findings found give support to our hypothesis.

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### In-Vitro Assessment on the Effect of Silver Diamine Fluoride on Natural Carious Dentin Microhardness

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This study aimed to assess (1) the effect of silver diamine fluoride (SDF) on natural carious dentin microhardness and (2) the correlation between tactile sensation (TS), fluorescence (F) and microhardness (VMH) on carious dentin. Carious teeth ( $N = 90$ ) scored ICDAS 4-6 were sliced longitudinally exposing carious dentin on one side and sound dentin on the other. Both sides were assessed by TS (soft, leathery, firm), F (red, pink or no fluorescence) (SIROinspect, Dentsply Sirona, USA) and Vicker's micro-

hardness (VMH). Samples were randomized into three groups (n = 30): No SDF treatment (Control/Group A); 38% SDF (Advantage arrest, Elevate Oral Care, USA) (Group B); and SDF with KI (Riva Star, SDI, Australia) (Group C). All samples were stored for 1 week under 100% humidity at 37°C and re-assessed for TS, F and VMH. Average microhardness change ( $\Delta$ VMH) for sound and carious surfaces were statistically significantly higher for experimental groups (Group B sound: 20.22±11.98 HV; carious: 19.76±9.35 HV/ Group C sound: 14.26±10.11 HV; carious: 22.51±7.67 HV) than for the control group (sound: -7.34± 8.28 HV; carious: -0.69± 3.53 HV). A mixed ANOVA test showed only a statistically significant  $\Delta$ VMH difference between experimental and control groups (p<0.001). The Spearman rank order correlation showed a statistically significant negative correlation between  $\Delta$ VMH and  $\Delta$ T (rs = -0.588, p <0.001) and between  $\Delta$ VMH and  $\Delta$ F(rs = -0.269, p = 0.01). There was a statistically significant positive correlation between  $\Delta$ T and  $\Delta$ F(rs = 0.226, p = 0.032). Microhardness of SDF treated dentin surfaces increased as compared to non-SDF treated surfaces. Addition of KI had no impact on microhardness. Furthermore, an increase in dentin microhardness was correlated to decrease in fluorescence and firmer tactile sensation.

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### Fluoride Determination in Toothpastes with F-ISE by the Direct Technique

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Total fluoride (TF) and total soluble fluoride (TSF) concentration in toothpastes have been determined with fluoride ion-specific electrode (F-ISE) by the direct technique [Cury et al.: Braz Dent J. 2010;21(5):396-400]. However, the relevance of the parameters set in the protocol is unknown. Based on this protocol, we evaluated the effect of: (i) amount of toothpaste on TF concentration in the slurry (0.25% to 4% w/v), (ii) centrifugation speed (1,000 to 12,000 g for 10 min) and (iii) centrifugation time (1 to 10 min at 1,000 and 3,000 g) of 1% slurry on TSF concentration in the supernatant, and (iv) incubation time (15 to 60 min at 45°C) for  $\text{FPO}_3^{2-}$  hydrolysis and insoluble-F dissolution. TF and TSF concentrations of fresh and aged  $\text{Na}_2\text{FPO}_3/\text{CaCO}_3$  (1,450  $\mu\text{g F/g}$ ), fresh  $\text{NaF/SiO}_2$  (1,100  $\mu\text{g F/g}$ ), and  $\text{SnF}_2/\text{SiO}_2$  (1,100  $\mu\text{g F/g}$ ) were determined (n = 9 repetitions). Fluoride concentration was expressed as  $\mu\text{g F/g}$  and the % of hydrolyzed  $\text{FPO}_3^{2-}$  and dissolved insoluble-F for  $\text{Na}_2\text{FPO}_3/\text{CaCO}_3$  samples were calculated. Data was analyzed by one-way ANOVA followed by Tukey's test. The increase of the toothpaste slurry concentration significantly reduced TF concentration found (p<0.05). At 1% slurry, TF concentration ( $\mu\text{g TF/g}$ ; mean±SD) found was: fresh  $\text{Na}_2\text{FPO}_3/\text{CaCO}_3$ : 1,460.8±28.5, aged  $\text{Na}_2\text{FPO}_3/$

$\text{CaCO}_3$ : 1,477.5±26.0,  $\text{NaF/SiO}_2$ : 1,056.2±18.8, and  $\text{SnF}_2/\text{SiO}_2$ : 1,057.6±21.3. For  $\text{Na}_2\text{FPO}_3/\text{CaCO}_3$  toothpastes tested: centrifugation at 1,000 g for 5-min was enough to precipitate insoluble-F; at 30-min incubation, more than 99% of  $\text{FPO}_3^{2-}$  was hydrolyzed and around 97% of insoluble-F was dissolved. The present study confirms the validity of the parameters set in the direct technique to determine TF and TSF concentration in different toothpaste formulations and shows that the centrifugation speed and time, and the incubation time could be reduced for  $\text{Na}_2\text{FPO}_3/\text{CaCO}_3$ -formulations.

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### In Vitro Fluoride Release from Toothpastes as Indicator of Bioavailability during Toothbrushing

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How much fluoride a toothpaste-formulation releases during toothbrushing should be evaluated as a pre-test of its anticaries potential. We developed an in vitro test of fluoride release from toothpaste and conducted a pilot in vivo study to test if it could indicate oral fluoride bioavailability during toothbrushing. A crossover in vivo study was conducted in three phases during which three volunteers brushed their teeth for 1 min with 0.7 g of MFP/ $\text{CaCO}_3$ -based (1450 ppm F) and two  $\text{NaF/SiO}_2$ -based (1100 or 1450 ppm F) toothpastes. The slurry toothpaste-saliva produced during toothbrushing was collected for fluoride determination. The in vitro test (n = 6) evaluated how much fluoride was released when 4 g of each toothpaste placed in the bottom of a flask and immersed in 12 mL of purified water was mechanically disturbed for 1-min at 200 rpm. Total fluoride (TF) and total soluble fluoride (TSF) were determined in the samples with ion-specific electrode (F-ISE). TF and TSF concentrations were also determined in the toothpaste with F-ISE to calculate the amount of fluoride subjected to the tests and the respective percentage released. The data were analyzed by one-way ANOVA followed by Tukey's ( $\alpha = 5\%$ ), and by correlation between %F-released-in-vivo vs. in-vitro. The %FT-released-in-vivo (mean ± SD; p<0.05) ranged from 58.1 ± 2.0 to 80.1 ± 2.4; in-vitro from 54.7 ± 3.1 to 71.8 ± 2.0; and %FST-released-in-vivo ranged from 51.3 ± 2.0 to 86.8 ± 2.2; in-vitro from 57.0 ± 8.9 to 74.9 ± 2.0. Correlations high and statistically significant were found between in vivo vs. in vitro data, either for %FT (r = 0.84; p = 0.0044) or %FST (r = 0.91; p = 0.0005). The data suggest that the in vitro model developed may indicate fluoride bioavailability from toothpaste during toothbrushing.

## Twenty-Four Weeks Clinical Evaluation of the Efficacy of Three Different Biomaterials Used as Desensitizing Agents

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The present randomized clinical trial aimed to compare the efficacy in reducing dentin hypersensitivity (DH) of a dentifrice formulation containing nano-hydroxyapatite Cavex ExSense (CES), with a Calcium Phosphate Carbonate Citrate Fluoride dentifrice Curasept Biosmalto Mousse (CBM) and a placebo (PI). Ninety subjects were recruited to participate in the study. A computer-generated random table with blocking to one of the three study treatments was used in order to have 30 subjects per group:

CES; CBM; PI. All the test materials were applied for 10 min on the surface of sensitive teeth by means of a tray. DH was evaluated using the evaporative sensitivity, tactile sensitivity tests, and assessed using a visual analogue scale (VAS). Response was recorded before the application of the materials (Pre-1), immediately after (Post-0), at 1 week (Post-1), 4 weeks (Post-2), 12 weeks (Post-3) and 24 weeks (Post-4). Statistical differences in VAS were assessed using the Kruskal–Wallis analysis at the different time-points ( $P < 0.05$ ), adjusting statistical significances for multiple comparisons (Bonferroni correction). Significantly lower values of cold air sensitivity and tactile sensitivity ( $p < 0.05$ ) were found for the group CES and CBM until 24-weeks control. In addition, statistically significant ( $p < 0.05$ ) lower values of sensitivity were reported for CBM group compared to those groups until Post-4 control. All the test materials decreased DH after 24 weeks in comparison to Pre-1 and in the control groups. Control group (PI) has shown no efficacy. The application of biomaterial based toothpaste are effective desensitizing agents, and CBM has proven to be the most reliable material in the clinical relief of dentin hypersensitivity.

## Microbiology

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### Antibiofilm and Anti-Caries Effects of Experimental Mouthrinses Containing Natural Extracts Under Microcosm Biofilm on Enamel

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This work evaluated the effect of experimental mouthrinses containing natural extracts on the lactic acid production and CFU counting 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, at 5% CO<sub>2</sub> and 37° C, for 5 days. The biofilm was daily treated with the mouthrinses (1 min): 1. *Vochysia tucanorum* Mart. (2.5 mg/ml), 2. *Myrcia bella* Cambess. (1.25 mg/ml), 3. *Matricaria chamomilla* L. (20 mg/ml), 4. Malvatricin<sup>®</sup> Plus–Daudt, 5. PerioGard<sup>®</sup>-Palmolive (Positive control) and 6. PBS (Negative control) (n = 9/group). Lactate concentrations were evidenced by enzymatic method and CFU counting by mL of total microorganisms, total streptococci, *Lactobacillus* sp. and *Streptococcus mutans* was done. Enamel demineralization was analyzed by transverse microradiography. Malvatricin<sup>®</sup> Plus (1.10±0.24 mmol/l) and PerioGard<sup>®</sup> (0.60±0.18 mmol/l) mouthrinses significantly reduced lactic acid production compared to PBS (2.61±1.27 mmol/l) (ANOVA/Tukey, p<0.0001). Malvatricin<sup>®</sup> Plus (7.68±0.15 log<sub>10</sub> CFU/ml) and PerioGard<sup>®</sup> (6.99±0.23 log<sub>10</sub> CFU/ml) treated-biofilm presented significant lower CFU for total microorganisms compared to PBS (7.93±0.14 log<sub>10</sub> CFU/ml) (ANOVA/Tukey, p<0.0001). Only PerioGard<sup>®</sup> [6.56(0.29) log<sub>10</sub> CFU/ml] significantly reduced *Lactobacillus* sp compared to PBS [7.64(0.08) log<sub>10</sub> CFU/ml]. CFU for total streptococci was significantly reduced by *Matricaria chamomilla* L. [7.58(0.08) log<sub>10</sub> CFU/ml], Malvatricin<sup>®</sup> Plus [7.59(0.09) log<sub>10</sub> CFU/ml] and PerioGard<sup>®</sup> [7.16(0.19) log<sub>10</sub> CFU/

ml] compared to PBS [7.91(0.19) log<sub>10</sub> CFU/ml]. *Streptococcus mutans* was significantly reduced only by PerioGard<sup>®</sup> (p<0.0001). Malvatricin<sup>®</sup> Plus, PerioGard<sup>®</sup> and *M. chamomilla* L. were able to significantly reduce mineral loss (2245 to 3730 %vol.µm) compared to PBS (6151.3 %vol.µm) (ANOVA/Tukey, p<0.0001). *M. chamomilla* L. was the only experimental natural agent with antibiofilm and anti-caries effects under this model.

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### Antimicrobial Efficacy of S-PRG Containing Toothpastes on S. Mutans Biofilm Growth and Development

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Surface pre-reacted glass-ionomer particles (S-PRG) release ions that may promote antimicrobial effect on cariogenic biofilm, therefore, its addition to toothpastes would be beneficial for caries prevention. The objective of this study was to investigate the S. mutans biofilm inhibition potential and the antibacterial activity on newly-formed and mature S. mutans biofilms of experimental dentifrices containing different concentrations of S-PRG. Bovine enamel samples (35 for each experiment) were randomly allocated into the groups (n = 5): toothpastes containing 0% (placebo); 1%; 5%; 20% and 30% of S-PRG; positive control dentifrice (NaF+triclosan); and negative control (distilled water). To evaluate the biofilm inhibitional potential, samples were previously treated with toothpaste suspensions (5min) and placed in a 24-well plate containing artificial saliva for 4 h. Biofilm formation was performed by adding 1ml artificial saliva + 1 ml BHI broth + 225 µl of S. mutans derived from a clinical strain to each well. Samples were incubated for 48h at 37°C in 5%CO<sub>2</sub>. Biofilm was detached, seeded in Petri dishes, and CFU/ml was determined. To evaluate the effect

of dentifrices on the newly-formed and mature biofilms, treatments were applied after 4 h and 24 h of biofilm growth. Data were analyzed by ANOVA, followed by Tukey test (5%). The results of Tukey test for biofilm growth inhibition showed significant differences for the groups ( $p < 0.05$ ): water  $\leq$  placebo  $\leq 1\%$   $< 5\%$   $< 20\%$  = NaF+Triclosan  $< 30\%$ . All toothpastes containing S-PRG exhibited antibacterial effect on the newly-formed (water  $<$  placebo  $< 1\%$  = 5%  $< 20\%$   $< 30\%$   $<$  NaF+Triclosan) and mature biofilm (water = placebo  $\leq 1\%$   $< 5\%$   $< 20\%$  = 30%  $<$  NaF+Triclosan) compared to negative control group, but they were not superior than positive control dentifrice. Experimental dentifrices containing S-PRG exhibited antimicrobial activity, being promising agents to prevent the cariogenic biofilm growth and development.

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### Association of Breastfeeding Duration with Mother-to-Child Transmission of Streptococcus Mutans Strains with Collagen-Binding Activity

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Streptococcus mutans, a bacterial pathogen responsible for dental caries, is known to possess cell surface protein antigens associated with various pathogenicity. Among those, 120-kDa collagen-binding proteins (CBPs) are related to *S. mutans* collagen-binding activity and involved in not only adhesion to dentine, but also the pathogenesis of systemic diseases such as infective endocarditis. Though it is considered that *S. mutans* is generally transmitted vertically from mother to child, the route of transmission of CBP-positive strains remains unknown. In the present study, we evaluated CBP-positive *S. mutans* strains isolated from mother and child pairs, focusing on the influence of breastfeeding duration on transmission. A total of 400 *S. mutans* clinical strains were isolated from saliva samples of 40 Japanese mother-child pairs (5 strains per subject). First, PCR analysis using DNA extracted from the isolated strains was performed to identify of CBPs, followed by AP-PCR analysis to assess whether *S. mutans* obtained from a mother and her child were the same clone. In addition, the mothers were given a questionnaire concerning their history and duration of breastfeeding the child. AP-PCR demonstrated that the vertical transmission of *S. mutans* was observed in 32 pairs (80%) and complete matching of 5 obtained specimens was observed in 9 pairs (22.5%). In addition, it was demonstrated that children whose mother possessed CBP-positive *S. mutans* had a significantly higher detection rate of CBP-positive strains ( $p < 0.01$ ) and that children possessing CBP-positive strains had a significantly shorter breastfeeding period than those possessing CBP-negative strains ( $p < 0.05$ ). These results suggest that breastfeeding duration is associated with mother-to-child transmission of CBP-positive *S. mutans*.

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### A 3D printed Microfluidic Flow-Cell for Microscopy Analysis of *In Situ*-Grown Biofilms

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Metabolic processes inside dental biofilms are likely to be influenced by the velocity of the thin saliva film in contact with the biofilm. In addition, *in situ*-grown biofilms are likely to behave differently than *in vitro* biofilms. However, there is a lack of commercially available flow-cells able to incorporate *in situ* samples and adjust the saliva film to a match the oral cavity. Therefore, the aim of this study was to design and 3D-print such a microfluidic flow-cell. As a proof of concept, we studied pH developments inside three 96 h biofilms under a stimulated saliva flow. The microscope compatible flow-cell consists of an incoming port, out coming port and a central bottomless impression for the *in situ* sample. Sealing the flow-cell to a coverslip forms a bottom to the flow-cell and creates a defined flow-space between the *in situ* sample and the coverslip. The participant grew the *in situ* biofilms on custom-made glass slabs fitted into a removable splint. The splint was worn the entire time except during meals, and was immersed in 10 % sucrose three times a day. Unsterile stimulated saliva and 4 % sucrose were used as flow medium, and the pH were analyzed using the ratiometric dye C-SNARF-4. The results of this pilot study showed that after 30 min of static conditions pH ranged from 5.7 to 6.9 with differences both between fields of view (FOVs) and between biofilms. Confirming the presence of microenvironments. After the onset of flow (5 mm / min), pH rose to neutral or slightly alkaline values in all three biofilms. However, differences between FOVs were still present. In conclusion, incorporating flow is important when studying pH developments inside biofilms.

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### Effect of Differently Charged Biopolymers on Initial Bacterial Adhesion on Bovine Enamel *In Vitro*

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Fluoride and stannous ions (F/Sn) containing products are effective anti-erosive agents, which also provide anti-bacterial

properties. Some biopolymers are able to increase the anti-erosive efficacy of F/Sn, however, only little is known about their potential to inhibit initial bacterial adhesion on pellicle coated dental hard tissue. Study aim was to investigate, if differently charged biopolymers could influence the initial bacterial adhesion on bovine enamel *in vitro*. On bovine enamel specimens (ground flat, polished) a pellicle was formed (2h in pooled stimulated human saliva). After application of biopolymers (pos. charged: chitosan (Ch), neg. charged: hyaluronic acid (HA), chondroitin sulfate (CS)) or F/Sn for 2 min at 37°C, specimens were incubated for 2h at 37°C with different bacterial strains (aerob/Gram-pos.: *Streptococcus mutans*, *S. oralis*, *S. sobrinus* and total bacteria from human saliva pool (aerob/anaerob)). CHX (2%) served as positive control, NaCl (0.9%) as negative control. The microbial evaluation was done by counting colony forming units (CFU). After Ch application, initial adhesion of all investigated Streptococci could be significantly reduced (1.32-1.47 log<sub>10</sub> units,  $p < 0.05$ ) compared to the negative control, whereas HA and CS had no reducing effect on the CFU. CHX eradicated colonisation on the pellicle coated surfaces completely; reduction by F/Sn ranged from 2.96-3.83 log<sub>10</sub> units ( $p < 0.0001$ ) in Streptococci. However aerobic and anaerobic bacteria of saliva pools were not influenced compared to the negative control. In conclusion, the positively charged chitosan has potential to reduce bacterial adhesion on the pellicle of bovine enamel *in vitro*. To investigate, if the combination of differently charged biopolymers could further enhance this effect, a follow-up study was performed.

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### Combining Oppositely Charged Biopolymers on Pellicle Coated Enamel to Reduce Initial Bacterial Adhesion *In Vitro*

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Oppositely charged biopolymers can form multilayers, potentially usable to establish protective coatings on oral surfaces like teeth. Especially patients with high caries risk, like patients with xerostomia, could benefit from such coatings, provided that they inhibit colonisation with cariogenic bacteria. Study aim was to investigate the effect of surface modification with combinations of oppositely charged biopolymers on initial bacterial adhesion. On bovine enamel specimens a 2 h *in vitro* pellicle was established (pooled stimulated human saliva). Then chitosan (Ch) and either hyaluronic acid (HA) or chondroitin sulfate (CS) were applied consecutively for 2min each. Combinations and order of

application were: Ch+HA, HA+Ch, Ch+CS and CS+Ch. No biopolymer was applied in NegContr, CHX in PosContr. After biopolymer application specimens were incubated for 2h in one of four bacterial suspensions (*Streptococcus sobrinus*, *S. oralis*, *S. mutans* and mixed species from unstimulated human saliva). Afterwards bacteria were ultrasonically detached and dilution series of bacterial suspensions were prepared. Suspensions were plated on agar plates and incubated to count colony forming units (CFU). PosContr inhibited bacterial adhesion. Significant reductions of CFUs by 1.22-2.19 log<sub>10</sub> units ( $p < 0.05$ ) compared to NegContr were found in groups *Sobrinus*-CS+Ch, *Oralis*-HA+Ch, *Oralis*-CS+Ch, *Mutans*-HA+Ch and *Mutans*-CS+Ch. No differences between any biopolymer and NegContr were found for total salivary bacteria ( $p = 1.000$ ). In groups with application of the positively charged biopolymer (Chitosan) last, less CFUs of all streptococci strains were culturable compared to groups with the positive biopolymer applied first ( $p < 0.05$ ). The combinations of Ch/HA or Ch/CS showed some potential to inhibit initial bacterial adhesion of streptococci monocultures on enamel. The positively charged active agent seems pivotal to this effect, as Chitosan did reduce bacterial adhesion if applied last but not if applied first.

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### Fluctuations of the Oral Biofilm Microbiota Following Different Dietary Phases

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Caries is associated with shifts within the plaque microbiota. So far, there is not much knowledge of the direct influence of nutrition on the microbial community. Hence, this study investigated the influence of different dietary phases on the microbiota in the supragingival biofilm *in situ*. Eleven study participants underwent 5 different phases starting with their regular diet (phase I). Four phases, of 3 months each, followed, in which the study participants frequently consumed sucrose (phase II), dairy (phase III), fibres (phase IV) and their regular diet (phase V). Towards the end of each phase splint systems with bovine enamel slabs were worn 3 x 7 days to collect the oral biofilm. The microbiota was investigated applying Illumina sequencing of the 16S rDNA gene. The beta-diversity showed significant differences between phase I and all the following phases, and also phase II was significantly different from all other phases (II vs. I  $p = 0.003$ ; II vs. III, IV and V  $p = 0.001$ ). The genus *Streptococcus* showed fluctuations over the course of the five phases with its abundance increasing significantly in phase II ( $p = 0.009$ ), decreasing in phase III ( $p < 0.00001$ ) and increasing again towards phase V (I 30.09%, II 41.87%, III 19.48%, IV 22.95%, V 26.3%). Other taxa showed different fluctuations, e.g. the class

bacteroidia, with *Porphyromonas* spp. and *Prevotella* spp. being the main representatives in the oral biofilm, showed a decrease of abundance in phase II while increasing significantly in phase III ( $p < 0.00001$ ). The microbiota revealed fluctuations depending on the dietary phases which were still detectable after going back to the regular diet for three months. While sucrose consumption favoured the caries-promoting non-mutans streptococci, frequent dairy consumption suppressed these taxa.

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### Oral microbiota Associated with Different States of Dental Caries in Brazilian Children

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Bacterial composition of lesion caries may be dependent of the affected tissue, supporting the tissue-dependent hypothesis. This study aimed to investigate the variation of microbial composition in biofilm samples collected from Brazilian children, including caries-free and caries-affected patients by 16S rRNA gene Illumina sequencing. Biofilms were collected on surfaces from caries-free individuals ( $n = 11$ ) and from active carious surfaces according to the following groups: biofilm over enamel carious lesions ( $n = 13$ ), biofilm over dentine carious lesions ( $n = 11$ ) and carious dentine ( $n = 14$ ). DNA was extracted from all samples and amplification and sequencing of the V3/V4 regions of the 16S rRNA gene were performed (Illumina MiSeq instrument, 2x300 bp). Canonical correspondence analysis showed that the samples significantly clustered based on caries status ( $p = 0.001$ ). Microbial profile differences between enamel and carious dentine samples showed that *Fusobacterium*, *Gemella*, *Leptotrichia*, *Granulicatella*, *Aloprevotella* and *Capnocytophaga* were some of the genera found in statistically higher levels in enamel caries samples ( $p$ -adjust value  $< 0.02$ ; adjusted using Benjamini & Hochberg – FDR; Wilcoxon test). Interestingly, *Lactobacillus*, *Parascardovia*, *Propionibacterium*, *Cryptobacterium*, and *Shuttleworthia* were detected almost exclusively in samples related to dentine caries. At species level, significant differences among microbial compositions among samples were found. The relative abundance of *Lactobacillus johnsonii*, *Cryptobacterium curtum* and *Lactobacillus fermentum* was much higher in dentine carious samples than on enamel caries lesions ( $p < 0.001$ ). In this study, *Streptococcus mutans* was more associated to the biofilm of enamel caries than to the biofilm of caries-free

patients ( $p = 0.003$ ). Furthermore, dentine caries lesions had significantly higher abundance of *S. mutans* than enamel caries lesions ( $p = 0.001$ ). Results have shown that the bacterial composition is related to the tissue affected, confirming the tissue-dependent hypothesis. Grants: CNPq- Process#424723/2016-6; FUNCAP-DEP-0164-00088.01.00/19 SPU#:191050934.

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### Metagenomic Analysis of Microflora of Dental Plaque from Children with Severe ECC Using Oxford Nanopore

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Severe Early childhood caries is a prevalent public health problem among young children and little is known about the microbiota associated with it. Therefore, there is a need to determine the oral microbiome using sensitive methods such as Oxford Nanopore technology (ONT). To conduct a metagenomic analysis to identify microflora of dental plaque from children with Severe Early Childhood caries using Nanopore technology. Plaque samples were collected from 13 children with S-ECC and placed in Eppendorf tubes and transferred to the laboratory and DNA extracted using DNAeasy Blood and Tissue Kit from Qiagen. The DNA samples were barcoded and pooled into library using LSK 108 from ONT. The barcoded library was then loaded onto R9.4 Flowcell and sequenced on GridIon X5 device. The data generated was base called using Albacore 2.0 that was further converted to nucleic acid base sequences and analysed using WIMP of EPI2ME Desktop Agent for Megan based classification. The identified results were further confirmed by gene analysis against Human oral Microbiome database (HOMD). At the species level, *Actinomyces oris*, *Veillonella parvula*, *Prevotella intermedia*, *Streptococcus oralis*, *Pseudopropionibacterium propionicum*, *Fusobacterium nucleatum*, *capnocytophaga sputigena* etc were found to be among the most dominating bacteria in dental plaque. *Streptococcus Mutans* was isolated from only one sample and in lesser quantities as compared to other 12 samples. ECC is a polymicrobial disease and the oral microbiota associated with it includes species such as *Actinomyces*, *Veillonella*, *Prevotella*, *Pseudopropionibacterium*, *Fusobacterium* etc. Oxford nanopore technology has the potential to analyse metagenomically bacteria which were previously undetected and provide avenues for further research to evaluate the actual role of streptococcus mutans in dental caries.

### Microbiology of Active and Inactive Caries Lesions as Inferred by 16S rRNA Gene Sequencing

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Understanding the microbiological basis of caries lesion activity is crucial to provide a better knowledge of dental caries development and for proper diagnosis of caries lesions. However, little is known about the differences in bacterial composition between active and inactive caries lesions. The aim of this study was to investigate the microbiota composition related to different caries activity status in enamel and dentine carious lesions from children and to identify potential bacterial biomarkers of caries activity by 16S rRNA gene Illumina sequencing. A total of 81 samples were collected from: biofilm collected from dental surfaces in caries-free children; biofilm over active and inactive enamel carious lesions; biofilm over active and inactive dentine carious lesions; and active and inactive dentine lesions (cariou dentine). Multivariate and univariate analysis were performed with R package to study global and specific compositional differences. There were significant differences in overall bacterial composition of samples collected from active caries lesions, inactive caries lesions and biofilm from caries-free children ( $p = 0.0017$ ; Canonical Correspondence Analysis). The genera *Lactobacillus*, *Cryptobacterium*, *Atopobium*, *Shuttleworthia* and *Megasphaera* were found in significantly higher levels in active dentine lesions, whereas *Neisseria*, *Kingella*, *Streptococcus* and *Granulicatella* were more abundant in inactive dentine lesions ( $p < 0.01$ ; Wilcoxon test). *Lactobacillus johnsonii*, *Cryptobacterium curtum* and *Lactobacillus fermentum* were confirmed as bacterial biomarkers for dentin caries activity ( $p < 0.001$ ; Boruta R library algorithm). *Lachnoanaerobaculum saburreum*, *Corynebacterium durum* F0235 and *Streptococcus sanguinis* were associated to the arrestment of dentin lesions ( $p < 0.001$ ). When comparing enamel caries lesions, *Actinomyces odontolyticus* and *Streptococcus sanguinis* were identified as bacterial biomarkers for inactive lesions; and *Granulicatella elegans* and *Streptococcus gordonii* for active enamel lesions. The data demonstrate that the microbiota identified among active and inactive caries lesions differs substantially.

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### Simultaneous Exposure of Sucrose and Ovalbumin Decreases Sucrose-Mediated Cariogenicity of a *S. mutans* biofilm-caries model

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The modulatory role of non-cariogenic macronutrients, such as proteins, on the cariogenic potential of the most cariogenic sugar, i.e. sucrose, remains elusive. Applied after a sucrose exposure, ovalbumin has shown to reduce the cariogenicity of sucrose in-vitro. Whether ovalbumin reduces sucrose cariogenicity when simultaneously consumed, is unknown. Thus, we aimed to determine the cariogenic potential of sucrose combined with different concentrations of ovalbumin in-vitro. *S. mutans* UA159 biofilms were grown over saliva-coated bovine enamel slabs ( $n = 6$ /group) and treated 3x/day for 5 minutes with: G.1) 10% Sucrose (positive control), G.2) 0.1% ovalbumin, G.3) 10% Sucrose+0.1% ovalbumin, G.4) 10% Sucrose+0.05% ovalbumin, G.5) 10% Sucrose+0.025% ovalbumin, and G.6) 0.9% NaCl (negative control). Culture medium was replaced 2x/day and medium pH measured. After 5 days of biofilm growth, biofilms and slabs were collected. Viable microorganisms, biomass, and insoluble extracellular polysaccharides (IEPs) were assessed in the biofilms. The percentage of surface hardness loss (%SHL) was calculated on the slabs as an indicator of demineralization. Data were compared by ANOVA/Tukey ( $p < 0.05$ ). While ovalbumin alone was not different from the negative control in all the dependent variables, sucrose alone exhibited the highest levels of %SHL, biofilm biomass, viable microorganisms, and IEPs. All sucrose+ovalbumin groups (G.3, G.4 and G.5) increased the pH, reduced biomass and viable microorganisms, when compared to sucrose alone ( $p < 0.05$ ). Lower %SHL was observed for sucrose+ovalbumin, except for G.5, which was not different from sucrose alone ( $p > 0.05$ ). In conclusion, ovalbumin added to sucrose appears to modulate the cariogenicity of *S. mutans* biofilms, reducing enamel demineralization. These findings suggest that adding ovalbumin could decrease the cariogenic potential of sugary-food, but further clinically relevant studies are needed to confirm these findings.

### Calcium glycerophosphate and fluoride affect the inorganic composition and pH of dual-species biofilms

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This study evaluated the effects of calcium glycerophosphate (CaGP), associated or not with fluoride (F), on the concentrations

of F, calcium (Ca), CaGP and phosphorus (Pi), and on the pH of dual-species biofilms of *Streptococcus mutans* and *Candida albicans*, before and after exposure to sucrose. Biofilms were treated three times (72/78/96 hours after the beginning of their formation) at three CaGP concentrations (0.125/0.25/0.5%), associated or not with F (500 ppm). Solutions containing 500 or 1100 ppm F, and artificial saliva were tested as controls. Exposure of biofilms to 20% sucrose solution occurred after the third treatment (96 h). Biofilm pH was measured (micro electrode) and the concentrations of F (ion-specific electrode), Ca (Arsenazo III), Pi (colorimetric method), and CaGP (heating in acid medium) were determined in biofilm (solid and fluid phases). Data were submitted to 2-way ANOVA, followed by Fisher's LSD test ( $p < 0.05$ ). The pH values were higher for CaGP/F groups compared to the other groups, regardless of sucrose exposure ( $p < 0.001$ ). There was a dose-response between F concentration in the solutions and biofilm pH. Sucrose exposure reduced biomass and fluid components analyzed for all treatments ( $p < 0.001$ ). CaGP led to lower F in fluid compared to 500 ppm F, higher Ca in fluid compared to 1100 ppm F, and did not make Pi available. Higher CaGP concentrations led to higher availability of CaGP in the fluid and biomass. CaGP/F increased F and Ca in the biomass, but not Pi ( $p < 0.001$ ). It was concluded that CaGP/F favors the presence of more Ca and CaGP than F in the fluid, and more Ca, F and CaGP in biomass, besides having a buffering effect on the biofilm, even after sucrose exposure.

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#### Immunohistochemical Study of Innate Immune Responses of the Dental Pulp to Caries

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This study was undertaken to examine the presence and quantitative changes of class II antigen presenting cells, dendritic cells, macrophages and T lymphocytes in the dental pulp in healthy and carious human teeth. We have examined 60 maxillary/mandibular human premolars under 3 different clinical conditions: healthy teeth, shallow and deep cavities. Teeth were extracted and immediately cut longitudinally; pulp tissue was extirpated and fixed in formalin for 24 hours at 4 °C. 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. Cells were identified by using the following monoclonal antibodies: HLA-DR, CD68 and CD4. In healthy teeth, HLA-DR-positive cells were distributed mainly in and around the odontoblast layer, with few CD68 positive cells located more coronary around the blood vessels and with

single CD4 positive cells. In shallow cavities, HLA-DR-positive cells were slightly increased and located, for the most part just beneath the odontoblast layer. CD68 positive cells were present in a small number located coronary mainly around the blood vessels. The number of T lymphocytes showed an increase in teeth with shallow dental caries. Deep caries lesions caused an aggregation of HLA-DR-positive cells and macrophages in the dental pulp corresponding to the lesion. The number of T lymphocytes showed a remarkable increase in the number and aggregation with the progression of the carious lesion. These findings suggest that interaction of dendritic cells and macrophages with T lymphocytes are supporting the concept that both types of cells can function as antigen presenting cells in inflamed pulp. Human dental pulp is equipped with a functional immune response against microbial insults from dental caries.

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#### Analysis of Tooth Surface Calcification Caused by Release of Ion from S-PRG Fillers

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Surface Pre-Reacted Glass-ionomer (S-PRG) fillers are attracting attention as functional materials because of their bioactive actions, including six types of long-term sustained release of ions. Thus far, antibacterial and demineralization inhibitory actions as the effects of various ions have been reported, though few studies have investigated promotion of tooth materials calcification. In the present study, we examined biological reactions including calcification caused by use of S-PRG filler in various experiments conducted with a rat model, following approval from the Animal Care and Use Committee of Okayama University. Varnish containing S-PRG filler (40 wt.%) or without fillers was applied to the upper molar of Specific pathogen-free Sprague-Dawley rats (28-day-old males), which were subsequently euthanized 1 week, or 1 or 3 months later. The upper molars was obtained along with the maxilla, and subjected to EDAX standardless quantitative analysis using scanning electron microscopy to analyze the presence of various ions. All ions contained in S-PRG filler were detected in rat dentin at 1 week after applying with strontium in particular detected in high concentrations. For histopathological evaluation, hematoxylin-eosin staining, and immunostaining with a type I collagen antibody and Ki67 antibody were performed to observe cell activation. Activation of pulp cells was significant and type I collagen expression was increased. These results indicate that strontium released from S-PRG filler penetrates dentinal tubules and promote tooth surface calcification based on increased type I collagen expression.

### Towards Antibacterial Flowable Composites: An Ex-Vivo Evaluation

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Objective was to assess antibacterial activities of a novel flowable composite compared with commercial composites. Infinix antibacterial flowable composite (Nobio) containing quaternary ammonium silica-based nanoparticle filler (1.5%), was compared with Filtek Supreme (3M), Tetric EvoFlow (Ivoclar Vivadent) and G-aenial Flo (GC). Three tests were performed: (a) Direct Contact Test (DCT), testing overnight growth (37°C) of bacteria, inoculated on composite samples, in temperature controlled 96-well spectrophotometer, (b) testing pH changes caused by salivary bacteria from 6 volunteers placed each on the surfaces of tested composites, growing overnight in media supplemented with 2.5% dextrose and (c) live/dead analysis (BacLight staining kit) of samples taken 12 months after in-vivo placement of Infinix or Filtek Supreme on 1st premolars of 3 volunteers in a split-mouth design. DCT results showed in Infinix a reduction of 6 log<sub>10</sub> in bacterial growth on Infinix, while control composites exhibited no inhibition. Salivary bacteria, placed on Infinix composite caused no change in the pH values (7.0), whereas same bacteria placed on control composites reduced the pH to 5.0–5.5 ( $p < 0.001$ ). The live to total bacteria-ratio from Infinix surface was significantly lower than on the control composite ( $p < 0.05$ ). The Infinix flowable composite exhibit potent and long-lasting antibacterial activity that reduced significantly viability of saliva bacteria and hindered residual bacteria from fermenting carbohydrates.

### The Key Role of Saliva on the Redox Status of the Oral Cavity

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The saliva plays a key role in determining the redox status of the oral cavity. In physiological conditions, there is a slight balance between antioxidants (AO) and reactive oxygen species (ROS). The excessive production of ROS, as well as a reduced antioxidant capability is the pathophysiological basis of numerous diseases affecting several organs, including the oral cavity. Specifically, the reduced antioxidant capacity results in increased risk of tooth lesions. The aim of this study was double: first, to validate the

clinical efficacy of an ozone-based mouthwash on the antioxidant potential of saliva; secondly, to assess the correlation between salivary antioxidant power and decay prevalence. Fifty patients were evaluated. The decayed-missing and filled teeth (DMFT) index was used. Two salivary tests (Sat Test and Sat Self by H&D srl) were performed before and following the ozonated-oil mouthwash administration. The Pearson's coefficient correlation was performed. A positive correlation was found between all the measured variables ( $p < 0,01$ ). The SAT test could be added as a routine exam for all apparently healthy subjects, but daily exposed to oxidative stress and an increase and/or reduction of reactive species. The addition of supportive therapies through home treatments can allow correct maintenance of oral health by also reducing the possibility of further tissue damage caused by imbalance between cellular oxidation and reduction by antioxidant systems.

### In Vitro Testing of Antimicrobial Activity of Different Toothpastes Marketed in Uzbekistan

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The aim of this in vitro study was to determine the antimicrobial activity of different toothpastes against some oral pathogens, namely *Streptococcus mutans*, *Lactobacillus rhamnosus* and *Candida albicans*. A total of five toothpastes commercially available in Uzbekistan were selected for the study (Galaxy (A), Lesnoy balzam (B), Lavadasept Splat (C), Tourcia Nuga Best (D), Aqua fresh (E)). The antibacterial activity was assessed by measuring inhibition zones by agar well diffusion method. Microorganisms were cultured on 15 plates. The disk diffusion method was used to test antimicrobial activity, sterile paper disks were prepared from each tested materials (1 g of toothpaste mixed with 5 ml of water). Water served as control. The inhibition zones around the discs were measured in mm after 24 h. The average value and standard deviations of inhibition zones were calculated. All toothpastes showed some inhibition (inhibition zone between 0.9 and 2 mm) against *S. mutans* and *L. rhamnosus*, but not against *C. albicans*. When compared to control (water), all toothpastes showed a significant difference ( $p < 0.05$ ) against *S. mutans*, and none was different to control against *C. albicans*. In the case of *L. rhamnosus*, only toothpastes A, D and E showed a significant difference to control. In addition to previously presented results (64th ORCA Congress, abstract n° 5) none of the presently examined toothpastes showed antimicrobial activities to *C. albicans*. Against cariogenic species, there is a small activity, the relevance of which should be further examined in vivo. The role of toothpastes' action on oral microbiota need to be researched in vivo for clinical application.

### Inhibitory Effects of Toothbrush Filaments Containing S-PRG Filler on Streptococcus Mutans

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Surface pre-reacted glass-ionomer (S-PRG) filler, a new dental material that releases 6 ions, is used in various dental materials such as composite resins, cements and bonding agents due to its beneficial functions, including anti-plaque formation, acid neutralisation, and enamel remineralization. In the present study, toothbrush monofilaments containing S-PRG filler were produced and the inhibitory effects on Streptococcus mutans, a major pathogen of dental caries, were analysed. Two types of monofilaments for toothbrushes with a diameter of 200 µm were manufactured using nylon or polyester, and blended with 20.0% or 1.4% S-PRG filler. S. mutans strain MT8148 (one time ten to the seven CFU/ml) was added to Brain Heart Infusion broth containing 1% sucrose. At the same time, each monofilament was put into the broth, which was incubated at 37 °C for 18 h. Then, bacteria adhered to the monofilament was removed and cultured on Mitis-Salivarius-Bacitracin agar plates. Approximately one time ten to the six CFU of S. mutans adhered to both nylon and polyester monofilaments without S-PRG filler, whereas the amount of S. mutans adhering to the nylon and polyester monofilament containing S-PRG filler decreased to approximately 1/20 and 1/5, respectively. Next, to measure the adhesive properties of S. mutans, the monofilaments adhered S. mutans were vigorously vibrated for 3 s. Then, the bacterial number detached by vortexing and that remaining the monofilament were counted. S. mutans MT8148 organisms that adhered to filaments containing S-PRG filler were significantly more easily exfoliated than those adhering to filaments without the filler (P<0.001). These results indicate that monofilaments containing S-PRG filler have an inhibitory effect on S. mutans suggest that they may be an effective material for toothbrushes.

### Characteristics of Streptococcus Mutans Biofilm Grown Using Arenga Pinnata in a Dynamic Flowcell Model

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Arenga pinnata is a naturally occurring sweetener obtained from sap of palm trees in tropical countries. The aim of this study was to assess the characteristics of S. mutans biofilm grown using 10% A. pinnata (palm candy) in a dynamic flowcell model. Sterile human enamel slabs of 3x2x1 mm were used as biofilm substrate.

The biofilms were grown in flowcell conditions with sequential use of artificial saliva, S. mutans in BHI broth and 10% A. pinnata (AP) solution for 3 d. Sucrose (SC) was used as control. Biofilm cell mass was estimated. The EPS was assessed for total protein, carbohydrate and lipid quantity. The biofilm samples on enamel slabs were subjected to confocal microscopy (CLSM) and scanning electron microscopy (SEM) for the assessment of live and dead bacteria and biofilm morphology respectively. The optical density of cell biomass was 0.31 and 0.41 for AP and SC respectively. The total protein content of SC was higher (281.8 µg) compared to AP (223.1 µg). The carbohydrate and lipid content was higher in AP. SEM showed spars distribution of S mutans in AP biofilm. The proportion of live bacteria were 40% and 46% in AP and SC biofilms respectively. The S. mutans biofilm grown with AP resulted in lower biomass, protein and live bacteria compared to sucrose supplemented biofilm.

### Waste Medicine: The Effects of Polyphenolic Fractions Isolated from the Brewing Process Against Streptococcus Mutans

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There is increasing interest in identifying natural bioactive compounds that can improve overall health. The brewery industry generates wastewater that could yield a natural extract containing bioactive phenolic (BWPF) compounds. Streptococcus mutans (S. mutans) is the principal etiological agent of human dental caries. The aim of this study is to evaluate the effect of BWPF natural compounds on S. mutans activity. The total phenolic content obtained from brewery wastewater process, was determined by the Folin-Ciocalteu assay. The experiments were repeated in triplicate (all experimental conditions at concentrations 0, 50, 100, 200 and 400 µg/mL of BWPF). The effect of BWPF on the growth of S. mutans (ATCC 25175) was assessed by growth curve assay, instead the effect on S. mutans glycolysis pH drop was measured according to standard methodology. The Lactate Dehydrogenase (LDH) activity was measured by an LDH Activity Assay Kit (Merck/Sigma-Aldrich, Italy) according to the manufacturer's instructions. Statistical analyses were performed using SPSS Statistics 23.0 (IBM, USA). The results for groups with or without BWPF were statistically analyzed by one-way analysis of variance (ANOVA) with pre-post test. A p-value of < 0.05 was considered statistically significant. It was observed that in comparison to the control, the bacterial growth was significantly inhibited by

BWPF treatment. Moreover the results indicated that BWPF can inhibit LDH activity and *S. mutans* biofilm formation at different time points. Difference between the largest and the smallest observation in the data (measure of dispersion) showed statistically significant differences ( $P < 0.05$ ) in all measurements. Waste materials such as BWPF has shown an inhibitory effect on *S. mutans* cariogenic properties and it represents a promising anticariogenic agent, which could be used for many industrial or pharmaceutical purposes.

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### **In Vitro Effect of Chlorhexidine Mouthwash Versus Hydroxyapatite and Zinc-PCA Containing Mouthwash Against Oral Probiotic**

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The purpose of this study was to investigate the antibacterial activity of chlorhexidine-containing mouthwashes (either 0.20% and 0.12%) against oral probiotic *Streptococcus salivarius* M18 (SSM18), in comparison with a new chlorhexidine-free mouthwash containing hydroxyapatite micro clusters and Zinc-PCA (Pyrrolidone Carboxylic Acid). The antibacterial effect of the examined mouthwashes was tested in agar diffusion assay. A soft Mitis salivarius agar was prepared and added with 2% sucrose and with *Streptococcus salivarius* M18. All mouthwashes were tested in different concentrations (pure, 1:2, 1:5, 1:10, 1:50, 1:100) to evaluate their effectiveness. Inhibition of bacterial growth was assessed by the formation of halos with no visible bacteria, which were measured in mm. Each test was repeated five times, to evaluate specific antibacterial efficacy of each mouthwash. The Chlorhexidine-containing mouthwashes showed antibacterial activity at all tested concentrations against oral probiotic SSM18, even at low concentrations, mean values of inhibition halos being 12.0 mm (11.1–13.0) and 11.5 mm (10.9–11.9) for pure mouthwash and 5.8 mm (5.6–6.0) and 5.2 mm (5.0–5.3) for 1:100, at 0.20% and 0.12% respectively. On the other hand, the chlorhexidine-free mouthwash showed also an antibacterial activity against *Streptococcus salivarius* M18, but with lower rates and only at high concentrations, mean values being 5.4 mm (5.3 - 5.5) and 0.0 mm, pure and 1:100 respectively. The difference at each concentration was statistically significant (U Mann-Whitney test,  $p < 0.01$ ). The minimum concentration of chlorhexidine-free mouthwash at which inhibition against SSM18 could be detected was 1:50 (2.2 mm, range

0.0 – 2.5). Our preliminary results suggest that this new antiseptic chlorhexidine-free mouthwash containing hydroxyapatite micro clusters and Zinc-PCA might be prescribed both in the short term or during probiotic assumption.

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### **Design of a New Intraoral Splint with 3D-Printed Inserts for Dental Biofilm Collection**

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Biofilm models are important tools to study the impact of bacterial metabolism and the effect of therapeutic measures on the caries process. Laboratory models consisting of few bacterial species are useful for high-throughput testing, but in-situ models reflect the complexity of the oral environment much better. The increased complexity typically results in a large variation in the amount of biofilm produced, not only between individuals but also between biofilms collected from the same person. Here, we designed a lower-jaw-splint with 3D-printed silicon inserts that protect the biofilm samples during growth and provide a standardized recession for each individual biofilm sample. We hypothesized that a standardized splint geometry reduces variation between replicate biofilm samples from the same individual and increases the amount of biofilm produced, compared to an in-situ-model without standardized geometry. The splints consisted of a 3D-printed metallic arch and two acrylic vestibular extensions. The extensions contained 3D-printed silicon inserts for biofilm carriers (4x4x1.5 mm). Each individual carrier was surrounded by silicon walls, recessed by 1.5 mm and kept in place by an undercut. 48-h biofilms were collected in 6-tuplicate (2 technical, 3 biological replicates) from five healthy participants, both with standardized splints and with 'traditional' splints where the carriers were fixed with sticky wax. The biovolume was determined by confocal microscopy. The coefficient of intra-individual variation was high for both systems, but slightly lower for the standardized splints (69 vs. 80). The average amount of biofilm was slightly lower on the standardized splints (124599  $\mu\text{m}^3 \pm 70284$  SD vs. 163616  $\mu\text{m}^3 \pm 135033$  SD), with no statistically significant difference ( $p = 0.33$ , 2-sample t-test). The use of silicon inserts eased the handling of the biofilm samples.

### Cariogenic Potential of Bovine Milk Evaluated by IEDT Model

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Lactose, the carbohydrate present in milk, has low cariogenic potential compared with sucrose but bovine milk is consumed for people from all age and root dentine is more susceptible to caries than enamel. In this study, we evaluated in situ whether fermentation of bovine milk is capable of demineralizing enamel and dentine. The study had a 4-phase crossover, double blind design in which eight volunteers wore palatal appliances containing four enamel blocks and four root-dentine blocks of known surface hardness (SH). The blocks were covered with a test plaque of *Streptococcus mutans* Ingbritt-1600 and placed in palatal appliances according to the Intra-oral Enamel Demineralization Test (IEDT) model. The volunteers wore the appliance for 30 min followed by extra-oral 3 min immersion in one of following treatments: 0.9% NaCl solution (negative control), bovine milk (test), 4.5% lactose solution (active control) or 4.5% sucrose solution (positive control). Further, the appliances were replaced into the mouth and after 45 min the enamel blocks were collected. The demineralization provoked was determined by the percentage of SH loss (%SHL) in relation to baseline at each distance from the edge of the blocks (50 to 500 µm). The results were expressed by the area under the curve (AUC) of %SHL x µm. Enamel and dentine results were independently analyzed by one-way ANOVA and Tukey's test ( $\alpha = 5\%$ ). Greater demineralization (mean±SD; n = 8) was provoked by sucrose treatment in enamel (17,088.9±8,104.8) and dentine (26,113.6±3,998.1) than the other treatments ( $p < 0.05$ ). Milk, lactose and the negative control did not differ among them ( $p > 0.05$ ) either for enamel (7,952.1±4,302.7, 7,861.9±5,172.7, and 6,385.6±2,955.3, respectively) or dentine (15,237.3±6,426.3, 16,285.4±6,523.7, and 14,841.0±3,473.0, respectively). The findings, using this in situ model, suggest that bovine milk does not present potential to demineralize either enamel or dentine.

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### EPS-Rich Matrix Increases Cariogenic Potential of Less Cariogenic Carbohydrates

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Sucrose alters biofilm virulence due to extracellular polysaccharides synthesis (EPS). In this study, the aim was to investigate if an EPS-rich matrix could increase the cariogenic effect of less cariogenic carbohydrates. *Streptococcus mutans* UA159 biofilms (n = 6/group) were grown on saliva-coated enamel slabs in UTYEB (pH 7.0) at 37°C, 10% CO<sub>2</sub>. Firstly, they were exposed 8x/day for 48-h to a mixture of 5.25% glucose + 5.25% fructose (G+F = absence

of EPS) or 10% sucrose (S = presence of EPS). Afterward, carbohydrate exposures were interrupted (starvation) for 48-h, and in the subsequent 48-h, the treatments of the biofilms previously grown with G+F or S were shifted. Samples were collected after the initial 48-h of biofilm formation under G+F or S exposure, 48-h after starvation, and 48-h after G+F and S treatments shifting. The pH of culture medium (biofilm acidogenicity), enamel demineralization (%SHL), and EPS amount were assessed. Data were analyzed by Two-way ANOVA followed by Tukey's test ( $\alpha = 5\%$ ). Biofilms formed under S exposure were more acidogenic (pH 5.3±0.0) than G+F treatment (pH 5.7±0.1) ( $p < .05$ ). After starvation, biofilms showed the same acidogenicity (pH 6.5±0.1;  $p > .05$ ), however, EPS-rich biofilms exposed to G+F reached the lowest pH after treatment shifting (4.7±0.1;  $p < .05$ ). Biofilms formed under S exposure caused around 2-times more demineralization (29.5%±8.5) than G+F (12%±4.0) ( $p < .05$ ). However, after starvation and treatment shifting, G+F treatment of sucrose-previously grown biofilm increased enamel demineralization in 74%, while S treatment of G+F-previously grown biofilm increased demineralization in 178%. EPS amounts were greater in biofilms formed under S exposure ( $p < .05$ ), either during biofilm growth or after treatment shifting, but without changing during starvation ( $p > .05$ ). The data suggest that EPS-rich matrix biofilm may increase the cariogenic potential of less cariogenic carbohydrates.

### Anti-Cariogenic Effect of Anacardic Acid-Loaded Zein Nanoparticle Solution on Bovine Enamel near Orthodontic Brackets

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This study aimed to evaluate the effects of anacardic acid-loaded zein nanoparticle solution on *Streptococcus mutans* biofilms formed on bovine enamel near orthodontic brackets, by microbiological and mineral hardness analysis. Orthodontic brackets bonded on bovine enamel specimens (n = 36) were immersed once/daily (2 min), for 5 d, in following groups: 1) 35% hydroethanolic solution; 2) Zein nanoparticle (0.0714% w/v) not loaded; 3 and 4) 0.12% Chlorhexidine digluconate solution. Culture medium was changed daily, and medium pH was checked. *S. mutans* biofilm was collected on the 5th day and the colony-forming units per ml (CFU/mL) was established. This experiment was repeated 3 times. Specimens were submitted to cross-sectional microhardness analysis. Knoop indenter was used to measure the hardness KHN and microhardness surface change was calculated. Microbiological and microhardness data (mean ± standard deviation) were analyzed with ANOVA and Tukey test. It was observed a biofilm reduction (Log CFU/mL) on group 3 (4.06 ± 1,20) compared to group 1 (7.56 ± 0.18) and 2 (7.58 ± 0.11) ( $p = 0.0007$ ).

No biofilm formation was observed on group 4. Despite the difference in CFU counts between groups 3 and 4 ( $p = 0.0003$ ), this difference was not observed in the microhardness analysis ( $p = 0.20$ ), and both groups 3 ( $5105 \pm 6631$ ) and 4 ( $-3798 \pm 9705$ ) presented lower mineral loss than groups 1 ( $17862 \pm 4489$ ) ( $p < 0.05$ ). In addition, the average of culture medium pH of groups 1 ( $4.22 \pm 0.07$ ) and 2 ( $4.29 \pm 0.07$ ) was lower than groups 3 ( $6.61 \pm 0.22$ ) and 4 ( $6.84 \pm 0.01$ ). We conclude that anacardic acid-loaded zein nanoparticle has anticariogenic effects as it promoted a reduction of *S. mutans* biofilm formation and a lower loss of mineral hardness.

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### Transcriptome Analysis of Genes Responsible for Acid Tolerance in *Streptococcus Mutans* Present on Root Surfaces

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Considering the microbial complexity of dental biofilms and potential microbial interactions that occur in this microbiologically diverse environment, it is necessary to analyze which mechanisms of acid tolerance are being expressed in polymicrobial biofilm obtained from clinical samples. The objective of this research was to evaluate the differential expression of genes involved in *S. mutans* acid tolerance mechanisms by transcriptome analysis of natural biofilms of healthy root surfaces ( $n = 10$ , SRS) and active root caries lesion ( $n = 9$ , RC). Total microbial RNA was extracted and mRNA isolated and sequenced on the Illumina Hi-Seq2500 platform. Pooling of samples with values below 30 ng / RNA was formed for the construction of genomic libraries. Data generated by RNA-Seq sequencing was compiled into a table of reads (reads) and mapped to the reference genome (*S. mutans* UA159). For the calculation of gene expression level the data were normalized with the DESeq algorithm ( $\text{Log}_2$ fold change  $> 1$  and  $\text{Pd}_j < 0,001$ ). The genes that showed differential expression in CR were analyzed for a better understanding of its importance for adaptation of *S. mutans* to an acidic environment. While 69 genes showed differential expression on decayed root surfaces (RC) ( $p =$ , no gene showed differential expression on healthy root surfaces (SRS). The main mechanisms that showed differential expression related to *S. mutans* tolerance to the acid environment were: genes associated with sugar metabolism and transport, nucleotide and nucleoside metabolism, amino acid biosynthesis, plasma membrane activity, genes related to cellular communication and response to external factors, genes related to extracellular pH control and genes related to macromolecule repair mechanisms. These results elucidate the major genes responsible for acid tolerance of *S. mutans* in clinical specimens.

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### Effect of Diurnal Sucrose and Nocturnal Lactose Exposures on Enamel Demineralization Evaluated by Cariogenic Biofilm

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The association of human milk and increased risk for caries development in children with prolonged breastfeeding could be explained by a diurnal cariogenic diet combined with nocturnal lactose fermentation, which was evaluated *in vitro* in this study. *Streptococcus mutans* UA159 biofilms were formed on saliva-coated enamel slabs with known surface hardness (SH) in UTYE medium ( $37^\circ\text{C}$ , 10%  $\text{CO}_2$ ). Biofilms were exposed 8x/day for 3 min to 10% sucrose to mimic a diurnal highly cariogenic diet, or to 50 mM NaCl as control for this factor. After that, biofilms were exposed for 2 h in 0.7% lactose to simulate nocturnal milk retention in mouth after breastfeeding, or UTYE only as control for this factor. Four conditions were evaluated: Ctr, non-exposed to sucrose or lactose as control; Lac, exposed only 2 h to lactose; Suc, exposed only to sucrose (8x/day); and Suc->Lac, exposed to sucrose (8x/day) and 2 h to lactose. The culture medium was changed three times daily, at the beginning of the day, after treatments and after the 2 h period, being the pH evaluated. After 96 h of growth, biofilms were collected to evaluate biomass (dry-weight), CFU counts, and extracellular polysaccharides (EPS). The SH was assessed to calculate the percentage of SH loss (%SHL). Data were analyzed by one-way ANOVA and Tukey's test ( $\alpha = 5\%$ ). The %SHL differed among the groups ( $p < 0.05$ ), being higher in Suc->Lac ( $40.6 \pm 6.8$ ) when compared to the others, Suc ( $32.1 \pm 7.2$ ), Lac ( $6.6 \pm 4.5$ ) and Ctr ( $2.4 \pm 3.1$ ). For CFU data ( $\text{Log}_{10}$ ), only Ctr group ( $6.0 \pm 0.5$ ) showed lower counts ( $p < 0.05$ ) than the others, Suc->Lac ( $7.0 \pm 0.5$ ), Suc ( $6.7 \pm 0.3$ ), and Lac ( $6.4 \pm 0.4$ ). The groups Suc->Lac and Suc presented similar values ( $p > 0.05$ ), but higher than Lac and Ctr ( $p < 0.05$ ) for biomass ( $0.8 \pm 0.3$ ,  $0.6 \pm 0.3$ ,  $0.2 \pm 0.2$ , and  $0.1 \pm 0.1$ , respectively) and EPS ( $219.1 \pm 47.8$ ,  $188.6 \pm 47.0$ ,  $23.6 \pm 11.0$ , and  $9.2 \pm 4.7$ , respectively). The data suggest that biofilm formed under diurnal sucrose exposure may increase the cariogenicity of nocturnal lactose exposure.

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### Antimicrobial Photodynamic Therapy of Natural Compounds Against *Streptococcus Mutans*

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Plant extracts can be used as natural photosensitizers in antimicrobial photodynamic therapy (aPDT). The aim of this study was to evaluate the antimicrobial activity of essential oils and plant extracts in aPDT against *Streptococcus mutans*. Essential oils and plant extracts were selected based on light absorption spectrum, resulting in the selection of three essential oils and three plant extracts. *Streptococcus mutans* ATCC 35688 suspensions ( $5 \times 10^7$  CFU/mL final concentration) were treated with one of the follow-

ing groups: negative control (culture media), plant material, light, vehicle control (with or without light) and aPDT (light + plant material). Groups plant material and aPDT were pre-incubated for 5 min with 2% essential oils or 50 µg/ml plant extracts. Groups that received light were irradiated at 450 nm and 80 J/cm<sup>2</sup>. The effectiveness of therapy was assessed by counting viable cells after treatment. Data were analyzed using the IBM SPSS version 20.0 program, with a significance level of 5%. Presence and absence of light and natural products were independent factors. Comparative analysis was performed by estimating means with a 95% confidence interval. Three plant extracts promoted a statistically significant bacterial reduction ( $p < 0.0001$ ) that varied between 3.19 log CFU/ml (CI95% 0.011) to 8.02 log CFU/ml (CI95% 0.011) (total bacterial reduction). The three essential oils tested did not show any bacterial reduction either alone (plant material group) or combined with light (aPDT group). The results of the present study showed that aPDT mediated by different natural compounds was efficient reducing or eliminating suspensions of *S. mutans*.

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## Abstracts Applying for the Nathan Cochrane Young Investigator Award

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### Salivary and Plaque Mycobiome in Early Childhood Caries and Health

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The oral cavity harbors a high diversity of microbes. However, the fungal component and its role in oral health and disease is understudied. To characterize the salivary and plaque mycobiome in children with and without caries. This is part of the "Predicting Caries Risk in Underserved Toddlers in Primary Healthcare Settings" study. Unstimulated saliva and dental plaque samples were collected longitudinally at three timepoints: ~1-yo (T1), ~2.5-yo (T2) and ~4-yo (T3). Children (n = 266) were classified as healthy (n = 119, ICDAS = 0 at all timepoints) or with caries (n = 147, ICDAS ≥ 1, at any timepoints). The concentration of fungal DNA was determined using qPCR. Samples with at least 0.5 pg/μl fungal DNA (236 samples from 62 children) were processed for ITS1 amplicon sequencing by Illumina MiSeq. The merged reads were quality-filtered, clustered into the operational taxonomic units (OTUs) and taxonomy was assigned using the RDP and UNITE ITS database. In saliva, fungal DNA concentration decreased over time, while in plaque, 2.5-year-olds had the highest fungal DNA. Fungal DNA concentration did not differ by caries status. The top 3 OTUs in plaque belonged to *Candida albicans* (51% of all sequences), *Candida dubliniensis* (5%) and *Malassezia restricta* (4%), while in saliva – to *C. albicans* (24.5%), *M. restricta* (7%) and *Cladosporium* (5%). In saliva, the mycobiome composition changed over time (p = 0.0001, PERMANOVA): OTUs assigned

the taxonomic name *M. restricta* decreased in relative abundance, while *Fusarium* and *Saccharomyces cerevisiae* increased with time. At T2 both the salivary and plaque mycobiome differed significantly according to caries status. In plaque, healthy children had higher Shannon diversity and lower relative abundance of *C. albicans* and *C. dubliniensis* than children with caries.

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### Effect of Probiotic Metabolites on Survival of *Streptococcus Mutans* In Vitro

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Objective was to investigate the effects of *Lactobacillus reuteri* and *Streptococcus oligofermentans* metabolites on survival of *Streptococcus mutans*. *L. reuteri* and *S. oligofermentans* were cultured in different minimal media (MM) or media containing glucose (MM+Glu), glycerol (MM+Gly), lactic acid (MM+Lac), arginine (MM+Arg) or all full medium (MM+all) in vitro. After incubation, culture supernatants were obtained and metabolite concentrations (reuterin, ammonia, hydrogen peroxide, lactate) determined by colorimetric assays. *S. mutans* was similarly cultivated in MM, MM+Glu, MM+Gly+Glu, MM+Lac+Glu, MM+Arg+Glu, MM+all (controls), as well as supernatants of the described probiotic cultures (tests), and colony forming units (CFU) were used to analyze *S. mutans* survival. Independent-sample t-test was used for statistical comparison (p = 0.05). *L. reuteri* produced detectable amounts of reuterin in MM+Gly and MM+all, hydrogen peroxide in all six groups, ammonia in MM+Arg and lactate in MM+Glu and MM+all, respectively. *S. oligofermentans* produced hydrogen peroxide in all six groups, ammonia in MM+Arg and MM+all, and lactate in MM+Glu and MM+all; reuterin was not produced. The mean ± SD CFU of *S. mutans* cultured in *S. oligofermentans* supernatants were significantly reduced in MM+Lac+Glu ( $2.00 \pm 0.84 \times 10^6$  CFU/mL) and MM+all ( $4.58 \pm 2.58 \times 10^6$  CFU/mL) when compared with controls

( $7.98 \pm 2.22 \times 10^6$  CFU/mL and  $13.20 \pm 1.40 \times 10^6$  CFU/mL, respectively;  $p < 0.05$ ) After cultivation in *L. reuteri* supernatant, no CFU of *S. mutans* were detectable at all in MM+Gly+Glu and MM+all, respectively (controls:  $8.76 \pm 4.92 \times 10^6$  CFU/mL and  $13.20 \pm 1.40 \times 10^6$  CFU/mL,  $p < 0.05$ ). Different probiotics reduced the survival of *S. mutans* strain-specifically. *L. reuteri* provided these effects via reuterin produced from glycerol, while *S. oligofermentans* employed hydrogen peroxide produced from lactic acid. The metabolic foundation of probiotic effects should be considered during scientific evaluation and clinical application.

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### Treatment Success of Deep Carious Lesions in Primary Molars: Comparison of Two Treatment Techniques

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This retrospective study compared the treatment success (pulp and restorations status) of two management options to treat deep dentin caries in primary molars: the conventional approach including pulpotomy and restoration with a preformed metal crown (P+PMC) and the Hall technique (HT; no caries removal or tooth preparation and restoration with a PMC). Patients' records between 2010 and 2017 in the Preventive and Pediatric Department at Greifswald University, Germany were reviewed and analyzed. All patients presenting at baseline clinical examination with deep dentine carious lesions without symptoms of irreversible pulpitis and had at least 6 months follow-up were included. Treatments were carried out by specialist pediatric dentists or postgraduate trainees. Three hundred-fourty-three children (mean =  $5.94 \pm 1.91$ ) with a minimum follow-up of 6 months (mean =  $23.0 \pm 14.74$ ) were included for the analysis; 208 (60.6%) were boys and the mean d3mft/D3MFT was 6.81/0.18, respectively. In total, 588 teeth were treated: 267 (45.4%) with P+PMC and 321 (54.6%) using the HT. 548 restorations (93.2%; P+PMC = 242, 90.6%; HT = 306, 95.3%;  $p = 0.26$ ) were successful (Restoration intact without clinical signs/symptoms of pulpal pathology). Fourteen teeth (2.4%) showed at least one 'minor' failure (Restoration loss but the tooth is restorable, without requiring extraction; P+PMC = 11 [4.1%]; HT = 3 [ $< 1\%$ ]). Twenty-six teeth (4.4%) showed at least one 'major' failure (Irreversible pulpitis, abscess, unrestorable tooth): P+PMC = 14 [5.2%]; HT = 12 [3.7%]). The clinical efficacy was high ( $> 90\%$ ) and comparable in both the conventional method (P+PMC) and the less-invasive treatment modality (HT) for treatment of asymptomatic deep carious primary teeth. These results suggest that the use of Hall Technique could be the choice of option in deep carious lesions.

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### Experimental Evaluation of a Diagnostic Flow-Chart Questionnaire for MIH Diagnosis

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Molar Incisor Hypomineralization (MIH) is a common Development Defect of Enamel (DDE). Although its high prevalence and complex management, knowledge regarding MIH diagnosis is lacking among dental professionals. A diagnostic flow-chart could be used to guide the diagnostic process for the identification of MIH defects. The aim of this study is to evaluate the impact of a diagnostic flow-chart for the identification of MIH defects in a clinical study. A sample of 141 final-year degree dental students was randomly divided in two groups and received a link to an anonymous digital questionnaire containing 5 DDE pictures: 2 MIH cases, 2 fluorosis and 1 amelogenesis imperfecta. A group of students (test-group) answered a 5 steps flow-chart yes/no questionnaire before each diagnosis, the questions allow a step-by-step analysis of each picture including numbers, colour and structure of the defects. Participants were asked to diagnose each defect choosing from a list of 7 possible DDE. The other group (control-group) instead performed the diagnosis without the flow-chart support. Sensitivity, specificity and Diagnostic Odds ratio (DOR) were calculated. The gold standard considered (true positive) was the capacity of diagnose an actual MIH case. One hundred-seventeen subjects completed the questionnaire: 63 in the test-group and 54 in the control-group. The diagnostic flow-chart showed a statistically significant higher sensitivity (44.4%) than the control-group (24.6%) according to chi squared test ( $p < 0.01$ ). The specificity and DOR were 96.8% and 99.5% in the test-group and 97.5% and 31.6% respectively for the control-group. Findings showed the potential of a flow-chart to ease MIH diagnosis. Further studies with higher number of subjects or with clinicians with different clinical experience are needed to confirm results.

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### The Effectiveness of Early Referral of Children by Well-Child Clinics for their First Dental Visit

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In the Netherlands, a first dental visit for children was usually recommended at an age of 2 years. In 2011 the caries experience

was high in 5-year-olds (41%), showing a need for dental visits earlier in life. A promising way to realize this regards collaboration with well-child clinics since in many countries all children visit these clinics from birth. Aim was to assess the effectiveness of early referral of children at the well-child clinic for a dental visit in the first year of life. Children attending well-child clinics in The Hague (urban) and in Groningen (rural) were involved and assigned to intervention (n = 356) or control groups (n = 286). The intervention regarded advice at the well-child clinic at the age of 6 months to visit a dental clinic that was trained to work according to the Non Operative Caries Treatment Program (NOCTP). The control group received care as usual. We assessed the degree to which children had a dental visit in their first year, based on the written question: "At which age did your child had his or her first appointment at the dental practice?", filled out by parents of children around 2.5 years. About half of the children in the intervention group attended the dentist in their first year versus less than 10% in the control group. Children in the intervention group have higher odds of having a first dental visit in their first year than children in the control group (intervention versus control, OR = 16.1, 95% C.I. = 9.7 – 26.8). Use of well-child clinics to refer children for their first dental visit is an effective way to achieve an earlier onset of preventive dental care for children.

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### Sealing versus Restoration of Occlusal Dental Caries Lesions in Primary Molars: a 30-Month Follow-Up RCT

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The aim of this RCT was to evaluate the survival of sealing (S) and restoration (R) of occlusal surfaces of primary molars after selective caries removal (SCR). Thirty-three high caries experience children aged 3 to 9 years ( $5.8 \pm 1.4$ ) with high caries experience ( $ceo-d = 8 \pm 3.2$ ) were included and 62 primary molars with dental lesions were selected and randomly divided into two groups (28S and 34R): lesions included in the S group were sealed with lighted-cured glass ionomer cement (LCGIC; GC Fuji II LC capsule, GC Corporation – Tokyo, Japan) and in the R group were restored with the same material after SCR technique. After 30 months, clinical and radiographic examinations were performed to evaluate restorative failures (USHPS criteria) and lesions arrestment. Kaplan-Meier test and Cox regression were performed to assess risk factors association with restorative failure (group, age, gender, visual plaque index, gingival plaque index, dmft, tooth,

location, depth and lesion extent). Thus, 22 teeth (10 S and 12 R) were reevaluated. The survival rate was 95.45% (group R 100.0% and group S 90.0%,  $p = 0.45$ ). No association of any risk factors with restorative failure was observed. No patient had painful symptoms or radiographic alterations in the furcation or periapical region. It can be concluded that sealing of dental caries lesions in occlusal surfaces using LCGIC presented more restorative failures than the restorative treatment after SCR, however no cases of clinical or radiographic lesion progression were observed in any of the evaluated groups.

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### Silver Diamine Fluoride and Potassium Iodide Effect on Hypersensitive Carious Primary Teeth: An Observational Cohort Study

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This observational cohort study aimed to assess the clinical outcomes (hypersensitivity relief and caries arrest) following application of combined silver diamine fluoride and potassium iodide SDF+KI (Riva Star<sup>®</sup>, SDI Limited) on hypersensitive carious lesions (ICDAS 5) after a 3-month period. Fifteen consecutive 2-5 years old patients (mean age  $3.58 \pm 0.95$ ) who required a desensitizing treatment of hypersensitive carious lesions were recruited at the Department of Preventive and Pediatric Dentistry of Greifswald/University in Germany. After positive reporting of hypersensitivity, teeth were isolated, a dentin sample was obtained for activity testing and SDF+KI was applied. Only one tooth per child was included for the analysis. Clinical variables were recorded at baseline and 3 months after treatment. For statistical comparisons between baseline and follow-up, paired t-test, Wilcoxon signed rank test and McNemar test were used. From the 15 participants, 9 (60%) were boys and 6 (40%) girls with a mean dmft =  $8.07 \pm 2.79$ . Overall, out of 15 (100%) active carious lesions at baseline, after 3-months, 13 (86.7%) were inactive and 2 (13.3%) remained active (Bjorndal Criteria;  $p = < 0.001$ ). For hypersensitivity, pain assessment before treatment scored 4 and 5 according to the Visual Analogue Scale in 8 (53.3%) and 7 children (46.7%), respectively. After 3-months, 13 (86.7%) patients' parents reported no pain (score 0), 1 (6.7%) reported score 2 and 1 (6.7%) reported no change in pain (score 4). Decrease in pain scores after treatment was statistically significant ( $p = 0.001$ ). There was no statistical significant improvement in Plaque and Papillary Bleeding Index before and after treatment. The combination of SDF+KI contained in Riva Star<sup>®</sup> capsules has a potent effect in desensitizing and arresting caries in hypersensitive active carious lesions in primary teeth. This study was partially funded by SDI Limited.

### Cost-Analysis of Repairing Restorations in Primary Molars with Encapsulated Glass Ionomer Cement versus Composite Resin

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This trial-based cost-analysis aimed to compare the baseline treatment costs of repairing restorations with encapsulated high-viscosity glass ionomer cement (HVGIC) or bulkfill composite (BULK) in primary molars. Primary teeth (3-to-6-years-old children) with restorations presenting failures and scheduled to be repaired were included. The teeth were stratified according to the diagnostic strategy used (International Dental Federation - FDI / Caries Associated with Restorations and Sealants - CARS) and the size of the defect (up to 1mm or greater than 1mm) and then randomized into two groups: HVGIC (Riva Self Cure, SDI, Australia) or BULK (Filtek Bulk Fill and Filtek Bulk Fill Flow, 3M ESPE, USA). A payer perspective from the Brazilian health care system was adopted. Resources used for performing treatments were valued using a specific sheet. Bootstrapped quantile regression analyses were used to investigate the association between cost and the size and material used in the restoration repairment. Other independent variables, e.g. child's caries experience, were tested to check their influence on costs. Onehundred-eightyfive teeth of 106 children (3-10 years old) were included. Major repairments costed similarly despite the material used (BULK-\$9.18; 95%CI: 7.86-10.49; HVGIC: \$11.66; 95%CI: 10.16-13.17,  $p = 0.42$ ). Minor repairments using bulk flowable resins costed significantly less (\$5.99; 95%CI: 5.02-6.95) than those using HVGIC (\$9.07; 95%CI:9.19-9.96,  $p < 0.001$ ). In conclusion, the bulk flowable resin, in short-term analysis, seems to be a cheaper option to be indicated to minor repairments for restorations in primary teeth.

### Caries Detection Using Intraoral Scanner Under In Vivo and In Vitro Conditions

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This study aimed to assess the difference in the fluorescence response from the occlusal surface of posterior teeth under in vivo and in vitro conditions using the TRIOS 4 intraoral scanner (3Shape, TRIOS A/S, Denmark) and assess the diagnostic performance of this method compared to visual inspection (ICDAS criteria) and histological assessment as reference method. Fifty patients who had molars / premolars scheduled for extraction were included in this study. The examined teeth were scanned intraorally with the intraoral scanner (IOS) using both white and blue

light (415 nm) (IOS in vivo). One – two carious or sound examination sites per tooth were selected and marked onto the 3D models. Each selected examination site was classified according to ICDAS criteria. Immediately after extraction all teeth were scanned again with the IOS under controlled environmental light conditions with reduced external light (IOS in vitro). Finally, histological examination was conducted on all examination sites. Sum of sensitivity and specificity for both IOS and ICDAS was above 1.8, when assessing the presence or absence of caries lesions in vivo. McNemar's test resulted in no significant differences between IOS (in vivo) and ICDAS, and between IOS in vivo and IOS in vitro assessments ( $p > 0.05$ ). In conclusion, the IOS employing fluorescence is a promising caries diagnostic device with a diagnostic performance which corresponds to a high level with the visual inspection using ICDAS criteria. In vitro set-up is reliable for further research projects, as in vitro results are comparable to in vivo.

### The Effect of Different Parotid Saliva Metal Ions on Patients' Caries Score. A Correlation Study

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Saliva contains various metal ions, which influence the pathogenesis of caries by impact on; tooth chemical composition, bacterial adherence to tooth surface, and microbial growth. Parotid saliva is produced in response to various stimuli, and contains particular metal ions, and, is supersaturated with respect to enamel mineral. To better understand the role of parotid saliva metal ions on dental caries diagnosis, the aim was to correlate various parotid saliva metal ion concentrations with the International Caries Detection and Assessment System (ICDAS) score, for each of a group of healthy adults. Stimulated parotid saliva samples from 21 (11 male, 10 female) subjects, were collected using the Lashley cup method. Metal ion concentrations were measured using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Clinical caries scores for each subject were classified using ICDAS. Each metal ion concentration was correlated with the ICDAS score for each subject, using Pearson's Correlation. ICP-OES analyses determined the concentration of up to 25 metal ions. Only 8 were found in all subjects with mean concentrations: Al3+0.35±0.09ppm; Ca2+ 4.88±0.46 ppm; K+ 44.45±3.36 ppm; Li+ 0.30±0.08 ppm; Na+ 7.79±1.43 ppm; Sr2+ 0.16±0.04 ppm; Zn2+ 0.08±0.01 ppm, Mg2+ 0.13±0.02 ppm. ICDAS scores ranged between 0 and 3.48. Concentrations of Al3+( $r = -0.446$ ;  $p = 0.042$ ), Sr2+( $r = -0.465$ ;  $p = 0.034$ ) and Li+( $r = -0.465$ ;  $p = 0.034$ ) were correlated (negatively) with ICDAS score, whereas not for K+( $r = 0.264$ ;  $p = 0.247$ ), Na+( $r = -0.295$ ;  $p = 0.195$ ), Ca2+( $r = -0.059$ ;  $p = 0.8$ ), Zn2+( $r = -0.432$ ;  $p = 0.051$ ), Mg2+( $r = 0.286$ ;  $p = 0.208$ ). Only some metal ions were found in all subjects, suggesting that only these are required for overall salivary function. The correlation between ICDAS score and Al3+, Sr2+ and Li+ suggests a cariostatic role for these ions. There is no correlation between ICDAS score and K+, Na+, Ca2+, Zn2+, Mg2+. Measurement of Al3+, Sr2+ and Li+ concentrations could be used as an indicator for caries risk.

### The Effect of Occlusal Loading on Cervical Gap Deformation: A 3D Finite Element Analysis

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Secondary caries can be accelerated by hydrodynamic flow in a gap between the tooth and restorative material. This study investigated whether occlusal loading can lead to increased hydrodynamic flow by deforming a gap between tooth and restorative material. Three-dimensional finite element analysis was employed to model a molar containing a restoration with an interfacial gap. The model was loaded using direct cusp-to-restoration contact and using a rubber tube model simulating a food bolus. The object exerting pressure was moved across the molar from buccal to lingual side. The applied forces were 50, 100, 200 and 400 N. The elastic modulus of the restoration material was varied between 5, 10, 15.9 and 25 GPa to resemble different kinds of composite. The primary outcome parameter was the volume of the gap under occlusal pressure. The volume of the gap at baseline was 0.7404 mm<sup>3</sup>. Occlusal loading resulted in deformation of the gap area. Maximum deformation was seen when loading was applied in the middle of the restoration. Higher forces and lower restoration stiffness led to more deformation of the gap. Maximum deformation with a force of 100 N and composite modulus of 15.9 GPa was -0.0083 mm<sup>3</sup> (1.12%). Deformation of the gap between tooth and restorative material could lead to increased hydrodynamic flow and faster secondary caries lesion formation. The measured deformation is small. Further research needs to show whether gap compression through occlusal loading affects secondary caries formation to a clinically relevant degree.

### The Effectiveness of Silver Diamine Fluoride on De-remineralisation of Artificial Dentine Lesions In Vitro

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Aim was to investigate the effect different topical fluorides on progression/regression of artificial dentine lesions under acidic challenge in vitro. Twenty-five bovine dentine slabs with subsurface artificial lesion were randomly assigned into four treatment groups: 1-Silver Diamine Fluoride (SDF), 2-SDF with potassium iodide (SDF/KI), 3-Fluoride varnish (FV), 4-deionised water (DW-negative control) and exposed to a 28 days pH cycling regime. The acidic challenge comprised five 2 min exposures daily in acetic acid (pH 4.8). Throughout the cycling period the slabs were stored in artificial saliva at 37°C. At baseline and following cycling slabs

were analysed by cross-sectional Knoop microhardness (KHN) with a Duramin Indenter to assess changes in mineralisation. A number of the samples following cycling were assessed using Scanning Electron Microscopy (SEM) (HITACHI S-3400N) following gold sputter coating. Wilcoxon-Signed Rank test showed a statistically significant increase in hardness (KHN) at 20, 40 µm ( $p < 0.05$ ) after treatment with SDF (Mean difference  $\pm$  SE: 11.86 $\pm$ 1.34; 8.92 $\pm$ 1.29), SDF/KI (5.72 $\pm$ 1.12; 3.80 $\pm$ 1.48) and FV (3.25 $\pm$ 1.29, 3.30 $\pm$ 1.63). However, the lesions became softer at deeper levels from 120 to 300 µm across all groups when compared to baseline. Kruskal-Wallis test and posthoc Bonferroni corrections showed the difference in dentine hardness between SDF and DW were significant ( $p < 0.05$ ) at all levels except at 80 and 100 µm. SEM showed presence of silver and fluoride on the lesion surface after the acidic challenge. In conclusion, in our model SDF showed a protective effect leading to a reduction in dentine demineralisation. However, this protection effect was confined to the outer layers of the dentine lesions.

### Effect of TiF<sub>4</sub> Varnish After Proanthocyanidin/Chlorhexidine Pre-Treatment on Dentin Erosion Progression, with or without DOM

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This in vitro study evaluated the protective effect of TiF<sub>4</sub> varnish, after pre-treatment with proanthocyanidin or chlorhexidine, on the progression of erosive dentin loss, under the presence or absence of the demineralized organic matrix (DOM). Bovine root dentin samples were eroded for 30 min (0.1% citric acid, pH 2.5) and the dentin loss was measured by profilometry (erosion profile). Half of them were subjected to the DOM removal using collagenase for 4 days, while the other half remained immersed in water (with DOM). The removal of DOM was checked by profilometry (DOM profile). Samples were randomly divided into 24 groups (n = 15) according to the factors: 1- With or without DOM; 2- Pre-treatment with 0.012% chlorhexidine gel, 10% proanthocyanidin gel or untreated (control) for 60 s; 3-Final Treatment with TiF<sub>4</sub> varnish, NaF varnish, placebo varnish or untreated (control) for 6 h. The samples were submitted to a pH cycling for 5 days: 0.1% citric acid (4 x 90s/day) and artificial saliva between the challenges. The final profile was obtained for the calculation of erosive dentin loss (µm, three-way ANOVA/Tukey test). When DOM was preserved, the dentin loss was lower compared to the condition without DOM (with DOM: 3.54  $\pm$  3.01 µm and without DOM: 8.00

± 3.97 µm,  $p < 0.01$ ). The pre-treatment had no influence on the progression of erosive dentin loss regardless of the presence or not of DOM ( $p = 0.63$ ). The final treatment with TiF<sub>4</sub> varnish (with DOM: 6.2 ± 4.4 µm / without DOM: 7.4 ± 3.4 µm;  $p < 0.01$ ) was effective in reducing the progression of erosive dentin loss compared to control (with DOM: 6.8 ± 3.7 µm / without DOM: 10.9 ± 3.4 µm;  $p < 0.01$ ), regardless of the presence or not of DOM and the pretreatments. DOM has important protective effect on the erosive dentin loss progression. TiF<sub>4</sub> was the unique treatment with promising protective effect on the erosive dentin loss progression.

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### Acceleration of Remineralisation Through Intra-Lesion pH Modulation

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The process of remineralisation is relatively slow. Although remineralisation with CPP-ACFP has been shown to be most effective at pH 5.5, the use of a high pH pre-treatment prior to the CPP-ACFP pH 5.5 treatment of artificial lesions in vitro has been demonstrated to enhance the rate of remineralisation. The aim of this study was to assess the effect of cyclic intra-lesion pH modulation on remineralisation of artificially demineralised human enamel lesions by CPP-ACFP in vitro. For Experiment 1, demineralised lesions were immersed in solutions according to one of two cyclic regimes, A or B ( $n = 20$ ): Cycle A = 10 min in distilled deionized water followed by 50 min in 1% CPP-ACFP (pH 5.5), Cycle B = 10 min in 134mM NaOH (pH 12.9) followed by 50 min in 1% CPP-ACFP (pH 5.5). These cycles were repeated 7 times daily for 15 days. For Experiment 2, lesions were allocated to two treatments, C or D ( $n = 5$ ): Treatment C = a cycle treatment repeated 20 times consisting of 2 min in 300mM NaOH (pH 12.9) followed by 10 min in 10% CPP-ACFP (pH 5.5), Treatment D = 200 min in 10% CPP-ACFP (pH 5.5). TMR was used to measure % remineralisation and a two-sample student t-test assessed significance between treatments. Cycles A and B showed 28.2±5.8% and 43.8±6.9% remineralisation respectively and were significantly different ( $p < 0.001$ ). Treatments C and D showed 23.1±3.4% and 1.9±1.3% remineralisation respectively and were also significantly different ( $p < 0.001$ ). The use of a high pH treatment to periodically increase the intra-lesion pH was concluded to significantly increase the rate of remineralisation of artificial lesions in an in vitro model.

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### Dietary Fluoride Intake of Pregnant Women from the ELEMENT Cohort in Mexico

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Reports of pregnant women's current dietary fluoride intake levels are much needed to increase our understanding of fluoride intake during pregnancy and inform future dietary recommendations. The current recommendation for pregnancy is 3 mg F/day from all sources (US-Institute of Medicine). Sources of dietary fluoride in Mexico City include foods, beverages and fluoridated salt. The aims of this study were 1) to describe and compare dietary fluoride intake throughout pregnancy and 2) to determine how it relates to different levels of adherence to Mexican dietary recommendations. Women were recruited at the beginning of their pregnancy ( $n = 571$ ). At each stage (Early, Middle, Late) demographic questions and anthropometric measurements were collected. The daily dietary intake of fluoride (mg F-/d) and pregnancy's key nutrients from Mexican dietary guidelines (calcium, folate, iron and protein) were assessed with 3-month recall Food Frequency Questionnaires (FFQs). Demographic and nutritional data were summarized with non-parametric statistics. The comparison of dietary fluoride intake across pregnancy stages and levels of compliance with Mexican dietary recommendations was made with random effects' multiple linear regression models, adjusting for covariates. Median unadjusted dietary fluoride intake throughout pregnancy was 0.69 mg F-/d (0.01 mg F-/kg/d). After adjustment for covariates, the association between fluoride intake and pregnancy stage was significant ( $p < 0.05$ ). Predictive margins of fluoride intake during pregnancy increased from 0.72 [CI: 0.70-0.74] in the early-stage, 0.76 [CI: 0.74-0.78] in the middle-stage, to 0.80 [CI: 0.78-0.82] mg F-/d in the late-stage. Women who were highly compliant with Mexican dietary recommendations ingested in average, 0.08 mg F-/d more than non-compliant women ( $p = 0.004$ ). In conclusion, levels of dietary fluoride intake increased with the progression of pregnancy and in women who were highly compliant with Mexican dietary recommendations.

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### In Vitro Evaluation of the Antibacterial Activity of Two Hydrophilic Sealants

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The purpose of this study is to assess the antibacterial activity of EmbraceWetBond Pit&Fissure sealant, Pulpdent® and

UltrasalXTHydro-Ultradent® against *Streptococcus mutans* and two probiotics: *Streptococcus salivarius* and *Lactobacillus reuteri*. The antibacterial effects of sealants were tested in both planktonic growth inhibition assays (20,10 µL Embrace - 20,50 µL UltraSeal, measuring Optical Density and Inhibition Percentage after 24 h incubated at 37°C in a total of 130 assays) and 4 agar diffusion assays (20, 10 µL Embrace - 20,50 µL UltraSeal, measuring zones of inhibition) using *Streptococcus mutans*, *Streptococcus salivarius* and *Lactobacillus reuteri* (3x10<sup>4</sup> bacterial cells). The materials were applied on the side walls of 96-well microtiter plates in the former and in disks directly in the petri plate on the latter. All materials showed antibacterial effects only in contact with the agar in the diffusion test. In the planktonic growth inhibition test, Embrace had a strong and broad antibacterial action against all the bacteria tested, (inhibition percentage 96±2%, p<0.0001). UltraSeal demonstrated its antibacterial efficacy, though at a minimal rate, only against *L. reuteri* (inhibition percentage 19%, p<0.0001). Its antibacterial action, therefore, was not satisfactory. Within the limits of this in vitro research, it can be concluded that Embrace is able to inhibit the growth of both cariogenic bacterium and probiotics. As a result, in order not to make two ineffective anti-prophylaxis manoeuvres (the seal with fluorine release and the action of the probiotic), it is necessary to plan the timing for the administration of the one and the application of the other. UltraSeal has a minimal antibacterial action against the lactobacillus. In conclusion, the probiotics here considered can be administered while applying UltraSeal.

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### The Microbiota Associated with Early Childhood Caries Recurrence

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The aims were (1) to investigate the association of *Streptococcus mutans* and *Candida albicans* with early childhood caries (ECC) recurrence; and (2) to compare the salivary microbiome of children with ECC recurrence to that of caries free and caries experienced with no relapse. In this cross-sectional clinical study, saliva and dental plaque from healthy (H), white spot lesions (WS) and dentin (D) sites were collected from 142 children aged 6 to 60 months who were: caries-free (CF), treated for ECC with no signs of relapse within 6 months (CE-NR), and treated for ECC and relapsed within 6 months (CR). Samples were quantified for total *Streptococcus* sp. and *Candida* sp. These microorganisms were differentiated by PCR and chromogenic agar, respectively. The salivary DNA was extracted, barcoded and sequenced using Nanopore sequencing platform. CR subjects had significantly higher counts of *Candida* sp in saliva (p = 0.001) and in plaque (p = 0.0202) compared to CF. The prevalence of *S. mutans*, Cnm+ *S. mutans* and *C. albicans* was significantly higher in CR subjects

compared to CF and CE-NR (p<0.0001). *Streptococcus oralis* was the most abundant species in all groups. The most abundant genera found in the caries relapse group was *Veillonella*. *Streptococcus A12*, a health associated bacterial species, was found in CE-NR as well as in CF; but rarely found in the CR group. Alpha Diversity Plot revealed that the CR group has greater species diversity than the CF and CE-NR groups. Our data indicates a potential association of Cnm+ *S. mutans* and *C. albicans* with caries recurrence and it suggests the CR subjects has a more diverse microbiome with a higher abundance and prevalence for cariogenic taxa when compared to CE-NR and CF groups.

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### The Endocrine Disruptor DEHP Alters Enamel Structure, Mineralization and Mechanical Properties

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Tooth resistance to caries depends on genetics, lifestyle and environmental risk factors. These latter may alter enamel quality which is determinant for the susceptibility to caries. Also, in case of Molar Incisor Hypomineralization (MIH), a recently described enamel pathology, the susceptibility to caries is increased too. MIH etiology is still unclear but environmental factors such as medication and endocrine disruptors (EDs) are highly suspected to contribute to the pathological process. The aim of the present study is to characterize dental defects in mice chronically exposed to low-doses (0.5 to 50 µg/kg/d) of the omnipresent ED, di-(2-ethylhexyl) phthalate (DEHP), during 12 weeks. About 30% males and 15% females presented dental lesions including opacities, scratches, enamel breakdown and even broken incisors in 50 % of cases. Enamel analysis by scanning electron microscopy showed altered structure with abnormal prisms and decreased interprismatic enamel in treated groups. Micro-CT quantified a significant decrease in mineral density and showed dose- and location-dependent severities of alterations. The fact that the most important defects were localized in the most external part of enamel layer demonstrate mastication surface vulnerability. Nanoindentation showed a decrease in enamel hardness during all stages of enamel mineralization, from the Dentin-Enamel Junction to the enamel surface, with more pronounced alteration in the external part of enamel. In conclusion DEHP is able to alter enamel structure and quality as other EDs already reported, they can thus contribute to increase the susceptibility to dental decay. DEHP belongs to phthalate family that is widely present in our daily environment in plastics, cosmetics, consumer goods, etc. Considering the high degree of population contamination by DEHP and phthalates, studies should be carried out on their relations with MIH and caries.

## Public Perception of Community Water Fluoridation in the UK

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Community water fluoridation (CWF) schemes serve about 372 million people in about 24 countries. Almost 10% of the UK population currently receives artificially fluoridated drinking water and CWF proposals by local authorities in the UK are subject to public consultation. Aim was to examine the public's awareness and attitude towards CWF in the UK. A web survey was conducted and shared to as many UK residents above the age of 18 years, as possible through social media. The questionnaire was on basic demographic information, usage of fluoridated dental hygiene products, awareness, perceptions and the sources of information regarding CWF. Of the 715 respondents to the survey, 75% were aware of the practice of CWF but had limited awareness of whether their water supply was fluoridated. About 70% of the respondents reported that the purpose of adding fluoride to water is to impart dental health benefits. Though 50% of the respondents believed that fluoridated water was safe for consumption, majority (62%) believed that there should be an option to opt in or out of fluoridation for their individual water supply. The responses pledging support for CWF were equal to the responses opposing at 41%. Forty percent of the respondents chose the public to be the final decision makers on CWF followed by dental/health professionals at 31% and government bodies at 18%. About 46% of the respondents reported not knowing what kind of information the UK government provided on CWF and an overwhelming majority (76%) believed that the government was not communicating health research to the public effectively. There is a clear need for better communication and public engagement by the health authorities regarding water fluoridation in the UK.

## Composition and Potential Mode of Action of Hydroxyapatite in Products Against Erosive Tooth Wear

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Hydroxyapatite-containing formulations are marketed for the prevention of erosive tooth wear. However, little is known about the character of hydroxyapatite in such formulations and their mode of action. Aim was therefore to examine their potential to cause mineral precipitation on eroded enamel surfaces, to analyze their particle fracture and where Ca/P can be found. Five formulations were examined: two hydroxyapatite toothpastes (HAP-TP1/2), two hydroxyapatite/fluoride toothpastes (HAP-F-TP1,

1450 ppm F-/HAP-F-TP2, 1490 ppm F-) and a hydroxyapatite mouthrinse (HAP-MR). Potential precipitation was analyzed by treating pre-etched enamel samples (36% orthophosphoric acid; 2 min) with toothpaste slurries/mouthrinse (3x2 min); experimental areas were compared to reference areas of the same sample by scanning-electron-microscopy (SEM; x4000). The particle fraction of the toothpastes was isolated from slurries by centrifugation and dried in ambient air. The particles (unwashed/washed with 0.5 molar hydrochloric acid) were analyzed by SEM/Energy-Dispersive-X-Ray-Spectroscopy (mass%) and the Ca content (ppm) of the supernatant was determined by Inductively-Coupled-Plasma-Optical-Emission-Spectrometry.

Compared to eroded reference areas, no significant precipitation was found on treated areas. The amount of particles in the toothpastes varied between 21.6% (HAP-TP2) and 34.4% (HAP-TP1), but particles did not differ in size, form and texture. The Ca/P amounts on particle surfaces were 11.9±10.1%/3.5±3.1% (HAP-TP1), 5.7±6.6%/1.8±1.8% (HAP-TP2), 5.7±8.5%/1.7±1.9% (HAP-F-TP2) and 0.4±0.2%/0.1±0.1% (HAP-F-TP1). Washing removed Ca/P completely exposing a silica structure. In the fluid phase, Ca amounted to 436±3.2 in HAP-TP1, 80±4.0 in HAP-TP2, 8548±108.8 ppm in HAP-F-TP2, 680±5.6 ppm in HAP-F-TP1 and 497±5.6 ppm in HAP-MR. Ca/P was detected on silica or in the fluid phase (Ca), anyhow, no mineral precipitation occurred on eroded enamel.

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## Proteomic Profile of the Saliva of Professional Wine Tasters with Erosive Tooth Wear

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The objective of this study was to compare the proteomic profile of the saliva of professional wine tasters (PWT) with mild (GWL) and moderate (GWH) erosive tooth wear (ETW) and of volunteers not PWT, without ETW (GC). Twenty-two subjects participated in this in vivo study (3 GWL/ 9 GWH/ 10 GC) and were clinically evaluated according to the Basic Erosive Wear Examination (BEWE). After clinical examination, the unstimulated saliva was collected. Samples were processed for proteomic analysis (nLC-ESI-MS/MS). The PLGS software was used to compare the proteomic profiles of all groups. Proteomic profile of the saliva was quite different among the distinct groups. The total of proteins identified were 1183 (GWL vs GWH), 1290 (GWL vs GC) and 1852 (GWH vs GC). In the quantitative analyses, when GWL was compared with GWH, 29 proteins were increased in GWL, highlighting 4 Proline-rich proteins isoforms, Kalirin, Mucin-7 and Statherin. When GWL and GC were compared, 20 proteins were increased in the first, highlighting Basic salivary proline-rich protein 1 (up to 200-fold), Basic salivary proline-rich protein 2 (up to 156-fold), Kalirin and Statherin. When compared GWH and GC, 19 proteins were increased at GWH, highlighting Basic salivary proline-rich protein 1 and 2, Kalirin, Hemoglobin subunit

alpha and 2 Albumin isoforms. Profound alterations in the proteomic profile of the AEP were found in GWL when compared to both GWH and GC. These findings might play a role in the resistance to ETW found in GWL.

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### Effect of Single Application Fluoride Treatment on Step Height Formation in Natural Human Dentine

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In recent years, most studies have investigated enamel erosion and the effect of acids on dentine is less understood. The erosive protective benefits of using fluoride in high and low concentrations on dentine is unknown. The aim of this study was to investigate the effect of 1250 ppm and 5000 ppm sodium fluoride on dentine using citric acid. Dentine specimens (n = 150) were sectioned from the coronal aspect of previously extracted human molars. The samples were randomly divided into 15 groups (n = 10). Pre-treatment involved the immersion of 5 groups (n = 50) into either: 1250 ppm NaF solution, 5000 ppm NaF solution or deionised water for 3 min. All samples were then placed in artificial saliva for 30 min. Following that, each group was fully immersed for 0 (deionised water-negative control), 10, 15, 20 or 25 min, respectively, in 0.3% citric acid at pH 2.7. The mean step height was measured using confocal non-contact white light laser profilometry. The mean step height without NaF treatment for 10,15,20 and 25 min (um) was 4.35 (0.58),6.18(0.41), 7.21 (0.39) and 9.08 um (0.74). At 1250 ppm of NaF for 10, 15, 20 and 25 min were (um) 3.97 (0.57), 5.36 (0.44),7.1 (0.5) and 8.74 um (0.58), and for 5000 ppm NaF were (um) 3.47 (0.77),5.19 (1.05),6.41 (0.66) and 7.00 um (0.56), respectively. There were statistically significant differences in step height between groups with no NaF treatment and those treated with 5000 ppm of NaF.

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### Knowledge of and Attitudes Towards Erosive Tooth Wear Among Chinese University Students

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The aim was to assess the knowledge of and attitudes towards erosive tooth wear among Chinese dental, medical, and non-medical university students. A questionnaire on knowledge (15 questions) of and attitude (10 questions) towards erosive tooth wear was designed and psychometric properties (Cronbach's alpha, corrected item-total correlation, test-retest reliability, and factor analysis) were analysed in a pilot study (n = 120 students). The questionnaire was then distributed to 3 groups of university

students (n = 635): dental students (DSs), medical students (MSs), and non-medical students (NSs). Score differences between groups and genders was analysed by one-way ANOVA. The Pearson correlation coefficient was used to determine the association between the knowledge and attitude scores (p<0.05). The questionnaire was found to be reliable, valid and reproducible. A total of 435 students participated in this study (response rate: 69.6%). The knowledge score of the DSs (11.5±3.4) was significantly higher than those of the NSs (5.5±4.0) and MSs (6.1±4.0) (P<0.001). The attitude score of the DSs (45.2±6.5) was significantly higher than those of the NSs (41.1±6.9) and MSs (41.8±6.4) (p<0.001). The majority of DSs expressed attitudes that were more accurate and positive than those expressed by the other 2 groups. The attitude score was positively correlated with the knowledge score (r = 0.237, P<0.001).

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### The Influence of Neighborhood Characteristics on Dental Caries Experience in 6-Year Old Children

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The aim of this study was to investigate whether differences in dental caries and dental health care use exist at neighborhood level, and whether characteristics related to the neighborhood can explain those potential differences. This study is embedded in The Generation R Study, a prospective cohort study conducted in the Netherlands. In total, 5102 6-year old children, representing 156 neighborhoods in the area of Rotterdam, were included in this cross-sectional study. Information about individual, family and neighborhood characteristics was derived from questionnaires, and via open governmental data resources. Caries was assessed via intraoral photographs, and defined with the decayed, missing and filled teeth (dmft) index. Multilevel multinomial logistic regression models were used to analyse the associations between several neighborhood characteristics and dental caries experience as well as dental care use. The models were adjusted for individual characteristics including: age, gender, brushing frequency, sugar consumption, and socioeconomic factors. In this study population 34% of the children had one or more caries lesions, and 92.3% visited the dentist in the past year. Differences between neighborhoods explained 5.4% of the chances of getting severe caries. In the fully adjusted models, neighborhood deprivation was significantly associated with increased severe dental decay (OR: 1.10; 95%CI: 1.08-1.12). The availability of snack bars, supermarkets and dental care practices per neighborhood, were not associated with dental caries or dental care use. While children in deprived neighborhoods are at higher risk of developing caries during childhood, the individual characteristics seem most important in explaining difference in dental caries risk. Therefore, is of importance to review current local policies and to implement appropriate intervention methods in vulnerable neighborhoods.

### Obesity and Weight Change are not Risk Factors for Coronal/Root Caries: Prospective 4-Years Study

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This study evaluates the association between overweight and obesity and coronal/root caries among south Brazilian adults. This prospective cohort study reassessed after 4 years of follow-up 414 individuals. Questionnaires recorded socio-demographic variables, oral hygiene habits, behavioral factors and access to dental services. Oral examination assessed gingival bleeding, gingival recession, and coronal and root caries. Height and weight were collected to calculate the body mass index. Multivariable negative binomial (coronal caries) and Poisson (root caries) regression models were applied analysis (incidence rate ratio [IRR]/95% confidence interval [95% CI]). Height and weight were collected to calculate the body mass index. The main outcome of this study was the obesity status (1) the BMI at baseline and (2) weight change over the follow-up period. Multivariable negative binomial (number of teeth) and Poisson (incidence) regression analysis models were applied analysis. Regarding weight change over time, 18.0% of the individuals remained as normal, 9.8% lost weight, 34.4% remained or became overweight and 37.7% remained or became obese. Smoking and baseline BMI were associated with weight change over time. The percentage of individuals with incident coronal caries was approximately 45% among all BMI categories. Similar increment of DMFT and DMFS was observed among all BMI categories. The percentage of individuals having incident coronal caries was 39.4% among those classified as normal weight both at baseline and follow-up. For the other three categories of weight change, incidence varied among 43.5% (obese), 49.2% (overweight) and 44.4% (lost weight) ( $p>0.05$ ). There were no differences among categories of weight change in DMFT/S increment. Absence of significant associations was also found regarding root caries. Overweight and obese adults should not be considered as at higher risk for dental caries.

### Prevalence of Smell, Taste, Trigeminal Disorders and Quality of Life Among 65-Year-Olds in Oslo

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This study aimed to investigate the prevalence of smell, taste and trigeminal disorders in 65-year-olds in Oslo, Norway. Methods: Two hundred and twenty-one participants (123 males, 98 females) were randomly selected from the Norwegian tax register. The participants' subjective assessment of smell and taste ability (visual analogue scale - VAS 0; very bad - 10; very good),

burning mouth sensation, oral pain, distorted taste and their association to quality of life (QoL) was investigated. Sniffin'n Sticks and Taste Strips (Burghart Messtechnik GmbH) were used for objective testing of smell and taste function. Reduced smell and taste (hyposmia and hypogeusia) and complete loss of smell and taste (anosmia and ageusia) were evaluated. Results: According to objective tests, 34% had smell disorders (hyposmia; 28% and anosmia; 6%) and 28% had taste disorders (hypogeusia; 21% and ageusia; 7%). A significantly greater proportion of males than females had hyposmia, hypogeusia and ageusia ( $p<0.05$ , Chi-square and Fischer's exact test). Thirteen percent of participants had a combination of smell and taste disorders. A large proportion of participants with hyposmia (67%) and anosmia (36%) reported their subjective assessment of smell above VAS-score five. Seventy-eight percent of participants with hypogeusia and 67% with ageusia reported their subjective assessment of taste above VAS-score five. Three percent of participants reported burning mouth sensation and 7% reported oral pain. Five percent of participants reported distorted taste. Anosmia was associated with reduced smell-related QoL ( $p<0.05$ ). Conclusions: Hyposmia and hypogeusia are common in the general population of 65-year-olds in Oslo. It is more prevalent among males. Anosmia reduces smell-related QoL. However, this study indicated an overall low awareness of smell and taste disability among affected participants.

This study was supported by the University of Oslo.

### Are Xerostomia and Hyposalivation Common Problems Among 65-Year-Olds?

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Dry mouth is a common complaint among the elderly. The aim of this study was to assess the prevalence of dry mouth and medication use among a young-elderly population. Methods: A random sample of 65-year-olds living in Oslo, Norway, underwent a clinical examination and answered questions related to dry mouth, including the Summated Xerostomia Inventory (SXI, range 5–15) and the number of medications used. The clinical parameters were the Clinical Oral Dryness Score (CODS, range 0–10), unstimulated (UWS) and stimulated (SWS) whole saliva secretion rates. Results: Four hundred and sixty participants were enrolled in the study (234 males, 218 females, response rate 58%). The mean number of medications used was 2.3 (SD 2.2). Twenty five percent used four medications or more. Ten percent reported having dry mouth "Often" or "Always". The mean SXI score was 6.8 (SD 1.9) and 4% had a score  $>10$ . The mean CODS was 1.9 (SD 1.4) and 12% of participants had CODS $>3$ . The mean UWS was 0.4 ml/min (SD 0.3) and the mean SWS was 1.9 ml/min (SD 0.9). Eight percent had pathologically low UWS ( $\leq 0.1$  ml/min) and 4% had pathologically low SWS ( $\leq 0.7$  ml/min). A significantly higher proportion of those who used four medications or more had pathologically low UWS

(13%) and SWS (8%) compared to those who used less than four medications (6% and 3%, respectively) ( $p < 0,05$ , Chi-square test). Conclusions: Xerostomia and hyposalivation were not common problems in this population of 65-year-olds. However, pathologically low UWS and SWS were significantly more prevalent among participants who used four or more medications. These data contribute to the knowledge base on dry mouth in the general population of young-elderly. This study was supported by the University of Oslo.

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### Dental Caries Experience of 12 Year Old Children from the Eastern Region of the Macedonia

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Despite scientific advances and the fact that the caries is preventable, dental caries is still the most prevalent dental affliction of childhood within developing countries and continues to be major dental public health problem. The aim of this study was to assess dental caries in 12 year-old children from Kochani city from the Eastern region of the Republic of North Macedonia. Dental status was evaluated using the 2013 World Health Organization caries diagnostic criteria for Decayed, Missing and Filled Teeth (DMFT) by 2 calibrated examiners. Data obtained during the survey was stored in a database and statistical analysis with SPSS 20.0 for Windows was performed. Using the t-test, the data were tested for possible statistically significant differences. A  $p$  value  $< 0.05$  was considered statistically significant. This cross-sectional study was conducted during 2018 year within the primary school children from sixth grade from four different central primary schools located in Kochani city. Parental consent was obtained for each child. The total number of children ( $n = 159$ ) in the sample was comprising 75 (47.17 %) females and 84 (52.83 %) males. The mean DMFT score of the whole sample was  $1.48 \pm 1.51$ . The prevalence of caries free children was 39.62 %.

The intensity of dental caries is low in 12 year old children from the Kochani city.

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### Effect of Different Etching Systems on Enamel Surface After Bracket Debonding: SEM and FTIR Evaluations

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Orthodontic treatment using fixed appliances is frequently associated with enamel damages leading to increased risks of caries. Attachments are bonded for limited periods and adhesion forces should not be too strong to cause enamel loss after debonding. The purpose of this in vitro study was to investigate the effects of different etching systems on tooth enamel morphology and biochemical composition, after bracket debonding. Non-carious unerupted human third molars were cut in mesiodistal direction to separate buccal and lingual surfaces. Samples were divided ( $n = 6$ ) into: Control group (GC, not treated), Group 1 (G1, 15% orthophosphoric acid), Group 2 (G2, 25% orthophosphoric acid), Group 3 (G3, 37% orthophosphoric acid) and Group 4 (G4, 10% maleic acid). Etching agents were applied on teeth for 30 s; then samples were rinsed with water for 10 s and air dried. Metal orthodontic brackets were bonded, and samples were stored in freshly prepared artificial saliva for 15 and 30 days. After debonding, the effects of etching systems were investigated through Fourier Transform Infrared (FTIR), Raman spectroscopies and Scanning Electron Microscopy (SEM) to evaluate respectively the biochemical structure and the surface enamel morphology. FTIR showed similar results in terms of carbonates and phosphates between GC and G1, G2 and G4 ( $p > 0.05$ , GraphPad Prism). Conversely, G3 exhibited a higher amount of organic matter (total proteins and collagen) ( $p < 0.05$ ), suggesting a greater exposure of the dentin. SEM showed a higher exposure of prismatic enamel in G2 and G4. While, G3 displayed the interprismatic enamel loss and dentin exposure. In conclusion, etching agents can damage enamel structure. To reduce the risk of caries, it is advisable to use lower concentrations, since an acceptable bond strength is less than 40 MPa.

### In Vitro Evaluation of the Effect of Different Remineralizing Agents on Human Enamel

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This in vitro study aimed to qualitatively evaluate the effect of different remineralizing agents on the enamel pattern, after exposure to an acid solution. Fifteen human sound extracted third molars were collected for the present study. Tooth crowns were transversally separated from roots, stored in a 0,5% chloramine solution and randomly allocated into the following five groups: Control Group (CG), without treatment; Negative Group (NG), treated with lactic acid solution; Mousse Group (MG), treated using a mousse of casein phosphopeptide (CPP) and amorphous calcium phosphate (ACP), associated with 0,33% sodium monofluorophosphate; Biosmalto Group (BG), treated with a dentifrice

containing amorphous calcium phosphate functionalized with fluorine and carbonate-coated with citrate; Duraphat Group (DG), treated with a 5% sodium fluoride varnish. While CG remained immersed in chloramine solution for the entire period of the study, all the other groups were immersed in lactic acid for 2 h and then subjected to the appropriate treatment for additional 2 h, repeating the protocol 3 times a day, for 8 d. Finally, all samples were examined by SEM. Scanning electron micrographs showed that CG had the typical sound enamel morphology with intact enamel rods and interrods, while NG presented an irregular surface with early pattern of demineralization characterized by interprismatic tissue dissolution without apparent destruction of enamel prism cores. MG showed intact enamel prismatic arrangement and slight interprismatic dissolution, while BG and DG presented not defined margins of enamel prisms and partial loss of interprismatic substance, being more pronounced in DG-treated samples. Qualitative SEM evaluations demonstrate that topical applications of CPP+ACP associated with sodium monofluorophosphate could represent the most effective protocol for re-establishing the intact enamel structure, in the event of exposure to acid environment.

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