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## Abstracts

### Contents

- Session 1  
**291 Microbiology**
- Session 2  
**303 Hard Tissues/Epidemiology**
- Session 3  
**314 Clinical Studies–1**
- Session 4  
**326 Clinical Studies–2**
- Session 5  
**336 Diagnostics**
- Session 6  
**347 Epidemiology**
- Session 7  
**357 De- Re-mineralization**
- Session 8  
**366 Fluoride**
- Session 9  
**373 Epidemiology**
- 381 Author Index**

## Session 1 Microbiology

1

### Correlation Between Relative Bacterial Activity and Sucrose-Related H<sup>+</sup>-Concentration of *Lactobacillus paracasei* and *Fusobacterium nucleatum*

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Treatment decision of a caries lesion depends on its activity status. In our previous pilot-study, we revealed distinct trends of relative bacterial activity of caries-associated and non-caries-associated bacteria. Nevertheless this approach could not identify, if increased acid production is the main reason for high relative bacterial activity measurements. Therefore, the present study aims at correlating the relative bacterial activity of caries-associated and non-caries-associated bacteria with H<sup>+</sup>-concentration in co-culture. Well plates were prepared with modified BHI-medium (L-cysteine, hemine) and overnight cultures of *Lactobacillus paracasei* (caries-associated) and *Fusobacterium nucleatum* (non-caries-associated) maintaining anaerobic conditions throughout the whole experiment. Bacterial growth at 37°C was measured in intervals of 60–120 minutes using a microplate photometer (OD620 nm) before and after adding sucrose to the samples. One well plate at each time point (0 h, 1 h, 3 h, 6 h, and 10 h) was opened, pH was measured and co-cultures were sampled [with sucrose (n = 12) and without sucrose (n = 12)]. Real-time quantitative PCRs were applied targeting the 16S-gene and the 16S-rRNA. As the 16S-rRNA-abundance represents the number of ribosomes, while the 16S-gene-abundance represents the number of genomes, the quotient of the relative abundance functions as a measure for the relative bacterial activity. For *L. paracasei* an increase in relative bacterial activity [Mean, SE (62.8%±23.5%)] was observed within the first

three hours after sucrose-pulse, while *F. nucleatum* showed a clear decrease in relative bacterial activity (−35.0% ± 9.6%); simultaneously the H<sup>+</sup>-concentration increased (1.15E−05mol\*l<sup>−1</sup> ± 4,61E−07mol\*l<sup>−1</sup>). Consequently, a significant positive correlation was found between the relative activity of *L. paracasei* and H<sup>+</sup>-concentration (Spearman rank correlation, r = 0.638 ; p = 0.002), while *F. nucleatum* exhibited a negative correlation (Spearman rank correlation, r = −0.741; p ≤ 0.001). In conclusion, under conditions chosen a clear but moderate correlation between relative bacterial activity and sucrose-related acidogenicity could be observed.

2

### The Antibacterial Effectivity of Root Canal Irrigants' Gas and Redissolved Phases

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Success of endodontic treatment depends on the eradication of microbes from the root canal system. The complexity of the infected root canal system is the main obstacle in achieving complete cleaning. During the root canal treatment the presence of remaining, mechanically untouched, areas increases the importance of chemical disinfection. The irrigants should be able to penetrate into the side-canal. The aim was to study the antibacterial activity of root canal irrigants' gas phase and their ability to redissolve while preserving efficacy. We used inoculation loops to place 1 µL of *Enterococcus faecalis* suspension (containing 9×10<sup>7</sup> CFU/ml bacteria) above 37°C 10 mL of 0.12% hyperpure chlorine-dioxide (ClO<sub>2</sub>), 2% chlorhexidine-digluconate (CHX), 2.5% sodium-hy-

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pochlorite (NaOCl), 10% potassium-iodide (KI) or distilled water in airtight bottles. One and 10 minutes later the surviving bacteria were plated and two days later the growing colonies were counted. In a further experiment, a Durham tube was filled up with bacterial suspension. The Durham tube was put in a closed Eppendorf in which the level of irrigants' was lower than the orifice of Durham tube. After 10 minutes in 37°C, the number of surviving bacteria was determined. Wilcoxon/Mann-Whitney tests were used for statistical analysis. The gas phase of ClO<sub>2</sub> killed all the bacteria within one minute. After ten minutes the potassium-iodide agent reduced the amount of bacteria from 9×10<sup>7</sup> CFU/ml to 3×10 CFU/ml, sodium-hypochlorite decreased initial bacterial count only with one order of magnitude, while chlorhexidine-digluconate and distilled water did not significantly alter number of surviving bacteria. In the study of redissolved ClO<sub>2</sub> eradicated bacteria, potassium-iodide showed a slight reduction, while chlorhexidine-digluconate and sodium-hypochlorite were inefficient. The ClO<sub>2</sub> is volatile and has powerful antibacterial effects in both gas and redissolved phases.

We would like to express our thanks to our dental students for the laboratory work. This study was supported by FOK/DH/-2013 of the Dental Faculty of Semmelweis University and the Hungarian Scientific Research Fund (OTKA K112364). All authors declare no conflict of interest and the authors have not received any financial support from the hyper-pure ClO<sub>2</sub> (Solumium Dental and-Oral) producing company.

### 3

#### **Streptococcus oligofermentans Reduces Cariogenicity of Pre-Formed Microcosm Biofilms**

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*Streptococcus oligofermentans* was able to inhibit the growth of cariogenic *Streptococcus mutans* in biofilms. Hence it is considered as a potential probiotic to prevent caries. Since the onset of dental caries links to multi-species biofilms, it is essential to investigate whether *S. oligofermentans* can inhibit the cariogenicity of multi-species biofilms as well. To this end, microcosm biofilms were inoculated from human saliva and grown in a 96-well active attachment model in the presence of 0.2% sucrose for 24 h. Then either growth medium or *S. oligofermentans* LMG 22279 in growth medium (5×10<sup>6</sup> CFUs) was added to the 24-h microcosm biofilms. These biofilms were grown for another 48 h. All biofilms (before and after the addition of *S. oligofermentans*) were grown under either constantly neutral pH or pH-cycling conditions (8-h neutral pH and 16-h pH5.5). The biomass and lactic acid production of the biofilms were examined after 72 h. The pH regime greatly influ-

enced the biomass and acidogenicity of the biofilms. The microcosm biofilms (without *S. oligofermentans*) grown under constantly neutral pH condition showed significantly less biomass and less lactic acid production (3.42 ± 0.41 mM) than those grown under pH-cycling condition (9.32 ± 0.64 mM). The addition of *S. oligofermentans* reduced the biomass of the microcosm by 2-fold (neutral pH) or 3-fold (pH-cycling), as compared to the group without *S. oligofermentans*. Similarly, the presence of *S. oligofermentans* reduced the lactic acid production of the biofilms by 1.8–1.9 fold, irrespective of the pH regime. In conclusion, *S. oligofermentans* was able to inhibit the growth and lactic acid production of 24-h pre-formed microcosm biofilms. It can be a competent probiotic for caries prevention.

This research was funded by the Department of Preventive dentistry of Academic Center for Dentistry Amsterdam (ACTA).

### 4

#### **Radiographic Depth Assessed by ICCMS and Dentine Infection in Approximal Caries Lesions in Permanent Teeth**

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The aim was to investigate the relationship between depth (radiolucency) of approximal lesions, and dentine infection with aciduric bacteria in permanent pre-molar and molar teeth. 48 patients aged between 13–52 years (mean 32; SD = 11.9) with approximal lesions that received operative treatment, were included in this study. Dentine was sampled with a sterile bur just pulpally to the enamel dentine junction. The bur was placed in 5 ml of phosphate buffered saline water (pH = 7) and processed within one hour after they were collected. The suspension (100 µl) was inoculated onto pH agar plates (pH 7, 6.5, 5.5 and 4.5) and incubated anaerobically for 7 days and evaluated according to presence or absence of bacterial colonies. One examiner evaluated the lesions depth on bitewing radiographs twice using the ICCMS 4 point classification system (R<sub>ICCMS</sub>: 0 = no radiolucency, 1 = initial, (radiolucency at max limited to the outer 1/3 of the dentine); 2 = moderate, (radiolucency in the middle 1/3 of the dentine); or 3 = extensive (radiolucency in the inner 1/3 of the dentine). Unweighted Kappa (K) and percentage agreement (PA) was used to assess the intra-examiner agreement of R<sub>ICCMS</sub> and presence of bacteria on those agar plates adjusted to a pH at 5.5 (aciduric bacteria = infected dentine), respectively. The relationship between R<sub>ICCMS</sub> and infected dentine was tested by the Fisher exact test: K was 0.8 (95%CI = 0.65–0.95) and PA was 100%. 14, 24 and 10 lesions were radiographically assessed as initial, moderate or extensive, respectively. In 12 cases (86%) the dentine was not infected among those 14 lesions assessed as initial. The dentine was infected in 21 (86%) out of the 24 lesions assessed as moderate and in all 10 cases the dentine was infected in the extensive assessed lesions (p < 0.001). There is a significant correlation between the radiographic lesion

depth and presence of aciduric bacteria into dentine. The dentine in moderate/extensive lesions ( $R_{ICCMS} = 2$  and 3) seems to harbor aciduric bacteria whilst this is not the case for most initial assessed lesions.

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## 5

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

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The aim of this in-vitro study was to assess antimicrobial efficacy of different toothpastes against some oral pathogens isolated from dental plaque. A total of four toothpastes commercially available in Uzbekistan (Lacalut aktiv (A), Parodontol 3D effect (B), Miswak (C), Colgate Total Pro Gum Health (D)) with different antimicrobial ingredients were tested for their antimicrobial activity against *Streptococcus mutans*, *Lactobacillus rhamnosus* and *Candida albicans* obtained from native cultures isolated from a patient's mouth. Distilled sterile water was used as negative control (NC). The disk diffusion method was used to test antimicrobial activity, 5 disks were prepared from each tested materials (1 g of toothpaste mixed with 5 ml of water). Inhibition zones were measured in millimeter after 24 hr. Mean values and standard deviations of inhibition zones were calculated. Two-way ANOVA (microorganisms and product) followed by Dunnett's test (against water as control) was performed; p-value of less than 0.05 was considered significant. The results indicated that two of the toothpastes (B and D) produced extinction zones significantly different from control on *S. mutans* ( $10 \pm 0.8$  and  $9.5 \pm 1$  mm) and *L. rhamnosus* ( $14 \pm 0.7$  and  $13 \pm 1$  mm), whereas A and C were not significant from water (0 to 0.25 mm). On *C. albicans* no toothpaste achieved an effect that was different from control. In conclusion, in vitro the antimicrobial activity levels of toothpastes B and D against to *S. mutans* and *L. rhamnosus* were significantly higher comparing to toothpastes A and C. However, no differences between the toothpastes' antimicrobial activities against to *C. albicans* were revealed. The toothpastes with antimicrobial agents need better researched to determine the correct clinic therapeutic application.

## 6

### Preparation of Novel Gallium-Curcumin Nanocomplex to Treat *Streptococcus mutans* NCTC10449

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Curcumin and gallium are reported to have anti-caries characteristics. In this study a synergistic action of Curcumin and gallium was explored by preparing Curcumin complexed gallium (Cur-c-Gal) nanoparticles and evaluating antibacterial activity on *S. mutans* NCTC10449. Curcumin complexed gallium (Cur-c-Gal) nanoparticles were prepared by the reduction of gallium chloride utilising curcumin as a reducing and stabilizing agent. Complex formation was confirmed by UV-Vis absorption spectra and Fourier transform infrared spectroscopy (FTIR) analyses. Particle size distribution of Cur-c-Gal was investigated using dynamic light scattering (DLS) technique. Cur-c-Gal was investigated for its ability to inhibit *S. mutans* NCTC10449 growth using the agar diffusion method on Iso-Sensitest agar plates which were inoculated with standardised cultures of *S. mutans* NCTC10449 (OD 600=0.03), and incubated for 48 h anaerobically at 37°C. The diameters of zones of inhibition around test samples and controls were measured in triplicate. Statistical analyses were conducted using the GraphPad software (San Diego, USA) and Tukey-Kramer multiple comparison tests. Particle size analysis by DLS of Cur-c-Gal showed an average size of 62 nm (range 24–105 nm). UV-Vis absorption spectra of Cur-c-Gal nanoparticles shifted to 475 nm indicating involvement of the carbonyl group of curcumin in metal complexation. Absence of 1640  $\text{cm}^{-1}$  (C = O stretching), 1383  $\text{cm}^{-1}$ , 1233  $\text{cm}^{-1}$  and 962  $\text{cm}^{-1}$  peaks in the FTIR spectra of Cur-c-Gal suggests interaction of GaCl at these sites. Agar diffusion assay established zones of inhibition of *S. mutans* NCTC10449 to be  $18 \pm 0.5$  mm for Cur-c-Gal compared with 0.2% chlorhexidine ( $41 \pm 3.5$  mm) and curcumin (0 mm). Cur-c-Gal achieved a statistically significant ( $p < 0.05$ ) growth inhibition of *S. mutans* NCTC10449 compared with controls and may have potential use in caries treatment.

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## Bacterial Reduction in Sealed Lesions Depends on Initial Bacterial Loads, Sealing Periods and Coronal Treatment

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We aimed to evaluate the survival kinetics of sealed dentin bacteria. We additionally assessed how cavity liners like calcium hydroxide (CH) or mineral trioxide-aggregate (MTA) reduce the numbers of sealed bacteria. **Methods:** Human dentin discs were mounted in a novel dual-chamber device. The simulated coronal surface was submitted to a *Lactobacillus rhamnosus* biofilm-model to induce bacterially-contaminated carious lesions, which were then sealed using composite with or without CH or MTA lining. The numbers of sealed bacteria were varied, as were the sealing periods (between 7 and 56 days). During the simulated sealing period, a pulpal fluid circulation was simulated. Our outcome was the number of bacteria remaining per g dentin after sealing. Statistical evaluation was performed via Mann-Whitney-U-test and ordinary-least-square regression analysis (OLS). **Results:** Sealing bacteria was found to be significantly affected by the initial bacterial load ( $\beta = 0.53$  [95% CI: 0.43/0.62]  $\times 10^6$  CFU/g,  $p < 0.001$ /OLS) and the sealing period (regression coefficient =  $-0.03$  [95% CI:  $-0.04/-0.02$ ]  $\times 10^6$  CFU/g,  $p < 0.001$ ). The relative reduction (in % of CFU/mg) followed a first-order kinetics with the largest reduction occurring in the first two weeks. The remaining bacterial numbers were significantly lower when MTA (15% [14/16]%) instead of CH had been placed (17% [16/19%],  $p < 0.05$ /Mann-Whitney) and highest when the lesion was restored without any lining (45% [28/72%]  $p < 0.001$ ). **Conclusions:** The largest bacterial inactivation occurs early during sealing. Sealing larger number of bacteria will lead to more bacteria surviving. Cavity liners might be useful for bacterial inactivation beneath sealants or restorations.

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## Aggregation by *Streptococcus mutans* Strains in Presence of Blood Components Contributes to Pathogenesis of Infective Endocarditis

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*Streptococcus mutans*, a major pathogen of dental caries, is considered to be a causative agent of infective endocarditis (IE). Previous studies have characterized 120-kDa collagen-binding proteins (CBPs) on the cell surface of some *S. mutans* strains and approximately 10–20% of individuals are considered to harbor CBP-positive strains in the oral cavity. In addition, bacterial DNA encoding

CBPs is frequently detected from extirpated heart valve specimens obtained from IE patients. On the other hand, expression of a 190-kDa protein antigen (PA) was demonstrated to inhibit CBP activity. Some pathogenic bacteria have been reported to aggregate in the presence of blood components, such as plasma or serum, which is considered to be an important virulence factor for development of systemic diseases. Here, we analyzed the pathogenesis of IE induced by *S. mutans* strains with different expression patterns of CBP and PA by focusing on their aggregation with blood components.

A total of 45 *S. mutans* strains were selected from our laboratory stock (15 positive for CBP-/PA+, CBP+/PA+ and CBP+/PA- each). Intergroup differences were estimated using Bonferroni's method after analysis of variance. CBP+/PA- strains showed prominent aggregation rates in the presence of both serum and plasma obtained from a human volunteer (65.9% and 62.9%), which was significantly higher than those of CBP+/PA+ (9.6% and 15.8%) and CBP-/PA+ strains (2.8% and 2.1%), respectively ( $P < 0.001$ ). In addition, extirpated heart valves from IE model rats infected with a CBP+/PA- strain displayed prominent bacterial mass formation 7 days after infection, which was not observed following infection with a CBP+/PA+ or CBP-/PA+ strain. Furthermore, ex vivo biofilm assays of extirpated bovine heart valves infected with  $1 \times 10^9$  CFU of each strain in the presence of fetal bovine serum revealed that the average bacterial number of CBP+/PA- strains recovered from the heart valves was  $1.4 \times 10^8$  CFU, which was significantly higher than those of CBP+/PA+ ( $5.6 \times 10^7$  CFU) and CBP-/PA+ strains ( $8.8 \times 10^6$  CFU) ( $P < 0.05$  and  $P < 0.001$ , respectively). Together, these results suggest that CBP+/PA- *S. mutans* forms bacterial aggregates in the presence of blood components, contributing to the pathogenicity of IE.

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## Long-Term Observations of Non-Alcoholic Steatohepatitis Aggravated by *Streptococcus mutans* Infection in Mice

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*Streptococcus mutans*, a major pathogen of dental caries, is known to cause infective endocarditis after invading the bloodstream. In our previous study, administration of *S. mutans* strain TW871 aggravated non-alcoholic steatohepatitis (NASH) in mice fed a high-fat diet (HFD). Furthermore, after an initial 4-week HFD, we identified typical NASH-like findings 12 weeks following administration of that bacterial strain, though periodic observations thereafter were not performed. In the present study, following approval from the Animal Experiment Committee of Osaka

University Graduate School of Dentistry, we compared the effects of various HFD feeding periods in mice administered TW871. Six-week-old C57BL/6J mice were given an HFD for 4 weeks, then *S. mutans* TW871 ( $1 \times 10^7$  CFU) or phosphate-buffered saline (PBS) was intravenously administered under general anesthesia. Following euthanasia at 8, 12, 16, or 44 weeks after administration, conventional NASH evaluations were performed and histopathological findings of obtained tissue sections were evaluated using image J software. At 8 weeks after TW871 administration, no typical NASH-like findings were identified, whereas they were observed after 12 weeks, with progression of severity noted after 16 weeks and severe NASH after 44 weeks. In the PBS-administered group, only initial stage NASH-like findings were identified at 44 weeks and no typical findings of NASH were observed after 8, 12 or 16 weeks. These results suggest that administration of only specific *S. mutans* strains aggravated NASH conditions much earlier in NASH-model mice.

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## 10

### Confocal Microscopy Monitoring of pH Dynamics in a 5-Species Dental Biofilm Model Under Flow Conditions

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Measuring pH dynamics in dental biofilms is of utmost importance for a thorough understanding of the caries process. We have developed a confocal microscopy based methodology to monitor pH in biofilms at the microscale in real-time. Here, we measure pH developments in different layers of a 5-species biofilm model of dental caries under static and flow conditions. Biofilms were grown in the absence of sucrose in ibidi  $\mu$ -slides for 30 h or 120 h. Biofilms were then stained with the ratiometric probe C-SNARF-4 at pH 7.0, incubated with 0.4% glucose, and z-stacks were acquired in 9 microscopic fields of view/biofilm after 45 min of static incubation and again after 15 min and 45 min of flow (1 mm/min). Images were cleared for cells and pH in the extracellular matrix was determined ratiometrically. Experiments were performed in duplicate and analyzed with t-tests. Under static conditions, pH in 30-h-biofilms dropped to 5.5–6.5 in different microenvironments ( $\bar{\Phi} = 6.06$ ;  $sd = 0.27$ ). pH was lower at the top of the biofilm than at the bottom ( $p < 0.05$ ). Under dynamic conditions, average pH rose ( $\bar{\Phi} = 6.63/15$  min;  $sd = 0.20$ ;  $\bar{\Phi} = 6.69/45$  min;  $sd = 0.25$ ), but distinct microenvironments were conserved. pH at the biofilm bottom became lower compared to pH at the top ( $p < 0.05$ ). In 120-h-biofilms, pH had dropped more slowly after 45 min of static incubation (6.0–7.0;  $\bar{\Phi} = 6.84$ ;  $sd = 0.22$ ), without significant differences between top and bottom ( $p = 0.59$ ). Despite the onset of flow, pH continued to drop at different rates ( $\bar{\Phi} = 6.25/15$  min;  $sd = 0.22$ ;  $\bar{\Phi} = 6.19/45$  min;  $sd = 0.29$ ). No significant differences were observed between top and bottom of the biofilms ( $p = 0.52$ ). In conclusion, pH in 120-h model bio-

films drops more slowly upon exposure to glucose than in 30-h-biofilms, but low-pH-microenvironments are conserved better under flow conditions.

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## 11

### Effect of *Candida albicans* on the Cariogenic and Acidogenic Potentials of *Streptococcus mutans* Biofilms

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This study aimed to evaluate the effect of *Candida albicans* (ATCC 90028) on the cariogenic and acidogenic potentials of *Streptococcus mutans* (ATCC UA159) *in vitro* biofilms. Single-species (SS; *S. mutans*;  $n = 12$ ) and dual-species (DS; *S. mutans* + *C. albicans*;  $n = 24$ ) biofilms were grown on sound bovine enamel slabs with known baseline hardness in the presence of culture media supplemented with 20 mM glucose and 3 mM sucrose for 24, 48 and 72 hours. The medium was replaced daily and the pH of the spent media was recorded. Enamel slabs ( $n = 4$  for SS and  $n = 8$  for DS) were collected at the end of each experimental period. Counts of viable cells and the percentage of surface microhardness change (%SMC) were also assessed. %SMC was significantly lower in DS ( $21.3 \pm 8.3$ ;  $n = 24$ ) than in SS ( $48.0 \pm 16.2$ ;  $n = 12$ ) biofilms ( $p < 0.01$ ). Higher demineralization was found for SS at 72 h compared to 24 h (24 h:  $42.8 \pm 7.2$  a; 48 h:  $42.0 \pm 19.5$  ab; 72 h:  $63.9 \pm 6.0$  b) but similar %SMC was found for DS among the different biofilm time points (24 h:  $21.6 \pm 7.3$  a; 48 h:  $22.6 \pm 7.4$  a; 72 h:  $22.7 \pm 7.2$  a). Statistically higher pH values were found for DS ( $5.37 \pm 0.20$ ;  $n = 24$ ) than for SS ( $4.38 \pm 0.30$ ;  $n = 12$ ) biofilms ( $p < 0.01$ ). No difference on acidogenic potential was found for SS biofilms among the different experimental periods (24 h:  $4.29 \pm 0.03$  a; 48 h:  $4.26 \pm 0.23$  a; 72 h:  $4.35 \pm 0.03$  a), but statistically higher pH was found for DS at 48 h and 72 h compared to 24 h (24 h:  $5.08 \pm 0.08$  a; 48 h:  $5.73 \pm 0.23$  b; 72 h:  $5.89 \pm 0.29$  b) ( $p < 0.05$ ). The pH rise observed in DS biofilms strongly correlated with increased number of viable fungal cells over time ( $r = 0.86$ ;  $p < 0.01$ ). The present data suggest that *C. albicans* may decrease both the cariogenic and acidogenic potentials of *S. mutans* biofilms.

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## 12

### Fluorescence Characterization of Oral Bacteria for Classification

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The aim of this study was to investigate the fluorescence characteristics of seven anaerobic oral bacteria strains related to oral disease. The following bacterial strains were purchased from

Korean Collection for Type Cultures (KCTC, South Korea); *Actinomyces israelii*, *Enterococcus faecium*, *Fusobacterium nucleatum*, *Lactobacillus gasseri*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Veillonella parvula*. The organisms were grown on solid state Columbia agar medium containing hemin (5 mg/L), vitamin K1 (10 µL/L), and sheep blood (50 mL/L). All strains were incubated in an anaerobic system in an atmosphere of 80% N<sub>2</sub>, 10% CO<sub>2</sub>, and 10% H<sub>2</sub> at 37°C for four days. The fluorescence emission of all samples was measured with a fluorescence spectrometer. The spectrometer was programmed to sweep through an excitation range between 335 nm and 425 nm, at 10 nm intervals, thereby yielding a total of 10 emission spectra for each sample. At every excitation wavelength, an emission spectrum was recorded between 400 nm and 700 nm, with data points logged every 0.5 nm. The complete fluorescence data set collected for each sample was therefore a three-dimensional data cube, composed of emission intensity measurements at 6,000 excitation–emission wavelength combinations. An excitation–emission matrix was created with the data set using graphing software. The differences in the autofluorescence characteristics of the seven oral bacteria were determined by principle component analysis (PCA) of the fluorescence spectrum data using SPSS version 23.0. The PCA classified the seven oral bacterial strains into three distinct groups as follows: Group 1: *A. israelii*, *E. faecium*, *S. mutans*, and *V. parvula*; Group 2: *L. gasseri*, and *F. nucleatum*; Group 3: *P. gingivalis*. These results demonstrated that fluorescence spectroscopy, when coupled with PCA processing, has the potential to detect and classify these bacteria.

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### 13

#### Antibacterial Activity of S-PRG Eluate on Dental Caries-Related Microcosm Biofilms

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The aim of this study was to determine the antibacterial activity of S-PRG eluate on dental caries-related microcosm biofilms. Dental biofilms were grown on bovine enamel discs by initiating them from human saliva, followed by growth in 0.3% sucrose growth medium. After the biofilms were matured for 24 hours, each specimen was treated for 1 minute with S-PRG eluates at different concentrations (25, 50 and 100%), distilled water (negative control) and 0.1% chlorhexidine (positive control) twice daily for 6 days. On the 7th day, the red/green ratio (R/G value) of biofilm fluorescence images taken with a QLF-D Biluminator<sup>TM</sup> was calculated using an image analysis program, and the numbers of total and aciduric bacteria within the biofilm were counted to evaluate the antibacterial activity. The Vickers hardness was also evaluated by measuring the percentage change in surface microhardness

(%SHC) in the enamel. The difference between the treatment groups was analyzed by one-way ANOVA and Tukey's test. The R/G values (1.35; 1.25; 0.98), total bacterial log<sub>10</sub>CFU/ml (6.24; 5.81; 5.43), aciduric bacterial log<sub>10</sub>CFU/ml (5.34; 4.72; 4.05) and % SHC (33.37; 29.60; 22.38) of all S-PRG eluate groups, by each concentration, were significantly different from the distilled water group (R/G values: 1.67; % SHC: 73.48; total and aciduric bacterial log<sub>10</sub>CFU/ml: 7.19 and 7.08) ( $p < 0.0001$ ). A comparison of the S-PRG eluate groups revealed that decreased significantly as the S-PRG eluate concentration increased ( $p < 0.0001$ ). The 100% S-PRG eluate group showed significant reductions in R/G value and aciduric bacterial CFUs when compared with the 0.1% chlorhexidine ( $p < 0.0001$ ). In conclusion, S-PRG eluates showed concentration-dependent antibacterial activity, and they were effective in inhibiting the formation of dental caries-related microcosm biofilms.

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### 14

#### Photodynamic Inactivation of a Multi-Species Biofilm Cultured from Cariogenic Bacteria In Vitro

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For caries-active patients, supportive antimicrobial measures may be useful additionally to mechanical biofilm-removal. Hereby, antimicrobial photodynamic therapy (aPDT) may be an option. Aim of this study was to investigate the efficacy of aPDT with phenalenone-based photosensitizer (PS) SAPYR for inactivation of a multi-species biofilm cultured from cariogenic bacteria in comparison to chlorhexidine-digluconate (CHX). Multispecies-biofilms were formed from *Actinomyces naeslundii* (AN; DSM-43013), *Actinomyces odontolyticus* (AO; DSM-19120) and *Streptococcus mutans* (SM; DSM-20523) under anaerobic conditions in 96-well-plates after pellicle-formation using steril-filtered saliva. A mixture of 50% modified fluid-universal-medium, 40% saliva and 10% bovine calf serum was used as culture-broth (modified from Zurich biofilm-model). After a culture period of 72 h, biofilms were either incubated with SAPYR for 10 min and irradiated for 10 min (PIB 3000, Waldmann;  $\lambda = 380\text{--}440$  nm, 50 mW/cm<sup>2</sup>; controls: untreated, PS only, light only) or were incubated with CHX (0.2%, 2%) for 20 min. Antimicrobial efficacy was evaluated by colony-forming-units (CFU;  $n = 12$ ). Additionally, biofilms were exemplarily visualized by scanning electron microscopy (SEM) in low-vacuum-mode. Untreated biofilms showed an overgrowth of

SM ( $4.6 \times 10^7$ ) of about 1 log<sub>10</sub>-step compared to AN ( $7.5 \times 10^6$ ) and AO ( $7.7 \times 10^6$ ). aPDT with SAPYR reduced CFU of SM more (2.9 log<sub>10</sub>) than CFU of AN and AO (2 or 1.5 log<sub>10</sub>, respectively). CHX 0.2% inactivated all species by 3.6–4.3 log<sub>10</sub>-steps while CHX 2% led to eradication by >6 log<sub>10</sub> below detection limit. SEM showed porous structures in the bacterial cell walls after aPDT-treatment. Under these experimental conditions, aPDT with SAPYR led to a shift in microbial composition in the biofilms due to higher inactivation of SM compared to AN and AO. SEM-visualization gave reason that damaging bacterial cell walls may be the mechanism of action of aPDT with SAPYR.

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## 15

### Fluorescence Change of Oral Microorganisms in Different Culture Media

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Dental plaque emits red fluorescence under a blue visible light near ultra-violet light. The fluorescence characteristics of each microorganism were reported in several studies. The aim of this study was to evaluate fluorescence change of oral microorganism in different culture media in vitro. The gram-positive *Actinomyces naeslundii* (AN, KCTC5525) and *Lactobacillus casei* (LC, KCTC3109), and gram negative *Prevotella intermedia* (PI, KCTC3692) which are known to emit red fluorescence were used this study. Each bacterium was activated in broth and cultivated in different agar media at 37°C for 7 days. Tryptic soy agar with hemin and vitamin K<sub>3</sub> (TSA), TSA with sheep blood (TSB), and basal medium mucin (BMM) medium were selected in this study. Fluorescence feature according to bacterial growth was taken under 405 nm wavelengths light using the quantitative light-induced fluorescence-digital (QLF-D) device. The red, green, and blue value of fluorescent colony were obtained by image analysis software and the red to green ratio (R/G value) was calculated for quantitative comparison. This procedure was repeated with five times. The AN, LC, PI colonies showed red fluorescence in TSB medium. The R/G value was  $7.24 \pm 1.70$ ,  $7.5 \pm 0.60$ ,  $8.06 \pm 2.64$ , respectively. But red fluorescence of all bacteria was significantly reduced in TSA and BMM medium ( $P < 0.01$ ). Blood containing medium would be a significant factor to red fluorescence for oral bacteria. Based on this in vitro study, it could be concluded that red fluorescence of oral bacteria would be affected by growth components, especially blood. It is speculated that bleeding in the oral cavity can increase red fluorescence of dental plaque.

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## 16

### Short and Long Term Effects of a Single Rinse with Different Mouthwashes

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The use of an ideal antiseptic mouthwash that is effective against pathogen microbes, but is not harmful to human body, helps keep good oral hygiene and assures adequate environment for dental treatment.

Aim of the study was to examine the change in plate count and number of *S. mutans* of saliva after a single rinse with different mouthwashes. We collected 1 mL unstimulated saliva from volunteer patients at baseline (30 individuals in every group) whose oral hygiene was poor (DMF  $\geq 10$ , minimum 5 active caries, Periodontal Screening Recording Index (PSR)  $\geq 2$ ). The patients rinsed with hyper-pure 0.0015 % ClO<sub>2</sub>, Listerine Total Care, 0.12% chlorhexidine or BioGate Si\*Clean mouthwash for 1 minute. Five minutes later 1 mL saliva was collected. In another 13 patients 0.03% ClO<sub>2</sub> was used for 1 min and the saliva was gathered before and after 5 and 90 minutes. Results were compared to 0.12% chlorhexidine. After dilution, plating, incubation the total plate count on blood and *S. mutans* number (CFU/ml) on mitis salivarius agar, were determined before and after rinsing. Significant change was observed in total plate count (values in log<sub>10</sub>) after ClO<sub>2</sub> ( $9.2 \pm 8.6$  vs.  $8.7 \pm 8.3$ ) and chlorhexidine ( $9.5 \pm 8.8$  vs.  $9.1 \pm 8.8$ ) and in *S. mutans* number after ClO<sub>2</sub> ( $4.4 \pm 3.6$  vs.  $4.0 \pm 3.4$ ), chlorhexidine ( $4.3 \pm 3.7$  vs.  $3.0 \pm 2.4$ ) and Listerine ( $4.5 \pm 4.0$  vs.  $3.9 \pm 3.8$ ) treatment. ClO<sub>2</sub> showed the greatest total plate count reduction (68.6%). In case of poor oral hygiene or in high risk groups, daily hyper-pure ClO<sub>2</sub> mouthwash could lead to preventive and therapeutic benefits.

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### Antimicrobial Effect of Tissue Tolerable Plasma on In Vitro *Streptococcus mutans* Biofilms

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The objective of the present study was to determine how the twice-daily treatment with tissue tolerable plasma (TTP) affects the development of *Streptococcus mutans* biofilms, as well as the antimicrobial effect against *Streptococcus mutans* mature biofilms. Standardized cell suspensions (ATCC UA159) were prepared for biofilm culture on saliva-coated sterilized dentin blocs (4×4×2). In experiment 1, the biofilms were submitted to TTP treatment during 1, 5 and 10 minutes (3 mm of plasma tip-to-sample distance) twice-daily for 5 days. In experiment 2, the biofilms were treated one-time after 5 days of biofilm growing. Within the controls groups (C), dentin surfaces were treated with 0.12% gel chlorhexidine; argon gas for 1, 5 and 10 minutes respectively, and one group did not receive any treatment at all. Three independent replicates were performed. For microbiological analyses, dentin samples had the biofilms collected, serially diluted and CFU counts were estimated after 48 h of incubation. TTP produced a dose-dependent reduction in log<sub>10</sub> CFU/mg of biofilm counts when applied on *S. mutans* mature biofilms on one-time groups: (1 min 11.2), (5 min 10.4) and (10 min 9.98) compared to negative control groups (ST 12.6), (NaF 12.3), (Arg<sub>1</sub> 12.3), (Arg<sub>5</sub> 11.1), (Arg<sub>10</sub> 11.7) and (CHX 12.9) (mixed model ANOVA/ Dunnet,  $p < 0.05$ ). However, log<sub>10</sub> CFU/mg of biofilm values did not show significant differences among the treatment duration when the biofilms were treated with twice-daily TTP (1 min 11.9), (5 min 12.1) and (10 min 12.2) compared to negative control groups (ST 14.7), (NaF 14.2), (Arg<sub>1</sub> 12.9), (Arg<sub>5</sub> 13.8), (Arg<sub>10</sub> 12.6) and (CHX 0.0) (mixed model ANOVA/ Kruskal-Wallis tests,  $p < 0.05$ ). For disinfection of dentin surfaces, twice-daily TTP treatment is a promising mechanism for the inhibition of biofilm development, presenting antimicrobial dose-dependent effect against mature *S.*

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### The Fitness Costs of Fluoride Resistance in *Streptococcus mutans* Biofilms

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*Streptococcus mutans* can develop stable resistance to fluoride through genetic modification. Findings on the fitness costs incurred from this resistance have been inconsistent. The aim was to compare the fitness of biofilms of fluoride-resistant *S. mutans* UF35 and UA159-FR (UAFR) to that of wild-type UA159. Strain UF35 contains one single nucleotide mutation. Strain UAFR contains multiple mutations and was obtained by growing strain UA159 on agar containing increasing concentrations of fluoride. All biofilms were grown for 48 h in the presence of 0.2% sucrose for two cycles of neutral pH (8 h) and pH 5.5 (16 h). During the second 16 h incubation, NaF (0–1mM) was added. The non-F exposed 48 h biofilms were treated with chlorhexidine (0–0.04%) for 5 min or with solutions of pH 3.0 for 0 to 30 min. The UAFR biofilms behaved differently from UF35 and UA159 biofilms. The biomass of UAFR was 2–4 fold higher than that of the other two strains. Chlorhexidine treatments resulted in a decrease in lactate production in a dose-dependent manner in all biofilms. However, the reduction at highest concentration of CHX (0.04%) was much less in UAFR biofilms (19% ± 5%) as compared with the other two biofilms (UA159, 85% ± 4%; UF35, 79% ± 12%). No difference in response to low-pH killing was observed for the three biofilms. UAFR and UF35 biofilms were indeed more resistant to fluoride than UA159 biofilms. UAFR biofilms displayed stronger resistance to fluoride than UF35 biofilms. In conclusion, the resistance to fluoride did not incur fitness costs of *S. mutans* strains in biofilms. The UAFR strain could better cope with the adverse challenges than UF35 and UA159 strains. This might be related to the multiple mutations induced by the natural selection of the fluoride resistance.

### Osteopontin Reduces the Adhesion Force of Dental Bacteria Without Blocking Bacterial Cell Surface Glycoconjugates

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The bovine milk protein osteopontin (OPN) has been shown to reduce the adhesion of oral bacteria to saliva-coated surfaces, which reduces biofilm formation and may contribute to caries control. We now quantified the effect of OPN (Lacprodan OPN-10) treatment on the adhesion force of *Lactobacillus paracasei* subsp. *paracasei* DSM 20020 and *Actinomyces naeslundii* AK 6 using atomic force microscopy based single cell force spectroscopy (AFM-SCFS). Furthermore, we performed a fluorescent lectin binding analysis (FLBA) to investigate if OPN treatment resulted in the selective blocking of specific cell surfaces glycoconjugates of *L. paracasei* subsp. *paracasei*, *A. naeslundii* and *Streptococcus mitis* SK 24. For AFM-SCFS, saliva-coated tipless AFM cantilevers were approached on single cells. The cantilevers were then retracted and adhesion was quantified from the recorded force curves. Adhesion on the same cells ( $n = 10$ ) was compared before and after OPN treatment. Adhesion energy was found to be reduced by 94% for *L. paracasei* and 61% for *A. naeslundii* ( $p < 0.05$ ). For FLBA, the binding of 75 FITC-labelled lectins to the three bacteria was screened. Lectins BanLec, ConA, VGA and WGA bound well to *A. naeslundii*, lectins ABA and HPA to *L. paracasei*, and lectins VGA and WGA to *S. mitis*. Immobilized bacteria were incubated with these lectins in the presence and absence of OPN. For each combination, 12 confocal images were acquired with fixed microscope settings, and average fluorescence intensities were determined. Experiments were performed in triplicate and analyzed with two-sample t-tests. No statistically significant differences were observed between OPN-treated and OPN-free cells ( $p > 0.05$ ). OPN strongly reduces the adhesion force of oral bacteria to saliva-coated surfaces, but the effect cannot be ascribed to the selective blocking of cell surface glycoconjugates.

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### Identification of Genomic Mutations Related to Fluoride Resistance in *Streptococcus mutans*

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Extensive and prolonged use of fluoride can lead to fluoride resistance in *Streptococcus mutans*. Previously, we have shown that this resistance in the *S. mutans* C180-2FR strain is partly due to a single nucleotide polymorphism (SNP) in the promoter of fluoride antiporter-coding genes. This study aimed to identify genomic mutations in the fluoride-resistant strain *S. mutans* UA159-FR (UAFR), which was step-wise selected on agar plates, and predict mutations that are related to fluoride resistance. To this end, we compared the complete genomic sequence of UAFR (NCBI GenBank accession: NZ\_CP007016.1) with that of *S. mutans* UA159 (NC\_004350.2) to identify SNPs, using Mauve to align the genomes and the GenBank annotation to identify the SNP-containing genes. The effect on protein function of SNPs leading to amino-acid substitutions was predicted with SIFT (<http://sift.bii.a-star.edu.sg/>). We were able to identify 13 non-synonymous protein-coding SNPs, which caused 12 amino-acid changes, as well as 11 SNPs in intergenic regions. A comparison of these SNPs with those from the comparison of *S. mutans* C180-2 versus C180-2FR, showed that both fluoride-resistant strains have a mutation upstream of fluoride antiporters, as well as one in pyruvate kinase. In addition, they both have an SNP in the intergenic region between *pepX* and *glpF*. In UAFR, we also identified a mutation in enolase, which is sensitive to fluoride. According to the SIFT prediction, the amino-acid substitution (T287I) in enolase may affect its function, although this prediction had low confidence. The other non-synonymous coding SNPs were predicted to be tolerated. In conclusion, three mutations, found *in silico*, related to fluoride resistance are functionally conserved in two fluoride-resistant *S. mutans* strains. These mutations need to be further investigated.

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### Oral Bacteria and Link with Human Cardiac Tissue and Valve Stenosis: Molecular and Immunohistochemistry Study

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**Aim:** To perform molecular and immunohistochemical analysis of oral pathogens in healthy human heart tissue and in calcified aortic stenosis. **Methods:** Dental plaque and heart valve samples from 10 patients with calcified aortic stenosis were collected for molecular analysis of *Streptococcus mutans*, *Prevotella intermedia*, *Porphyromonas gingivalis* and *Treponema denticola* by real-time Polymerase Chain Reaction (PCR). Heart valve tissue from 05 healthy young patients submitted to necropsy was also collected. Other fragments of all samples were fixed in 10% buffered formalin for histomorphological study (Hematoxylin/eosin), histochemistry (Giemsa) and immunohistochemistry (anti-*Streptococcus mutans*). **Results:** Molecular analysis of supragingival and subgingival plaques revealed high frequency of *S. mutans* and *P. intermedia* (90.0% to 100.0%), whereas *P. gingivalis* and *T. denticola* were present in a smaller number of samples (10.0% to 67.0%). *S. mutans* was found in 80.0% of calcified aortic stenosis by PCR and immunohistochemical analysis. Areas of calcification, fibrosis and myxoid degeneration were present in diseased valves, sites where we visualized streptococcus by histochemical reaction. In healthy valves, the DNA of *S. mutans* was also identified (80.0%), however, the histomorphological and immunohistochemical studies did not demonstrate tissue alterations or bacterial colonization, respectively. **Conclusion:** DNA of *S. mutans* was found at high frequency in patients with calcified aortic stenosis as well as in healthy cardiac tissue. However, visualization of this microorganism through immunohistochemical analysis only in calcified aortic stenosis may suggest viability and / or increased pathogen density in the disease, as well as a possible relationship with the histological changes in the cardiac valve.

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### Effect of Tissue-Tolerable Plasma on Oral Biofilms Formed In Situ

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The aim of this study was to evaluate the antimicrobial effect of Tissue-Tolerable Plasma [TTP] on oral biofilms formed *in situ* and also verify if this treatment damages the structure of enamel under treated biofilm. Twenty-two volunteers used palatine intraoral devices containing bovine enamel slabs that were treated 10 times per day with a 10% sucrose. After 7-day intraoral period, the biofilm formed over the enamel slabs were treated for 5 minutes, as follows: TTP, argon flow, 0.12% chlorhexidine, or 0.89% NaCl solution. Biofilms samples were collected, diluted in decimal series and plated in culture medium for the growth of total microorganisms, total streptococci, mutans streptococci and lactobacilli. In order to analyze whether the treatments damaged the enamel structure, the slabs were analyzed by scanning electron microscopy (SEM) and Raman spectroscopy. ANOVA/Holm-Sidak or Kruskal-Wallis/Dunn ( $p < 0.05$ ) were used for analyzing the data. TTP produced a significant reduction in  $\log_{10}$  CFU/mg of biofilm of the total microorganisms (from 8.560 to 7.383), total streptococcus (from 7.690 to 7.054) and mutans streptococci (from 6.469 to 5.692). Argon flow also significantly reduced the mutans streptococci counts (from 6.469 to 6.289). The plasma treatment did not influence on the viability of the lactobacilli under the conditions tested ( $p = 0.497$ ). SEM revealed smooth and homogeneous regions on the surface of the treated enamel, unobserved topographical differences between the enamel subjected to different treatments. In Raman spectroscopy, four major bands were presented in the inorganic phase. No statistical differences in the Raman spectra were observed for enamel of different treatments. In conclusion the plasma was effective in reducing viable bacteria present in mature oral biofilms produced *in situ* and it did not modify the morphology and composition of the enamel.

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### Association of *Lactobacillus casei/paracasei* with Caries Activity in Dentinal Lesions

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Molecular analyses of the microbial diversity have revealed that *Lactobacillus* spp. are frequently detected in dentinal caries lesions. However, differences in the microbiota between active and arrested cavities have been scarcely addressed. This study aimed to identify and to quantify the prevalence of *Lactobacillus casei/paracasei* (LCP) in active and arrested dentine carious lesions in children with early childhood caries. Dentine samples were collected from 29 active and 16 inactive carious lesions that were diagnosed in 40 preschool children aged 2–5 years. Dentinal-cavitated lesions were evaluated by the Nyvad criteria for the assessment of caries lesion activity. Total RNA was extracted from the dentine samples, purified and converted to cDNA. Reverse transcription quantitative real-time PCR analyses were performed for the identification and quantification of LCP. Serial dilutions starting from 300 to 0.0003 ng of reference bacterial DNA concentrations were used for relative quantification of the targeted bacteria. The results of the concentrations were normalized relative to the total bacterial load estimated using the primers for total bacteria 16S rDNA. The microbial data were evaluated using SPSS 17.0 software ( $\alpha = 5\%$ ). For identification data, the  $\chi^2$  test was used. For quantification data (identification and virulence), the data were analyzed by Student t-test or Mann-Whitney test. Total oral microorganisms (TM) and LCP were identified and quantified in dentinal samples. TM average quantity was not statistically different in active and arrested groups ( $p > 0.05$ ). LCP was identified in 48.14% of active dentin samples and in 33.34% of inactive lesions ( $p > 0.05$ ). However, the relative quantification revealed that LCP was significantly more abundant in active dentin lesions ( $p = 0.05$ ). This study provides insights about the oral microbiota related to dentin caries activity status, indicating that metabolically active *Lactobacillus casei/paracasei* may be linked to dentin lesion activity.

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### Effect of Sucrose Exposure Interruption on Biofilm Cariogenicity

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We evaluated the effect of sucrose exposure interruption on biofilm composition and enamel demineralization. *Streptococcus mutans* UA159 biofilms ( $n = 4$ ) were grown on whole human saliva-coated slabs in LMW medium, pH 7.0, 37 °C, 10% CO<sub>2</sub>. During the first two days, the biofilms were treated 8x/day with a mixture of 5.25% glucose + 5.25% fructose as control or 10% sucrose solutions. In the next two days, the exposure to carbohydrates was interrupted and the biofilms were treated 8x/day with NaCl 0.9% to simulate a fasting condition. Samples of biofilm and enamel slabs were collected after 56, 80 and 104 h of biofilm growth. The medium's pH was analyzed at the beginning and at the end of the day during all the experimental time. Intracellular and extracellular soluble and insoluble polysaccharides and CFU counts were evaluated in biofilms. Enamel demineralization was quantified by percentage of enamel surface hardness loss (%SHL). Data were analyzed by ANOVA two-way and Tukey's test, or t-test ( $\alpha = 5\%$ ). Sucrose group presented lower pH (5.6) than glucose+fructose group (6.0) after 48 h of cariogenic challenge ( $p < 0.05$ ). After carbohydrate interruption, sucrose and glucose+fructose group presented similar pH (6.4) at 80 and 104 h, but lower than the LMW medium (6.8). At all time points (56, 80 and 104 h), sucrose caused higher ( $p < 0.05$ ) demineralization (23.1, 17.1 and 17.9%) than glucose+fructose (8.2, 7.5 and 10.9%), but the effect of harvest time was not significant ( $p > 0.05$ ). The biofilm exposed to sucrose presented higher CFU counts and polysaccharides concentration ( $p < 0.05$ ), however no decrease on polysaccharides concentration was observed after sucrose interruption ( $p > 0.05$ ). In conclusion, the findings suggest that biofilm composition does not change after sucrose exposure paralization, but enamel demineralization is interrupted.

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**Anti-Biofilm Activity of *Anacardium occidentale* Chestnut Extract**

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This study aimed to determine the minimum inhibitory and bactericidal concentration (MIC and MBC) of a cashew chestnut hexane extract formulation and analyze the effect of this extract in *S. mutans* UA159 biofilm formation *in vitro*. To determine the MIC and MBC for *S. mutans* suspensions, the following formulation was prepared: hexane extract of cashew chestnut (0.0093% w/v) + dispersing agent (0.0714% w/v) + 35% hydroethanol solution. It was tested extract concentrations from 0.022 µg/mL to 93

µg/mL. *S. mutans* biofilms were formed on hydroxyapatite discs. Immediately after saliva-coat formation, discs were immersed in these solutions for 2 minutes: Group 1 – hydroethanol solution; Group 2 – Dispersing agent + hydroethanol solution; Group 3 – hexane extract of cashew chestnut + dispersing agent + hydroethanol solution; Group 4 – 0.12% chlorhexidine digluconate. After that, discs were placed in batch cultures at 37°C in 5% CO<sub>2</sub> for 5 days. Biofilms were grown in tryptone yeast-extract broth containing 1% sucrose. Culture medium was replaced once daily. On the 5th day, the biofilm was collected, sonicated, diluted and plated on BHI agar for counting of colony forming units (CFU) (48 h, 37°C, 5%CO<sub>2</sub>). The results were expressed as CFU/mg of biofilm (dry weight). MIC and MBC extract formulation concentration was 0.36 µg/mL. MIC of formulation vehicle was 89.25 µg/mL and MBC was 178.5 µg/mL. There was no biofilm formation on groups 3 and 4. There was no statistically difference in CFU levels between groups 1 (6.39 ± 0.13) and 2 (6.72 ± 0.11) (p > 0.05). Results show the potential anti-plaque effect of this cashew chestnut extract formulation. Studies should be conducted in order to analyze the effect of this formulation in other concentrations and multi-species biofilm models.

## Session 2 Hard Tissues/Epidemiology

26

### Abrasion Properties of Diamond Dentifrices on Dentine and Enamel

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This study aimed to analyze the abrasive wear of diamond-loaded dentifrices on dentine and enamel surfaces *in vitro*. Bovine dentine and enamel specimens ( $n = 120$ ) were brushed with 1) a diamond-loaded dentifrices (2 g/kg, particle size: 2.5  $\mu\text{m}$ ) without additional fillers, 2) a diamond-loaded dentifrices (1.5 g/kg, particle size: 2.4  $\mu\text{m}$ ) with 25% additional fillers, 3) a diamond-loaded dentifrices (3 g/kg, particle size: 2.4  $\mu\text{m}$ ) with 25% additional fillers, 4) Colgate Total (CT), or 5) Elmex Sensitive Plus (ES). Brushing was performed with a cross brushing machine ( $F = 2.5 \text{ N}$ ; 60 brushing strokes/min). Abrasive wear of specimens ( $n = 12$ ) was measured profilometrically at baseline. Enamel specimens were re-measured after 21600 and 43200 brushing strokes (BS); dentine specimens (under moist conditions) after 3600 and 7200 BS. Data were compared between groups using one-way ANOVA and post-hoc pairwise tests with Tukey correction,  $\alpha = 0.05$ . Diamond loading significantly ( $p < 0.05$ ) influenced enamel wear with increased abrasion potential corresponding to particle size and concentration (1:  $1.79 \pm 0.45 \mu\text{m}/10 \text{ kBS}$ ). Conversely, addition of conventional filler reduced these values (2:  $1.06 \pm 0.28 \mu\text{m}/10 \text{ kBS}$ ; 3:  $1.61 \pm 0.3 \mu\text{m}/10 \text{ kBS}$ ). CT and ES revealed a similarly low abrasion potential on enamel (CT:  $0.27 \pm 0.13 \mu\text{m}/10 \text{ kBS}$ ; ES:  $0.19 \pm 0.11 \mu\text{m}/10 \text{ kBS}$ ). On dentine specimens, diamond-loaded dentifrices and ES showed no difference (1:  $7.74 \pm 2.64 \mu\text{m}/10 \text{ kBS}$ ; 2:  $10.06 \pm 2.32 \mu\text{m}/10 \text{ kBS}$ ; 3:  $10.09 \pm 2.62 \mu\text{m}/10 \text{ kBS}$ ; ES:  $7.37 \pm 1.13 \mu\text{m}/10 \text{ kBS}$ ), while CT-brushed specimens exhibited significantly higher abrasion (CT:  $30.99 \pm 7.7 \mu\text{m}/10 \text{ kBS}$ ). These data suggest that abrasion caused by diamond fillers in experimental toothpastes is differentially affected by particle size and concentration. Supplementing conventional fillers seem to induce a masking effect on the diamond fillers thus reducing abrasion.

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27

### Functionalised Calcium Carbonate Particles to Occlude Dentin Tubules

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Functionalised calcium carbonate particles (FCC), surface coated with hydroxyapatite (HAP), have been investigated for their potential of occluding dentin tubules counteracting dentin hypersensitivity. The particles were incorporated in a hydroxymethyl cellulose (HMC) suspension and toothpaste formulation (TP) for treating dentin specimens and were compared to negative controls. Focus was on correlations between morphological and element specific properties as function of particle concentration to visualize tubules occlusion. Two types of FCC were applied (A: 5.5  $\mu\text{m}$ , B: 2.0  $\mu\text{m}$  average diameter). In total ten treatment groups (5, 10, 20, 30% A vs. 5% B in HMC; 5, 10% A vs. 5% B in 15 and 20% silica containing TP; HMC and toothpaste X), with three specimens each, were generated. Dentine specimens were obtained from human retained molars, embedded and wet grinded prior treatment. One mL of suspension was applied with a brushing simulator for 2 min followed by rinsing with deionized water. Scanning electron microscopy and energy dispersive x-ray analyses were performed on surfaces and cross-sections. Morphology images and element analyses were consistent revealing 5% B in HMC was as sufficient in tubules occlusion as 30% A. The presence of FCC within the tubules was confirmed by element ratios and respective standard deviations of Ca/P (30% A:  $2.71 \pm 1.84$  and 5% B:  $4.64 \pm 2.18$ ) and Ca/O (30% A:  $0.36 \pm 0.09$  and 5% B:  $0.38 \pm 0.22$ ) to be

close to the ratios determined for raw particles of A (Ca/P:  $3.50 \pm 1.85$ ; Ca/O:  $0.68 \pm 0.34$ ) and B (Ca/P:  $3.29 \pm 3.36$  and Ca/O:  $0.33 \pm 0.11$ ). Future investigations will address whether silica plus HAP-coated FCC, expected to be attractive for fluorine adsorption, are more sufficient against acid attacks than solely silica based tubules occlusion.

The study was supported by Omya International AG.

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## 28

### Indirect Pulp Treatment in Primary Teeth: A Mixed Treatment Comparisons Meta-Analysis

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We aimed to systematically review the literature to address the question regarding the influence of different materials in the clinical and radiographic success of indirect pulp treatment in primary teeth. Literature searching was carried out until December 2016 in PubMed/MEDLINE, CENTRAL, Scopus, TRIP and Clinical Trials databases selecting randomized clinical trials that compared materials used for indirect pulp treatment in primary teeth. Two reviewers independently selected the studies and extracted the data. The effects of each material on the outcome (clinical and radiographic failures) were analyzed using a mixed treatment comparisons meta-analysis. The ranking of treatments according to their probability of being the best choice was also calculated. From 1,088 potentially eligible studies, 11 were selected after screening titles and abstracts. The studies were excluded because were other interest area ( $n = 957$ ), literature review ( $n = 116$ ) or did not compare different materials used for indirect pulp treatment in primary teeth ( $n = 5$ ). After full-text analysis, one study did not perform random allocation of subjects, two papers had follow-up less than six months, one study showed dropout higher than 30%, four trials did not assess clinical and radiograph success as outcome, one study did not evaluate only deep caries lesion, other study did not remove infected dentin in the control groups and two trials were published twice times. Finally, 4 papers were included in meta-analysis. In all papers, calcium hydroxide liner was used as control group *versus* adhesive system, resin-modified glass ionomer cement and placebo. The follow-up period ranged from 24 to 48 months, with dropouts of 0–25.7%. The material type did not significantly affect the risk of failure of the indirect pulp treatment. However, calcium hydroxide presented higher probability to failure. Concluding, the current evidence does not support the recommendation of any material instead of other for indirect pulp treatment in primary molars, since they present similar efficacy in the included studies. There is also no evidence that the use of a liner, as recommended in AAPD Guidelines, could be more efficacious than restore cavities directly using other adhesive materials.

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## 29

### Tooth Wear in High Performance Team Sportive

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High performance sportive have a particular life style considering their diet, their training and overall being. Competitions bring on a lot of pressure and stress, especially in higher levels of performance. This study analyses how stress affects team players as far as their oral health, focusing mainly on tooth wear condition.

**Material and Method:** The study was conducted at the end of competition season 2015–2016 and involved a male volleyball team and female handball team. Both teams were running in Romanian National Championship, and the male volleyball players actually became national champions of the year 2016. The clinical examination was performed by two calibrated examiners, using just probe and mirror. The whole oral status was evaluated taking in consideration mainly tooth wear, VDO was measured as well. The examination included the coaches of the teams. Out of the 14 players of the volleyball players 9 presented tooth wear in various degrees. Considering BEWE index we found: 2 players with score 2, 4 players with score 3 and 3 players with score 4. The score was higher in older players. One of the coaches also had severe tooth wear, score 4 BEWE, diagnosed bruxism both day and night time.

The general oral status of the female handball team was better than the male volleyball players. Out of the 16 players we found 10 players with tooth wear: 4 players with score 1, 4 players with score 2 and 2 players with score 3. 8 players were aware of the problem, and 3 of them were using night guards for bruxism.

The conclusion of this study was that stress condition during high level competition is a direct factor to developing tooth wear regardless the healthy life style and diet.

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## 30

### Findings on Dental and Dietary Habits of School-Aged Amateur Athletes

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The aim of present study was to examine the dietary habits and frequency of dental erosion in school aged children who do amateur sports. A total of 107 children (aged between 6–15) including 51 girls and 56 boys, who were dealing with amateur sports (53 swimming, 54 other sports such as basketball or volleyball) participated in the study. A questionnaire, which contained 22 questions on socio-demographical properties, types and durations of the sports, the consumption of the drinks and food that caused erosion in teeth, was asked to the children participated in the study.

The examinations were performed in clinical conditions, caries were recorded according to the WHO criteria whereas dental erosion was determined by BEWE index. The clinical examinations were performed by one pediatric dentist. The intra-examiner reliability was tested by repeating the examination of 10 participants (10% of the study population), within the 1 week interval between the examinations. Cohen's Kappa was calculated at 0.88 representing good agreement for reproducibility of erosion. Data were analysed with SPSS 22 program. For possible statistical differences chi-square test, Fisher's exact test and continuity (Yate's correction) were used.  $p$  value  $<0.05$  was determined to be statistically significant. The rate of the consumption of energy and sports drinks was determined as 0.9% and 32.7% and dental erosion was 17%; and the DMFT and dmft values were  $6,81 \pm 6,34$ ,  $1,95 \pm 4,13$ , respectively. The dental erosion in children who dealt with swimming (28.3%) was found higher than in children who dealt with other sports (5.7%) ( $p:0.004$ ;  $p < 0.05$ ). However, no significant difference was observed in children who dealt with swimming according to the duration of sports in terms of erosion frequency ( $p:0,800$ ;  $p > 0.05$ ). When the effect of sports drinks on erosion and DMFT and dmft scores was evaluated, no significant difference was detected between groups ( $p:0,160$ ,  $p:0,978$ ,  $p:0,467$ ;  $p > 0.05$ ). In conclusion, swimming is a risk factor increasing the dental erosion in children; and prevention measures are of importance for children who do sports.

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31

### **Prevalence of Erosive Tooth Wear and Associated Risk Factors Among 15-Year Old Adolescents: A Cross-Sectional Study in Flanders, Belgium**

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Although Belgium has recently been ranked as the second consumer of soft drinks in Europe, no data on Erosive Tooth Wear (ETW) are currently available. Therefore, the aim of this cross-sectional study was to determine the prevalence and associated risk factors of ETW in adolescents residing in the region of Flanders, Belgium. Convenience sampling was used to recruit participants from different types of schools present in the Belgian educational system: General secondary education (GSE, 3 schools,  $n = 295$ ) and Vocational/Technical secondary education (VSE, 5 schools,  $n = 318$ ), mean age  $15.1 \pm 0.8$  years. Three calibrated dentists performed dental examination and a self-administered questionnaire was applied to evaluate risk factors associated with ETW. Presence or absence of ETW was determined using the Basic Erosive Wear Examination (BEWE) index and the overall prevalence was calculated using  $BEWE >0$ . Logistic regression analysis was performed to evaluate risk associations. From 613 recruited adolescents, 48.6% presented at least one affected tooth surface ( $BEWE >0$ ).

Logistic regression analysis found an association with school type (VSE) and dietary factors ( $\geq 1$  daily consumption of soft drinks, consumption of acidic beverages before night-time brushing). School type may be explained by a lower parental socio-economic status, influencing dietary choices and less health education subjects in school curricula. The presence of ETW at early stages, together with the high consumption of soft drinks in Belgium, may indicate the need of prevention and setting up of public health measures to educate Belgian adolescents in all school types about the consequences of ETW.

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32

### **Erosive Tooth Wear in Athletes with Intellectual Disabilities**

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Despite the increase of research related to Erosive tooth wear (ETW) in the past decade, little is known about this type of wear in patients with intellectual disabilities (ID) and in related subgroups such as those with Down Syndrome (DS). Therefore, the aim of this study was to determine the presence of ETW in athletes with ID, with and without DS, who participated in Special Olympics (SO) Belgium 2016. A total of 234 SO athletes were effectively recruited for this study ( $n = 97$  DS), and two calibrated examiners performed dental examination of the entire group of athletes with ID using the Basic Erosive Wear Examination index (BEWE). Pearson Chi-square test and Mann-Whitney U test were used to detect significant differences (level of significance  $p < 0.05$ ). The prevalence of ETW was 62.97% for the entire group of SO athletes, 46.3% for athletes with ID (not DS) and 84.5% for DS athletes. Athletes with DS presented significantly higher mean BEWE ( $4.67 \pm 5.64$  SD) and prevalence of ETW (69.2%  $BEWE >0$ ) than athletes with ID without DS (Mean BEWE:  $1.96 \pm 3.47$  SD and 46.3%  $BEWE >0$ ;  $p = 0.002$  and  $p = 0.012$ ). In conclusion, the higher prevalence of ETW found in athletes with DS, which differentiated substantially from ID athletes without DS, shows the need of implementing preventive and treatment plans that specially target this group of patients.

### The Impact of Mouthwashes on the Efficacy of Fluoride Toothpastes in Preventing Dental Erosion/Abrasion

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Toothbrushing with fluoride toothpaste followed by rinsing with mouthwash is a routine procedure to maintain good oral hygiene. The aim of this *in vitro* study was to investigate the effect of commonly used mouthwashes (essential oils-EO, cetylpyridinium chloride-CPC, chlorhexidine-CHX, sodium fluoride-F) with deionized water (DI) as negative control on two fluoride dentifrices (sodium fluoride-NaF, stannous fluoride-SnF<sub>2</sub>) in their ability to prevent enamel and dentin erosion/abrasion in a factorial design. Polished bovine enamel and dentin specimens (n = 8, each) were subjected to a 5-day pH cycling model with twice-daily treatments, with or without abrasion, with fluoride toothpaste (1 min) followed by mouthwash exposure (1 min). Erosion (0.3% citric acid, pH2.6) was performed 5×/d. Specimens were exposed to artificial saliva during remineralization periods. After 5 d, enamel and dentin surface loss (SL) was determined using non-contact profilometry and the efficacy of each treatment combination (toothpaste+mouthwash) compared (three-way ANOVA; factors: toothpaste, mouthwash, abrasion or not). For both dentin and enamel there were no statistically significant two- or three-way interactions as SL was only affected by toothpaste and mouthwash. NaF caused less SL than SnF<sub>2</sub> (4.60 vs. 5.83 μm; p < 0.0001) in dentin, whereas the opposite was found in enamel (5.20 vs. 3.56 μm; p < 0.0001). Toothbrushing abrasion caused comparatively more SL in enamel (6.53 vs. 2.23 μm; p < 0.0001) than in dentin (6.06 vs. 4.38 μm; p < 0.0001). None of the tested mouthwashes affected SL, although the toothpaste × mouthwash (p = 0.066) and toothpaste × mouthwash × abrasion (p = 0.052) interactions were approaching significance for dentin, but not for enamel (p = 0.182 and p = 0.472, respectively). Considering the limitations of laboratory research, it can be concluded that commonly used mouthwashes containing antimicrobial agents or additional fluoride, do not impair the erosion/abrasion protection afforded by fluoride toothpastes. Further studies on dentin, however, are warranted.

### Modification of Acquired Pellicle with CaneCPI-5 Reduces Initial Enamel Erosion

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Cystatin-B was identified as an acid-resistant protein in the acquired enamel pellicle; it could therefore be included in oral products to protect against erosion. However, its cost is high. Recently, a cystatin derived from sugar cane (CaneCPI-5) was cloned, recombinantly expressed and shown to reduce initial enamel erosion *in vitro*. The present study evaluated the effect of pellicle modification, by treatment of enamel with solutions containing different concentrations of CaneCPI-5, on the protection against initial erosion *in vitro*. Sixty bovine enamel specimens (4×4 mm) were treated for 2 h (under stirring at 30°C) with one of the following solutions: 1) deionized water (control); 2) 0.5% mucin + 0.27% casein; 3) 0.025 μg/μL CaneCPI-5; 4) 0.1 μg/μL caneCPI-5; and 5) 1.0 μg/μL CaneCPI-5. Stimulated saliva was collected from three volunteers, pooled and used to form an acquired enamel pellicle on the specimens for additional 2 h. All specimens were then incubated in 0.65% citric acid (pH 3.4) for 45 sec at 30°C. Treatment was done once/day for 3 days. Surface hardness was analyzed at baseline and after days 1 and 3 and percentage of surface hardness change (%SHC) was calculated. Data were analyzed by Kruskal-Wallis and Dunn's test (p < 0.05). At day 1, treatment with 0.1 μg/μL (1.8 ± 4.3%) and 1.0 μg/μL CaneCPI-5 (1.0 ± 5.3%) completely prevented SHC in comparison with control (24.4 ± 6.0%) that did not significantly differ from mucin+casein (18.1 ± 2.7%) and 0.025 μg/μL CaneCPI-5 (10.4 ± 7.2%). At day 3, %SHC increased for all groups, but those treated with 0.1 μg/μL (27.0 ± 3.3%) and 1.0 μg/μL (27.0 ± 2.2) significantly differed from control (41.5%±4.6%), mucin+casein (37.8%±9.1%) and 0.025 μg/μL CaneCPI-5 (33.3 ± 3.3%). Thus, CaneCPI-5 at 0.1 μg/μL seems to be a good candidate to be added to oral products to protect against erosion.

This study was supported by The Royal Society (Newton Fellowship, NA 140459).

### Evaluation of the Calcivis Imaging System's Ability to Assess Erosive Challenge to Enamel Over Time

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**Aim:** To evaluate the capability of the Calcivis Imaging System as a method for detecting and monitoring acid erosion on enamel surfaces over time.

**Methods:** Previous ORCA posters reported a luminescent assay for assessing caries lesion activity and erosive challenges (2008/9/10/13/14/15/16). The system consists of a specialised intraoral camera capable of imaging very low level, transient luminescence when a calcium activated photoprotein is applied to enamel surfaces of teeth or enamel blocks. Using a dark box, images were obtained from five sample extracted teeth, and five bovine enamel blocks. The teeth and enamel blocks were subject to erosive conditions (30% phosphoric acid) at regular intervals over a period of up to one hour (brushing with water between exposures). Images were taken of each successive acid-challenge to monitor the ongoing demineralisation of the enamel surface. The images obtained from sample extracted teeth using the Calcivis Imaging System clearly show elevated luminescence in areas exposed to erosive conditions, which increases in intensity upon successive acid challenge over time. A similar effect is observed in the case of the enamel blocks, where the increase in luminescence intensity can be measured using image analysis software. Sound enamel block surfaces exhibit a mean luminescence value of 13, whereas acid challenged surface exhibit values of 35, 48, 57, 56, 63, 57 and 56 for 1, 5, 10, 15, 20, 25 and 30 s exposures (SD of 8, 9, 18, 16, 8, 19 and 24 respectively).

In conclusion the increased luminescence observed in this study was caused by an increase in free calcium ions liberated from within the surface enamel layer following erosive challenge. The Calcivis Imaging System was able to detect and monitor acid erosion of enamel surfaces over time.

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### Impact of Heating from 100–500°C on Enamel Erosion Resistance

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As previous studies have shown that CO<sub>2</sub> laser irradiation can both increase enamel resistance to erosion as well as heat up the surface to temperatures over 100°C, it was the aim of the present study to investigate the effect of several temperature lev-

els on enamel erosion resistance. One hundred and twelve polished bovine enamel samples were randomly allocated to one of seven groups (n = 16): no treatment, control (C); daily application of fluoride solution (F); heating up to 100°C, 200°C, 300°C, 400°C, and 500°C. The fluoride solution contained AmF/NaF/SnCl<sub>2</sub> (500 ppm F<sup>-</sup>, 800 ppm Sn<sup>2+</sup>, pH = 4.5). Heating in oven was conducted at a rate of 1 K/min and each temperature level was maintained for 1 min. After surface treatment samples were submitted to an erosive cycling over six days, including immersion in citric acid (2 min/0.05 M/pH = 2.3) six times daily and storage in remineralization solution (≥1 h) between erosive attacks. After treatment and at the end of each two cycling days (Baseline, D2, D4, D6) enamel surface loss was measured using a 3D laser profilometer. Data were statistically analyzed with a two-way repeated measures ANOVA and post-hoc comparisons (α = 0.05). All temperature levels (100–500°C) and fluoride treatment caused significant increase in enamel erosion resistance as compared to C (–24.6 ± 1.3 μm, p < 0.05). However only groups 300 °C (–8.3 ± 2.7 μm) and 400°C (–5.6 ± 4.2 μm) significantly decreased enamel surface loss also as compared to daily fluoride treatment (–12.3 ± 2.3 μm, p = 0.015/0.000). Heating enamel to 300°C and 400°C levels indeed significantly increased enamel erosion resistance in vitro. This finding may help to explain the erosion protective effect of the CO<sub>2</sub> laser irradiation in future studies.

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### Salivary Pellicles on Deproteinized Enamel Provide Lower Protection Against Erosion Than Pellicles on Sound Enamel

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Deproteinized enamel has previously been shown to erode faster than untreated enamel. In this study, we analysed and compared the erosion protection conferred by salivary pellicles on deproteinized and sound enamel.

We prepared 64 specimens from human molars, grinding away 100 μm from the surface. Half of these specimens were deproteinized by individually immersing them in hydrazine hydrate for two weeks at 70°C. The other half of specimens served as controls and was incubated for two weeks at 70°C in mineral solution. We then determined the exposed surface area, measured the initial surface reflection intensity (SRI) and surface microhardness (SMH), and placed large Knoop indentations on the surface of each specimen to determine surface loss. The deproteinized and control groups were each divided into two subgroups (n = 16), either receiving a salivary pellicle or not. All specimens underwent five cycles of a treatment consisting of salivary pellicle formation or incubation in a humid chamber, erosion in 10 ml of citric acid (1%, pH 3.6) for 1 min, and removal of the pellicle leftovers (2 min, 3% NaOCl). After each cycle, we measured SRI, SMH, surface loss, and calcium

released during the erosion, and performed Wilcoxon rank sum tests to analyse the differences.

Pellicles on deproteinated enamel were not able to protect from SMH loss. SMH decreased by  $28.9 \pm 5.0\%$  without and  $27.8 \pm 6.1\%$  with pellicle. Contrary to the control group, pellicles on deproteinated enamel were not able to offset the beginning of significant surface loss (final surface loss  $57 \pm 67$  nm ( $p = 0.008$ ) for deproteinated,  $21 \pm 58$  nm ( $p = 0.651$ ) for control group). By SRI, we detected a significant protection from the pellicle after 2 cycles in the control group ( $p = 0.032$ ), while it took 3 cycles to detect such a protection in the deproteinated group ( $p = 0.016$ ). Furthermore, pellicles on deproteinated enamel released in total  $1.9$  mmol/mm<sup>2</sup> less calcium than pellicles on control enamel. We conclude that pellicles on deproteinated enamel are not able to protect against erosion to the same extent as pellicles on untreated enamel, suggesting differences between these pellicles.

The authors appreciate the support of the Department of Preventive, Restorative and Pediatric Dentistry, University of Bern.

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38

### Different Apparent Erosion Protection of Toothpastes Caused by Measuring Methods

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The aim of this study was to compare three current measurement methods to determine the protective effects of toothpastes on enamel. Bovine enamel samples ( $n = 6$ ) were subjected to five erosive attacks per day (1 min citric acid pH 2.7). The anti-erosive properties of Biorepair Plus (BR), Elmex Sensitive Plus (ES) and Colgate Total (CT) toothpaste were investigated by placing the samples (experiment 1,  $n = 6$ ) into the respective slurry or artificial saliva ([mmol/l] NaCl: 9.92; CaCl<sub>2</sub>: 1.53; NH<sub>4</sub>Cl: 2.99; KCl: 17.0; NaSCN: 1.97; KH<sub>2</sub>PO<sub>4</sub>: 2.42; CO(NH<sub>2</sub>)<sub>2</sub>: 3.33; Na<sub>2</sub>HPO<sub>4</sub>: 2.40; pH 6.4), which served as control, for 3 min twice a day. The influence of brushing was tested (experiment 2,  $n = 6$ ) by additional abrasion with 20 toothbrush strokes. Knoop hardness measurements and profilometry were carried out after 5 test days. Results were tested for statistical differences by Wilcoxon rank sum tests and were considered significant at  $p < 0.05$ . After experiment 1, the control only reached 43.4% of its base Knoop hardness, whereas a significant increase was observed for BR (53.7%) and ES (71.0%). Surface Hardness Recovery (SHR%), calculated from the indentation lengths, was also significantly increased for BR (28.9%) and ES (63.9%). With profilometric measurement no significant differences could be observed between the groups (0.7–1.0  $\mu$ m substance loss). After additional abrasion (experiment 2) significantly more substance loss was seen for BR (1.4  $\mu$ m) and CT (1.5  $\mu$ m) in comparison to the control (1.1  $\mu$ m). A significant increase in hardness appeared after abrasion for CT (72.8%) and ES (76.4%) in comparison to the control (54.4%). This was also the case for the SHR% for CT (66.4%) and ES (72.3%). In conclusion, profilometric quantification of substance removal shows the total irreversible substance loss. Calculations from hardness measurements only show

the remaining condition of the surface after the treatment. The partial abrasion of the softened surface may pretend a re-hardening that has not taken place.

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39

### Effect of Soft and Harsh Movements During the Erosive Challenges on Initial Enamel Erosion

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Two studies from our group (Lussi et al.: PLoS ONE 2015;10:e0143957, and Carvalho et al.: Clin Oral Invest 2016;20:1973–1979) had been carried out using the same methods: deciduous teeth were immersed in different drinks (mineral water – pH 6.53, orange juice – pH 3.63, energy drink – pH 3.35, Coca-Cola – pH 2.55, and candy spray – pH 2.14) for 2 and 4 mins, at 30°C and shaking at 95 rpm. However, the shaking movements were made with two different devices (travel paths of 22 mm or 50 mm). This led to two distinct types of movements during the erosive challenges: soft (4180 mm/min) and harsh (9500 mm/min). Percentage surface hardness (%SH) was calculated, and the Mann-Whitney Test was used to verify any differences between the two types of movements. We hereby discuss these differences on initial enamel erosion. Harsh movements cause significantly more erosion than soft movements ( $p \leq 0.001$ ), after 2 min immersion in all drinks, except mineral water. This trend is also maintained when specimens are immersed for 4 min in orange juice (%SH reduces to 90% and 72%), energy drink (75% and 53%), and Coca-Cola (66% and 46%, for slow and harsh movements, respectively;  $p \leq 0.001$ ), but not for mineral water or candy spray. We observed no enamel hardness loss for mineral water, whereas candy spray caused a reduction of %SH to 18% and 15%, for soft and harsh movements, respectively, with no significant differences between the two types of movement ( $p = 0.064$ ). The type of movement during the erosive challenge, therefore, plays a significant role on initial enamel erosion, leading to distinct differences on enamel surface hardness loss, but this difference diminishes when either a non-erosive or a strong erosive substance is used for longer erosion times.

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### Food Acid Content and Erosive Potential of Sugar-Free Confections

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Dental erosion is an increasingly prevalent problem associated with the frequent consumption of acidic foods and beverages. The aim of this study was to measure the food acid content and the erosive potential of a variety of sugar-free confections. Thirty sugar-free confections were selected and extracts analysed to determine pH, titratable acidity, chemical composition and apparent degree of saturation with respect to apatite. The effect of the sugar-free confections in artificial saliva on human tooth enamel was determined in an *in vitro* dental erosion assay using change in surface microhardness. The change in enamel surface microhardness (% $\Delta$ SMH) was used to classify the confections as high, moderate or low erosive potential. Seventeen of the thirty sugar-free confections were found to contain high concentrations of food acids, exhibit low pH and high titratable acidity (TA) and have high erosive potential (24% to 52% % $\Delta$ SMH, that was significantly higher than that of the low erosive potential confections ( $p < 0.05$ ) as shown by ANOVA and a *post hoc* Dunnett T3 test). Significant Pearson's correlations were found between % $\Delta$ SMH and pH ( $-0.892$ ,  $P < 0.001$ ) and between % $\Delta$ SMH and TA ( $0.82$ ,  $P < 0.001$ ) of the confections. Ten of these high erosive potential confections displayed dental messages on the packaging suggesting they were safe for teeth.

In conclusion this study showed that many sugar-free confections, even some with "Toothfriendly" messages on the product label contain high contents of food acids and have erosive potential. Sugar-free confections are thought to be "safe for teeth", however those with high contents of food acids, may cause erosion in at risk patients with poor quantity and/or quality of saliva.

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### De and Remineralization Cycles and Fluoride Effect on Microhardness and Roughness of Enamel Surface

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Dental erosion, which is evaluated by surface microhardness (SMH) and surface average roughness (Ra), is caused by the demineralization of enamel. De and remineralization occur daily on enamel surface, fluoride accelerates remineralization. The aim of this study was to evaluate how de and remineralization cycles with

and without fluoride affect the SMH and Ra. 48 polished human enamel samples, 12 in each group, were used in the study. Group 1 was exposed to demineralized and remineralized solutions 4 times for 5 minutes respectively. Group 2 was exposed to demineralized and 225 ppmF fluoridated remineralized solutions 4 times for 5 minutes respectively. Group 3 was exposed to demineralized and remineralized solutions 20 times for 1 minute respectively. Group 4 was exposed to demineralized and 225 ppmF fluoridated remineralized solutions 20 times for 1 minute respectively. After being immersed in these solutions, SMH measurements were performed with Knoop diamond a load of 50 g on a hardness tester and the average roughness (Ra) was measured by using Atomic Force Microscope. Group 2 ( $134.9 \pm 37.1$  HK) shows the highest SMH and Group 3 ( $84.1 \pm 20.4$  HK) shows the lowest. Group 1 ( $205.9 \pm 46.6$  nm) shows the highest Ra and Group 4 ( $139.3 \pm 56.9$  nm) was the lowest. 2-way ANOVA was used to analyze SMH and Ra. For SMH, both de and remineralization cycles and fluoride addition were significant factors ( $p < 0.05$ ). For Ra fluoride addition was the only significant factor ( $p < 0.05$ ). The results indicated that remineralized enamel crystal was different from crystal before demineralization; and fluoride helped prevent enamel erosion.

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### Bond Stability to Eroded/Abraded Dentin After Deproteinization

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This study investigated the combined effect of erosion and toothbrush abrasion associated or not to deproteinization (10% sodium hypochlorite) on the bond strength stability ( $\mu$ TBS) to dentin, using a universal adhesive system by means of total and self-etching techniques. Bovine dentin specimens were divided into five groups according to the organic matrix condition ( $n = 20$ ): Control (C); Erosion (E); Erosion + Abrasion (EA); Erosion + Sodium hypochlorite (EH); Erosion + Abrasion + Sodium hypochlorite (EAH). The groups were further divided ( $n = 10$ ) according to the application of a universal adhesive system (total-etching or self-etching). After bonding procedure, resin blocks were built up with resin composite and cut to obtain sticks for  $\mu$ TBS test. One half of the sticks of each specimen was immediately tested and the other half was tested after artificial aging (5,000 thermocycles/5 and 55°C). ANOVA 3 way ( $\alpha = 5\%$ ) showed significant difference for the triple interaction ( $p = 0.0007$ ). The higher  $\mu$ TBS means were obtained by EH ( $27.27 \pm 5.33^A$ ) and EAH ( $27.35 \pm 5.96^A$ ) groups, followed by C ( $19.39 \pm 7.04^B$ ), E ( $16.52 \pm 8.82^{BC}$ ) and EA ( $14.53 \pm 6.80^C$ ) groups. It was concluded that erosion and erosion/abrasion did not significantly influence the  $\mu$ TBS to dentin. The artificial aging reduced  $\mu$ TBS values only for groups control, erosion and erosion/abrasion using the total-etching technique. The deprot-

einization maintained the bond stability to the eroded and eroded/abraded dentin, especially when using the total-etching technique.

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43

### Effect of Whitening Toothpastes on Dental Enamel In Vitro

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The aim of this in vitro study was to examine the effect of whitening toothpastes on enamel surfaces, since a change in surface wettability may affect enamel susceptibility to acids. Sixty four enamel specimens were made from 16 bovine teeth and specimens from each tooth were divided into 4 groups. Slurries (5 g toothpaste: 10 ml H<sub>2</sub>O) were made of the following toothpastes: A (iWhite Instant Teeth Whitening Toothpaste, Sylphar, pH 5.3), B (Solidox Hvite tenner, Lilleborg AS, pH 9.6), and C (Colgate Caries Control, Colgate Palmolive, pH 7.2). Deionised water was used as the control. Wettability of the enamel was measured using drop shape contact angle analysis with a 2 µL deionized water sessile drop. Samples were treated by gentle agitation in the slurries for 5 min. Baseline contact angle measurements were performed and then repeated after slurry treatment. Data were analysed by one way ANOVA, and Tukey HSD multiple comparisons. The results showed that average baseline contact angles did not differ significantly between groups (range: 56°–59°). After treatment mean changes in contact angles were for A (Δ39°), B (Δ23°), C (Δ22°) and control (Δ-3°), with significant between-group differences: ΔA vs. ΔB (p = 0.017) and ΔA vs. ΔC (p = 0.012). Toothpastes B and C were not significantly different from each other, and the control was significantly different from A, B and C. In conclusion, the toothpaste slurries significantly reduced the contact angles, suggesting increased wettability of the enamel surfaces. However, one of the whitening toothpastes had a much greater impact than the other toothpastes. Use of whitening toothpastes may significantly increase enamel susceptibility to acids and this may have clinical relevance for patients at risk of erosive wear.

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44

### Protective Effect of Including Casein Phosphopeptide Amorphous Calcium Phosphate Varnish on Enamel Erosion In Vitro

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In recent years, different agents for inhibiting erosion have been widely investigated, such as casein phosphopeptides with amorphous calcium phosphate complex (CPP-ACP). The aim of this study was to investigate the *in vitro* effect of MI Varnish on preventing enamel erosion by Atomic Absorption Spectroscopy (AAS) and Atomic Force Microscopy (AFM). A total of 28 enamel samples were prepared from extracted permanent molars, divided into 4 groups. Group-1: GC MI Varnish (5%NaF +CPP-ACP; GC Corporation, Japan), Group-2: Clinpro White Varnish (5%NaF +TCP; 3M, ESPE, USA), Group-3: Positive Control Colgate® Duraphat® Fluoride Varnish (NaF 5%), Group-4: Negative Control (deionized water). Before enamel surface treatment, the samples were immersed stimulated pooled human saliva for 1 hour. After treatment, varnishes were cleaned with cotton tip and deionized water; all enamel samples exposed to 15 ml. Coca-Cola for 10 min. After this, Ca<sup>++</sup> released in Coca-Cola was determined by AAS. Data were analyzed with repeated measures ANOVA. The surface topography was evaluated by AFM from three specimen in each group.

Deionized water demonstrated a significantly higher Ca<sup>+2</sup> release compared to those of groups Duraphat > Clinpro White Varnish > MI Varnish, respectively (p < 0,01). AFM images of enamel surface treated with MI Varnish resulted in less morphological changes of the tooth substrate when compared with Duraphat and Clinpro White Varnish; thus, indicating that MI Varnish promoted remineralization.

None of the agents provided protection against the development of erosion; on the other hand CPP-ACP and F containing MI Varnish was able to reduce enamel surface softening after the erosive challenge.

### Mineral Phase Changes in Dental Enamel after Furnace Heating and CO<sub>2</sub> Laser Irradiation

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Loss of tooth substance due to dental erosion or caries is still a major health problem. To protect dental enamel from erosion or caries a new approach of ceramic laser coating was planned. Nevertheless the effects on phase changes of dental enamel after laser irradiation are still not clear. So the aim of this study was to identify the effects of laser irradiation and furnace heating on phase changes of dental enamel. Ground polished bovine enamel blocks were irradiated with a CO<sub>2</sub> laser at two different parameters: 1) G20 (600°C average surface temperature, 20 μs, λ = 10.6 μm) and 2) G490 (1700°C average surface temperature, 490 μs, λ = 10.6 μm). Enamel powder was ground from sixty bovine incisors and used at room temperature, as negative control, or heated in an electric furnace at 10 temperature levels: 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000°C. After scraping off the material from the surfaces of the irradiated samples, the obtained enamel powders (grain size ≤45 μm) were subjected to XRD analysis (2θ range from 5–70°, CuKα, 40 kV and 40 mA, step size: 0.01°, 96 s counting time, Bragg-Brentano geometry). Phase analysis was conducted based on the standards from the International Centre for Diffraction Data (ICDD). XRD analysis showed that a second phase of β-tricalcium phosphate at 700°C after furnace heating emerged. After laser irradiation with G20 no new phases and after irradiation with G490 phases of α-tricalcium phosphate and tetracalcium phosphate could be detected. CO<sub>2</sub> Laser parameter G20 (20 μs/ 1.3 J/mm<sup>2</sup>) did not cause negative side effects on dental enamel regarding mineral phase changes. Whereas parameter G490 (490 μs/ 17.5 J/mm<sup>2</sup>) or temperatures above 700°C caused remarkable phase changes in dental enamel, which are known to have a higher solubility than natural enamel apatite.

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### Genome-Wide Association Study (GWAS) of Erosive Tooth Wear: Results from The Northern Finland Birth Cohort

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The aim of the work was to accomplish a GWAS on erosive tooth wear (ETW) firstly in a sample of the Northern Finland Birth Cohort 1966, and secondly in the sample stratified by gender. ETW was assessed clinically on 1,962 (61.7% of all invited) individuals using the Basic Erosive Wear Examination (BEWE sum, 0–18) in the University of Oulu. Severe ETW (BEWE sum 9–18) was selected as a cut-off point for GWAS and members affected by severe ETW were considered as cases in the study population. Those with BEWE ≤8 were considered as controls. An Illumina Infinum Human CNV370-Duo marker set was used for the genome-wide association genotyping. The following quality control steps were applied; SNPs with the p-value from a test of Hardy-Weinberg Equilibrium <1E-7, call rate <95% or minor allele frequency <0.05 were excluded from the study along with individuals with genotyping success rate <95%. In addition statistically estimated, HapMap2 and 1000 Genomes imputed, markers were available. For every SNP meeting statistical (P-value ≤10E-8) or suggestive significance (P-value ≤10E-5), we explored any nearby genes and whether they had known biological functions relevant to erosive wear using NCBL Variation Viewer. The performed GWAS scans implicated several loci and SNPs putatively associated with severe erosive wear. In a full sample GWAS, one genome-wide significant loci was observed on the chromosomal region 2p25. When the sample was stratified by gender, altogether 24 statistically significant SNPs were observed – the strongest signals in males located on the regions 5q31, 8p11 and 20q13, and in females on 4q21, 5q13 and 13q34. These results cautiously suggest that the individual variance, as well as the gender difference in the severity of erosive wear may have a genetic contribution.

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### Effect of Toothpastes with Desensitizing and/or Anti-Erosion Claims on Initial Enamel Erosion and Abrasion

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To investigate the effect of desensitizing and/or anti-erosion toothpastes on initial erosion and abrasion, 150 enamel specimens were prepared and randomly allocated into 10 groups (n = 15): Artificial Saliva (AS) and Colgate Cavity Protection (CP), as controls; desensitizing toothpastes (Blend-a-Med Pro Expert; Elmex Sensitive Professional; Sensodyne Rapid Relief; Sensodyne Repair and Protect); and toothpastes with anti-erosion claims (Candida Protect Professional; Elmex Erosion Protection; Sensodyne Pronamel; Regenerate). The specimens were submitted to 5 erosion-abrasion cycles: 60 min in artificial saliva (37°C), 3 min in 1% citric acid (pH 3.6, 25°C, 70 rpm), and 2 min immersion in toothpaste slurry with 25 s of toothbrush abrasion (200 g, 120 strokes/min, 40 mm/s). After 5 cycles, we calculated surface loss (SL) values (in  $\mu\text{m}$ ) and verified differences between groups using Kruskal-Wallis and Mann-Whitney tests ( $\alpha = 0.05$ ). SL results are presented as median and interquartile range. For the control groups, AS presented the lowest SL (1.69; 1.48–1.95) and CP presented 2.20; 1.74–2.68. Among the desensitizing toothpastes, Sensodyne Repair and Protect showed the lowest SL (1.71; 1.43–1.96), which was not different from AS ( $p = 0.820$ ). Blend-a-Med Pro Expert showed the highest values (3.98; 3.67–4.25). Regarding the anti-erosion toothpastes, Sensodyne Pronamel (2.26; 1.93–2.69) and Elmex Erosion Protection (2.30; 1.93–2.43) presented the lowest SL values, with no difference between them ( $p = 0.694$ ). Regenerate had the highest SL (3.47; 3.22–3.95), which was not different from Blend-a-Med Pro Expert ( $p = 0.071$ ). In conclusion, none of the toothpastes protected against surface loss. Enamel surface loss occurred to different extents, regardless of the desensitizing and/or anti-erosion claim of the toothpastes.

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### Mechanism of Action of Sn/F Solutions as Agents Against Enamel Erosion

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Sn/F-containing solutions reduce erosive tissue loss effectively, but the mode of action is only poorly understood, in particular considering clinically relevant application modes. The study thought to investigate whether tin is retained on/in enamel from marketed Sn/F-mouthrinses compared to an experimental concentrated SnF/solution and when applied according to recommendations for daily use.

Human enamel specimens (n = 15/group) were exposed to alternating cycles of erosion (6×5 min/day, 1% citric acid, pH 2.4) and remineralisation (1 h between cycles and overnight) and treated (1×30 s after the first erosion/day) with the following Sn/F solutions: (a) concentrated solution (1900 ppm Sn<sup>2+</sup> as SnCl<sub>2</sub>, 500 ppm F<sup>-</sup> as NaF/AmF), (b) Elmex Erosion Protection (800 ppm Sn<sup>2+</sup> as SnCl<sub>2</sub>, 500 ppm F<sup>-</sup> as NaF/AmF), (c) Meridol (400 ppm Sn<sup>2+</sup> as SnF<sub>2</sub>, 250 ppm F<sup>-</sup> as SnF<sub>2</sub>/AmF); (d) negative control: nihil (erosion only). After 7 days, tissue loss was quantified profilometrically; energy-dispersive-x-ray-spectroscopy measured tin amounts on surfaces and as depth profile on cross sections (layers of 10  $\mu\text{m}$ ). Statistics: ANOVA with Tamhane's post hoc, Pearson correlation coefficient. Compared to negative control all solutions reduced tissue loss significantly ( $p \leq 0.001$  each); loss values were  $27.8 \pm 6.0$ ,  $36.2 \pm 4.2$ ,  $40.4 \pm 1.0$  and  $72.2 \pm 6.0$   $\mu\text{m}$  after treatments a-d resp. Amounts of tin on surfaces were  $3.7 \pm 0.3$ ,  $2.4 \pm 0.6$  and  $2.1 \pm 0.6$  wt% after treatments a-c resp. with a linear negative correlation to loss ( $r = 0.53$ ,  $p \leq 0.001$ ). Tin was incorporated in enamel to a depth of 20  $\mu\text{m}$  (first 10  $\mu\text{m}$ :  $0.9 \pm 0.4$ ,  $0.7 \pm 0.2$  and  $0.6 \pm 0.1$  wt% in groups a-c resp.), but there was no correlation to loss. In conclusion tin is retained on and incorporated in enamel even after several erosive challenges and when applied according to clinical recommendations. Sn-ions in the hydroxyapatite structure decrease acid solubility of enamel in a dose-dependent way.

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### Impact of Differently Characterized Particles in Toothpastes and Gels on Abrasion of Eroded Enamel

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Toothpastes usually contain mixtures of particles modulating viscosity and cleaning/polishing properties. The abrasivity of particles, however, causes also wear and the effects of different types of such particles in the context of enamel erosion are only poorly understood. Aim was therefore to investigate whether experimental toothpastes each containing a single type of particle cause different abrasion effects on eroded enamel. Syloblanc 81, Sylodent 850c, Perkasil SM660, Sorbosil AC77, Sorbosil AC36, Sorbosil AC39, and Apyra l24 representing different abrasivity, particle sizes, and types of silica/alumina were investigated. In experiment 1 these particles were used in professionally produced formulations and in experiment 2 added to a gel (Lavera Neutral Zahngel). All formulations contained no active ingredients and were used as slurries (1:3 by weight). Human enamel specimens ( $n = 16/\text{group}$ ) were eroded and remineralised (1 cycle:  $6 \times 2 \text{ min}$  0.5% citric acid, pH 2.8, alternated by  $6 \times 15 \text{ min}$  storage in a remineralisation solution), after the first and last erosion of each cycle immersed in slurries (2 min) and within this time brushed (15 s, brushing machine). Controls were erosion only and brushing in distilled water. Substance loss was measured profilometrically after 3, 6, 9 and 12 cycles. Statistics: ANOVA and Tamhane's post hoc. In experiment 1 loss values after erosion only were  $2.7 \pm 1.0$ ,  $7.1 \pm 1.5$ ,  $11.3 \pm 2.3$  and  $15.2 \pm 2.8 \mu\text{m}$  resp.; brushing in distilled water increased values to  $5.9 \pm 1.4$ ,  $12.9 \pm 2.0$ ,  $19.0 \pm 2.1$  and  $25.4 \pm 2.4 \mu\text{m}$  resp. ( $p \leq 0.001$  each compared to erosion only). Brushing with toothpastes did not increase values compared to distilled water, with only minor differences between formulations. Experiment 2 revealed very similar values confirming findings from experiment 1. Differently characterised types of abrasives in formulations without active agents had no impact on abrasion of eroded enamel neither in professionally formulated toothpastes nor when added to a gel.

Dr. Theiss Naturwaren, Homburg, Germany, provided the experimental toothpastes as well as the particles under study. Otherwise, the study was funded by Justus-Liebig-University Giessen, Germany

### The Effect of Different Erosion Times on Surface Roughness of Natural and Polished Enamel

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Surface roughness measurements are becoming increasingly used within dental research and have been used to characterise changes in enamel that has been polished flat. However, there are few studies investigating enamel in its natural form, specifically what level of erosion is required to initiate structural changes. The aim of this study was to investigate the effect of three erosion times on the surface roughness of polished and natural enamel. 60 enamel samples were prepared using buccal sections of human molars (REC: 12/LO/1836) embedded in Protemp4 (3M, UK). 30 were fully submerged and polished until optically flat and 30 were left with their outer enamel surface uncovered. Samples were divided between three groups ( $n = 10$  polished  $n = 10$  unpolished). Group 1 underwent 3 cycles of 5 minutes' immersion in orange juice drink (Sainsbury's' Basic) at 62 rpm agitation using an orbital shaker (Stuart Scientific, Mini Orbital Shaker S05, Bibby), group 2 for 10 minutes per cycle and group 3 for 15 minutes, the groupings were known to the operator. The samples were imaged at baseline and after completion of erosion using a non-contact profilometer (Taicaan, Southampton, UK) and images analysed for Sa roughness by levelling and applying a  $25 \mu\text{m}$  Gaussian filter. Median (IQR) Sa of polished enamel significantly increased for all three erosion groups from  $0.08$  ( $0.10$ )  $\mu\text{m}$  to  $0.26$  ( $0.02$ )  $\mu\text{m}$ ,  $0.15$  ( $0.11$ )  $\mu\text{m}$  to  $0.25$  ( $0.07$ )  $\mu\text{m}$  and  $0.10$  ( $0.08$ )  $\mu\text{m}$  to  $0.27$  ( $0.04$ )  $\mu\text{m}$  respectively (U- Mann Whitney;  $P < 0.001$ ). However, there was only significant change in unpolished enamel for group 3, median (IQR) Sa decreased from  $0.50$  ( $0.29$ )  $\mu\text{m}$  to  $0.42$  ( $0.14$ )  $\mu\text{m}$  ( $P < 0.05$ ). In conclusion natural enamel becomes significantly smoother after erosion and is more resistant to erosive changes compared with polished enamel which becomes rougher.

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## Session 3 Clinical Studies–1

51

### A Retrospective Clinical Study on the Longevity of Gold Inlays

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**AIM** To investigate the longevity and reasons for failure of posterior Class-II gold inlays among a group of Norwegian adults. **METHODS** A cohort of 138 patients (81 male and 57 female) regularly attending a general practice for regular check-up were examined by one clinician (GS) in 2016. The patients had a total of 391 posterior Class II gold inlays placed in the period 1970 to 2015. The inlays were categorized as acceptable or failed. Information on the longevity and reasons for failures was collected from the patient records. Reasons for failure were classified as either “secondary caries”, “fracture”, “lost inlay” or “other”. Participation was voluntary and no compensation was given. The implementation was approved by the Regional Committee for Medical Research Ethics in Norway (ID: 2015/1324). **RESULTS** The mean age of the patients at placement was 50.8 years (SD: 12.7 yr). Most gold inlays were placed in molars (85.9%) and 14.1% were placed in premolars; 49.4% of the inlays were in the maxilla and the 50.6% in the mandibula. Average length of follow-up was 11.6 years (range: 1–46 years, SD: 7.9); 93.4% were classified as successful and 6.4% as failed. Reasons for failure were secondary caries (41.3%), lost inlay (25.4%), fractures (23.8%) and other (9.5%). Mean annual failure rate (AFR) of the inlays was 0.57%. **CONCLUSION** The present retrospective clinical study demonstrated a still annual failure rate of Class II gold inlays. Thus, gold inlays can still be considered as a good treatment option in posterior teeth.

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52

### ICCMS™ vs. Colombian NHS Caries-Management Schemes in Schoolchildren. One-Year Results with a Multicenter RCT Approach

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The ICDAS-derived International-Caries-Classification-and-Management-System (ICCMS™) focuses in maintaining health, controlling caries and preserving tooth structure. **Aim:** To compare in a multicenter RCT setting the 1-year effectiveness of A) adjusted ICCMS™ and B) Colombian-standard NHS caries-management schemes. After ethical approval and parent-informed consents, 240 7-year olds from 6 Colombian dental-school clinics were assessed by 6-trained examiners for: -ICCMS™ caries-lesion likelihood (low/moderate/severe); -caries lesions in terms of ICCMS™ Initial ( $d_I$  and  $D_I$ :ICDAS-1/2) and conventional dentine/cavitated Moderate/Extensive caries lesions ( $d_{M/E}$  and  $D_{M/E}$ :ICDAS-3/6) plus fillings and missing teeth to account for the ICCMS™ caries experience ( $d_{IMEfs}$  and  $D_{IMEMFS}$ ), and -caries activity assessment (active/inactive). Randomly allocated children (A/B) received management of caries by trained dentists. After one year children were reassessed by 2-blind trained external examiners. Caries progression was defined as a follow-up score increment (1-Sound, 2-Initial-inactive-caries-lesion, 3-Moderate-inactive-caries-lesion, 4-Sealant, 5-Filling, Extensive-inactive-caries-lesion, 6-Initial-active-caries-lesion, 7-Moderate-active-caries-lesion, 8-Extensive-active-caries-lesion, 9-Extracted-due-to-caries).

The 1-year follow-up sample (n = 205; A:104; B:101) presented a prevalence and mean of  $d_{\text{IMEFs}}$  and  $D_{\text{IMEFMS}}$  of around 93% and 11, respectively. Mean number of initial caries lesions ( $d_{\text{IS}}/D_{\text{IS}}$ ) (A:6.79 ± 4.85; B: 7.33 ± 5.25) corresponded to around 66% of  $d_{\text{IMEFs}}$  and  $D_{\text{IMEFMS}}$  index, and significantly decreased from baseline (A:9.42 ± 8.72; B:9.90 ± 7.93) (Mann Whitney;  $p < 0.05$ ). From available number of surfaces at follow-up (n = 22140; A:11232; B:10908), most remained within the same code (A:80.6%; B:81.5%), around 1/10 regressed (A:10.7%; B:10.3%) and 8% progressed (A:8.0%; B:8.2%). Thus both groups showed significant differences with respect to baseline (Wilcoxon signed-rank test;  $p < 0.05$ ). ICCMS™ caries-lesion likelihood percentage distribution improved significantly after one year ( $\chi^2$ ;  $p < 0.05$ ): most were high at baseline (A:62.1%; B:57.4%) and moderate after 1 year (A:53.9%; B:52.5%). Mean number of appointments was similar (A:6.6 ± 3.8; B:6.6 ± 3.3). No significant differences were found after one year between groups ( $\chi^2$ ;  $p > 0.05$ ). Both caries-management schemes were effective after one year with an overall caries-lesion likelihood decrease and >91% surfaces stabilizing/arresting.

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### 53

#### **Demineralization of Enamel Discs in Subjects Rinsing with Various Amounts of Sugar Four Times Per Day**

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It is generally thought that sugar in the diet causes caries. However, a dose-response relationship between the amount of sugar consumed and enamel caries has never been univocally demonstrated. The aim of this intra-oral model investigation was to compare demineralization of pre-demineralized enamel discs, exposed to a sugar rinse regime with a fixed frequency and duration (30 s), but with various amounts of sugar by a 10% sugar solution in a cross-over design. Fifteen subjects wore a removable partial prosthesis carrying an enamel disc (baseline lesion: 2856 vol% × μm) covered with a Dacron gauze for four consecutive periods of one month each. The subjects rinsed four times per day between meals 25 ml of 10% sugar solution either 1 × 30 s (total amount of sugar per day: 4 × 2.5 g = 10 g), 2 × 15 sec (20 g per day), 3 × 10 s (30 g per day) or 2 × 15 s 25 ml water without sugar (0 g per day). After one month on the 10 g sugar per day regime, the net mineral loss measured with transverse microradiography was 4018 vol% × μm, after the 20 g regime 4444 vol% × μm and for the 30 g regime 3088 vol% × μm, while without sugar the net mineral loss was 2259 vol% × μm. Statistical analysis using a linear mixed model did not find a significant effect of the rinsing regime on net mineral loss. This study does not support the idea that changing the amount of sugar but not frequency nor rinsing time is related to the extent of enamel caries development.

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### 54

#### **Caries Preventive Effect of One-Year Use of Low-Dose Xylitol Chewing-Gum in High-Caries-Risk Adults**

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This Randomised Clinical Trial investigates the caries preventive effect of low-dosage (2.5 g/die) xylitol chewing-gum administered for one-year in a high-caries-risk adult population. 179 subjects (≥1 cavitated lesion and salivary concentration of mutans streptococci (MS) ≥10<sup>5</sup> CFU/mL) were recruited and randomly assigned to two groups. Polyols group received for one-year gums containing 28% isomalt, 31% sorbitol, 9% mannitol and 1% maltitol syrup, while xylitol group received gums containing 26% sorbitol, 11% mannitol and 30% xylitol. Caries status, using the International Caries Detection and Assessment System, salivary MS, and plaque-pH were evaluated at baseline, at the end of the administration and re-evaluated after one year in 66 xylitol and 64 polyols subjects. All participants reported to use a F-toothpaste (1000/1450 ppm) on a regular basis. The net caries increment for initial, moderate and extensive lesions and for the caries experience was evaluated using the nonparametric Mann-Whitney-U-test. The total caries experience increment was 1.25 ± 1.26 in the xylitol group and 1.80 ± 2.33 in the polyols group ( $p = 0.01$ ). Subjects treated with xylitol had a reduction of Risk Rate at tooth level of 23% respect to those treated with polyols with a number needed to Treat of 55 teeth. The area under the curve at pH<sub>5.7</sub> was statistically significant lower ( $p = 0.02$ ) in the xylitol group. A decrease of the concentration of salivary MS was noted in xylitol group ( $p < 0.01$ ).

**Conclusions:** Subjects using the low dose xylitol chewing-gum showed a significant lower MS concentration, plaque acidogenicity and a lower caries increment compared to subjects using a polyols chewing-gum. One-year administration of low dosage of xylitol through a chewing-gum provided a caries preventive effect in high-caries-risk adults.

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### Evaluation of the Difference in Caries Experience in Diabetic and Non-Diabetic Children – A Case Control Study

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The aim was to evaluate the difference in caries experience and different caries-related variables between diabetic and non-diabetic children. A further analysis was carried out on diabetic children after they were divided in two groups based on their metabolic control. A total of 204 individuals participated in this study: 68 children diagnosed with type 1 diabetes and 136 non-diabetic control children. Diabetic children were then divided in: a) 20 children with adequate metabolic control (Hb1ac  $\leq$ 7.5) and b) 48 children with poor metabolic control (Hb1ac  $>$ 7.5). Data on dietary and oral habits were collected by a self-compiled questionnaire. Saliva samples were carried out after chewing a piece of paraffin for 5 minutes. Genomic probes were prepared from 10 bacterial strains and analyzed using the checkerboard DNA-DNA hybridisation method. Plaque acidogenicity was recorded using pH indicator strips up to 30 min after a sucrose rinse. ICDAS index was also calculated. One-way ANOVA was used to compare mean differences in the caries experience and bacterial scores in the two groups. Caries prevalence varies from 54% in diabetic subjects with good metabolic control to 70% in diabetic subjects in bad-metabolic control; no statically significant difference in caries status was found in the two study groups. No statistically significant difference was found for tooth brushing frequency, use of fluoridated toothpaste, mouthwash and other fluoride supplements and the pattern of dental check-ups between the groups examined. Plaque-pH (minimum pH, AUC<sub>6,2</sub> and AUC<sub>5,7</sub>) was statistically different in diabetic respect to the non-diabetic children ( $p < 0.01$  or  $p < 0.05$ ) were found. The bacterial counts differed significantly between diabetic and non-diabetic subjects regarding *S. mutans*, *S. sobrinus*, *L. salivarius* and *L. fermentum* ( $p < 0.05$ ). In conclusion, Type 1 Diabetes Mellitus patients showed a more cariogenic bacterial environment and a direct effect on plaque pH reducing it from normal levels was detected.

### Non-Operative Caries Treatment Program in Five and Six-Year Old Children in Rotterdam, The Netherlands – Practice-Based Study

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Aim of this practice-based study was to investigate the efficacy of the Non-Operative Caries Treatment Program (NOCTP) in five and six-year old children enrolled for dental care at youth dental care practice Sanakids, located in a deprived area of Rotterdam, the Netherlands. In this practice some dentists have adopted NOCTP, while others use a standard recall scheme of dental check-ups at 6 month intervals. A survey was conducted among parents of five and six-year old children registered as patients at the youth dental care practice Sanakids. DMFT values were obtained from the patient-records. Children were treated according to NOCTP or the standard protocol. The questionnaire consisted of items regarding dental knowledge (7 items, low = knowledgeable), Locus of control (17 items, high = internal) and parental tooth brushing behavior of the child's teeth (7 items, low = poor). Differences between groups were explored using t-tests. Complete questionnaires and DMFT values were obtained from 128 participants (64 per group, age  $5.67 \pm 0.78$  NOCTP,  $5.73 \pm 0.65$  controls). DMFT in the NOCTP group ( $0.12 \pm 0.11$ ) was significantly lower than in the control group ( $0.34 \pm 0.23$ ,  $p < 0.01$ ). Parents in the NOCTP group had more knowledge about the oral health of their child ( $13.78 \pm 3.65$  versus  $17.81 \pm 5.01$ ,  $p < 0.01$ ), a more internal locus of control ( $60.87 \pm 8.71$  versus  $49.09 \pm 10.90$ ,  $p < 0.01$ ) and better brushing habits towards the child ( $8.81 \pm 2.05$  versus  $6.78 \pm 1.99$ ,  $p < 0.01$ ). Parents in the NOCTP group have more dental knowledge, feel more responsible and play a more active role in the maintenance of the teeth of their children, resulting in lower caries prevalence in these children. Whether this difference is a result of NOCTP or these parents being more inclined to enroll their child at a dentist practicing NOCTP is unknown.

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### Glass Ionomer Sealants for Controlling Moderate Caries Lesions on Occlusal Surfaces: A Preliminary Analysis

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This is preliminary analysis of an ongoing study to verify the efficacy of sealing moderate caries lesions with glass ionomer cement (GIC) as an alternative to restoring these lesions (NCT03005405). We included in this analysis 2/3 of the entire sample (n = 63 children) that had been followed in the first year of this study. Children were divided into 2 groups, according to the intervention on moderate caries lesions: GIC restoration, after opening using high-speed burs or GIC sealant. Children were evaluated at the baseline and every 6 months after treatment. Teeth were assessed using WHO criteria for caries detection and also the criteria for ART restorations/sealants (Frencken and Holmgren, 2001). As outcomes, we considered: 1. any need of reintervention (repair/replacement/caries progression), 2. need of replacement of restoration/sealant and 3. caries progression (change in lesion severity). Cox regression with shared frailty was performed to verify the influence of type of treatment on described outcomes and Hazard Ratios (HR;95%CI) were calculated. Independent variables were used to adjustment. All children (128 teeth) were examined at least once after the treatment. Approximately 20% of this sample needed reintervention, but no difference was observed between treatments (HR = 1.25; 0.47–3.32). Irrespective of the group, 40% of these failures did not require the replacement of restoration/sealant and occurred in the first 6 months. Although groups were also similar in need of replacement of restoration/sealant (HR = 1.30; 0.34–4.98), the need of reintervention tended to increase for GIC sealants, after 12 months. No differences; however, were noticed for caries progression between treatments (HR = 1.35; 0.29–6.35). Analysing these preliminary results, sealing moderate lesions using GIC seems to have similar longevity than GIC restorations, but failures in GIC sealants may increase after 12 months, demanding longer follow-ups.

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### The Effect of an Anti-Sensitivity Product in Relieving Dentine Hypersensitivity

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**Objective:** To evaluate the efficacy of an anti-sensitivity product instantly after 1 application and 1, 4 and 8 weeks usage in a double-blind, parallel 3 groups RCT. Subjects with 2 sensitive teeth (Schiff 2 or 3) from cervical erosion/abrasion/gingival recession were measured with Yeaple Probe and Schiff Score. Test products were:

Group1: Negative control (fluoride paste 1450 ppm F); Group2: Pepsodent Expert Sensitive Serum (2% Hydroxyapatite and 3% Potassium Citrate) + fluoride paste; Group3: Regime (Pepsodent Expert Sensitive Serum + anti-sensitivity Toothpaste). Subjects brushed with 1.5 g of allocated pastes 1 min, followed by rinsing; for Groups 2 and 3, subjects were instructed to apply 1 drop of the product per sensitive tooth with applicator for 10 s after each toothbrushing throughout the 8 weeks testing period. The 1, 4 and 8 weeks measurements were collected 8 hours after subjects' last usage. The outcome variable per subject were the averaged values of the two sensitive teeth. Between test group differences were assessed for statistical significance by Wilcoxon Rank Sum test due to slight departure from normality by SAS V9.3 (SAS Institute Inc.), statistical significance was  $p < 0.05$ .

157 subjects (52 to 53/group) completed the study. The Means (SE) of scores ( $*p < 0.05$ ) were presented at baseline, instant, W1, W4 and W8 respectively.

Yeaple (grams) Group1: 11.21 (0.28), 10.86 (0.30), 11.27 (0.35), 11.92 (0.59), 15.00 (1.68); Group2: 10.76 (0.27), 12.71 (0.89)\*, 16.25 (1.47)\*, 23.94 (1.76)\*, 46.76 (3.32)\*; Group3: 10.83 (0.24), 12.08 (0.52)\*, 16.08 (1.26)\*, 26.93 (2.46)\*, 50.94 (3.04)\*. Schiff (sensitivity scale) Group1: 2.63 (0.05), 2.52 (0.06), 2.54 (0.06), 2.30 (0.08), 2.21 (0.08); Group2: 2.69 (0.04), 2.28 (0.06)\*, 2.11 (0.07)\*, 1.98 (0.08)\*, 1.64 (0.09)\*; Group3: 2.61 (0.05), 2.33 (0.05)\*, 2.27 (0.07)\*, 1.92 (0.08)\*, 1.67 (0.10)\*.

Both product (Group2) and Regime (Group3) gave significantly greater reduction in hypersensitivity than Negative control group at all the time points as evaluated by both Yeaple and Schiff. In conclusion Pepsodent Expert Sensitive Serum was an efficacious product in reducing dentine hypersensitivity instantly and after 1, 4, 8 weeks.

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## Caries Risk Factors in 1-Year-Olds Predicting Dentin Caries at 6 Years of Age

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The aim was to identify risk factors in one-year-olds predicting dentin caries at 6 years of age in a region with low caries prevalence. Caries risk was assessed by 3 different dental hygienist or dental assistant in 804 one-year-olds. The oral mutans streptococci (MS) score was performed from a tooth surface or (in pre-dentate children) from oral mucosa. A parental questionnaire with questions regarding family factors (siblings with or without caries), general health, food habits (night meals, breastfeeding, other beverage than water), oral hygiene habits and emerged teeth were answered by parents of the 1-year-olds. Dentin caries was assessed when the children were 6 years old by 7 different dentists. Simple and multiple logistic regression analyses identified caries-associated risk factors.

Five percent of the one-year-olds were assessed to have an increased caries risk. At 6 years of age dentin caries was found in 16 % of the children. Forty-six percent of the children with high caries risk at 1 year had caries at 6 years age. Fourteen percent of 1 year olds with non caries risk had caries at 6 years age. Simple regression analysis found the 1-year-variables Caries in sibling ( $P = 0.001$ ), Night meal ( $P \leq 0.00$ ), Beverages other than water ( $P \leq 0.00$ ), Presence of MS ( $P = 0.004$ ) and male Gender ( $P = 0.032$ ) to be associated with dentin caries at 6 years. Multiple regression analysis found the 1-year-variables Beverages other than water ( $P \leq 0.000$ ), Night meal ( $P = 0.003$ ), Caries in sibling ( $P = 0.014$ ), male Gender ( $P = 0.034$ ) and Presence of MS ( $P = 0.046$ ) to be associated with dentin caries at 6 years of age. In conclusion, behavioural, family, and microbial factors are important when assessing caries risk among 1-year-olds in a region with low caries prevalence.

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## Caries Experience and Risk Assessment in Children and Adolescents with Cerebral Palsy

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The aim was to compare the caries experience and risk assessment in children and adolescents with cerebral palsy (CP) and non-morreactive (NR) living in Northeast of Brazil. Sixty individuals aged 6–12 years, being 30 with confirmed diagnosis of CP and 30 NR were paired by sex and age. Dental caries was registered by one calibrated examiner using DMFT criteria. Oral hygiene was estimated by visual plaque index. Dietary information and fluoride usage were collected from a questionnaire. Seven factors from Cariogram computer model were included: caries experience, diet (content and frequency), hygiene index, related diseases, fluoride usage and clinical judgment. Clinical judgment was standardized for both groups allowing the other factors to determine the caries risk. The percentages of “chances of avoiding new lesions” (caries risk) were obtained from Cariogram, and the subjects were classified into four risk groups. Caries experience for NR and CP were  $3.3 \pm 3.1$  and  $4.4 \pm 5.0$ , respectively, with no significant difference (Mann-Whitney;  $p > 0.05$ ). Caries risk assessment classification for NR/CP was found 13.3%/17.2% low risk, 46.7%/41.4% intermediate risk, 30.0%/20.7% high risk and 10.0%/20.7% very high risk, respectively. There was no statistically difference between the groups (Chi-square;  $p > 0.05$ ). The average caries risk profile for “chance to avoid dental caries” for NR and CP was  $41.6 \pm 18.3$  and  $41.7 \pm 18.3$ , respectively, with no significant difference (t-Student;  $p > 0.05$ ). The dominant sector was “susceptibility and the last one “circumstance” in both groups. Significant differences were observed for “diet” (Mann-Whitney;  $p < 0.05$ ) and “circumstance” (Mann-Whitney;  $p < 0.05$ ) sectors between the groups. In conclusion, dental caries experience was high and the caries risk pattern varied from intermediate to high in children and adolescents with CP and NR.

### Responsiveness of the Child Perception Questionnaire to Oral Health Care Treatment Program for Adolescents

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Responsiveness refers to the sensitivity of an instrument to detect clinical changes. The aim of the present study was to assess the responsiveness of the Child Perception Questionnaire (CPQ<sub>11-14</sub>) to an oral health care treatment program for non-privileged 10–15 years old adolescents in the region of Itapoã, Federal district of Brazil. Adolescents who answered the CPQ<sub>11-14</sub>, were clinically and radiographically examined at baseline (n = 618) and after 1 year (n = 560). The program was based on intensive patient oral health care education and training in addition to professional toothcleaning (prophylaxis with or without scaling) according to individual requirements. Educational and training activities focused on risk factors for development and arrest of caries, and for development and remission of gingivitis, together with appropriate brushing technique of posterior erupting teeth with gel 1.23% F<sup>-</sup>. These activities were assured for all adolescents every 3 months during the study period. Further training was given to adolescents with active lesions and or gingivitis. Operative procedures such as fillings, endodontics and extractions were also included in the program. Responsiveness of the instrument was tested by relating changes in the number of surfaces with cavitated dentine lesions (D<sub>3</sub>S) after 1 year to differences in mean CPQ<sub>11-14</sub> scores and subscale scores (n = 560). No responsiveness of the CPQ<sub>11-14</sub> was found when groups of adolescents that decreased in D<sub>3</sub>S scores, that did not change in D<sub>3</sub>S scores or that increased in D<sub>3</sub>S scores were compared (ns, ANOVA). However, responsiveness was observed between the groups that showed a decrease and an increase in D<sub>3</sub>S scores (p = 0.023, ANOVA followed by post hoc analysis (LSD)). When changes in shallow lesions and in moderate to deep caries lesions were considered, significant results were found for the latter (p = 0.016). Finally, significant decreases in the means CPQ<sub>11-14</sub> total and subscale scores were found (p < 0.001, t-test). The CPQ<sub>11-14</sub> was sensitive enough to detect clinical changes related to increase and decrease of cavitated dentine lesions and changes in moderate to deep caries lesions. A beneficial effect of the oral health care treatment program was observed.

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### Comparator Choice in Caries Prevention and Management Trials: Systematic Review and Network Analysis

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**Objectives:** The robustness and applicability of the overall evidence is affected by the comparators in networks of trials. We analyzed networks of trials on the prevention/management of caries/carious lesions, hypothesizing that certain comparators are over- and others underproportionally investigated, and that comparisons within comparator classes are preferred over comparisons between classes. **Methods:** A systematic review of randomized controlled trials on caries prevention or management of existing carious lesions was carried out. All comparators were classified at each of three levels of granularity, becoming more detailed with each level: (a) degree of invasiveness (non-, micro- or invasive), (b) the specific non-invasive, micro-invasive or invasive approach, (c) the actual material or technique used. Social network analysis was used to evaluate trial networks. **Results:** Searching electronic databases found 4,774 articles of which 765 were relevant and 605 were included. The networks for all levels were polygonal. There was a high degree of separation of comparisons in prevention versus management trials. Invasive comparators were tested most frequently (number of comparators: 611), mainly in management trials. Non-invasive comparators were tested next often (474), mainly in caries prevention. Micro-invasive strategies were tested next often (233), in both, prevention and management trials. On more granular levels, few interventions dominated the networks. Regardless of the level, the majority of trials compared within, not between classes. Prevention trials were mainly conducted in children (number of trials in adults/children/both: 37/241/11), while those on management in both children and adults (117/179/21). **Conclusions:** Comparator choice in cariology trials is driven by indication, and limits conclusions on the true comparative effectiveness of all strategies. There are a variety of comparators that have not been, but should be, compared to one another.

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### Is Health-Related Quality of Life Correlated to Oral Status in Primary Sjögren's Syndrome Patients?

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Primary Sjögren's syndrome (pSS) is an autoimmune disease characterized by reduced lacrimal and salivary secretions, fatigue, and muscle and joint pain. The aim was to investigate any relationship between health-related quality of life (HRQoL), dental status, chemosensory status, oral dryness and other subjective clinical symptoms in pSS patients. Informed consent was obtained from patients (n = 34, 53 ± 12 y) and healthy controls (n = 32, 49 ± 12 y). HRQoL was assessed using the combined domains Physical Component Summary (PCS) and Mental Component Summary (MCS) of the 36-item short-form health survey (SF-36). Caries experience (D<sub>3-5</sub>MFT) was recorded, and gustatory/olfactory functions were assessed using impregnated taste strips and odorant-filled felt-tip pens. Symptoms of oral dryness were recorded using the Summated Xerostomia Inventory (SXI) and Clinical Oral Dryness Score (CODS). Self-reported complaints of a burning sensation in the mouth, dysgeusia and halitosis were registered. T-test and Spearman's rho were used in the data analysis. Patients with pSS had lower SF-36 scores than controls (PCS: 39.8 ± 10.3 vs. 56.8 ± 5.3, MCS: 44.8 ± 11.9 vs. 55.1 ± 6.5, p < 0.001), higher D<sub>3-5</sub>MFT (18.5 ± 6.9 vs. 12.2 ± 6.8, p < 0.001), lower gustatory and olfactory scores (gustatory: 9.4 ± 3.5 vs. 12.8 ± 2.1, p < 0.001, olfactory: 9.1 ± 3.1 vs. 10.8 ± 1.1, p = 0.003), and more oral dryness (CODS: 4.9 ± 2 vs. 0.59 ± 0.9, p < 0.001; SXI: 12.1 ± 2.5 vs. 5.9 ± 1, p < 0.001). Significant correlations were found between MCS and gustatory scores (r = 0.37, p = 0.03) and dysgeusia (r = -0.38, p = 0.026). CODS was negatively correlated with halitosis (r = -0.35, p = 0.045) and burning sensation (r = -0.39, p = 0.025). SXI was negatively correlated with burning sensation (r = -0.52, p = 0.002) and dysgeusia (r = -0.36, p = 0.037). Furthermore, taste score was negatively correlated with DMFT (r = -0.34, p = 0.046). In pSS patients, significant correlations were found between HRQoL, dental status and chemosensory scores, oral dryness and other subjective clinical symptoms, highlighting the complexity of oral components of this disease.

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### The Effect of Anxiety Body Odour on Dental Students' Performance

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Dental anxiety is a common phenomenon in both young and adult patients. Human body odours (BO) can transfer anxiety-related signals, with consequences for conspecifics emerging at the behavioural level. The aim of this study was to evaluate the effect of human body odour chemosignals on the performance of dental students. Twenty-four 4th year dental students donated their body odour during a clinical session (Anxiety BO) and a classroom session (Rest BO). Twenty-four age and gender-matched 3rd year dental students [17 females, age: 24.8 y (± 5.6)] performed dental procedures while exposed to three odor conditions: Anxiety BO, Rest BO and Masker (consisting of a clean t-shirt), all masked with 50 µl of eugenol. During three sessions at the preclinical laboratory restorative dental procedures were performed on KaVo dental training models mounted on phantom heads: tooth 34 MO minibox, tooth 35 MO box, tooth 46 MOD box. The restorative procedure was scored on a range from 0 (poor) to excellent (50) based on: damage to neighbouring teeth, pulpal depth, gingival height, occlusal depth and breadth, preparation on wrong teeth. Results from linear mixed models indicated that the different odour conditions could not be perceptually distinguished by the participants (ps > 0.08). However, results revealed that the dental students' performances were significantly poorer when exposed to Anxiety BO compared to Rest BO and control: Anxiety BO: 28 < Rest BO: 33 < Masker: 35). The results were supported by Bayesian statistics, indicating that there was a strong (BF = 19) to very strong (BF = 92) difference between the dental students' performances under the different odour conditions. These findings suggest the need for increased awareness of the anxiety-inducing effects of BO on dental professionals.

### Cariostatic Co-Operative Action of Statherin and Histatin-1 on Hydroxyapatite Disc Demineralisation Rate Kinetics

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Statherin, and histatin1 are salivary phosphoproteins involved in enamel homeostasis. These proteins are integral to the organised bio-molecular pellicle on exposed enamel surfaces, statherin binding directly to enamel. Previous studies demonstrate reductions in enamel (and HAP discs used as enamel analogues) demineralisation kinetics following exposure to full length statherin, and

truncated peptides, under artificial caries conditions. The initial aim was to investigate if histatin1 had similar caries reducing efficacy, and, if statherin and histatin1 (HTN1) in showed synergistic cariostatic effects. Synthetic Nterminal 21 residue statherin like peptide (STN21), and histatin1 peptide (HTN1), were produced using Fmoc chemistry. Each were dissolved in PBS at concentrations of 0.2 mM. A third test solution containing both STN21 and HTN1 peptides each 0.1 mM was also prepared (total peptide concentration 0.2 mM). HAP discs (2 mm diameter, 20% nominal porosity) were mounted within SMR environmental cells and exposed to artificial caries like demineralising solution (0.1 M acetic acid, pH 4.0) for 48 h. The samples were rinsed and exposed to 2.0 ml of each of the three test peptide solutions (STN21, HTN1, and STN21 + HTN1) for 24 h. Then, demineralising solutions were further circulated for 48 h. Real-time measurements of HAP disc demineralisation kinetics (RDHAP) was performed using Scanning Microradiography (SMR) throughout. Each experiment was repeated 6 times. The reduction in RDHAP following treatment individually with STN21, and HTN1, was 38.9 ( $\pm 1.9$ ) % and 33.4 ( $\pm 1.9$ ) % respectively. However, following treatment with both STN21 and HTN1 together, the reduction was 53.1 ( $\pm 3.7$ ) %. In conclusion, the HAP treated with both statherin-21 and histatin1 further reduced HAP demineralisation kinetics than each individually. This result suggests a cooperative action of statherin and histatin1 in resisting enamel caries progression by the salivary pellicle.

66

### Edentulism and Health Related Risk Factors in Italian Elderly Living in Nursing Home

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No data on oral health in the older Italian adult population are available. This paper reports the prevalence of oral health conditions, in a population-based study of older subjects. A population-based cross-sectional study was undertaken among Italian individuals aged more than 60 years. Physical examination among 1976 subjects (74.04% females and 25.96% males) including the number of teeth and the type of prosthetic rehabilitation was carried-out. Moreover, Body Mass Index (BMI), the Mini-Mental State Examination (MMSE) to estimate the severity and progression of cognitive impairment and The Mini-Nutritional Assessment (MNA) as a screening to identify who are malnourished or at risk of malnutrition were collected. Cross-tabulation between clinical, demographic and anamnestic variables was performed and analyzed with chi-square test. 883 subjects (44.69%) were edentulous, while 89.07% of the sample has <21 functional teeth. 55.61% of the subjects has some prosthetic rehabilitation. Females

have more remaining teeth than males ( $p < 0.01$ ). MMSE was not statistically associated with the number of teeth present in the entire sample, but in the older segment of the sample the association became evident. Both BMI and MNA were significantly associated with the number of remaining teeth ( $p = 0.02$  and  $p < 0.01$ , respectively). In conclusion these findings highlight the high burden of poor oral health in older Italian population. The determinants of these oral health problems in older populations merit further research to reduce the burden and consequences of poor oral health in older people.

67

### Dry Mouth and Dry Eye Examinations of a Norwegian Cohort of Primary Sjögren's Syndrome Patients

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The aim of this study was to carry out comparative dry mouth and dry eye examinations of a Norwegian cohort of patients with primary Sjögren's syndrome (pSS) and age- and gender-matched controls. **Methods:** Thirty-four female patients with pSS (53  $\pm$  11.9 yr) and 32 healthy controls (49  $\pm$  11.5 yr) were recruited to the study. All participants underwent comprehensive ocular and oral examinations. Oral examinations included recording decayed, missing, and filled teeth ( $D_{3-5}MFT$ ), Summated Xerostomia Inventory (SXI) and Clinical Oral Dryness score (CODS). Unstimulated (UWS) and stimulated saliva (SWS) secretion rates were also recorded. Comprehensive dry eye work-up included patient reported dry eye questionnaire – McMonnies Dry Eye Index (MDEIS) and clinical tests -tear osmolarity, Schirmer I test, tear film break-up time (TFBUT) and ocular surface staining (Oxford Grading Scheme). Mann-Whitney U test was used for inter-group comparisons. **Results:** Compared to the control group, pSS patients had higher  $D_{3-5}MFT$  index (18.5  $\pm$  6.9 vs. 12.1  $\pm$  6.8,  $p < 0.05$ ), SXI score (12.5  $\pm$  2.5 vs. 5.94  $\pm$  1.0,  $p < 0.05$ ), CODS (4.9  $\pm$  2 vs. 0.6  $\pm$  0.9,  $p < 0.05$ ), and lower salivary secretion rates (UWS: 0.08  $\pm$  0.07 vs. 0.3  $\pm$  0.2, SWS: 0.6  $\pm$  0.4 vs. 1.5  $\pm$  0.7,  $p < 0.001$ ). Moreover, patients with pSS had higher MDEIS scores (17.6  $\pm$  3.8 vs. 4.1  $\pm$  2.0,  $p < 0.05$ ), higher tear osmolarity levels (334.8  $\pm$  21.6 vs. 319.7  $\pm$  15.8,  $p < 0.05$ ), lower Schirmer I values (4.8  $\pm$  4.0 vs. 16.2  $\pm$  11.6,  $p < 0.01$ ), shorter TFBUT (2.4  $\pm$  2.6 vs. 5.4  $\pm$  3.3,  $p < 0.05$ ) and higher ocular surface staining scores (3.9  $\pm$  2.3 vs. 0.8  $\pm$  1.2,  $p < 0.01$ ). Moderate correlations between SWS and Schirmer I test ( $r = 0.56$ ,  $p = 0.03$ ), SXI and MDEIS questionnaires ( $r = 0.456$ ,  $p = 0.04$ ) were observed. **Conclusion:** The findings of the current study revealed more severe dry mouth, higher caries experience as well as more severe dry eye symptoms in the pSS patient group. The results suggest that pSS patient examination

should include both comprehensive dry eye and dry mouth examinations.

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68

### Variables that affect Parental Perception and Acceptance of Silver Diamine Fluoride (SDF) Staining

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SDF presents a minimally invasive and inexpensive intermediate treatment for children with difficulties to receive conventional treatment. However, it permanently stains decayed enamel and dentin and this may limit acceptance of this treatment.

The aim was to assess parental perceptions of SDF staining, and how location, need for advanced behavioral management methods and demographics influence acceptance of the treatment. With a web-based instrument, we surveyed parents of children who had experienced dental decay visiting NYUCD Pediatric Dental Clinic and private offices to assess demographics, perceptions of pictures of SDF-treated decay, and treatment acceptability under different management scenarios. Differences in proportions were compared by means of the McNemar test, Pearson correlations were used to quantify the relationships among demographic indicators and a linear mixed model was used to compare variations of mean acceptance levels.

98 mothers and 22 fathers participated. Their median age was 40 y; approximately 42% White, 11% Black, 36% Hispanic and 10% Asian; with diverse socioeconomic backgrounds. In a scenario where the child could cooperate to get restorations, 32% of parents reported they were 'somewhat likely' or 'very likely' to choose SDF to treat anterior teeth and 61% posterior teeth. Acceptance rose to 70% ( $p < 0.001$ ) for anterior teeth and to 76% ( $p < 0.001$ ) for posterior teeth when faced with the scenario of requiring general anesthesia. We found higher levels of acceptance, and less difference between anterior and posterior acceptance, among those with less than a college education, lower income, younger age and Hispanic ethnicity.

In conclusion, parental acceptance of SDF is higher for posterior than anterior locations and it increases with the need of general anesthesia. Lower SES was associated with a greater acceptance of SDF. About a third of parents find the treatment unacceptable under any circumstance.

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69

### QLF-D as Oral Hygiene Evaluation Tool to Assess Plaque and Demineralisation in Orthodontics

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**Objective:** To assess the use of Quantitative Light-induced Fluorescence-Digital Biluminator TM (QLF-D<sup>TM</sup>) to detect plaque coverage and demineralisation in patients with poor oral hygiene prior to start of orthodontics. **Design and Setting:** A 1:1 parallel arm RCT was conducted at Liverpool University Dental Hospital. 60 patients with poor oral hygiene who were referred to hygienist or a dentist before they are added on the waiting list to have fixed orthodontic appliance treatment were randomly allocated to receiving oral hygiene reinforcement (OHR) at three consecutive appointments using white light (WL) or QLF-D images taken with the QLF-DTM device as visual aids. For both groups, plaque coverage,  $\Delta R30$  and change in demineralisation, measured by the degree of fluorescence loss,  $\Delta F$ , were assessed on QLF-D images from the baseline to the final appointment. A questionnaire was used to ascertain the patients' perspectives of the images being used as oral hygiene aids. There were no significant differences in plaque accumulation ( $P = 0.81$ ) or demineralisation ( $P = 0.69$ ) between the WL and QLF-D groups. There was no significant change in demineralisation over the three visits in either group, however there was a significant reduction in plaque in both groups ( $P < 0.001$ ) with a mean percentage change in  $\Delta R30$  of 51.8% and 95% CI of 40.36% to 63.26%. 92.5% of the QLF-D group and 76.7% of the WL group expressed it would be useful to be given such OHR for the full duration of orthodontic treatment. In conclusion OHR using WL or QLF-D images as visual aids were effective in reducing plaque coverage. There was no difference in the level of demineralisation or plaque coverage between the QLF-D and WL groups. More patients reported that the QLF-D images were useful than patients shown WL images.

University of Liverpool is funding this RCT.

### Self-Administered Non-Invasive Treatment with High-Fluoride Toothpastes for Root Caries Lesions in Community-Dwelling Elders, a RCT

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Self-administered non-invasive therapy with high-fluoride dentifrices is an attractive alternative to traditional restorative treatment, but the available evidence is scarce. The aim of this randomized controlled trial (RCT) was to compare the effectiveness of toothbrushing with 5,000 ppm versus 1,450 ppm fluoridated toothpaste (F-toothpaste) on preventing and arresting root carious lesions, in community-dwelling elders.

A 2 12-month double-blinded RCT was devised with a calculated sample size of 345 independently-living older adults of 60 years of age or older. To be enrolled, volunteers had to have at least 5 teeth in the mouth and one root carious lesion. Participants were randomly assigned to the arms of the study: toothbrushing twice daily with high-fluoride (5,000 ppm F) or regular F-toothpaste control (1,450 ppm F) dentifrice. Incidence and arresting of existing root carious lesions were recorded at the 12-month follow-up and compared with baseline data. Lesions were detected and diagnosed by ICDAS II criteria for presence, and Nyvad's criteria for caries activity. Baseline and follow-up clinical examinations were performed by one calibrated examiner. Data were compared between both arms using Z test for two independent populations and differences considered significant if  $p < 0.05$ . At 12-month, root caries incidence was 40% and 3% in the 1,450 ppm and 5,000 ppm F-toothpaste group, respectively, with a relative risk (RR) of 0.064 (CI: 0.05–0.08). The percentage of root caries activity in the control group was 61.7% at baseline and 53.3% at the 12-month follow-up period. In the high-fluoride toothpaste treatment with 5,000 ppm F, the 68.7% of active lesions at baseline decreased to 3.8% after one year. **Conclusion:** Self-administered noninvasive therapy with high-fluoride dentifrices appears to be highly effective in arresting active root carious lesions and reducing the onset of new lesions in community-dwelling elders. ClinicalTrials.gov NCT02647203.

Funded by the Interdisciplinary Excellence Research Program on Healthy Aging (PIEI-ES), University of Talca, Talca, Chile.

### One-Year Outcomes of Dental Caries Management Using Different Approaches in Primary Molars of Lithuanian Children

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This study is part of an ongoing multi-centre trial testing different approaches of caries management in primary molars. **Aim:** to compare clinical efficacy of three caries treatment methods for occluso-proximal cavities in primary molars (caries removal following conventional restoration, CR; sealing in caries with stainless steel crowns as Hall Technique, HT; opening up a cavity, applying fluoride and training oral self-care, NRCT) after one year in a group of Lithuanian children. **Methods:** 122 3–8-yr-olds (mean 5.69, SD 1.23) with one occluso-proximal cavity each were treated following random allocation to treatment groups (CR = 52; HT = 35; NRCT = 35). Treatment was carried out in a university paediatric clinic by post-graduate trainees. Mean baseline dmfs of all children was 13.57 (SD 9.28) with no statistical differences among groups. Outcome measures were success rates, and clinical failure rates defined as minor (restoration loss/need for replacement, reversible pulpitis, caries progression) and major failures (irreversible pulpitis, abscess). Chi-square and Kruskal–Wallis tests served for statistical analysis. **Results:** 120 children with a minimum follow-up of 11 months (mean 12, SD 0.54) were examined. Success rates (no major and/or minor failures) were 73%, 94%, and 47% in CR, HT and NRCT groups, respectively ( $p < 0.05$ ). Minor failures were recorded in 20 teeth: 12 (35%) in NRCT, 8 (16%) in CR, and 0 in HT. More minor failures occurred in NRCT than in CR and HT, and in CR than in HT group ( $p < 0.05$ ). Major failures were recorded in 16 teeth: 6 (18%) in NRCT, 6 (12%) in CR, and 2 (6%) in HT group, with no significant differences between groups ( $p > 0.05$ ). **Conclusion:** Hall technique appeared to be the most successful treatment modality in these children after one year of follow up.

### Influence of Somatotropin Therapy on Dental Condition and Serum Vitamin D Level in Short Stature Children

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The objective of this clinical study was to investigate the connection between growth hormone therapies in short stature children and vitamin D serum level related to teeth conditions. The experimental approach: The examined group comprised 110 children (27 girls; 83 boys) who were hospitalized due to growth hormone deficiency at the University Children Hospital in Lublin, Poland. In this group there were 47 children who just started treatment with somatotropin and 63 children who started treatment 2–3 years ago. The control group consisted of 41 healthy children. To assess primary and permanent teeth condition of children the DMFT (dmft) index was used. Vitamin D in the examined groups has been marked in the peripheral blood. The obtained results were compared between the groups by used of statistical tests (Mann-Whitney test, t-Student test and Pearson's correlation). The main results: Vitamin D level was 25.67 ng/ml in the group of children with GHD and 16.75 ng/ml in the control group ( $p < 0.0001$ ). The mean DMFT (dmft) index was 5.0 (3.37) for short stature patients and respectively 4.31 (3.39) for healthy children. Statistical analysis revealed that both in the control group and in the group of short stature patients, there was no significant correlation between the DMFT (dmft) index and the level of serum vitamin D. Also there was no significance in mean blood level of vitamin D depending on phase of treatment with somatotropin. The conclusions: The level of vitamin D in the blood of examined children with growth hormone deficiency and healthy does not correlate with their teeth condition. A few years somatotropin therapy does not influence on serum vitamin D level but short stature children have its level higher comparing to healthy one.

### Caries Preventive Effect of One-Year Use of a Chewing-Gum Containing Magnolia in High-Caries-Risk Adults

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This Randomised Clinical Trial investigates the caries preventive effect of a chewing-gum containing magnolia bark extract administered for one-year in a high-caries-risk sample compared to a polyols chewing-gum. 182 high-caries-risk adults ( $\geq 1$  cavitated lesion and salivary concentration of mutans streptococci (MS)  $\geq 10^5$  CFU/mL, age range 30–45 years) were randomly assigned to two groups. Polyols group ( $n = 90$ ) received for one-year gums containing 28% isomalt, 31% sorbitol, 9% mannitol and 1% maltitol syrup, while magnolia group ( $n = 92$ ) received gums containing 26% sorbitol, 11% mannitol, 30% xylitol and 0.17% of magnolia bark extract (magnolol 0.10% and honokiol 0.07%). Caries status using the International Caries Detection and Assessment System, salivary MS using the dip-slide technique, and plaque-pH using the pH indicator strips, were evaluated at baseline, at the end of the administration (one-year) and one-year after the chewing gum cessation in 63 magnolia and 64 polyols subjects. All participants reported to use F-toothpastes (1000/1450 ppm) on a regular basis. The net caries increment for initial, moderate and extensive lesions and for the caries experience was evaluated using the nonparametric Mann-Whitney-U-test. The total caries experience increment was  $1.09 \pm 1.21$  in the magnolia group and  $1.80 \pm 2.33$  in the polyols group ( $p = 0.01$ ). Subjects treated with magnolia had a reduction of Risk Rate at tooth level of 39% respect to those treated with polyols, with a number needed to treat of 31 teeth. The area under the curve at pH 5.7 and 6.2 and MS concentration were statistically significant lower ( $p < 0.01$  for both) in the magnolia group.

Subjects using chewing-gums containing magnolia bark extract showed a significant lower caries experience increment, lower cariogenic bacteria and plaque acidogenicity compared to subjects using polyols chewing-gums.

The study was supported by Perfetti vanMelle Italia srl, Lainate (Milan), Italy.

**Three-Dimensional Visualization App on Smart Phone May Improve Tooth Brushing in Small Children**

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Performing proper toothbrushing is a complicated process for children to become competent. Competence possibly could be improved with simple technical monitoring and motivating system. The aim was to investigate the effect of a smart phone app for improving manual toothbrushing via a gravitation sensor. **Method:** In this prospective, controlled, single-blinded, randomized clinical trial, 49 children (mean age  $5.1 \pm 0.6$  years) were randomly as-

signed to the test ( $n = 26$ ) and control group ( $n = 23$ ). All children were provided with manual toothbrushes (Rainbow Vigilant™) and oral health instructions. Only the children of the test group received a smart phone app with a gravitation sensor to visualize and reward good brushing. At baseline and recalls after 6 and 12 weeks, plaque and gingival indices (QHI, PBI) were recorded and transferred to SPSS 14.0. Friedman-Test was used for intra-group analysis and Mann-Whitney-U-Tests for analysis between the two groups.

**Results:** At baseline, there were no significant differences between test and control group regarding plaque and gingival indices (QHI:  $2.36 \pm 0.7$  and  $2.42 \pm 0.8$  resp.,  $p = 0.94$ ; PBI:  $0.42 \pm 0.2$  and  $0.47 \pm 0.3$  resp.,  $p = 0.59$ ). At the 6- and 12-week recalls, the test group showed statistically significant better oral health indices than the controls. 6-weeks: QHI,  $0.8 \pm 0.5$  and  $1.88 \pm 0.9$  resp.,  $p < 0.001$ ; PBI,  $0.08 \pm 0.1$  and  $0.26 \pm 0.2$  resp.,  $p < 0.001$ . 12-weeks: QHI  $0.44 \pm 0.5$  and  $1.49 \pm 0.7$  resp.,  $p < 0.001$ ; PBI  $0.05 \pm 0.18$  and  $0.21 \pm 0.1$  resp.,  $p < 0.001$ . In conclusion, tooth brushing supported by a smart phone app may present a successful approach for improved toothbrushing in children.

Approved by the Ethic Committee/University of Greifswald, informed consent from the parents of the children was obtained.

## Session 4 Clinical Studies–2

### 76 Withdrawn

### 77

#### Erosive Tooth Wear in Brazilian Adolescents Enrolled in an Oral Health Care Treatment Program

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The present study assessed the occurrence and severity of erosive tooth wear (ETW) in 618 10–15 years old adolescents enrolled in an oral health care treatment program in the region of Itapoã, Federal District of Brazil. In the framework of this school based program, two mobile dental offices were available for examination and treatment of the adolescents. A questionnaire about dietary, self-performed oral health care and other life style habits was answered by the participants. Parents answered another questionnaire about socio-economic data. Clinical and radiographic examinations were performed including registration of dental caries, fluorosis and erosive tooth wear. The Basic Erosive Wear Examination Index (BEWE) was employed to assess the occlusal surfaces of permanent first molars and the palatal and buccal surfaces of maxillary anterior teeth. Erosive tooth wear was observed in 17.3% of the adolescents, the majority (16.5%) presenting initial loss of surface texture (score 1). Distinct erosive wear (score 2) was observed in five participants only. There were significant differences in erosive tooth wear between age groups (10–12 year olds <13–15 year olds;  $p = 0.002$ ,  $X^2$  test), but not between genders ( $p = 0.288$ ). More than half of adolescents presenting erosive tooth wear reported consumption of fruits or industrialized juices more than once per day and 37.4% also reported consumption of acid carbonated beverages at least once daily. Adolescents who reported a consumption of acid carbonated beverages more than once weekly presented significantly higher erosive tooth wear than those who

reported occasional consumption ( $p = 0.030$ ). An effective management of ETW is required for the population studied, both for preventing the development of new erosive lesions and controlling the progression of existing active erosive lesions.

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### 78

#### In the Quest for Understanding the Genetic Role in Dental Caries

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We have previously shown that AQP5 genetic variation and expression in whole saliva are associated with caries experience suggesting that this gene may have a functional role in protecting against caries. To further explore these results, we tested if variants in and flanking this gene are associated with subclinical dental enamel mineral loss. DNA and enamel samples were obtained from 53 individuals. Enamel samples were analyzed for Knoop hardness indentation length of sound enamel, integrated mineral loss after subclinical carious lesion creation, and change in integrated mineral loss after remineralization. DNA samples were genotyped for single nucleotide polymorphisms using TaqMan chemistry. Chi-square and Fisher's exact tests were used to compare individuals above and below the exact mean sound enamel micro-

hardness of the cohort with alpha of 0.05. Furthermore, 4,983 subjects from our Dental Registry and DNA Repository project were studied. Of these patients, 100 of them reported having kidney disease. Codes used for insurance purposes were evaluated to determine which patients were treated with composite resins in their anterior teeth. The G allele of AQP5 marker rs3759129 and A allele of AQP5 marker rs296763 were associated with enamel more resistant to demineralization ( $p = 0.03$  and  $p = 0.05$ , respectively). Also, a total of 2,115 individuals had composite resins in anterior maxillary teeth (43%), in contrast with 52 individuals among those being in the subgroup of patients with kidney disease ( $p = 0.05$ ). AQP5 genetic variations influence the initial subclinical stages of caries lesion formation in the subsurface of enamel. Patients who have kidney disease will more often need anterior composite resin restorations that are possibly surrogates of enamel aesthetic concerns (i.e. enamel hypoplasia) or higher caries experience and they may be a good human model to study individual susceptibility to caries.

The Dental Registry and DNA Repository project is funded by the University of Pittsburgh School of Dental Medicine.

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79

### Evaluation of Brazilian Red Propolis Toothpaste against Salivary *Streptococcus mutans* in Orthodontic Patients

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The objective of this pilot study was to compare the efficacy of two toothpaste (1500 ppm), the first one containing 1% Brazilian Red Propolis (BRP) extract and the second one a common toothpaste against salivary *Streptococcus mutans* (SM) in orthodontic patients. This was a randomized and Double-Blind study. Forty participants, caries-free, aged 12–18 years, from both genders were selected and randomized into two groups. The first group received BRP toothpaste and the second group received fluoridated common toothpaste. The participants brushed their teeth for 1 min thrice a day for 30 days. Saliva was then collected before starting treatment (D0) and 30 days after day 1 (D30) to verify the effectiveness of BRP toothpaste treatment in the reduction of SM. The microbiological analysis was repeated twice, establishing dilutions of 1:100 mL and 1:1000 mL on Mitis salivarius-bacitracin agar (MSB). SM isolates were identified by its characteristic colony morphology and the values were expressed as  $\log_{10}$  (CFU/mL). Comparisons between different times within the same dilution were carried out by repeated measures analysis of variance associated with Tukey's multiple comparisons test. The paired t test was used to compare the two dilutions at each evaluation time. The proportion

of patients who showed a confirmed SM reduction in the BRP group ( $0.10 \pm 0.19$ ) was significantly higher than that in the Control group on D30 ( $0.40 \pm 0.28$ ). There were no reported side effects. In conclusion these data indicate that the BRP toothpaste showed antimicrobial activity against SM for up to 30 days in orthodontic patients.

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80

### Impact of the "2x2x2 Social Marketing Strategy" on Children's Oral Health Indicators and Knowledge

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Social marketing is the application of commercial marketing principles to the design, implementation and evaluation of programs in order to improve people's behaviours and their personal welfare. This study aimed to assess differences in the Gingival Index (GI), Simplified Oral Hygiene Index (OHI-S) and knowledge about tooth brushing, among children who received education under a traditional model and those who experienced the "2x2x2 Social Marketing Strategy". The "2x2x2 Social Marketing Strategy" refers to children brushing their teeth twice a day for two minutes using 2 centimetres of fluoridated toothpaste. Children aged 6–8 years old attending schools in Municipality of Santiago, Chile, participated in the study. The sample consisted of 264 children of 1<sup>st</sup>-2<sup>nd</sup> grades of Miguel de Cervantes School. They were divided into an Intervention group ( $n = 134$ ) who experienced "2x2x2 Social Marketing Strategy" and a Control group ( $n = 130$ ), who received education in oral health under the traditional care model. Clinical examinations were performed by two calibrated examiners at baseline and 6 weeks after intervention, GI and OHI-S were determined. A questionnaire was applied to the children to evaluate knowledge of the duration, frequency and amount of dentifrice to be used during tooth brushing. Means were calculated for oral health variables (GI and OHI-S). To assess for differences between groups, t student test was performed for  $\Delta$ GI and  $\Delta$  OHI-S (at the  $p < 0,05$  level). Variables related to knowledge were measured as proportions (duration, frequency and amount of dentifrice). The differences among groups were tested by Chi<sup>2</sup> test (at the  $p < 0,05$  level) for " $\Delta$ Correct Responses". The Intervention group significantly increased their knowledge ( $\Delta$ Correct Responses) about the amount of dentifrice to be used by 50% while the Control group did so by 28% ( $p < 0,05$ ). The difference between final and initial OHI-S in the Intervention group ( $\Delta$  OHI-S) was 0,28 whereas in the Control group was 0,07 ( $p < 0,05$ ). The difference between the final and initial GI in the Intervention group ( $\Delta$ GI) was 0,17, whereas in the Control group was 0,19 ( $p = 0,56$ ). In conclusion, the "2x2x2 Social Marketing Strategy" significantly increased children's knowledge regarding the amount of toothpaste to be used during tooth brushing. It also, significantly reduced dental plaque. Both the "2x2x2 Social Marketing Strategy" and traditional education decreased gingival inflammation.

## Egg Ovalbumin Modulates Dental Biofilm and Salivary pH After Sucrose Exposure, *In Vivo*

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We have previously reported that egg ovalbumin has a promising anticaries potential, but an *in vivo* effect has not been shown. The aim of this study was to determine if ovalbumin has a modulating effect on the sucrose-induced acidogenicity of the dental biofilm. 12 healthy volunteers participated in this double-blinded clinical trial of 4 sequential phases with a wash-out period. Volunteers refrained from oral hygiene from 48 h and fasted 8 h before the experiments and were randomly-assigned to one of the 4 study groups. For all groups, subjects rinsed with a 10% sucrose solution for 1 min followed by the assigned treatment for 1 additional min: distilled water (caries-control) (Group 1), 0.2% ovalbumin solution (Group 2), 2% ovalbumin solution (Group 3) or a 0.5% NaF solution, (anticaries control) (Group 4). Biofilm pH was monitored at baseline and 2, 5, 10, 15, 20 and 30 min after the last rinse with pH-sensitive strips located in the proximal area of upper molars. Salivary pH was measured at each time interval using a pH-meter and the area under the curve (AUC) was calculated. A two-way ANOVA test was used to compare data from the study groups followed by Mann-Whitney U test. Differences were considered significant if  $p < 0.05$ . When compared with sucrose followed by distilled water, ovalbumin was effective to reduce pH drop, in a dose-dependent manner, at an even higher level than NaF. For both saliva and the biofilm, ovalbumin showed significantly higher AUC than the caries-control at 2, 5, 10 and 15 min after rinsing ( $p < 0.0001$ ), which means about 35%, 45%, 50% and 60% reduction in acidogenicity, respectively. At 20 and 30 min, pH returned to basal levels, without differences among the groups. In conclusion, rinsing with ovalbumin seems to inhibit sucrose-mediated acidogenicity from the dental biofilm. Ovalbumin could be a novel anticaries agent.

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## The Effects of Oral Health Education Using Qraycam™

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The purpose of this study is to evaluate the effects of oral health education using Qraycam™ and to prevent dental caries of middle school students through visual feedback. 118 middle school students were divided as follows into two groups; control group (only oral health education) and intervention group (oral health education with feedback using Qraycam™ image). Both groups were educated on oral health and had their dental plaque scores identified by using Qraycam™. The Qraycam™ image was sent the intervention group only by text message. After four weeks, both the intervention group and the control group re-evaluated their plaque scores and then filled out the questionnaire. The results between the groups were analyzed by repeated measures ANOVA and independent-sample t-test using the SPSS 18.0 statistical package program (IBM Co., Armonk, NY, USA). This study was evaluated the effects of oral health education by  $\Delta R$  value and Simple Plaque Score (SPS).  $\Delta R$  value is percentage of the ratio of the red and the green component with respect to that ratio of sound tissue. The high  $\Delta R$  value means high risk. SPS is a numerical value that can be used to evaluate the amount and quality of dental plaque by using Qraycam™ analysis program, ranging from 0 to 5 summarizes this: 0 = very good oral hygiene, 5 = extremely high risk. Both the  $\Delta R$  value and SPS value were statistically significantly reduced in the intervention group ( $p < 0.001$ ). The amount of change, before and after the intervention, was also higher in the intervention group compared to the control group, the value of  $\Delta R$  was statistically significant ( $p < 0.05$ ). Tooth brushing time and the importance of oral health were significantly increased in the intervention group compared to the control group ( $p < 0.001$ ). The use of the Qraycam™ image as a visual feedback tool in oral health education is a positive method for improving oral health.

### Clinical Efficacy of Chlorhexidine Mouthrinse versus Chlorhexidine Gel on Plaque, Gingival Inflammation: A Randomized Controlled Trial

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The aim of this study was to compare the effect of chlorhexidine solution and gel on dental plaque inhibition and gingivitis relief by randomized clinical trial. All subjects were randomly divided into two groups: experimental group (1% chlorhexidine gel) and control group (0.1% chlorhexidine solution). Before the study, all subjects were given consent and were scaling to control the oral environment as similar as possible. Clinical results were evaluated by the BANA test and dental plaque index (PI), sulcus bleeding index (SBI), gingival index (GI). They were given chlorhexidine products suitable for each group and were instructed to use each product in the morning and evening for 1 week. All subjects were to re-evaluated 4 weeks and 8 weeks after using chlorhexidine products. The results were analyzed using repeated measured ANOVA and t-test using the SPSS 18.0 statistical package program (SPSS Inc. U.S.A).

**Results:** PI, SBI and GI were significantly significant differences according to treatment duration in all groups ( $p < 0.001$ ). The GI was statistically significantly decreased in chlorhexidine gel group compare to chlorhexidine solution group at 4 weeks and 8 weeks. In both groups, the BANA score was significantly decreased ( $p < 0.001$ ), and after 8 weeks, the BANA score was significantly lower the chlorhexidine gel group ( $0.44 \pm 0.61$ ) than the chlorhexidine solution ( $1.05 \pm 0.51$ ).

**Conclusion:** 1% Chlorhexidine gel is more effective in reducing gingival inflammation than 0.1% chlorhexidine solution and it is effective in reducing microbial bacteria in the mouth.

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

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This split-mouth randomized controlled clinical trial had the main purpose to evaluate the efficacy of caries infiltration (CI) in controlling the progression of non-cavitated proximal lesions in primary molars. Fifty healthy children (5 to 9 years, mean age 6.2 yr), presenting with at least two primary molars with proximal lesions on radiographs (radiolucency reaching the inner half of the enamel -E2 or outer third of dentin -D1) were included in the study. Lesions were randomly allocated to one of the groups: Test (resin infiltration with Icon<sup>®</sup>, DMG, Hamburg, Germany) or Control (brushing + flossing). Caries risk assessment was based on the Cariogram model. The main outcome after 1-year was assessed blindly by pair-wise reading of the standardized bitewing x-rays. After 1-year follow-up, 42/50 patients were reassessed (dropout rate = 16%). The majority of the sample (92.9%) corresponded to high or medium caries risk. At baseline, test group comprised 29 E2 and 13 D1 lesions and Control group, 35 E2 and 7 D1 lesions. The proportion with caries progression was 11.9% (5/42) in the Test group compared to 33.3% (14/42) in the Control group (McNemar Test:  $p = 0.012$ ). Progression to cavitation was observed in one Control lesion. Caries progression to D2, was observed in 3/42 (7.1%) test lesions and 8/42 (19%) control lesions were indicated for restoration. No unwanted side effects were observed. The results indicate that caries infiltration is an applicable method to be used in children and efficacious in controlling proximal caries lesions in primary molars, since test lesions progressed significantly less in the control group.

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### Dentin-Pulp Complex Response after Selective Caries Removal in Permanent Teeth: A Randomized Clinical Trial

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The aim of this randomized clinical trial was to analyze the dentin-pulp complex response in permanent molars after the selective caries removal (SCR) compared to the total caries removal (TCR) during the 6-month period. Methodology: Sixteen permanent molars (12 subjects) with active caries lesions in the inner half of dentin and vital pulp were randomly divided into two groups: 1) TCR group (n = 5), in which the decayed tissue was completely removed with the aid of dye, and 2) SCR group (n = 11), in which carious dentin was completely removed from the dentino-enamel junction and lateral walls, keeping the affected dentin in pulpar wall. The main outcome was percentage of the level of oxygen saturation (%SaO<sub>2</sub>) of pulpal blood flow assessed by a pulse oximeter adapted for human dental anatomy (before and immediately after restoration of the cavity, 7 days, at 1 and 6 month posttreatment). Secondary outcomes were intraoperative pulp exposure and pulp necrosis. **Results:** There was no difference in %SaO<sub>2</sub> values in the comparison into the group or between groups, before and 6 months posttreatment: SCR (90.28 ± 3.81; 90.00 ± 2.38) and TCR (87.00 ± 1.83; 91.66 ± 0.57) (p > 0.05). Pulp exposure occurred in 1 tooth of SCR group and necrosis cases were not registered in any group. **Conclusion:** The dentin-pulp complex response assessed by %SaO<sub>2</sub> did not differ between SCR and TCR after 6 months follow-up.

This study was supported by Foundation for the Support and Research to Scientific and Technological Development of Maranhao (FAPEMA).

### ART versus Hall Technique in Primary Molars: 1-Year Survival and Cost Analysis of a RCT

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The aim of this study was to evaluate the survival rate and cost-analysis of ART versus HT in occluso-proximal carious lesions in primary molar. After securing approval from local Ethical Board (#1.293.935) and registration in ClinicalTrials.gov (NCT02569047),

children aged 5–10 yo (n = 131) presenting at least one occluso-proximal cavitated caries lesion in a primary molar were selected from schools of Tietê, Brazil. One tooth per child, fitting inclusion criteria, was randomly allocated to receive an ART restoration using high viscosity glass ionomer (Equia Forte, GC Corp.) or a stainless steel crown placed using the HT. Restorations were evaluated after 1, 6 and 12 months. Primary outcome is the treatment survival. The time spent in each treatment, direct (material and professional) and indirect costs (procedure and equipment depreciation) were recorded and calculated. Incremental cost assessments for failures were not performed. Kaplan-Meier survival analysis and log rank tests were carried out. Cox regression test investigated associations between survival and other variables while Poisson regression analysis was used for testing association with total cost of each treatment (α = 5%). The overall restorations survival rate was 78.6% (HT = 98.5% and ART = 58.5%) after 12 months. There was a statistically significant difference between interventions' survival rates (HR = 29.6; p = 0.001, CI = 4.01 to 218.9). The difference between treatments in relation to the total cost per restoration performed was statistically significant, being higher for HT (p < 0.001). However, the mean cost of an ART restoration was 10.53€ (SD = 2.7) and for HT 12.43€ (SD = 2.38). Occluso-proximal cavities restored using crowns placed with the Hall Technique reveal better survival rates after 1 year. Hall Technique restorations show higher cost when compared to ART, with a difference lower than 2€. Costs of treatment failure were not evaluated.

This study was supported by FAPESP (#2015/18098-2).

### Photobiomodulation of Salivary Glands with Polychromatic Polarised Visible Light in High Caries Risk Patients

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The aim of the present pilot study was to assess how photobiomodulation therapy of the salivary glands with polychromatic polarised visible light affects caries risk factors.

15 patients with high caries risk, according to Cariogram were randomly assigned into two groups. Experimental group (N = 9) was treated with polychromatic polarised visible light (Bioptron AG, Switzerland, wavelengths 480–3400 nm, average power density 40 mW/cm<sup>2</sup>) and control group (N = 6) with non-therapeutic visible light. The light was applied extraorally bilaterally on the parotid and submandibular glands and intraorally on the sublingual glands for 25 min, 3 times a week, for 4 weeks. Caries was assessed according to ICDAS criteria at the start and the end of therapy. Salivary risk factors were determined at the start, after two weeks, and at the end of therapy. There were no statistically significant differences between experimental and control group in age, gender, dental status and caries, stimulated and unstimulated salivary flow

rate, salivary pH and buffering capacity and *Streptococcus mutans* (SM) and *Lactobacillus* (LB) CFU/ml counts (t-test). During the therapy, buffering capacity in the experimental group increased ( $1.9 \pm 0.6$  vs.  $2.7 \pm 0.5$  vs.  $2.6 \pm 0.5$ ). At the end of therapy, bacterial counts and caries risk were lower than at the start of therapy (SM  $3.7 \pm 0.5$  vs.  $2.0 \pm 1.1$ ; LB  $3.8 \pm 0.4$  vs.  $1.9 \pm 0.8$ ; caries risk  $3.7 \pm 0.5$  vs.  $2.3 \pm 1.2$ ) (one-way repeated measures ANOVA or paired t-test,  $p < 0.05$ ). In the control group all the parameters assessed remained unchanged during the therapy (one-way repeated measures ANOVA or paired t-test). Photobiomodulation of the salivary glands with polychromatic polarised visible light might positively affect salivary risk factors in high caries risk patients.

88

### Photobiomodulation of Salivary Glands with Light-Emitting Diode Light in High Caries Risk Patients

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The aim of this pilot study was to elucidate how photobiomodulation therapy of the salivary glands with light-emitting diode (LED) light affects caries risk factors.

18 patients with high caries risk, according to Cariogram were randomly assigned into three groups. Experimental group A (N = 6) was treated with continuous LED light (Ortholumm ML 5/1, Votan d.o.o., Slovenia, wavelengths 625, 660 and 850 nm, average power density  $16 \text{ mW/cm}^2$ ), experimental group B (N = 6) with the same LED light in pulsed mode, and control group (N = 6) with non-therapeutic visible light. Light was administered extraorally bilaterally on the parotid and submandibular glands and intraorally on the sublingual glands for 25 min, 3 times a week, for 4 weeks. Caries was assessed according to ICDAS criteria at the start and the end of therapy. Salivary risk factors were determined at the start, after two weeks, and at the end of therapy. There were no statistically significant differences between experimental and control group in age, gender, dental status and caries, stimulated and unstimulated salivary flow rate, pH and buffering capacity and *Streptococcus mutans* (SM) and *Lactobacillus* (LB) CFU/ml counts (t-test). During the therapy, bacterial counts decreased in group A (SM  $3.0 \pm 0.9$  vs.  $1.8 \pm 1.5$  vs.  $1.2 \pm 1.2$ ; LB  $3.0 \pm 0.9$  vs.  $1.0 \pm 0.6$  vs.  $1.5 \pm 0.5$ ) and unstimulated salivary flow rate increased in group B ( $2.3 \pm 1.0$  vs.  $4.3 \pm 2.3$  vs.  $3.8 \pm 2.6$ ) (one-way repeated measures ANOVA, Dunnett's test,  $p < 0.05$ ). Caries risk decreased in both experimental groups (A  $3.7 \pm 0.5$  vs.  $1.8 \pm 0.4$ ; B  $3.5 \pm 0.8$  vs.  $2.0 \pm 1.1$ ) (paired t-test,  $p < 0.05$ ). Assessed parameters did not change in the control group (one-way repeated measures ANOVA or paired t-test).

Photobiomodulation therapy of the salivary glands with LED light might positively affect caries risk factors in high caries risk patients.

89

### Early Childhood Caries Association to Caries in Permanent Teeth

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Early childhood caries (ECC) is the presence of one or more caries lesions in children <6 years old. ECC may place children at a higher risk of developing caries in permanent teeth (PT). The aim was to assess if ECC is associated with caries in PT in children who participate in a school-based sealant program (SBSP). As part of the SBSP, children receive a dental exam to determine presence of caries using the ICDAS criteria. The program returns to sites to provide follow-up care once a year. Electronic records from 7/01/2005 to 5/31/2015 were searched. Children <6 years old at initial exam, examined >4 times, and >7 years old at last follow-up were included. Data were analyzed using Pearson Chi-Square test. **Results:** 95 records qualified and were evaluated for ECC at initial visit and presence of new caries lesions in PT and primary teeth (pt) at follow-up visits. Children were followed for an average of 4.4 years (min 3 – max 8); 51 were males; mean age at initial visit was 5.3 years old (min 1); and 9.7 years old (max 12) at the last follow-up visit. 27 (28%) children had ECC at initial visit. 7 (26%) of these developed new caries lesions in both pt and PT, 1 (4%) only in PT, and 13 (48%) only in pt. At the end of study period, 22 (23%) children not initially diagnosed with ECC had new caries only in pt, 7 (7%) only in PT, and 7 (7%) in both PT and pt at follow-up. Pearson Chi-Square test did not show a significant association between ECC and subsequent caries in PT ( $p > 0.5$ ). **Conclusion:** Presence of ECC may not be associated with PT caries in this group of children.

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90

### Root Caries Management Adjacent to Restorations of Head-and-Neck Patients After Radiotherapy: 6-Month Follow-Up

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Radiation-therapy associated with reduced salivary flow is particularly determinant for high-risk root caries, as they cause composition and morphological changes in dentin. This randomized

clinical controlled study aimed to evaluate the restorative performance of a resin-modified glass-ionomer-cement (RMGIC) in irradiated patient and the prevention of new caries lesions adjacent to restoration, comparing the effect of high-fluoride (F), arginine-based and conventional concentration toothpastes. Patients presenting post-radiation root caries (n = 38) were recruited and randomized distributed into three treatments groups: CP- Clinpro® (5,000 µg F/g + tricalcium-phosphate) (n = 10 per treatment, n = 1 per subject), N- Colgate Neutraçucar® (1,450 µg F/g + 1,5% arginine + CaCO<sub>3</sub>) (n = 15 per treatment, n = 1 or 2 per subject) compared to CT- Colgate Total 12® (1,450 µg F/g- control group) (n = 13 per treatment, n = 1 or 2 per subject). Based on eligibility criteria, all patients were enrolled after completed the radiotherapy. Two calibrated operators performed the restorative procedures (RMGIC – Vitremer). Salivary flow was determined for patients, who received the respective toothpastes, an extra-soft toothbrush (CS 5460B, Curaprox) and artificial saliva (1% carboxymethylcellulose and distilled water). Two calibrated examiners (Kappa = 0.94) evaluated the restorations based on modified USPHS criteria at baseline and 1, 3 and 6-month follow-up. The biofilm index was also verified. Data was collected and statistically assessed with Kruskal-Wallis test ( $p < 0.05$ ). There were no statistically significance differences among the performance of the restoration among the three groups regarding the criteria retention, marginal adaptation, marginal staining, post-operative sensitivity, adjacent caries, color alteration, anatomic form and surface texture ( $p > 0.05$ ). **Conclusions:** Even with oral complications caused by radiation therapy, if the restorations are properly performed and patients are under professional control and supervised, conventional concentration toothpaste can be as effectiveness as high-F and arginine-based toothpastes to prevent secondary caries.

91

### Quantification of Fungiform Papillae in Children and Its Association with Dental Caries

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In this study, we aimed to quantify Fungiform Papillae of the tongue in children with primary and mixed dentitions and to correlate FP count to caries experience of these children. Ninety-four healthy children (45 girls-49 boys; aged 5–10 years) who applied to Marmara University Pediatric Dentistry Department participated in our study. The ethical approval was obtained from MU Dental Faculty Clinical Studies Ethics Committee. DMFT/dft index, brushing habits, ethnicity, socioeconomic status, taste preferences were evaluated. The participants were categorized into three groups as low (0–5 caries), moderate (6–9 caries) and high ( $\geq 10$  caries). Then Brilliant Blue FCF (E133) was applied to the tongue. It was adhered filter paper with a 10-mm diameter circular cutout punched in it to the tip of the anterior of the left side of the tongue next to midline. It was taken close-up images of the tongue. Each photo was scored and compared by two calibrated researchers according to Denver Papillae Protocol using software ImageJ 1.46r 32bit. The data collected was analyzed statistically using chi-square test. Low, moder-

ate and high caries groups were 28 %, 35%, 37% respectively; low and high FP count groups were 46% and 54% respectively. Girls' FP counts were higher than boys ( $p = 0.006$ ) and children with primary dentition had higher FP count than children with mixed dentition ( $p = 0,005$ ). Mean FP count for low, moderate and high caries groups were  $28,69 \pm 11,21$ ;  $36,54 \pm 17,86$ ;  $36,01 \pm 12,06$  respectively. Mean DMFT/dft values of the low and high FP count groups were  $6,51 \pm 4,22$  and  $9,65 \pm 4,79$  respectively and the difference between FP count and caries experience groups were statistically significant ( $p = 0,049$ ). In this study we found a relationship between FP count and caries experience groups which may demonstrate an evidence for the connection between taste buds and dental caries experience.

92

### Determination of First Permanent Molar Extraction Frequency and Its Reasons from Two Dental Faculties in Turkey

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The aim was to determine the first permanent molar [FMP] extraction frequency and its reasons by the findings of state Universities in two different regions in Turkey. **Methods:** The study was carried out by using 4868 panoramic radiograph from healthy children aged between 7–12 years (mean age  $9.81 \pm 1.43$ ). 2948 radiographs from Marmara University (MU, İstanbul) and 1927 radiographs from Adnan Menderes University (AM, Aydın) Faculty of Dentistry were obtained. The study was approved by the Ethics Committee (22.02.2016–26). The patients' information was recorded by the same dentist (DY). The reasons for extraction and treatment status before extractions were recorded on an evaluation form. Data obtained during the survey were stored in a database (SPSS) and tested for possible statistical differences using the chi-square test, fisher's exact chi-square test and continuity (Yates) correction.  $p$  value  $< 0.05$  was considered to be statistically significant. **Results:** The total number of children who required extraction was 471 (332 from MU and 139 from AM University), comprising of 245 females and 226 males. Caries with poor prognosis was the main reason for extraction (52.6%), followed by apical lesion (31.7%). About 95% of cases did not have any previous treatment of the FPMs. There were no significant differences in extraction frequency and in the number of FPMs extracted between MU and AM. There was no significant difference between two Universities' suggested extraction reasons for tooth 16, tooth 26, tooth 46; except a significant difference was found for tooth 36 ( $p:0.009$ ;  $p < 0.05$ ). The extraction rate for tooth 36 (6.7%) in MU was found to be lower than in AM (12.8%) due to MIH (molar-incisor hypomineralisation); whereas the extraction rate for tooth 36 (53.4%) in MU was found to be higher than in AM (36%) due to apical lesion. **Conclusion:** Due to the early eruption and the importance of the occlusion, FPMs need an extensive prevention program in children with high caries risk.

### Association of Fungiform Papillae Count with Hypodontia and Caries Experience

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Animal studies suggest that teeth and taste buds are co-patterned from common epithelium [Bloomquist et al: PNAS 2015; 112:E5954–62] In this study we investigated fungiform papillae (FP) count of the tongue in children with or without hypodontia and whether or not it had any association with the caries experience of these children.

A total of 134 children (71 girls-63 boys; 40 hypodontia, 94 normal; aged 5–12 years) who applied to Marmara University Pediatric Dentistry Department participated in our study. The ethical approval was obtained from MU Dental Faculty Clinical Studies Ethics Committee. DMFT/dft index, brushing habits, ethnicity, socioeconomic status, taste preferences were evaluated. The participants were categorized into three groups as low (0–5 caries), moderate (6–9 caries) and high ( $\geq 10$  caries). Then Brilliant Blue FCF (E133) was applied to the tongue. Photos were taken close-up images of the tongue. Each photo was scored and compared by two calibrated researchers according to Denver Papillae Protocol using software Image J 1.46r 32bit. The data collected was then analyzed statistically using chi-square and Student's t-tests.

The primary molars existed in hypodontia patients and the number of teeth was similar to control group in the mixed dentition stage. Mean numbers of FP count were  $24,64 \pm 10,75$ ,  $34,17 \pm 14,43$  for hypodontia and normal patients respectively and the difference was statistically different ( $p = 0,0021$ ). Mean DMFT/dft values were  $5,35 \pm 4,04$ ,  $6,51 \pm 4,22$  hypodontia and normal patients respectively and the difference was statistically different ( $p = 0,0006$ ). In hypodontia group no statistically significant difference was found between gender or age. In this study we found early evidence for lower FP count in patients with the absence of tooth germs which may reflect as lower level of caries experience.

### Instructing to Floss: Questionnaire and Video Observation Results from a Randomised Intervention Study

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Flossing requires dexterity and is difficult to learn. The aim was to investigate the effects of a video instruction with or without an individualised hands-on training on self-evaluation of flossing

skills and performance of flossing. A convenience sample of students ( $24 \pm 2.9$  years) not involved in medicine/dentistry was included. Group 1 ( $n = 37$ ) watched a video demonstrating correct flossing of the whole dentition followed by an individualised hands-on training, group 2 ( $n = 36$ ) watched the video only. At baseline, after instruction and after re-instruction (7-day intervals) subjects flossed while being video-filmed through a mirror and answered questionnaires. Videos were analysed with the observation software INTERACT. Questionnaires collected information about flossing habits at baseline; on a scale from 0–10 subjects agreed/disagreed that flossing is easy, time-consuming or painful and that they reach all interdental spaces. Fifty-five subjects had already used floss but only 12 had instructions from a dentist. At baseline, video analysis revealed that only 18.8% of the subjects flossed completely whereas 29.0% flossed fewer than half of all interdental spaces. Questionnaires revealed that 68.5% strongly agreed (scores 8–10) that anterior flossing, but only 20.5% that lateral flossing is easy; 37.0% strongly agreed that they reach all interdental spaces. These numbers improved significantly after instruction (video: 26.9 and 6.0% resp.; questionnaire: 83.6, 31.5 and 61.6% resp.) and after re-instruction (video: 30.9 and 2.9% resp.; questionnaire: 90.4, 52.1 and 67.1% resp.). Instructions had only minor effects on scores for pain and time-consumption; hands-on training had no effect over video instruction alone. These results show that a video instruction distinctly improves self-evaluation and performance of flossing. Nevertheless, even after re-instruction and hands-on training, correct flossing was not fully implemented indicating the need for new learning strategies.

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### The Prebiotic Effect of CPP-ACP Sugar-Free Chewing Gum in Healthy Individuals

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The primary anticariogenic effect of CPP-ACP is its ability to provide bioavailable calcium and phosphate to promote remineralisation. Recent clinical studies suggest that regular CPP-ACP use reduces plaque *Streptococcus mutans* levels, a species that is a biomarker of caries risk. The aim of this study was to determine if regular use of CPP-ACP chewing gum had a prebiotic effect promoting the growth of beneficial bacterial species in supragingival plaque. Twenty participants were recruited for a three leg cross-over, randomised, controlled trial. Each leg was two weeks duration and participants either chewed no gum, a sugar-free chewing gum or a CPP-ACP sugar-free chewing gum. Participants abstained from oral hygiene during treatment periods and had wash-out periods of two weeks. After each treatment period supragingival plaque was collected and analysed for bacterial composition by sequencing the V4 variable region of the 16S rRNA gene. Data

were analysed using a linear mixed modelling approach with treatment group as a fixed effect term and participant as a random effect term. The CPP-ACP gum produced a significant ( $p < 0.01$ ) increase in the proportions of *Streptococcus sanguinis* (212%), *Rothia dentocariosa* (227%), *Corynebacterium durum* (180%) and *Streptococcus mitis* (155%) when compared to no gum. Although the proportion of these species increased there was no significant increase in plaque index. All the species promoted by the CPP-ACP gum possessed one or both of arginine deiminase and/or nitrate reductase. These species are known to be associated with health (low caries activity). In conclusion, this clinical study demonstrated that regular chewing of a CPP-ACP gum promoted prebiosis by significantly increasing the proportion of health-associated bacterial species in supragingival plaque.

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96

### Comparison of Oral Hygiene Advices Given by Dentists and Dental Students in Chile and Latvia

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The aim was to compare recommendations about oral hygiene given by Chilean (CL) and Latvian (LV) dentists and dental students to patients and their attitude about relevance of oral hygiene advice. **Methods:** A self-administered, modified and validated questionnaire [Morgan et al: Int J Dent Hyg. 2013;11:121–5; Maldupa et al.: J Oral Res 2014; 4:88–94] was randomly distributed to dentists and students from Valdivia, Chile and Riga, Latvia. Data were analyzed with chi-square, significant level was 5%. **Results:** Completed questionnaires were returned by 387 dentists (117 CL; 270 LV) and 277 dental students (225 CL; 52 LV). Differences ( $p < 0.05$ ) were found for both dentists and students for give hygiene advice to all patients (CL = 85.5%, LV = 47.8%), amount of toothpaste for adults (less than pea-size in CL = 21.3% vs. no-recommendation in LV = 47.4%). Respondents from both countries reported similar values of value of oral hygiene for general health (CL = 89.7%, LV = 99.0%), recommendations can improve oral health (CL = 94%, LV = 97.4%) and that dentists does not give enough recommendations (CL = 83.6%, LV = 61.5%) Both groups thinks that few people from their countries have good oral health (CL = 9.4, LV = 14.7). Respondents from Chile give low priority to other dental staff in the role of give advice to patients (36.6% from CL vs. 87% from LV). **Conclusion:** This study found significant differences in recommendations given by dentists and students from Chile and Latvia. The fact, that Chile has no Dental Hygienists may explain these differences.

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97

### Occlusal ART Restorations Using Three Different Filling Materials: 2-Years Follow Up of a RCT

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The aim was to compare survival rates of three different fillings materials for Atraumatic Restorative Treatment (ART) restorations in occlusal cavities in primary molars placed in school environment. **Material and methods:** Ethical clearance to conduct the study was obtained from the Ethical Committee of the University of São Paulo (# 464.863) and the study is registered at Clinical Trials website (NCT02217098). Two hundred and eighty one (281) children, aged between 4 to 8 years, were randomly allocated in 3 different groups: GIC – restoration using high viscosity glass ionomer (Equia Fil – GC Corp), COM – restorations using Compomer (Dyract – Dentsply) and CAR – restorations using Glass Carbomer Cement (Glass Carbomer – GCP Dental). All treatments were performed following the ART premises and all restorations were evaluated after 2, 6, 12, 18 and 24 months by one calibrated evaluator. Restoration survival was evaluated using Kaplan-Meier survival analysis and Log-rank test, while Cox regression analysis was used for testing association of clinical factors ( $\alpha = 5\%$ ). **Results:** The overall survival rate of occlusal restorations was 75.8% (GIC = 83%; COM = 77%; CAR = 69%). There was difference in survival rate between the materials tested, with a poorer performance of CAR (HR = 1.90; CI = 1.01–3.61;  $p = 0.048$ ). No difference was found between GIC and COM ( $p > 0.05$ ). The main reasons for failure of restorations after 24 months were: rough surface defect over 0.5 mm deep and secondary caries ( $n = 17$ ), the restoration was missing or disappeared (almost) completely ( $n = 38$ ) and pulp inflammation ( $n = 11$ ). **Conclusions:** The new developed material Glass Carbomer cement is less suitable for occlusal ART restorations in primary molars when compared to high viscosity glass ionomer cement and compomer.

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98

### 18 Month Follow Up of the Hall Technique for Managing Carious Primary Molars: A Pilot Study

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The Hall Technique (HT) is one biological approach to managing caries in primary molars Dental caries is sealed under preformed metal crowns (PMCs) without any caries removal, tooth preparation, or local anesthesia. The aims of this study were to evaluate outcomes for carious primary molars managed by PMCs fitted using the Hall Technique. Teeth were randomized to HTs or Control restorations. The 13 PMCs fitted on 8 children, using the

HT, were placed when there was clinical evidence of approximal caries into dentine. PMCs (3M™ ESPE™ Stainless Steel Crowns) is cemented over the tooth without caries removal, tooth preparation or local anaesthesia. Compomer fillings (Voco Glasiosite) were placed in 13 teeth as control restoration. Every 6 months clinical/radiographic follow-up data were recorded. Restorations and HT scored as satisfactory was considered “successful,” while those presented Minor and/or Major Failures will be considered as “failed”. Statistical analysis was performed with SPSS 16.0 for Windows. The comparison of data was made using the chi-square. For all tooth types, only one of the PMC was lost. At 18 months, ‘Major’ failures (irreversible pulpitis) were recorded only one HT (92.3%). There was no minor failures for HT. There was no major failures for control restorations ‘Minor’ failure (restoration wear/fracture; or secondary caries) for control restorations was 6 (46.10%). The success rate of Hall Technique was statistically significant than compomer in 18 months ( $p = 0.034$ ). In conclusion, after 18 months, Hall technique showed more favourable outcomes for pulpal health and restoration longevity than compomer restorations. The technique however requires further evaluation through clinical trials before its use could be generally recommended.

and partial sealant retention. Visual and fluorescence assessments were consistent and feasible. Incomplete sealant retention occurred in 5% of cases at 6 months and 9% of cases at 12 months and was probably due to procedure imperfections. The combined use of transparent sealant and a fluorescence camera shows clinical effectiveness and diagnostic efficacy for occlusal surface monitoring.

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99

### Monitoring Pit and Fissures Using Transparent Sealant and Fluorescence Intraoral Camera, 12 Months Follow Up

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The aim of this *in vivo* study is to report on the combined use of a fluorescence intraoral camera and transparent sealant for the clinical monitoring of pits and fissures. 96 permanent molars with a ICDAS II code 0, 1, or 2, (in 48 patients aged 12–14) were registered at the First Observation Unit (Oral and Maxillofacial Sciences Department), Sapienza University, Rome. Clinically selected teeth were double-checked using a VistaCam iX Proof (Dürr Dental AG) and sealed with a transparent sealant (ControlSeal, VOCO GmbH), following the established indications for use if a pit and fissure condition was confirmed within the camera’s internal cut-off point of 1.5 (“early enamel demineralization”). Clinical follow-up was performed using VistaCam at 6 and 12 months to assess sealant retention and any demineralization trend. At baseline, 57.4% of the registered teeth were sound, both visually and when using the fluorescence camera, 42.6% presented an early demineralization (<1.5 with VistaCam and ICDAS II 1–2). Subsequent VistaCam assessment of surfaces underlying the transparent sealant totally confirmed initial evaluations. Complete sealant retention rated 95% at 6 months, and 91% at 12 months. No case of complete detachment was observed. At the 12-month follow-up, VistaCam measurements resulted stable in the whole sample, except for one permanent molar, which presented a demineralization increment

## Session 5 Diagnostics

**100****Caries-Activity Assessment: Agreement Among Examiners from Clinical Assessments Ex-Vivo and Assessments from Bio-Luminescent Marker Images***S. Martignon<sup>a,b</sup>, A. Cortés<sup>\*a</sup>, N. B. Pitts<sup>b</sup>, K.R. Ekstrand<sup>c</sup>, D. F. Cabrera<sup>a</sup>, V. Avila<sup>a</sup>, G.A. Castiblanco<sup>a</sup>, L.F. Gamboa<sup>a</sup>**cortesandrea@unbosque.edu.co*

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The assessment of caries activity remains a challenge with no current available gold standard. This study quantified the level of agreement among examiners for assessments of caries activity made a) clinically ex-vivo on freshly extracted teeth and b) by examining same teeth images disclosed with a caries activity bio-luminescent marker using a prototype benchtop laboratory system (Calcivis<sup>®</sup>). Following IRB and patients/caregivers' consents, 12 subjects, with teeth scheduled to be extracted, participated. An externally-trained examiner selected a range of surfaces to be examined and took corresponding clinical photographs. Ex-vivo clinical activity assessments were conducted 2 and 9 days after extractions independently and in a random sequence by 3 examiners trained with ICCMS<sup>™</sup> using a points system; a sum of <sup>37</sup> points was considered an active lesion, otherwise surfaces were considered inactive lesion/sound [Ekstrand et al.: *Oper Dent*, 2007;32:225–235]. Obtained bio-luminescent Calcivis<sup>®</sup> images and corresponding available clinical images were randomly organized in slides indicating the assessment site. Six examiners independently assessed and 7 days later reassessed these images in a new random sequence. Calcivis<sup>®</sup>-image activity is denoted as a related bright blue/white signal on the image. 44 surfaces from 5–10-year-old children and 18–22-year-old adults were included: sound surfaces (n = 17), initial lesions (ICDAS 1–2; n = 15), and moderate-extensive lesions (ICDAS 3–6; n = 12). Results are given in perfect agreement (PA). Proportions of ex-vivo activity inter-/intra-examiner PA for sound

surfaces, initial lesions and moderate-extensive lesions were: 90%–91% and 88%–90%; 88%–94% and 83%–94%, and 77%–85% and 62%–80%, respectively. Correspondent bio-luminescent-image activity inter-/intra-examiner PA were: 32%–79% and 51%–89%; 43%–57% and 47%–68%, and 67%–84% and 67%–92%, respectively. In conclusion, examiners showed moderate-good inter-/intra-examiner agreement for ex-vivo and fair-good inter-/intra-examiner agreement for bio-luminescent activity assessment. Increasing agreement was achieved when the 2 methods were combined.

This study was partially funded by Universidad El Bosque, Colombia; King's College London, UK; and University of Copenhagen, Denmark, and Calcivis<sup>®</sup>.

**101****Does the Detection of Initial Caries Lesions Make the Examination More Time-Consuming and Expensive?***I. Floriano<sup>\*a</sup>, L.P. Antonio<sup>a</sup>, R.A. Gomes<sup>a</sup>, I.C. Louzada<sup>a</sup>, N.M. Ladewig<sup>a</sup>, G.M. Machado<sup>a</sup>, I.M. Pedroza-Uribe<sup>a</sup>, R.A. Pereira<sup>a</sup>, E.S. Rocha<sup>a</sup>, T.K. Tedesco<sup>b</sup>, M.E. Viganò<sup>a</sup>, L. Yoshioka<sup>a</sup>, D.P. Raggio<sup>a</sup>, F.M. Mendes<sup>a</sup>, M.M. Braga<sup>a</sup>, CARDEC Collaborative Group**isabelafloriano@usp.br*

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The aim of this study was to evaluate the impact of detecting initial carious lesions in primary teeth, regarding time and costs. Caries detection was performed using visual inspection (ICDAS) in 258 3-to-6-years-old children according to two strategies: including all caries lesions severity (G1, n = 130) and excluding initial caries (G2, n = 128) (NCT02473107). The time of the examination was registered and the costs of the session (tooth cleaning + caries examination) were calculated. Other independent variables were also registered. Linear regression was performed to investigate the association between the strategy of caries detection performed and outcomes, as well as other possible independent variables. The co-

efficients were calculated with 95% of confidence interval. Detecting all lesions, with any severity, required, on average, 40 seconds more than for not detecting initial lesions (mean  $\pm$  standard deviation) (G1: 435.8 s  $\pm$  128.4, G2: 397.0 s  $\pm$  103.1;  $p = 0.004$ ). However, this difference in the duration of examinations did not cause significant differences on their costs (G1: 19.80  $\pm$  10.52 reais, G2: 18.31  $\pm$  9.63 reais;  $p = 0.30$ ). Longer examinations were observed for children with higher caries experience ( $\beta$  [95% CI],  $p$ -value) (5.70 [3.55 to 7.86],  $p < 0.001$ ), bad oral hygiene (156.88 [95.37 to 218.39],  $p < 0.001$ ) or presented bad behaviour during the appointment (43.45 [11.07 to 75.83],  $p = 0.009$ ). Finally, the higher the dmfs, the higher the cost of the session, independently of the strategy for caries detection (0.19 [0.04 to 0.34],  $p = 0.014$ ). The detection of initial caries lesions results to slightly more time. However, it does not impact on costs of the examination. Actually, the more carious or restored surfaces child has, the more expensive the session, regardless the strategy for caries detection.

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102

### Does Children's Caries Experience Influence the Performance of Examiners in Detecting Approximal Caries Lesions?

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This study aimed to evaluate if the children's caries experience exerts influence on the performance of visual and radiographic methods for the detection of approximal caries lesions in primary molars. Eighty children aged from 3 to 6 years were selected and classified according to the past caries experience considering cavitated lesions (WHO criteria). Two calibrated examiners evaluated 526 approximal surfaces for the presence of caries lesions using visual inspection and radiographic methods. As reference standard, two other examiners checked the surfaces by direct visual inspection after the temporary separation with orthodontic rubbers. Poisson multilevel regression analyses were conducted to evaluate the influence of the caries experience on the performance of diagnostic strategies. Prevalence ratios (PR) and 95% confidence intervals (95%CI) were calculated. Significant influence of caries experience was observed only on the visual inspection, since a higher proportion of false-positive results was observed in children with higher caries experience at non-cavitated lesions threshold (PR = 4.01; 95%CI = 1.12 to 14.39) and for false results in general at cavitated threshold (PR = 2.78; 95%CI = 1.05 to 7.38). The detection of non-cavitated caries lesions in children with higher caries experience was overestimated (specificity = 0.696), compared with children with lower caries experience (specificity = 0.918), probably due to a cognitive bias (confirmation bias). However, the examiners underestimated the detection of cavitated car-

ies lesions in children with higher caries experience (sensitivity = 0.143) compared with lower caries experience children (sensitivity = 0.222), probably because of representativeness bias. Radiographic method was not influenced by the children's caries experience. In conclusion, the child's caries experience exerts influence on visual inspection in detecting approximal caries lesions in primary teeth, suggesting the occurrence of cognitive bias when examiners conduct visual inspection for caries lesions detection in children.

The study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Process 2012/24243-7) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

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103

### Caries Status of Black Stained Teeth Using ICDAS System

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Black stain (BS) is generally seen on cervical area of a tooth. The aim was to analyse caries status by ICDAS System and to compare BS with non-BS teeth. 10 patients with BS from 185 patients attended to Marmara University aged 16–51 years were included to study. Detailed medical histories were recorded and informed consent taken. A total of 111 teeth with BS and 144 with non-BS teeth of patients were recorded with ICDAS Scores after visual (naked eye, mirror-sound and digital photography) and radiographic examinations by single examiner. ICDAS Score 0 indicated 'non-carious' ICDAS 1-2 were reversible lesion (White spot lesion) and ICDAS 3-4-5-6 were saved as 'cavitated carious' teeth. All teeth with restorations and crowns were excluded from study. Chi-square test was used for statistical comparisons. The proportion of teeth without caries is significantly higher among teeth with black-stain than without BS (62.2% and 36.1% respectively;  $\chi^2=18.231$ ,  $p < 0.001$ ; Kappa = 0.216,  $p < 0.001$ ). Detailed analysis showed statistically significant difference between three groups namely ICDAS = 0, ICDAS = 1-2 and ICDAS = 3-4-5-6. Additionally, six of total 10 patients were reported as suffering from Fe+ deficiency at different degrees. In conclusion, black stained teeth showed less caries scores compared to non-BS. Kappa statistics indicate that, there is a weak (Kappa = 0.216) and significant ( $p < 0.001$ ) agreement between presence of BS and absence of decay. While it was tooth-specific formation, ICDAS System gave tooth-specific and exact results. We think, further studies are necessary to examine besides any relation whether BS can protect against caries or not; and the mechanism between Fe+ deficiency and BS development.

### Comparison of Different Plaque Assessment Algorithms with QLF

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Qraycam is a tool which can be used to identify early intra-oral anomalies. To capture and quantify the plaque tooth coverage, program Qray (v1.24 Inspektor Research Systems) was used. In some cases the automatic analysis results are not intuitively correct. New analysis methods were introduced. The aim of this study was to compare different analysis algorithms to quantitatively assess plaque coverage of fluorescent in vivo frontal Qraycam images. Fluorescent frontal images were made of 9 patients. All analysis produced plaque coverage (P) proportional to the tooth area size. Manual analysis required manual construction of a contour of the teeth. All pixels in the contour were plotted in a graph according to their red and blue values. The plaque area was achieved by interactively moving a line in the graph. All pixels above the line where qualified as pixels where plaque was present. Instant visual feedback was provided by coloring the plaque pixels in blue in the original image. Automatic analysis was performed by using the standard plaque analysis. The region of interest (ROI) was automatically set by the program and the plaque coverage was calculated at a cutoff of  $\Delta R > 30\%$  in the ROI by the automatic analysis algorithm in the program. Semi-automatic analysis used the same routine as automatic analysis. The ROI was manually adjusted to make a better fit with the tooth area. The mean PManual was 15% (SD 13%), mean PAutomatic was 14% (SD 13%) and mean Psemi-automatic was 14% (SD 14%). Correlation of automatic analysis with manual was ( $r^2=0.94$ ) and for semi-automatic analysis ( $r^2=0.94$ ). It was concluded that manual, automatic and semi-automatic plaque analysis produced comparable results with the same accuracy and precision.

## 105

### Reproducibility and Accuracy of ICDAS In Assessing Coronal Caries Lesions. A Systematic Review

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We performed a systematic review to evaluate the overall accuracy of the ICDAS in assessing coronal caries lesions. Additionally, the reproducibility of ICDAS was also evaluated in these

studies. A previous search strategy was adopted [Gimenez et al., 2015], up to July 2016 to identify laboratory and clinical studies using visual examination and published in English. Studies considered for analyses were those that 1) assessed primary caries lesions using ICDAS; 2) were performed at coronal surfaces and 3) used a reference standard, permitting the accuracy of the method to be calculated. Fifty-six studies were included and different subgroups were analyzed regarding setting and type of teeth. From these studies, data concerning Spearman correlation coefficients (Rs), intra- and inter-reproducibilities was collected. Meta-analyses summarized the results and considering different parameters: INTRA- and INTER-rater reliabilities (RR) and accuracy (Rs, summary ROC-curves (SROC) and diagnostic odds ratio (DOR). The two latter were expressed at D<sub>1</sub>/D<sub>3</sub> levels. Heterogeneity of the studies was assessed by Cochran's Q and I-squared tests. For primary teeth, the INTRA-RR was 0.81 (CI 95%: 0.71–0.96; n = 8) and INTER-RR was 0.68 (CI 95%:0.6–0.76; n = 17). For permanent teeth, corresponding figures were:0.78 (CI 95%:0.71–0.85; n = 20 and: 0.67 (CI 95%:0.58–0.75, n = 15). The pooled Rs for studies in the primary dentition was 0.78 (CI 95%:0.73–0.84; n = 5) and for the permanent dentition 0.68 (CI 95%:0.58–0.78; n = 12). Most areas under the SROC-curves were higher than 0.75. Laboratory (LS) and clinical (CS) studies presented similar results at D<sub>1</sub>-level both in primary (LS: (0.86; SE = 0.02, n = 16) and CS: (0.90; SE = 0.03, n = 24) or permanent teeth (LS (0.79; SE = 0.02, n = 5) and CS (0.78; SE = 0.04, n = 4)). Similar values were found at D<sub>3</sub> level, except for permanent teeth with very few and varied results. The DOR values were very high (range 6.1 to 59.6). I<sup>2</sup> values varied between 40%–90%, indicating a high heterogeneity between studies. In conclusion, the ICDAS presented substantial/high level of reproducibility and accuracy when primary coronal caries lesions were assessed.

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## 106

### Caries Excavation with Fluorescence Aided Caries Excavation, Caries Detector or Conventional Excavation

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This in vitro study aimed to investigate the effectiveness of three techniques to assess minimal invasive excavation. Fluorescence Aided Caries Excavation (FACE), Caries Detector and conventional excavation were compared. **Method:** In a single blinded cross-sectional study sixty extracted human (pre)molars were assigned to three experimental groups: FACE, Caries Detector (Kuraray) or conventional excavation (n = 20, per group). Molars and pre-molars with an overall ICDAS score of 3, 4 or 5 were included. Caries were excavated using the assigned excavation aid. The cavity preparation process was assessed by two calibrated dentists-lecturers and one calibrated dental hygienist/epidemiologist-lecturer.

turer using a VAS-scale of 0 to 10, where 5 was considered correct minimal invasive preparation. Assessed were photographs of the elements during the preparation process and the final tangible elements. Cavity preparations were carried out by four dental hygiene bachelor students of Inholland in their 4<sup>th</sup> year. **Results:** Mean VAS scores for caries preparation were 5.46 (SD 1,6 ) for FACE, 5.65 (SD 0,9) for caries detector and 6.04 (SD 1.1) for conventional excavation. FACE resulted in the best minimal invasive preparations and Conventional method had the largest scores related to over-excitation although differences between groups were not significant ( $F = 1,060$ ,  $p = 0,354$ , One-Way ANOVA). In conclusion, FACE, Caries Detector or conventional excavation can all be used for minimal invasive excavation, according to this study. In addition, dental hygiene students show a small tendency to over-excitation (preparation), but within limits of acceptance.

107

### Impact of Radiographic Examination on Treatment Decision Related to Dental Caries: A Before and After Study

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This study evaluated the impact of radiographic examination on changes of treatment decision related to dental caries lesions compared to the treatment decision planned after visual inspection alone. For this, 126 children (3 to 6 years-old) were selected and examined by two calibrated examiners through visual inspection using ICDAS, and treatment planning was made based on this clinical examination. Then, the same examiners evaluated bitewing radiographs and made another treatment planning, now based on the association of both visual and radiographic methods. Each dental surface of primary molars received a treatment decision as follow: (i) no treatment, (ii) non-operative treatment, and (iii) operative treatment. Frequency of changes in the treatment decision after the radiographic examination was calculated. Multilevel Poisson regression analysis was performed to evaluate the variables that influenced these changes. Prevalence ratios (PR) and 95% confidence intervals (95%CI) were calculated. Changes from no treatment decided with visual inspection alone to non-operative treatment after radiographic examination were observed in 52 surfaces (3.2%), and changes to operative treatment occurred in 46 surfaces (2.8%). Moreover, 50 surfaces (6.2%) with a first decision of non-operative treatment changed to operative treatment after the radiographic evaluation. Changes in the treatment decision were more frequent in children with higher caries experience (PR = 2.30; 95%CI = 1.39 to 3.79) and on approximal surfaces (PR = 2.68; 95%CI = 1.69 to 4.25, compared to occlusal surfaces), but they were less frequent in second primary molars (PR = 0.60; 95%CI = 0.43 to 0.84) than in first molars. In conclusion, the impact of radiographic examination on changes of treatment decision compared to the treatment planning made by visual inspec-

tion is small, but changes were more significant in children with higher caries experience and in approximal surfaces.

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108

### Approximal Caries Between Neighbor Teeth in Bitewing Radiographs: Frequency and Relationship

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Approximal caries is a common phenomenon. The disease begins at the zone below the contact point of neighbor teeth. Approximal lesions may begin simultaneously in both neighboring teeth at the same time or a lesion is established and progressed in one tooth surface, possible increasing the risk for occurrence of caries on approximal surface of the neighbor tooth surface. The frequency for this situation has not been examined yet. The aim of this study was to determine the frequency of initial caries lesions in the approximal surface of posterior teeth, which are neighbor to an approximal caries lesion with radiographical depth beyond the outer third of dentin and needs to be restored. 368 bitewing radiographs from adults' patients (over 12 years), were analyzed. Teeth with at least one approximal caries lesion cavitated or deep enough to be restored, and a neighbor tooth next to it were included. The radiographs were analyzed by one observer with experience in caries diagnosis, recording if the neighbor surface of the tooth next to the caries has or has not an initial caries lesion in the enamel/outer third of the dentine. Fisher exact test was used for statistical analysis of differences between presence/absence of initial approximal lesion and presence/absence of deep caries on neighbor tooth.

A total of 108 (30%) out of 368 teeth had a deep/cavitated approximal lesion. The neighbor surface to the 108 surfaces in need of restorative treatment were carious (initial) in 105 cases (97.2%,  $p < 0.001$ ). There is a need to detect and establish a treatment protocol for initial approximal caries lesion by taking advantage of the direct view created during the operative treatment of deep caries in the neighbor tooth.

### Treatment Decision for Hypomineralized First Permanent Molars Among MIH Experts

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There is no consensus about the best preventive/restorative strategies for hypomineralized teeth. This study aimed to evaluate the treatment decisions for first permanent molars (FPM) presenting hypomineralization of different levels of severity among MIH experts. To be included in the sample, researchers should have at least one study about MIH published in English in journals indexed in MEDLINE (last 5 years) traced using the terms: Molar Incisor Hypomineralization; Hypomineralization, Molar Incisor. A questionnaire based on 17 clinical cases was sent electronically to 30 researchers from 17 different countries amongst 5 continents. Cases included: 2 cases of post-eruptive breakdown (PEB) not exposing dentin, 12 PEB exposing dentin, and 2 PEB exposing dentin with pulp involvement. Information about pain was provided. Treatment options were: no treatment needed (NT); non-invasive (NI) (fluoride or sealant); restorations (REST) (amalgam, resin, glass ionomer cement (GIC), indirect restorations or stainless steel crown (SSC)); endodontic treatment/restorations; or extraction (EXT). Response rate was 76.6%. Statistical methods were based on descriptive analyses of frequency. For PEB not exposing dentin, the majority decided for NT (13%) or NI (42%), although direct REST was the decision in 30.4% of the cases and EXT in 8.7%. For PEB exposing dentin, REST was the most common decision (59.1%) (41.3% direct, 17.8% indirect, incl. SSC) followed by EXT (21.4%). For PEB exposing dentin with pulp involvement, EXT was the treatment choice in 80.4% of the cases. When direct REST was indicated, resin was the primary choice (65%) followed by GIC (35%). The presence of pain did not reflect on more invasive treatments. Even among researchers who are directly involved in investigations of MIH, a great disparity between treatment decisions for hypomineralized FPM was observed.

### Do Bitewing X-Rays Add Information to the Clinical Registrations?

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The aim was to demonstrate caries distribution in adults and extent of bitewings' contribution to clinical caries diagnostics. After stratification with respect to gender and age groups from the original 4,402 patients [Kongstad et al.: Acta Odont Scand 2013;7:1560–1569], 240 persons were randomly selected equally distributed in gender and age groups (18–34-years-old, 35–44-years-old, 45–54-years-old, 55–64-years-old, 65–74-years-old and >75-years-old). Clinical (C) and radiographical (R) registrations were categorized as sound, carious, restored and missing on occlusal and approximal surfaces of first and second molars, and premolars. Lesions were scored as primary or secondary. Lesion depth and quality of the restorations were recorded on radiographs. Chi-square and Fisher Exact tests were used for statistical analysis of differences between clinical and radiographical registrations. The level of agreement between the registrations was measured using kappa. 11,520 surfaces (3,840 occlusal and 7,680 approximal) were analyzed. Overall agreement (A) between C and R was 58% (occlusal:71%, kappa = 0.5; approximal:51%, kappa = 0.3). Sound surfaces was C:54%, R:25%, A:24%, primary caries C:1%, R:6%, A:0.3%, secondary caries C:0%, R:7%, A:0.02%, restored C:45%, R:35%, A:33%. Sound surfaces were most frequent in 18–34-years-old, and least in >75-years-old. Primary caries was most frequent in 35–44-years-old and least in >75-years-old. Secondary caries was most frequent in 65–74-years-old and least in 18–34-years-old and 35–44-years-old. Restored surfaces were most frequent in 65–74-years-old and least in 18–34-years-old. Missing surfaces were most frequent in >75-years-old and least in 18–34-years-old,  $p < 0.001$ . Frequency of sound, carious, restored and missing surfaces scored clinically and radiographically differed in relation to gender, age group, tooth type and surface ( $p < 0.001$ ). Primary caries was limited to enamel (81%) and secondary caries in dentin (93%). Insufficient restorations were found in younger age groups, males, and restorations in dentin ( $p < 0.05$ ). Data about lesion activity was unavailable. In conclusion the Distribution of sound, carious and restored surfaces varied between clinical and radiographical registrations. Radiographs supplemented with diagnostics of primary and secondary caries which were “overlooked” in clinical examination. Especially regarding approximal caries is important to complement clinical records with BW. Radiographs also added information about quality of restorations.

### The Optimum Laser Fluorescence Cut-Off Values for the Detection of Approximal Caries in Primary Molars

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Currently there are no validated thresholds for the interpretation of laser fluorescence pen values for primary teeth. Therefore, the aim of this in-vivo study is to find the appropriate cut-off values for interpretation of the laser fluorescence pen (LF pen) (DIAG-NOdent pen, Kavo Biberarch, Germany) for the detection of approximal caries in primary molars. **Methods:** Eighty-two children (aged 5–10 yrs) were recruited. Initially 1030 approximal surfaces were examined clinically using the LF pen according to the manufacturer's instructions. Subsequently 356 primary molars were extracted yielding 542 surfaces for serial sectioning and histological validation. Optimum cut-off values for detection of approximal caries in primary teeth were identified using the Youden's J statistic. **Results:** The optimum cut-off value at D<sub>1</sub> (enamel and dentine caries) diagnostic threshold was found to be 10. The sensitivity and specificity of the LF pen at this threshold were 0.65 and 0.89 respectively. For caries penetrating the outer third of dentine the optimum cut-off value was 14. The sensitivity and specificity of the pen at this threshold were 0.77 and 0.85 respectively. For caries penetrating the middle third of dentine the optimum cut-off value was 15. The sensitivity and specificity of the LF pen at this threshold were 0.82 and 0.81 respectively. The cut-off values found for sound, enamel caries, outer dentine caries and middle dentine caries are: 0–9, 10–13, 14 and ≥15, respectively. Compared to the manufacturer's cut off values which are; sound = 0–7, Enamel = 8–15, Dentine = ≥16. **Conclusion:** The optimum cut-off values for the detection of approximal caries in primary molars are different from those suggested for permanent molars by the manufacturer. The threshold values presented here provide a higher sensitivity without compromising the specificity.

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### Reproducibility of Calcivis Activity Imaging System Measurements in Primary and Permanent Teeth

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The Calcivis<sup>®</sup> Caries Activity and Demineralisation Imaging System (Calcivis) is a device which applies a photoprotein to the tooth surface which binds free calcium ions specific to active caries lesions. This photoprotein emits a blue light signal which is proportional to the amount of calcium present. The integrated camera

visualizes the elevated calcium ions as luminescence spots on the tooth surface. This study aimed to evaluate the reproducibility of a prototype Calcivis System (Calcivis black box) to assess caries lesion activity in-vitro. **Material and method:** Investigation sites of 149 freshly extracted teeth (55 primary and 94 permanent teeth) were assessed visually for lesion activity. The sites were photographed using Calcivis and the images were analysed for presence or absence of luminescence spots (t1). Teeth were stored in deionized water and second Calcivis measurements were performed after four weeks (t2). Agreement of visual and Calcivis findings (activity yes/no) was assessed by kappa statistics. McNemar test was used to test whether Calcivis measurements varied significantly between the t1 and t2 ( $\alpha = 0.05$ ). **Results:** Kappa-values for agreement between visual classification and Calcivis were: all teeth 0.62 (t1 and t2); primary teeth 0.30 (t1) and 0.44 (t2); permanent teeth 0.80 (t1) and 0.74 (t2). Kappa values for agreement between Calcivis measurements between t1-t2 were: all teeth 0.80; primary teeth 0.79; permanent teeth 0.81. No significant differences between both Calcivis measurements were found (p-values >0.05). **Conclusion:** The agreement of Calcivis measurements with visual criteria regarding lesion activity was substantial for permanent teeth only. Reproducibility between two Calcivis readings after four weeks of teeth storage was substantial and without any significant differences.

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

### Evaluation of the Calcivis Imaging System for Persistence of Images on Tooth Enamel Surfaces

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The aim was to investigate the persistence and reproducibility of images of demineralising tooth surfaces produced by the Calcivis Imaging System, using repeat applications of a calcium-sensitive photoprotein. **Methods:** The Calcivis Imaging System consists of a specialised intraoral camera capable of imaging very low level, transient luminescence when a calcium activated photoprotein is applied to enamel surfaces of teeth. Using a dark box, five extracted teeth were imaged repeatedly (ten times) using the application of the photoprotein until the luminescence observed significantly decreased. The samples were then rinsed and rehydrated using deionised water and re-imaged. **Results:** The images obtained of teeth following successive applications of the Calcivis Imaging System photoprotein clearly show reproducible and persistent images with sequentially decreasing luminescence from the enamel surface. This decrease in luminescence can be measured using image analysis software which shows a reduction in mean luminescence intensity value from 12 to 6 over 10 sequential applications of photoprotein. The sample teeth were then immersed in deionised water for a period of 30 minutes before a further treatment with photoprotein. The images from teeth after immersion show all samples exhibit levels of luminescence comparable to the original baseline images. **Conclusions:** Following successive applications of a calci-

um-sensitive photoprotein, the Calcivis Imaging System was able to detect and monitor the level of free calcium ions from within the enamel surfaces. Images were reproducible up to around the fifth or sixth photoprotein application, before the luminescence exhibited a significant decrease from the initial image. The decreased luminescence indicates a reduction in the concentration of free calcium ions. Once depleted, the calcium ions are replenished upon rehydration of the enamel surface.

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114

### Performance of CAST and ICDAS Criteria for the Detection of Occlusal Caries Lesions in Permanent Teeth

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The aim of this *in vitro* study was to compare the performance of CAST (Caries Assessment Spectrum and Treatment) instrument and ICDAS (International Caries Detection and Assessment System) in detecting occlusal caries lesions in permanent teeth. One occlusal site on each 120 extracted permanent molars was visually assessed twice by two examiners using CAST instrument (codes 0, 3, 4, 5 and 6) and ICDAS (codes 0–6), with one week interval between evaluations. These teeth were then histologically prepared and assessed for caries extension. Weighted kappa coefficient was used to assess inter- and intra-examiner reproducibility. Sensitivity, specificity, accuracy and area under the receiver operating characteristic (ROC) curve (Az) were calculated for each examiner at D1 (enamel and dentine lesions) and D3 (dentine lesions) thresholds. The intra-examiner Kappa values were 0.868–0.937 (CAST) and 0.804–0.811 (ICDAS) and inter-examiner values were 0.671 (CAST) and 0.695 (ICDAS). At D1 threshold the sensitivities were 0.79–0.93 (CAST) and 0.80–0.96 (ICDAS), specificities were 0.79–0.86 (CAST) and 0.76–0.86 (ICDAS), and accuracy were 0.79–0.93 (CAST) and 0.80–0.93 (ICDAS), respectively. At D3 threshold the sensitivities were 0.74–0.75 (CAST) and 0.79–0.81 (ICDAS), specificities were 0.93–1.00 (CAST) and 0.90 (ICDAS), and accuracy were 0.84–0.87 (CAST) and 0.86–0.87 (ICDAS), respectively. The Az values were 0.85/0.88 (CAST) and 0.84/0.89 (ICDAS) at D1 threshold and 0.81/0.89 (CAST) and 0.86/0.91 (ICDAS) at D3 threshold. There was no statistically significant difference between the performance values ( $p > 0.05$ ). Spearman correlation coefficients with histology were 0.70 (CAST) and 0.72 (ICDAS). In conclusion, ICDAS and CAST instrument presented similar performance for the detection of occlusal caries lesions in permanent teeth, demonstrating good suitability for visual inspection.

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115

### Comparison of Validities for Detecting Enamel Caries by the Generations of QLF Devices

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Quantitative light-induced fluorescence (QLF) technology-based devices introduced in the 1980s have been developed as various types, but no study has compared the different generations of QLF devices. The aims of this *in vitro* study were to compare fluorescence properties between each generation and to evaluate their validities for detecting non-cavitated enamel caries lesions. A total of 52 human permanent premolars and molars were used. Fluorescence images were captured by three QLF devices (Inspektor Pro, QLF-D, and Qraycam). Fluorescence parameters—mineral content ( $\Delta F$ ) and bacterial metabolites ( $\Delta R$ )—were calculated. The severity of lesions was categorized into the following 3 scores using polarized light microscopy: normal (S), enamel demineralization to outer half of enamel (E1), and inner half of the enamel up to the dentin-enamel junction (E2). The Kruskal-Wallis test was used to compare the fluorescence parameters between QLF devices. Correlations between two parameters and histological scores, sensitivity, specificity, and area under the ROC curve (AUC) were calculated. The teeth were categorized into 19 sound, 19 E1 lesions, and 12 E2 lesions by histological analysis (2 were lost). The medians of the parameters between three QLF devices showed no significant difference ( $P < 0.017$ ). The correlations were acceptable for Wallis test was used to compare the fluorescence parameters between QLF devices. The correlations were acceptable for  $\Delta F$  ( $r > 0.78$ ) and  $\Delta R$  ( $r > 0.73$ ). The AUCs of  $\Delta F$  and  $\Delta R$  at the cut-off (lesion presence or not) for all the devices were also higher than 0.91 and 0.82, respectively. In the case of  $\Delta R$ , the sensitivities were higher for the QLF-D and Qraycam (87.10) instruments than for the Inspektor Pro (64.54), and the specificities were higher for QLF-D and Inspektor Pro (100.0) than for Qraycam (90.48). The validities of all QLF devices did not differ significantly for the detection of non-cavitated caries lesions.

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### Stimulated Raman Scattering Microscopy for Label-Free Imaging of Demineralized Enamel

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The objective was to evaluate the ability of stimulated Raman scattering microscopy (SRS) to quantify enamel demineralization. 15, 5'5'2.0-mm bovine-enamel blocks were used. Surfaces were divided into four equal areas. Each area was chemically demineralized for 0, 8, 16 or 24 h, respectively. The spectral coverage from ~850 to 1150 cm<sup>-1</sup> was tested. SRS images were obtained with 10 μm increment up to 90 μm from surface to dentin-enamel junction at two locations (0.48×0.48-mm) between sound (0 h-demin) and 8 h demineralization (8 h demin) and 8 h demineralization (8 h-demin) and between 16 h (16 h demin) and between 16 h (16 h-demin) and 24 h demineralization (24 h-demin). SRS intensity from water, phosphate and carbonate was measured. The water intensity against depth decay curve was fitted with exponential decay. A decay constant (water-content) was obtained. The spectra profile with two Lorentzian functions was fitted to obtain the peak heights of phosphate and carbonate groups. Peak ratio was divided the phosphate peak height by carbonate peak height (P/C-ratio). P/C-ratio was normalized relative to 90 μm (P/C-ratio-norm). For each area, Knoop hardness value (SMH) was obtained before (SMHB) and after demineralization (SMHD). SMH-Change [(SMHD-SMHB)/SMHB] was determined. Comparisons were made using repeated measures ANOVA. SMH-Change was increased as demineralization time increased. All demineralization groups were significantly different from each other. For water-content, 24 h-demin was significantly higher than all other groups; 8 h-demin and 16 h-demin were significantly higher than 0 h-demin. For P/C-ratio and P/C-ratio-norm, 0 h-demin was significantly higher than 8 h demin was significantly higher than 8 h-demin at 0 and 10 demin at 0 and 10 μm; 16 h-demin was significantly higher than 24 h-demin at 0 μm. For P/C-ratio-norm, 0 h-demin was significantly higher than 24 h-demin at 0 demin at 0–40 μm. There was moderate-to to-strong correlation (r = 0.72) between SMH-Change and water-content. At 0–20 μm, there was weak-to-moderate negative correlation of water-content and P/C-ratio and P/C-ratio-norm. It can be concluded that SRS has potential to quantify enamel demineralization.

### Quantitative Evaluation of Tooth Wear With QLF Technique

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The aim of this study was to use quantitative light-induced fluorescence (QLF) to determine the relationship between the serial grinding stage of occlusal surfaces and the intensity of fluorescence under 405 nm visible light irradiation in vitro. Sixteen permanent premolars were collected and subjected to artificial wear. SS-OCT images were used to determine the measurement point where enamel outline was closest to the DEJ of the cusp. The length from that point to the base of the specimen was then measured. Each specimen was then serially ground in an apical direction. Serial QLF-D and SS-OCT images were acquired over the grinding depth (per 100 μm). The QLF images were converted to 8-bit gray scale to calculate the fluorescence intensities of the ground regions using brightness measurements. The maximum brightness (MB) was measured using gray scale images of the sound regions prior to grinding (MB<sub>baseline</sub>) and then again after grinding (MB<sub>worn</sub>). The grinding depth and phased average MB values were compared using Pearson's correlation analysis and the average MB<sub>baseline</sub> and each average MB<sub>worn</sub> over the grinding depth were compared using an independent t-test. A total of thirteen samples were included in final results. Three samples were limited in the ability to observe the DEJ contour by OCT due to the crack lesions near the measurement points. The average MB<sub>worn</sub> values gradually increased in parallel with the grinding depth and showed a strong positive correlation (r = 0.992, P < 0.001). Significant differences were observed between the average MB<sub>baseline</sub> and each serial MB<sub>worn</sub> at serial grinding depths of 200 μm (P < 0.05). In conclusion, the fluorescence intensity of teeth, determined by QLF at 405 nm of visible light increased with grinding stage with a strong positive correlation.

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### In Vitro Assessment of Quantitative Light-Induced Red Fluorescence Related to Activity of Caries Lesions

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The present study aimed to evaluate whether red fluorescence and changes in the degree of red fluorescence following remineralization are influenced by the activity status of caries lesions. A total of 62 smooth surface caries lesions on extracted human teeth (ICDAS codes 1–4) were divided into either active or inactive lesions based on Nyvad criteria (unweighted kappa = 0.67). The lesions were then treated with NaF solution (367 ppm), artificial saliva, and demineralizing solution using a pH-cycling model for 12 days. Fluorescence variables associated with red fluorescence and fluorescence loss were measured using a quantitative light-induced fluorescence (QLF) digital system. Prior to the pH-cycling treatment, active lesions exhibited a significantly greater increase in red fluorescence intensity ( $\Delta R$ ;  $p = 0.049$ ), red fluorescence area ( $\Delta \Delta R$ ;  $p = 0.018$ ), and fluorescence loss ( $\Delta F$ ;  $p = 0.045$ ) relative to inactive lesions. Multiple logistic regression analysis revealed that, among the red fluorescence variables, the square root value of the fluorescence area exhibited a significant positive association with the discrimination of active lesions (odds ratio = 1.023, 95% CI = 1.005–1.041). Following the pH-cycling treatment, significantly greater changes in  $\Delta \Delta R$  and lesion volume ( $\Delta Q$ ) were observed for active lesions relative to inactive lesions ( $p = 0.018$  and 0.038, respectively). In conclusion, our findings indicate that the area of red fluorescence differs between active and inactive lesions, and that the square root of this area is associated with lesion discrimination. Moreover, significantly greater recovery of lesion volume and red fluorescence area was observed for active lesions following treatment, relative to inactive lesions. These results indicate that the area of red fluorescence detected by a QLF system may be useful for the discrimination of activity status in caries lesions.

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### Ultrasonic System as a Diagnostic Method on Smooth Surface Caries with Different Depths

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The purpose was to investigate the ultrasonic system (USG), a non-destructive measurement method, on diagnosis of smooth surface caries lesions at different depths. 200 extracted teeth were cleaned and stored in refrigerator in saline solution containing 0.1% thymol. Following ICDAS evaluation, initial measurements of each tooth were recorded for mineral loss and lesion area using the FluoreCam (Daraza Corporate Headquarters, Indiana, ABD). The initial lesion depth measurements were also made with USG (Novascope 4500, Harisonic, Staveley NDT, Kennewick, Wash, ABD). Finally, all lesions were evaluated histologically (Leica Stereomicroscope, Leica Microsystems GmbH, Germany). Statistical analysis was performed using two-way ANOVA. When compared with the gold standard (histological evaluation) for detection of tooth decay, the intraclass correlation coefficient (ICC) was 0.97 (95% GA: 0.97–0.98) for the USG, 0.65 (95% GA: 0.54–0.74) for the ICDAS classification and 0.59 (95% GA: 0.45–0.69) for FluoreCam ( $p < 0.001$  for all). ICC values for the ICDAS-I group were 0.96 (95% GA: 0.93–0.97) for USG, 0.67 (95% GA: 0.46–0.80) for FluoreCam when layers were stratified according to ICDAS classification. In ICDAS-II group, 0.97 (95% GA: 0.96–0.98) for USG, 0.58 (95% GA: 0.34–0.74) for FluoreCam, in the ICDAS-III group; In the ICDAS-IV group, 0.97 (95% GA: 0.90–0.99) was found for USG ( $p < 0.05$  for all). ICC in ICDAS-IV group was not statistically significant for FluoreCam. In conclusion a very high level of agreement was found between histological diagnosis and USG. Ultrasonography and FluoreCam may be used clinically for the detection of enamel caries.

### Caries Detection Using ICDAS Is Important and Easy? The Undergraduate Students' Perspective – A Multicentre Study

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We aimed to verify how easy and important the undergraduate students judged caries lesions assessment using ICDAS and identify variables regarding the students' perspective. Undergraduate students enrolled in a bigger multicentre randomized controlled study reported, in a 10-point scale, the levels of easiness and clinical importance of performing caries detection using ICDAS. They also reported their level of self-confidence in their knowledge about the topic in a 4-point scale. Six included centres represented different contexts/backgrounds regarding the use of ICDAS in education. Multilevel Poisson analyses were performed to identify the association between students' perspectives and the educational institution, the students' year, type of training, performance on practical and theoretical tests. The centre, the class and the student were considered as levels. Rate ratios were calculated (RR; 95%CI). 865 students were included (response rate:95%). Differential trends were observed among the centres, but they are adjusted in the models. The level of importance reported for caries lesion assessment using ICDAS was, on average, 9.1 (SD±1.4) and the easiness was 7.3 (SD±1.8). Most students considered themselves as prepared to this task. Those students who considered the task more important were those who also considered it easier (RR = 1.05; 1.03–1.08). Final-year students (RR = 1.31; 1.17–1.47) and those who reported a higher level of easiness (RR = 1.05; 1.02–1.09) were more self-confident in performing caries detection using ICDAS, independently of training. Laboratorial training, students' knowledge or ability in assessing caries lesions did not influence students' perspectives about the issue ( $p > 0.05$ ). Concluding, from the undergraduate students' perspective, caries lesion assessment using ICDAS is an important task which is moderately difficulty. Nevertheless, the students' perspective on this issue seems to be more linked to contextual characteristics than to students' training or abilities.

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### Evaluation of Chemiluminescence for Detection of Enamel Caries Lesion Activity

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The aim was to evaluate the capability of the Calcvivis Imaging System to assess caries lesion activity and remineralisation on enamel surfaces. The Calcvivis System consists of a specialised intraoral camera capable of imaging very low level, transient luminescence when a calcium activated photoprotein is applied to enamel surfaces. The luminescence level can be measured using image analysis software which returns a value between 0 and 255 depending on intensity. Using a dark box, images were obtained from five sound enamel samples and five samples displaying active caries on the buccal aspect. A further ten inactive caries samples were also imaged for comparison. Active caries lesions were prepared using a validated multi-species bacteria model, and inactivated through a standardised 100 day remineralisation/abrasion protocol (Neuhaus et al.: Caries Res 2016;50:220). Images clearly showed significantly increased luminescence from four out of the five active lesion samples when compared to both the sound and inactive samples. The remaining active sample showed a slight increase in luminescence compared to background. This analysis showed sound and inactive samples both to have a mean value ( $\pm$ SD) of 0.2 ( $\pm$ 0.1), while the active samples had measured luminescence of between 14 and 40, depending on the status of the lesion (mean value  $32.5 \pm 12.6$ ). **Conclusions:** Of particular note is the lack of observed luminescence from inactivated lesions, confirming arrest of net demineralisation within the lesion site. The chemiluminescence method was able to detect active caries lesions created using a validated model and could potentially be used to monitor remineralisation therapies.

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### Performance of Undergraduate Dental Students on ICDAS Occlusal Caries Detection After Different Learning Strategies

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This study aimed to evaluate the performance of undergraduate dental students in detecting occlusal caries lesions using ICDAS after different learning strategies. Sixty-four students, who never had any experience on ICDAS, assessed 80 occlusal surfaces of permanent teeth according to ICDAS, initially using a table describing all seven codes. After two weeks, students were allocated in three groups and trained according to the learning strategies used: G1 (n = 21): online ICDAS *e-learning* program; G2 (n = 22): online ICDAS *e-learning* program + digital learning tool (DLT) [Luz et al.: J Dent Educ 2015;19:235–241]; G3 (n = 21): no learning strategy (control). Two weeks later, G1 and G2 reassessed the teeth. Teeth were histologically prepared and assessed for caries extension. Sensitivity, specificity and area under ROC curve were obtained at D1, D2 and D3 thresholds (McNemar test). An ANOVA test was used to compare the results among the groups in each threshold. G1 and G2 showed statistically significant higher values of sensitivity at D2 threshold after training (G1: before: 0.87; after 0.91/G2: before: 0.87; after 0.94) and specificity (G1: before: 0.47; after: 0.49/G2: before: 0.46; after: 0.47). Comparing groups, G2 showed higher sensitivity than G1 at D2 threshold (0.94 and 0.91, respectively). It can be concluded that the performance of undergraduate dental students was improved by the association of the digital learning tool to the online ICDAS program for the detection of occlusal caries lesions *in vitro*.

### Correlation of Inflammatory Markers in Saliva and Early Childhood Caries: Analysis of the Mother-Child Binomial

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The aim of this study was to evaluate the levels of inflammatory markers (IL-6, TNF- $\alpha$ , VEGF) in the saliva of mother/child binomials, correlating them with the presence of caries, maternal/child adiposity and sugar consumption. Salivary samples from 40 maternal/child pairs were analyzed to determine the levels of VEGF, IL-6, TNF- $\alpha$  and these were correlated with maternal and child variables. The maternal variables were: Waist circumference, dental caries index (DMF-T) and sugar consumption. The child variables were: Body mass index (BMI), caries index (dmf) and child sugar consumption. The data was collected through a socio-economic questionnaire, a food frequency questionnaire and a dental clinical examination. Maternal DMFT ( $r = 0.35$ ,  $p = 0.03$ ), maternal waist circumference (MWC) ( $r = 0.35$ ,  $p = 0.02$ ) and maternal sugar consumption ( $r = 0.32$ ,  $p = 0.04$ ) were correlated with salivary VEGF levels. Maternal DMFT ( $r = 0.45$ ,  $p = 0.004$ ) and MWC ( $r = 0.32$ ,  $p = 0.04$ ) were also correlated with the highest salivary levels of IL-6. In children, higher dmft values were correlated with the highest salivary levels of VEGF ( $r = 0.42$ ,  $p = 0.008$ ). The mothers of children with early childhood caries (ECC) had higher mean values of IL-6 in saliva compared to mothers of children free of ECC ( $p = 0.03$ ). The group of children with ECC had mean values of VEGF in the saliva 63% higher and twice the values of salivary IL-6 than caries-free children, but with no statistical difference. Inflammatory markers VEGF and salivary IL-6 were correlated to caries severity in the mother-child binomial. Obesity and high consumption of sugars appear to underlie relationships between metabolic markers and dental caries.

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## Session 6 Epidemiology

125

### Who Is Using ICCMS™: A Comparison Between Students, Teachers and Clinicians

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To achieve best practice in caries management, the ICDAS group has developed The International Caries Classification and Management System (ICCMS™). This system includes four elements (4D): D1- Determine (Patient-level Caries Risk); D2- Detect & Assess (Caries Staging and Activity plus Intraoral-level Caries Risk); D3- Decide (Personalised Care Plan: Patient & Tooth Levels), and D4- Do (Prevention Strategy according to Caries Lesions Likelihood and Tooth-preserving Non-Operative/operative Lesion's Management). **Aim:** To determine, with regard to ICCMS™ the extent to which clinicians, teachers, and students perceive that they have Capability in, Opportunity for, Motivation for, and Behaviour related to, caries diagnosis and management strategies. Experimental approach: A total of 139 clinicians, 213 teachers and 605 dental students from different cities of Colombia completed the 79-question survey. The Capability and Opportunity scales

were measured on a five-point scale (1 to 5), whereas the Motivations scores were measured on a six-point scale (0 to 5). For both cases, higher scores indicate a greater engagement with each area of ICCMS™. **Results:** The median scale scores showed generally moderate to high levels across respondents of perceived Capability, Opportunity, and Behaviour for both caries diagnosis and management (3.75 to 4.88). In contrast, there were low levels of Motivation related to caries diagnosis and management for clinicians and teachers (2.00 to 3.20). There were statistically significant differences between the respondents in the scale medians for Motivation diagnosis and management (Kruskal-Wallis test with Dunn-Bonferroni correction,  $p < 0.001$ ), with clinicians and teachers obtaining lower median scores than students. **Conclusions:** Clinicians, teachers, and students show good levels of Capability in, Opportunity for, and Behaviour regarding caries diagnosis and management approaches. However, the Motivation of clinicians and teachers needs to be improved.

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126

### Caries Experience and Need for Restorative Treatment Among Finnish Adults

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The aim of this study was to analyze caries experience and need for restorative treatment among Finnish adults. Experimental approach: The study population was a subgroup of the epidemiological research program 'Northern Finland Birth Cohort 1966' (NFBC 1966). NFBC 1966 offers longitudinal data on the individuals whose expected date of birth was in 1966 and who were born in the two northernmost provinces of Finland. In 2012–2013 those living near or in the city of Oulu ( $n = 3,181$ ) were invited to the

general and oral health examinations. Of those, 61.7% agreed to participate and formed the present study group (n = 1,962). Cariological examination was performed by trained and calibrated dentists in fully equipped dental units using WHO gingival probes and fibre-optic transillumination. Caries lesions were classified using ICDAS criteria; using score 4 as the cut-off point for restorative treatment need. Caries experience was assessed using DMFT. The data were analyzed with SPSS 20.0 (IBM, Illinois, USA) and the differences between the genders were tested with t-tests. **Results:** On average, 39.8% of the study population had need for restorative treatment (males 46.6%, females 33.9%,  $p < 0.001$ ). The mean DMFT-score was 14.9 (SD 5.15), the difference between males (15.3, SD 5.42) and females (14.6, SD 4.88) being statistically significant ( $p = 0.006$ ). The mean number of decayed teeth among males was 1.2 (SD 2.00, min 0, max 15) and among females 0.79 (SD 1.68, min 0, max 19),  $p < 0.001$ . **Conclusions:** Need for restorative treatment is fairly high among Finnish adults, especially males, in northern Finland. As for controlling caries, there is obvious need for continuous oral health promotion and preventive procedures even among adult population, future dentulous seniors.

127

### Changes in Dental Caries in Preschool Children in Northwest Russia between 2007 and 2016

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The prevalence of caries among preschool children has been decreasing over recent decades in most European countries. To the contrary, we reported a significant increase in the prevalence and experience of caries among Russian children from 1997 to 2007. **Aim:** To study changes in the prevalence and experience of caries among 6-years-old children from 2007 to 2016 in Northwest Russia. **Design:** This study is a part of a nation-wide cross-sectional study. Altogether, 1232 children at the age of 6 were randomly selected in the Arkhangelsk region in 2016. Caries experience of primary teeth was assessed by the same two calibrated dentists in accordance with the WHO-2013 criteria at the d3 level. The dmft index and the Care Index (ft/d(3)mft\*100) were calculated. Dichotomous and continuous data from 2016 were compared with earlier published results from 2007 from the same region using Z-tests for dichotomous- and one-sample t-tests for continuous data. **Results:** The prevalence of caries decreased from 95.0% in 2007 to 90.8% in 2016 ( $p < 0.001$ ). Reduction in the overall caries experience did not reach the level of statistical significance (6.7 in 2007 vs. 6.5 in 2016,  $p = 0.118$ ). The number of decayed teeth decreased from 5.5 to 4.7,  $p < 0.001$ . The number of missing and filled teeth increased from 0.4 to 0.6 ( $p < 0.001$ ) and from 0.8 to 1.2 ( $p < 0.001$ ), respectively. The Care Index increased from 5.8% to 18.5% ( $p < 0.001$ ). **Conclusion:** The prevalence of caries in Northwest

Russia significantly decreased over the last decade. While the average number of decayed teeth decreased the mean number of treated teeth and the Care Index increased. Our findings reflect minimal improvements in both prevention and dental services in Northwest Russia over the last decade.

128

### Caries Trend (2011–2016) Among 12-Year Old Palestinian Refugee Children. Experience of the UNRWA Project

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The aim was to determine the trends in dental caries prevalence and severity among Palestinian refugee schoolchildren in the different refugees' camps between 2011 and 2016. Secondly, to investigate the relationships between clinical results and several behavioural and socio-demographic indicators. **Materials and Methods.** During the academic school years 2010/2011 the United Nation Relief and Work Agency for Palestine refugees in the near east (UNRWA) commissioned a survey on oral health to be conducted among 12-year-old Palestinian schoolchildren (n2011= 2758). The same survey was repeated in 2016 (n2016=1823). Data were collected in the five UNRWA fields (Gaza, Lebanon, Jordan, Syria, West Bank) by 11 calibrated exam-

iners. Demographic information, socio-economic level of the family, dietary habits, oral hygiene habits, dental attendance of child and parents were also collected. Logistic regressions were performed among caries experience, caries prevalence and the most relevant behavioural and socio-demographic indicators. **Results.** The overall mean caries experience expressed as DMFS was 3.922 in 2011 and 3.692 in 2016. A statistically significant trend was observed through 2011 and 2016 about DS component (mean DS2011=3.19; DS2016=2.95;  $p < 0.01$ ). Anova test between total DMFS in 2011 and 2016 was statistically significant ( $p < 0.01$ ) showing a greater variation between the two surveys. Both in 2011 and in 2016 caries experience was linked to the different five UNRWA fields (OR 1.13, 95% CI = 1.07  $p < 0.01$ ; OR 0.93; 95% CI = 0.87,  $p = 0.05$ ) and to the consumption of soft drinks (OR 0.821, 95% CI = 0.69,  $p = 0.02$ ; OR 1.27, 95% CI = 1.04,  $p = 0.01$ ). **Conclusions:** Oral health is one of the greatest health problems among Palestinian refugee children. An effective combined prevention and disease management programme should be implemented and monitored.

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129

### Caries Prevalence and Risk Factors Among 12-Year-Old Latvian Schoolchildren

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The aim was to evaluate dental caries prevalence, severity and risk factors in 12-years-old Latvian schoolchildren. **Methods:** Sixth-grade pupils in 95 schools ( $n = 3691$ ) were selected by the stratified cluster sampling method for a cross-sectional study. Clinical examinations (merged ICDAS) and survey using validated questionnaire was carried out from January to March 2016 by seven calibrated examiners (0.718–0.767 Kappa) at the school premises on a mobile examination table in a sleeping position, with forehead lamp lighting (80 lumens), using dental mirrors and CPITN probes. Caries risk factors were assessed by self-administered questionnaires. Potential risk factors were analyzed by logistic regression with R program. **Results:** We examined 2682 children from 92 schools (response rate = 74.5%); 2138 of them were 12-year-olds. The prevalence of caries at D<sub>1</sub> level is 98.5%, D<sub>3</sub> level 79.8%, D<sub>5</sub> level 71.9%. The average value of D<sub>3</sub>MFT is 3.35, D<sub>5</sub>MFT – 2.5 and D<sub>1</sub>MFT – 9.21. Only 6.5% of children have sealants. Half of the 12-year-olds brush their teeth twice per day, but only 21,3% of them are certain that their toothpaste contains fluoride. Caries prevalence is slightly lower in high family affluence group (78.21%) than in low affluence group (83.77%;  $p = 0.07$ ). Almost all children (99.3%) eat food containing added sugar at least once per week and 84.7% daily, but there is no significant difference in caries among groups with different eating habits. Dental and dental hygiene visits less than once per year, as well as brushing teeth less than once per day were significant caries risk factors. **Conclusion:** Caries prevalence in Latvia is very high and it can be explained by lack of dental care and usage of fluoride toothpastes.

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130

### Prevalence of Dental Caries and Fluorosis in Two Towns of Edirne, Turkey

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The aim of this study was to investigate the prevalence and the severity of the dental caries and dental fluorosis in children of two small counties of Edirne known to have detected as endemic fluoride area in the 1970's. Tap water samples were collected from a total of 17 rural settlements in two neighbouring counties of Edirne (Süloğlu and Havsa), 3 schools established in this area were reached and 178 children aged between 8 and 13 (101 boys, 77 girls) were screened for dental caries, dental fluorosis and other enamel defects using dft/DMFT, TF, DDE indices respectively. Saliva samples were collected for the investigation of fluoride content using fluoride electrode. All data was analyzed statistically using SPSS v21 software. Eight villages had fluoride concentration between 0,5–1,15 ppm (Group 1: mean 0,703 ± 0,201 ppm) whereas 9 villages had between 0,3–0,5 ppm (Group 2: mean 0,357 ± 0,053 ppm). In group 1 fluoride area, 29% of 51 children were caries-free, mean dft/DMFT scores was 3,14 ± 2,77 and 10%, 8%, 2% of these children had TF1, TF2, TF3 scores whereas in group 2 fluoride area 14% of 127 children were caries-free, mean dft/DMFT scores was 5,76 ± 3,68 and 3%, 4% of these children had TF1, TF2 scores respectively. The difference between dft/DMFT of 2 groups was statistically significant ( $p = 0,0000$ ) No difference was found between both fluoride area groups in the salivary fluoride contents of children. This study is the preliminary report of dental findings of the children living in an area previously determined as higher fluoride content in the water. We found that caries levels were lower and dental fluorosis were higher in the optimal fluoride water levels than below-optimal fluoride water levels. We are currently extending the area for a larger investigation.

### Caries Progression in a Cohort of Brazilian School Children Assessed by the Nyvad Criteria

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The aim of this study was to describe permanent tooth surface level caries progression over 2 years in a deprived community of Brazil (0.7 ppm F in the drinking water). **Methods:** Data were collected from 144 schoolchildren aged 7–11 years old who had previously been instructed in brushing the teeth twice daily with a fluoride toothpaste. Dental examinations were performed in 2014 (t0) and 2016 (t1) by the same trained and calibrated examiner (intra-examiner kappa 0.95). Immediately after brushing and drying the teeth, dental caries was assessed using the Nyvad criteria. Conditional probabilities were performed to evaluate caries lesion transition patterns at the tooth surface level. **Results:** 52.08% of the sample was female and 47.9% male (mean age 9.41 ± 1.41). Caries prevalence at t0 was 80.5% including enamel and dentine lesions and 44.4% including only dentine lesions. At t1, caries prevalence was 96.5% and 68.7%, respectively. Over 2 years, 92.2% (n = 8.624) of sound surfaces remained sound, while 5.6% (n = 521) progressed to non-cavitated enamel lesions and 2.2% (n = 212) to cavitated lesions. A detailed analysis of the caries lesion transition patterns showed that 44.6% of the active non-cavitated lesions at baseline became inactive or regressed to sound, 44.7% remained active and 7.6% progressed to cavitated lesions during the observation period. Similarly, 89.3% of the inactive non-cavitated lesions at baseline remained inactive or regressed to sound, and only 2.5% became active or progressed to cavitated lesions (7.5%). **Conclusion:** A low proportion of enamel lesions progressed to cavitation in this population, which might be explained by the presence of fluoride sources.

### National Oral Health Survey of 3-Year-Old Children in Germany 2015/2016

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Due to the lack of a national caries prevalence study of young children, this study aims to evaluate the oral health status of 3-year-olds in Germany. **Methods:** For the first time, 3-year-olds were included in the National German Oral Health Survey in

Children performed regularly since 1994/95 under the responsibility of the German Association for Dental Prevention in Children and Adolescents (DAJ). Employing a randomized cluster selection from lists of kindergartens (2/17 German regions) or a full survey (8/17), 95127 3-year-olds were examined in a total of 10 out of 17 German regions, by calibrated community or study dentists. The expected drop-out rate had been set between 5%–45% depending on the legislation of each German region, but the data showed to be in line with existing regional data. The examination included the WHO criteria [2013] for the dmft and the number of teeth with initial carious lesions. **Results:** 86.3% of children were caries-free at the dmft-level, 81.3% including initial caries lesions. The mean weighted dmft in 3-year-olds was 0.48 with single components of dt = 0.35, mt = 0.05 and ft = 0.08. The care index on a tooth level corresponded to 26.1%, leaving 11.4% of children in need of seeing a dentist for treatment. The mean number of affected teeth in children with dmft >0 was 3.57. Among the 10 regions, the mean dmft ranged between 0.38 and 0.59. **Conclusion:** Due to the legally defined structure of the DAJ and its regional subdivisions, a National Health Survey on 3-year-olds was feasible with reasonable effort. It revealed a considerable amount of untreated caries lesions present in a smaller risk group, which most likely cannot be treated by general dentists in a practice setting. This calls for further risk-based preventive efforts in early childhood.

The study was funded by the Deutsche Arbeitsgemeinschaft für Jugendzahnpflege e.V. (DAJ) and was approved by the Ethics Committee of the University of Greifswald (BB48/10a).

### Influence of Examining 12-Year-Olds in 6th Grade Only on Caries Experience in National Oral Health Survey in Germany 2015/2016

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The objective of this analysis was to evaluate the influence of restricting the examinations of 12-year-olds within the German National Oral Health Survey on 6th graders only. **Methods:** In a subanalysis, the data on 12-year-olds in 6th grade from Saxony-Anhalt (n = 6391) examined within the National German Oral Health Survey in Children performed under the responsibility of the German Association for Dental Prevention in Children and Adolescents (DAJ) were compared to the caries data of 12-year-olds in other grades. **Results:** The mean DMFT for 12-year-olds in 6th grade in Saxony-Anhalt was 0.52 (Germany 0.5; IDZ 2016). The 1230 12-year-olds examined in 5th grade exhibited clearly higher caries levels of 0.96 DMFT resulting in a common mean DMFT of 0.59. The inclusion of another 236 pupils in special needs schools (4th, 7th and 8th grade, mean DMFT 1.2–1.75) increased the overall DMFT to 0.61 DMFT.

**Conclusion:** The restriction of 12-year-olds in 6th grade within the German National Oral Health Survey excludes some children

with lower learning performance and higher caries levels. The overall caries levels for all 12-year-olds are underestimated by about 17% of the values for 12-year-olds in 6th grade.

The study was funded by the Deutsche Arbeitsgemeinschaft für Jugendzahnpflege e.V. (DAJ) and was approved by the Ethics Committee of the University of Greifswald (BB48/10a).

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134

### Effect of the Sampling Procedure on Caries Experience of 6–7-Year-Olds in Berlin: Analysis from the National Oral Health Survey in Germany 2015/2016

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The aim of this analysis was to evaluate the impact on the caries data of two sampling procedures: a random cluster selection from the state schools' lists (RCS) performed by the German Association for Dental Prevention in Children and Adolescents (DAJ) compared to the full-census examinations (FC) performed regularly within the framework of the health report at the country level of 6–7-year-old first graders in the state of Berlin/Germany.

**Methods:** 6–7-year-old first graders were examined in Berlin as a part of the National German Oral Health Survey in Children performed regularly since 1994/95 under the responsibility of the DAJ. In a subanalysis, the caries levels of examined children using a RCS (n = 4524) were compared to the results obtained using a FC sampling (n = 21934, 42.2% of all children in first grade).

**Results:** In the RCS sample, 53.4% of children were caries-free at the dmft level and 46.4% when initial lesions were included, while this corresponded to 49.4% and 43.5% in the FC data. In the RCS the mean dmft was 1.88 with single components of dt = 0.69, mt = 0.25, and ft = 0.94 as compared to 2.13 in the FC (dt = 0.78, mt = 0.29, and ft = 1.05), which represented a difference of 13% in the dmft value. 328 children (1.5%) in special schools (high caries risk children: dmft = 2.55) were not examined in the RCS sampling, but in the FC, therefore improving the oral health results in the RCS.

**Conclusion:** In general, the caries levels for all 6-7-year-old first graders using the RCS sampling were lower as compared to the FC data. Sampling bias was attributed to the lack of examining the required special schools, which gather schoolchildren who present with learning difficulties, physical disabilities or behavioural problems.

The study was funded by the Deutsche Arbeitsgemeinschaft für Jugendzahnpflege e.V. (DAJ) and was approved by the Ethics Committee of the University of Greifswald (BB48/10a).

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135

### Impact of the Educational Level on Caries Experience in 12-Year-Old Children in Germany

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The objective of this study was to evaluate the impact of the type of school on caries experience in 12-year-olds. **Methods:** The National German Oral Health Survey in Children performed regularly since 1994/95 under the responsibility of the German Association for Dental Prevention in Children and Adolescents (DAJ) allows a comparison of caries experience according to the type of attended school. In Germany, great variations exist in the school system. Therefore, data from one representative region (Saxony-Anhalt, Germany) with a regional mean DMFT very close to the national DMFT (0.5; IDZ 2016) and a high sample size (n = 6391) was chosen for analysis. The examination was performed by calibrated community dentists including the WHO criteria [2013] for the DMFT assessment. **Results:** 6391 12-year-old children in 6th grade were examined in Saxony-Anhalt, with a caries prevalence of 23.3%. The overall mean DMFT of this sample was 0.52 ( $\pm 1.23$ ) with single components of DT = 0.13  $\pm$  0.58, MT = 0.02  $\pm$  0.24, and FT = 0.36  $\pm$  0.95. In children attending "Gymnasium" (academic high school, n = 43.4%) the mean DMFT was the lowest with 0.29  $\pm$  0.85, followed by 0.33  $\pm$  0.87 DMFT in "Gesamtschule" (comprehensive school; n = 6.9%). In the "Sekundarschule" (high school, n = 44.2%) children presented a mean DMFT of 0.68  $\pm$  1.36, and in "Förderschule" (school for children with special needs, n = 5.6%) the mean DMFT was 1.18  $\pm$  2.2, which was about 4.1 times higher than in "Gymnasium". **Conclusion:** Despite the generally very low levels of caries prevalence and experience, still up to about 4-fold higher caries experience was observed with respect to the educational level of 12-year-olds. These results show that in order to reduce dental health inequality and to achieve further caries reductions in this age group, risk-based preventive efforts addressing children in risk schools are needed.

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### Cariology Education in Canadian Dental Schools: A Survey

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The aim of the survey was to document the Cariology curriculum for undergraduate dental students across Canadian Dental Schools. **Methods:** Ten academic members, one from each dental school, who supervise Cariology education were recruited. An adapted version of ORCA/ADEE Cariology curriculum group questionnaire was used. **Results:** Participants from all 10 dental schools completed and returned the questionnaire. In four schools Cariology and Restorative dentistry were taught by the same department. Five schools had didactic/laboratory courses focusing primarily on Cariology as well as a specific written curriculum for Cariology. In eight schools the didactic/laboratory education in Cariology was provided during the first two years of education. Six schools provided Cariology related hands-on workshops/laboratories before students started working with patients. In teaching Cariology, seven institutions included dental hard tissues defects (eg., abrasion, attrition) and dental erosion. The following caries detection methods were addressed in the Cariology curriculum: visual (10/10), tactile (9/10), ICDAS criteria (6/10), caries activity assessment (9/10), radiographic (10/10), other detection tools (e.g., fluorescence, 8/10). Seven schools charted caries lesions' activity in clinic. Four institutions use the 'watch' term for caries charting. While caries risk assessment was dealt with in the curriculum by all 10 schools, only one school applied this concept regularly in clinical practice. Clinical Cariology teaching is carried out mostly by part time clinical instructors (7/10) and faculty members (9/10). Faculty members' calibration for caries detection criteria was reported by only one school. The main concern reported by all institutions was the difficulty of implementing didactic knowledge into clinical training of dental students. **Conclusions:** Cariology curriculum concepts are well implemented in didactic education across Canadian Dental Schools. All Canadian Institutions lack appropriate integration of Cariology curriculum into clinical practice.

### Correlation Between the DMFT Score and The Concentration of Fluoride in Drinking Water from Macedonia

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The aim of the study was to determine the correlation between the DMFT score of 15-years old children and the concentration of fluorine in drinking water. The DMFT of 414 students (226 (54,6%) males and 188 (45,4%) females) from settlements (six different cities and fourteen different villages) in the Eastern region of the Republic of Macedonia, attending 7 secondary schools. Twenty water samples were taken in the study area to determine their fluoride concentration by electrochemical method using a special ion Analyser (pH/ISE meter-Thermo-Orion) with a F-specific electrode (Thermo Orion Ion Plus Fluoride Electrode) at the Institute of Public Health. The Spearman Correlation was used for statistical analysis. **Results:** The mean DMFT was 5.77, with standard deviation (SD) of 4.02. The maximum concentration of fluorine was determined in drinking water from village Star Karaorman (0,87 ppmF) and the minimum concentration (0.1 ppmF) from the village Grad. The correlation between the value of the DMFT score of the children from the Eastern region, and the concentration of fluoride in drinking water was  $r=-0.27$  ( $p < 0.01$ ). **Conclusion:** The correlation between the value of the DMFT score and the concentration of fluoride in drinking water was negative indicating an association between increased water F concentration and reduced caries score.

### Body Weight and Behavioural Factors on Dental Caries in Mexican Rural and Urban Adolescents

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The aim was to elucidate the role of bodyweight and behavioural factors on caries prevalence in two different adolescent populations, rural and urban, living in Mexico, the country with the highest childhood obesity rate in the world. **Methods:** Adolescents from the rural area of Tepanacan and the city of Veracruz were enrolled. Caries was recorded using the International Caries Detection and Assessment System (ICDAS) and the Body Mass Index was calculated (kg/m<sup>2</sup>) and classified as normal weight or overweight. Oral habits (toothbrushing, flossing, dental attendances) and dietary patterns (sweet intake) were assessed. A dummy variable between Body Mass Index and living area (BMI/Area) was generated. Data was analysed using STATA and a multinomial logistic regression model was run, using the International Caries Detection and Assessment System as dependent variable. **Results:** Four hundred sixty-four 12–15-year-old subjects participated. Body Mass Index and area of residence were significantly associated ( $\chi^2 = 12.59$   $p < 0.01$ ). Area was also associated to caries severity ( $\chi^2 = 24.23$   $p < 0.01$ ) with the highest number of caries in dentine recorded in the rural area. The dummy variable BMI/Area was related to caries severity ( $\chi^2 = 27.47$   $p < 0.01$ ): overweight adolescents with caries in dentine were most frequently found in rural area. A higher prevalence of caries in enamel and, conversely, a lower prevalence of caries in dentine ( $p < 0.01$ ) was recorded in adolescents from the urban area, where better oral habits, but higher sweet intake ( $p = 0.04$ ) were encountered. According to the multinomial logistic regression model, BMI/Area was significantly associated with caries severity ( $p < 0.01$ ). **Conclusions:** Body weight was not associated to caries prevalence in the overall population. Nevertheless, when BMI was associated to the area of residence, overweight became a statistically significant risk factor for caries severity in adolescents living in rural area.

### Longitudinal Associations between Dental Caries Incidence and Risk Factors in Late Childhood and Adolescence

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The aim was to assess longitudinal associations between permanent tooth caries incidence and modifiable risk factors. **Methods:** The Iowa Fluoride Study has followed a cohort of newborns from birth. Standardized dental caries exams without radiographs were conducted at about ages 5, 9, 13, and 17 years, with permanent tooth caries incidence (DFS) determined from 5–9, 9–13, and 13–17 years. Detailed questionnaires were sent bi-annually about fluoride exposures and intakes, dietary intakes (water and other sugar-free beverages, milk, sugar-sweetened beverages, and 100% juice), and toothbrushing frequency. Averages for the exposure variables were determined for ages 7.0, 7.5, 8.0, 8.5, and 9.0 years; 11.0, 11.5, 12.0, 12.5, and 13.0; and 15.0, 15.5, 16.0, 16.5, and 17.0. Generalized Linear Mixed Model analyses related period-specific caries increments to period-specific averaged exposures, subject age, sex, and mother's educational level ( $n = 392$ ). The Akaike Information Criterion (AIC) was used in model building to assess best penalized fit of predictors. **Results:** From age 5 to 9, 9 to 13, and 13–17, 22%, 32%, and 55% of subjects had positive DFS increments, respectively. Ten models had AIC values within 2 units of the lowest AIC model. Greater age, being female, lower maternal education, and lower brushing frequency were associated with higher caries incidence in all 10 models, while lower milk intake, lower juice intake, and lower income were in 7, 7, and 2 models, respectively. **Conclusions:** Being older, being female, lower mother's education, and lower brushing frequency were consistent risk factors for greater permanent tooth caries in longitudinal mixed model analyses.

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### Impact of Dental Caries Morbidity on Oral-Health Related Quality of Life in Children and Adolescents

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The aim of this study was to evaluate the impact of dental caries morbidity using the Caries Assessment Spectrum and Treatment (CAST) on oral-health related quality of life (OHRQoL) in children and adolescents from public schools of Patos-PB, Brazil. A cross-sectional study was carried out on a sample of 461 children aged 8–10 years and 454 preadolescents/adolescents aged 11–14 years. Cluster sampling was used to select the participating schools. Dental caries was registered by two calibrated examiners using the CAST instrument that was grouped in stages according to the maximum CAST code per subject as: 0–2 healthy, 3 premorbidity, 4–5 morbidity, 6–7 severe morbidity and 8 mortality. OHRQoL was measured using Portuguese versions of Child Perceptions Questionnaires (CPQ) for children (CPQ8–10) and preadolescents/adolescents (CPQ11–14). Univariate and multivariate logistic regression analyses were used to identify items associated with CPQ and global scores. Dental caries status for the children aged 8–10 years and preadolescents/adolescents aged 11–14 years were 14.8%/14.5% healthy, 18.9%/24.9% premorbidity, 40.8%/38.1% morbidity, 19.1%/16.1% severe morbidity and 6.5%/6.4% mortality, respectively. The mean CPQ8–10 and CPQ11–14 scores were  $15.4 \pm 11.2$  and  $17.5 \pm 8.9$ , respectively. Factors associated with higher CPQ8–10 scores in the multivariate regression analysis after adjustments were age (9 years; PR = 1.24, and 10 years; PR = 1.32) and dental caries status (morbidity; PR = 1.23, and severe morbidity; PR = 1.30) ( $p < 0.05$ ). Factors associated with higher CPQ11–14 scores were dental caries status (morbidity; PR = 1.20) ( $p < 0.05$ ). In conclusion, dental caries morbidity was associated with a negative impact on OHRQoL in children and adolescents in this Brazilian population.

### Caries Increment and Oral Hygiene Changes in 6 and 12 Years Old Children in Riga, Latvia. A Three Year Follow Up Report

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This study investigated the caries increment and oral hygiene changes in 6 and 12 years old children in Riga, Latvia over a 3-year period. **Methods:** Thirty-eight 6 and thirty-nine 12 years old children were examined visually and with bitewing radiographs for dental caries at baseline and after 3 years. Decayed, missing, and filled surfaces (dmfs/DMFS) in all teeth were scored by one calibrated examiner using the Radike's caries scoring criteria. Oral hygiene level was determined using Green-Vermillion index (G-Vind.) at baseline and after 3 years. The data was analyzed using t-test and Wilcoxon test ( $\alpha = 0.05$ ). **Results:** The mean (SD) values of oral hygiene and caries experience at baseline/3-year period in 6- vs.12-year olds were as follows. G-Vind: 1.14 (0.80)/1.48 (0.89) [ $p = 0.4768$ ] vs. 0.99 (0.45)/1.45 (1.22) [ $p = 0.0337$ ]. DMFS: 0.72 (1.02)/3.13 (3.13) [ $p = 0.0000$ ] vs. 6.79 (5.14)/14.79 (9.86) [ $p = 0.0000$ ]; dmfs: 11.26 (8.71)/7.74 (4.86) [ $p = 0.0780$ ] vs. 3.57 (2.03)/1.5 (0.71) [ $p = 0.3173$ ]; all surfaces: 95.52 (8.21)/100.4 (9.14) [ $p = 0.0073$ ] vs. 118.92 (9.66)/125.23 (4.88) [ $p = 0.0003$ ]; all approximal: 42.68 (3.46)/44.47 (3.93) [ $p = 0.015$ ] vs. 52.31 (3.91)/54.87 (1.99) [ $p = 0.0002$ ]; all occlusal: 10.16 (1.64)/11.11 (1.57) [ $p = 0.013$ ] vs. 14.26 (1.86)/15.49 (0.91) [ $p = 0.0000$ ]; all bucca/lingual: 42.68 (3.46)/44.47 (3.93) [ $p = 0.0152$ ] vs. 52.31 (3.91)/54.87 (1.99) [ $p = 0.0002$ ]; bucca/lingual filled: 0.87 (2.35)/1.32 (2.12) [ $p = 0.0264$ ] vs. 0.72 (1.08)/1.79 (2.11) [ $p = 0.0008$ ]. Approximal decayed: 4.08 (3.48)/2.32 (2.37) [ $p = 0.0020$ ] only in 6-year olds. Approximal filled: 0.87 (1.54)/2.18 (2.30) [ $p = 0.0004$ ]; occlusal filled: 2.34 (1.98)/4.13 (2.90) [ $p = 0.0000$ ]; bucca/lingual decayed: 0.69 (1.45)/0.26 (0.64) [ $p = 0.0089$ ] only in 12 year olds. **Conclusions:** The result of the present study suggests that the increased caries experience within 3 years among the 6 and 12 years olds in Riga may be due to the concurrent decreased level of oral hygiene.

### Change in Caries Knowledge of Parents of 6–8-Month-Olds Involved in Punctually Applied Caries Prevention in Moscow: 1 Year Results

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As caries prevalence in 3-yr-olds in Moscow is moderately high, punctually applied prevention strategies at the earliest age have been initiated in one district in Moscow since 2015. According to previous interviewing, toddlers' parents required oral health education. The aim of this study was to describe change of knowledge of parents of young children during first year of the program. **Methods:** In 2015 and 2016, 55 children and 60 children, respectively, aged 6–8-months-old were involved in the program, where children/parents came to the clinic at certain dental ages. Parents received intensive dental education and training in toothbrushing relating to their child's tooth status. Parents were interviewed about their child's oral health and feeding habits at baseline and after 1 year. Plaque accumulation and caries were recorded. Chi-Square-test was used to discover difference in answers at baseline and after 1 year. **Results:** From 2015–2016, 117 out of 232 children (50%) dropped out of the study. After 1 year 50% of children had visible plaque; 4% had initial caries lesions. The number of parents brushing their child's teeth every day increased from 15% to 100%. Eighty-six percent of parents brushed their child's teeth twice a day; 72% chose toothbrushes according to dentist's advice. Tooth-paste use increased from 9% to 100%. The number of parents using bottle feeding for child decreased from 67% to 14% ( $p < 0.05$ ); night feeding decreased from 88% to 69% ( $p < 0.05$ ). After 1 year no parents used sugar-containing formula; 72% didn't breastfeed. The number of children fed more than 8 times per day decreased from 48% to 14% ( $p < 0.05$ ) and none had feeding problems. **Conclusion:** The study showed significant improvement in parents' oral health knowledge after the 1 year of program.

### Long-Term Follow-Up of Inactive Occlusal Caries Lesions: 4–5-Year Results

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The aims of this prospective cohort study were: (1) to assess the clinical behavior of inactive caries lesion on the occlusal surfaces of permanent molars over 4–5 years and (2) to estimate the risk of caries progression in these surfaces compared with sound occlusal sites. Clinical examinations were conducted at baseline ( $n = 258$ ) and after 4–5 years and included the recording of dental plaque at the occlusal surfaces, tooth cleaning, air drying, detection of caries lesions and recording of the eruption stage of permanent molars. Lesion activity was assessed according to clinical characteristics of surface color and texture. Logistic regression models with generalized estimating equations were used to estimate the risk of caries incidence and progression over the study period. After 4–5 years, a total of 193 schoolchildren were followed (response rate of 74.8%), totaling 1,152 teeth [554 (48.1%) sound molars and 598 (51.9%) molars with inactive occlusal enamel lesions]. According to the criterion of activity, 30.6% ( $n = 59$ ) of children presented at least one molar with active lesion, filled or extracted; inactive lesions were around at a 2-fold increased risk for caries progression than sound surfaces ( $OR = 2.34$  95%CI = 1.51–3.62). According to the criterion of severity, 13% ( $n = 25$ ) of children presented at least one molar progressing to dentine cavity, filling or extraction; inactive caries lesions also presented a significantly higher risk for caries progression when compared with sound surfaces ( $OR = 2.69$ , 95% CI = 1.50–4.83). In conclusion, this longitudinal study showed that the vast majority of lesions (85–90%) identified as inactive enamel caries at baseline have not progressed over 4–5 years. Despite this fact, it was possible to detect an increased risk for caries progression in caries-inactive occlusal sites compared with the sound ones.

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### Association between Overweight/Obesity and Coronal and Roots Caries in Adults from South Brazil

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The aim was to assess the association between overweight/obesity and coronal and root caries in adults. **Methods:** This cross-sectional study used a multistage sampling strategy to draw a representative sample of individuals aged  $\geq 35$  years from Porto Alegre, South Brazil. A total of 1,002 individuals were included in the study. Questionnaires recorded gender, age, educational status, tooth brushing frequency, interproximal cleaning frequency, and access to dental services. Oral examination included gingivitis, gingival recession, and coronal and root caries. Anthropometric measures (weight and height) were collected to calculate the body mass index. Negative binomial regression and Poisson regression were used to assess the relationship of weight status with coronal DMFT and root caries index, respectively. **Results:** Prevalence of overweight and obesity were 39.80% (95%CI = 35.18–44.41) and 31.33% (95%CI = 26.50–36.16), respectively. The coronal DMFT was 18.68 (95%CI = 18.24–19.13) and the root caries index was 11.03% (95%CI = 9.84–12.21). No significant differences in coronal and root caries were found among categories of weight status. After adjustment for potential confounders, weight status was not associated with coronal [overweight (IRR = 0.99, 95%CI = 0.94–1.03); obesity (IRR = 0.98, 95%CI = 0.92–1.05)] and root caries [overweight (PR = 0.93, 95%CI = 0.67–1.29); obesity (PR = 0.88, 95%CI = 0.59–1.32)]. **Conclusion:** Overweight and obesity should not be regarded as risk indicators for coronal and root caries in adults from South Brazil.

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### Polish School Nurses' Knowledge of Oral Health: An Initial Report

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Polish school nurses duties include, among others, maintaining oral health. The aim of the study was to describe the baseline knowledge of oral health presented by Polish school nurses. A cross sectional study with the use of a self-administered questionnaire was conducted on randomly selected school nurses working in Polish provinces. The questionnaire included education, rural, urban place of work, self-assessment of knowledge regarding dental caries, providing supervised toothbrushing, cooperation with a dentist, knowledge of the causes of dental caries, products promoting dental caries. Data were analyzed with the Kruskal Wallis and Chi square tests with the level of statistical significance at  $p < 0.05$ . The final sample consisted of 165 school nurses, 72.7% had secondary education, 42.4% worked in small towns, 78.8% performed supervised toothbrushing, and 81.2% declared that their knowledge regarding dental caries and prevention of dental caries was sufficient. Supervised toothbrushing, chats with pupils about the prevention of dental caries took place more often in larger agglomerations ( $p = 0.021$ ,  $p = 0.033$ ); significantly more pupils from larger towns reported oral health problems to school nurses ( $p = 0.025$ ), nurses working in rural areas or small towns statistically more often ( $p = 0.023$ ) gave oral hygiene instructions. School nurses who have been working over twenty years 23.55 (25.00) years agreed that carbohydrates contributed to the development of dental caries ( $p = 0.001$ ), and disagreed that infrequent dental visits ( $p = 0.047$ ) and the lack of fluoride prevention ( $p = 0.024$ ) were responsible for dental caries. Although the nurses believed their knowledge of dental caries and prevention of dental caries to be sufficient, the results suggest the need of further education.

## Session 7 De- Re-mineralization

146

### Caries-Preventive Effect of High Fluoride Varnishes on Artificial Dentine Caries Assessed With pH-Cycling

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The aim of this study was to compare the caries preventive effect of different high fluoride varnishes on artificial dentine caries-like lesions. Bovine dentine specimens ( $n = 220$ ) with one artificial caries lesion (mean baseline mineral loss (95% CI): 2,365 (2,298;2,432) vol% $\times\mu\text{m}$ ) were prepared and randomly allocated to eleven groups. Interventions before pH-cycling were as followed: application of a varnish containing sodium fluoride (22.600 ppm F<sup>-</sup>; positive control; Duraphat [NaF]); NaF + tricalcium phosphate (22.600 ppm F<sup>-</sup>; Clinpro White Varnish Mint [TCP]); NaF + casein phosphopeptide-stabilized amorphous calcium phosphate complexes (CPP-ACP) (22.600 ppm F<sup>-</sup>; MI Varnish [CPP]); silver diamine fluoride (35.400 ppm F<sup>-</sup>; Cariestop 30% [SDF]) and no intervention (negative control [N]). During pH-cycling (14 days, 6 $\times$ 120 min demineralization/day) half of the specimens in each group were brushed (10 s; 2x/d) with either fluoride-free (0) or 1,100 ppm F<sup>-</sup> NaF (1) dentifrice slurry (named e.g.:TCP<sub>0</sub>, TCP<sub>1</sub>). In another subgroup specimens were pH-cycled but not brushed (N<sub>NB</sub>). Differences in integrated mineral loss ( $\Delta\Delta Z$ ), lesion depth ( $\Delta LD$ ) were calculated between values after pre-demineralization and after pH-cycling using transversal microradiography and photographic images. After pH-cycling N<sub>NB</sub>, N<sub>0</sub> and N<sub>1</sub> showed significantly increased  $\Delta Z/LD$  values, indicating further demineralization ( $p < 0.001$ ; two-tailed paired t-test). Contrastingly, CPP<sub>0</sub>, CPP<sub>1</sub>, SDF<sub>0</sub> and SDF<sub>1</sub> showed significantly decreased  $\Delta Z/LD$  values, indicating remineralization ( $p \leq 0.004$ ; two-tailed paired t-test). CPP<sub>0</sub>, CPP<sub>1</sub>, SDF<sub>0</sub> and SDF<sub>1</sub> [ $\Delta\Delta Z$ : CPP<sub>0</sub>: 747 (271;1,223); CPP<sub>1</sub>:815 (457;1,173); SDF<sub>0</sub>: 1,031 (750;1,312); SDF<sub>1</sub>: 1,153 (847; 1,460)] showed significantly higher changes in  $\Delta\Delta Z/\Delta LD$  than N<sub>NB</sub>, N<sub>0</sub>, N<sub>1</sub> and NaF<sub>0</sub> [ $\Delta\Delta Z$ : N<sub>NB</sub>: -1,284-(1,567;-1,000); N<sub>0</sub>:-1,214-(1,555;-874); N<sub>1</sub>:-978-

(1,462;-494); NaF<sub>0</sub>: -104-(437;229)]. In conclusion, under the conditions chosen, all fluoride varnishes hampered further demineralization. However, only the application of CPP and SDF could remineralize artificial dentine caries-like lesions under net-demineralizing conditions.

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147

### Effect of Remineralizing Gels on Color, Microhardness and Wear of Bleached Dental Enamel

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The aim of this study was to evaluate the effect of remineralizing gels on color, microhardness reduction and wear of bleached enamel. **Methods:** 240 bovine enamel samples were prepared. The baseline color, microhardness and surface profile were evaluated. Samples were assigned into six groups ( $n = 40$ ): Group NC (negative control) – no treatment; Group PC (positive control) – bleaching (BL) with 40% hydrogen peroxide gel (Opalescence boost-Ultradent) for 60 min; Group BL/Rs – After bleaching a calcium silicate/phosphate remineralizing gel (Rs-Regenerate Boosting Serum-Unilever) was applied; Group Rs/BL – Rs application was followed by BL procedure; Group Rs/CL/Rs – Rs application was followed by BL and Rs again; Group BL/SF – BL was followed by 2% sodium fluoride gel (SF) application. After treatments the microhardness and color evaluations were performed again. Then the specimens were subjected to 100,000 abrasive cycles in a brushing machine. Each group was divided into two subgroups according to the toothpaste used ( $n = 20$ ): Ct–Close Up toothpaste (Unilever); Rt-Regenerate toothpaste (Unilever). The final profiles of the enamel surface were evaluated and the wear calculated. The data were analyzed using one and two-way ANOVA and Tukey's test. **Results:** For color change (Delta E), non-

significant differences were observed among the bleached groups. Significant differences were observed for microhardness after treatments ( $p = 0.0001$ ): NC-( $323.28 \pm 10.96$ )<sup>a</sup>, PC-( $246.28 \pm 30.46$ )<sup>b</sup>, BL/Rs-( $264.06 \pm 28.54$ )<sup>c</sup>, Rs/BL-( $265.22 \pm 23.58$ )<sup>c</sup>, Rs/BL/Rs-( $263.55 \pm 18.66$ )<sup>c</sup>, BL/SF-( $269.10 \pm 25.66$ )<sup>c</sup>. In relation to wear the two-way ANOVA showed significant differences for remineralizing gels and toothpastes ( $p < 0.005$ ). For gels: NC-( $2.85 \pm 0.98$ )<sup>a</sup>, PC-( $3.36 \pm 0.84$ )<sup>b</sup>, BL/Rs-( $2.23 \pm 0.91$ )<sup>c</sup>, Rs/BL-( $2.13 \pm 0.85$ )<sup>c</sup>, Rs/BL/Rs-( $2.19 \pm 0.70$ )<sup>c</sup>, BL/F-( $2.12 \pm 0.70$ )<sup>c</sup>. For toothpastes: Rt-( $2.04 \pm 0.80$ )<sup>a</sup>, Ct-( $2.92 \pm 0.88$ )<sup>b</sup>. **Conclusions:** Bleaching reduced microhardness and increased the abrasive wear. The remineralizing gels produced a similar increasing of the bleached enamel microhardness and reduction of the wear, without interfering on color change.

148

### Synergistic Activity Between CPP-ACP and SnF<sub>2</sub> in a Polymicrobial Biofilm Model

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The anticariogenic activity of CPP-ACP and SnF<sub>2</sub> has been well-documented. SnF<sub>2</sub> has also been reported to demonstrate antibacterial activity. The aim of this study was to determine the activity of CPP-ACP with and without SnF<sub>2</sub> on lesion formation in sound enamel blocks which acted as substrata for the culture of a polymicrobial biofilm. **Methods:** Six representative supragingival bacterial species (*Actinomyces naeslundii*, *Fusobacterium nucleatum*, *Lactobacillus casei*, *Streptococcus mutans*, *Streptococcus sanguinis* and *Veillonella dispar*) were grown as biofilms on sound enamel blocks in a constant depth film fermenter using artificial saliva medium for 19 days. During the experiment, 1% sucrose was pulsed four times daily for 10 min. Three treatments were applied to the biofilms: SnF<sub>2</sub> (290 ppm F), 2% CPP-ACP and CPP-ACP/SnF<sub>2</sub>. Each treatment was pulsed twice daily for 10 min and was compared with a control. 12 enamel blocks with intact biofilms were removed on Days 6, 12 and 19 and assessed for mineral loss using transverse microradiography. Biofilm composition was determined using 16 s rRNA gene sequence analysis. Differences in mineral loss and microbial composition were analysed using Kruskal-Wallis and post-hoc Wilcoxon Rank sum tests. **Results:** Between days 6 and 19 the demineralisation rate in the control, based on regression analysis, was 225 vol%min.µm/day. CPP-ACP and SnF<sub>2</sub> both significantly reduced the demineralisation rate by 50% ( $p < 0.05$ ). CPP-ACP and SnF<sub>2</sub> combined reduced the rate by 72% and was significantly different compared with all other groups. At day 19, CPP-ACP/SnF<sub>2</sub> treatment showed the greatest changes in biofilm composition; with substantial increases in the acid-sensitive species *F. nucleatum* and *S. sanguinis*. In conclusion, CPP-ACP/SnF<sub>2</sub> treatment significantly reduced the demineralisation rate of enamel and modulated biofilm composition.

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149

### Erosive Potential of Green Smoothies In-Vitro with Specimens from Human Dental Enamel

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The objective of this in vitro-study was to determine the erosive potential of selected green smoothies using dental enamel specimens. Twenty-four caries-free extracted human front teeth were included in this study. The crowns were separated from the roots, polished with SiC and aluminium oxide discs of decreasing particle size, cut in half, fixed and embedded in 12-well plates with Venus (Heraeus Kulzer, Hanau, Germany) with an average exposed enamel area of 38 mm<sup>2</sup>. One half per tooth ( $n = 6$  per smoothie) was incubated for 18 h at 37°C with 4 ml of a green smoothie (Grüner Smoothie Rote Beete pH = 4.1, True fruits, pH = 4.0; Vegan to go, pH = 4.2, Saftig Smoothie, pH = 3.7); the second half (controls;  $n = 24$ ) were incubated with saline. Calcium released into the supernatants, was measured with kits (Randox, Laboratories, Krefeld, Germany), the amounts were corrected for calcium in the smoothies and expressed in mg/38 mm<sup>2</sup>. Surface roughness (Ra, Rq, Rz, Rmax, Rt,) was determined ( $n = 48$ ) profilometrically (Perthometer, Mahr, Göttingen, Germany), using the MarSurfX20 software. The enamel surfaces were also visually examined in a digital microscope (VHX, Keyence, Neu-Isenburg, Germany) at magnifications of x300. The following amounts of calcium were released (mean±SD, mg/38 mm<sup>2</sup>): Grüner Smoothie Rote Beete  $0.57 \pm 0.12$ , True fruits,  $0.38 \pm 0.11$ ; Vegan to go  $0.23 \pm 0.12$ ; Saftig Smoothie  $0.9 \pm 0.28$ . The amount of calcium released by Saftig Smoothie was statistically significantly higher ( $p < 0.001$ ; one-way ANOVA, Tukey post-hoc test). Significant differences in the values for surface roughness between controls and specimens exposed to smoothies were only found for Grüner Smoothie Rote Beete (paired t-test) where a slight decrease was observed (e.g.  $-0.77 \mu\text{m}$  for Rq). In conclusion, in-vitro-exposure to green smoothies for 18 h caused only a minor loss of dental enamel and minimal alterations in surface topography.

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### Comparative Evaluation of Remineralizing Efficacy of Biomimetic Self-Assembling Peptide on Artificially Induced Enamel Lesions

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The aim was to investigate the efficacy of biomimetic self-assembling peptide (P<sub>11-4</sub>) on enamel remineralization compared to casein phosphopeptide amorphous calcium phosphate (CPP-ACPF) and fluoride based delivery systems. 40 enamel specimens were divided randomly into four groups (n = 10) according to the remineralizing agent; G1: artificial saliva (control), G2: fluoride varnish (Bifluorid 10, VOCO, Germany), G3: CPP-ACPF varnish (MI Varnish, GC Corporation, Japan), G4: self-assembling peptide agent (CURODONT Repair, Credentis, Switzerland). To induce artificial enamel lesions, the specimens were immersed in a demineralizing solution for 96 hours. All products were applied according to manufacturer's instructions and specimens were stored in a daily renewed artificial saliva. The surface microhardness was assessed at baseline, after demineralization, 1-week and 4-weeks remineralization. The mean surface microhardness recovery percentage (SMHR%) was calculated. Statistical analysis was performed with IBM®-SPSS® (IBM Corporation, USA) Statistics Version 20 and the significance level was set at  $p \leq 0.05$ . Two-way ANOVA analysis for the effect of different variables on mean SMHR%. One-way ANOVA followed by Tukey's post-hoc test to compare between more than two groups. Student's t-test to compare between two groups. The highest SMHR% was found in self-assembling peptide ( $81.12 \pm 9.22$ ) followed by fluoride ( $47.72 \pm 8.85$ ) and CPP-ACPF ( $47.41 \pm 14.15$ ) with the lowest SMHR% in artificial saliva ( $7.53 \pm 4.50$ ) at  $p < 0.001$ . No statistically significant difference was found between fluoride and CPP-ACPF at  $p = 0.99$ . There was a statistically significant difference in SMHR% between 1-week ( $39.71 \pm 25.95$ ) and 4-weeks ( $52.18 \pm 28.76$ ) at  $p < 0.001$ . In conclusion, self-assembling peptide confers the highest remineralizing efficacy compared to fluoride and CPP-ACPF; showing a promising, non-invasive regeneration potential. Also, extended period of time helped to attain more benefits from the remineralizing regimens applied.

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### Remineralization Potential of Galla Chinensis, and Grape Seeds Extracts

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This study was carried out to evaluate remineralization potential of both Grape seeds extracts (GSE) and Galla Chinensis extracts (GCE). Furthermore evaluate if the addition of fluoride had a synergistic effect with (GSE) and (GCE). Artificial carious lesions were created on 60 sound human molars. The specimens were divided into six groups according to remineralizing agents used. G1, the specimens were immersed in artificial saliva (control), in G2 sodium fluoride 0.24% NaF. G3, 0.4% GCE. G4, 6.5% GSE. While in G5, 6 NaF+GCE and NaF+GSE respectively. The surface microhardness (SMH) and Environmental Scanning Microscope (ESEM) in each group was assessed prior to creation of artificial carious lesion, after creation of artificial carious lesion, after remineralization with 2, 4 weeks and after exposure to acidic challenge. Data was analyzed using ANOVA and Tukey test.

Demineralized enamel showed the lowest (SMH). After 2 weeks remineralization SMH were ranked as follows, NaF, GCE, GCE+NaF and GSE+NaF ( $238.88 \pm 14.32$ ,  $244.77 \pm 9.36$ ,  $246.72 \pm 11.29$ ,  $237.93 \pm 10.79$  HV) > GSE and control groups ( $220.81 \pm 13.55$ ,  $212.58 \pm 16.78$  HV). However after 4 weeks remineralization showed the following, NaF, GCE and GCE+NaF groups ( $261.44 \pm 15.90$ ,  $268.02 \pm 15.82$  and  $269.28 \pm 13.94$  HV) > GSE+NaF ( $249.74 \pm 10.36$  HV) > GSE = control group ( $230.54 \pm 13.54$  HV). After exposure to acidic challenge, SMH NaF, GCE, GCE+NaF groups were ( $244.01 \pm 17.49$ ,  $247.90 \pm 17.62$ ,  $255.37 \pm 14.19$  HV) > GSE+NaF group ( $220 \pm 10.92$  HV) > control and GSE group ( $168 \pm 14.19$ ,  $192.65 \pm 11.48$  HV). (ESEM) showed mineralized coating at variable degree according to remineralizing agents used. In conclusion, GCE has equal remineralization potential to fluoride based remineralizing agent. Addition of fluoride enhance the remineralization potential of GSE, meanwhile it did not affect the remineralization potential of GCE.

### Effects of Xylitol Varnishes on the Inhibition of Demineralization In Vitro

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The present study aims to evaluate the efficacy of a new varnish containing xylitol alone or combined with fluoride on the inhibition of bovine enamel demineralization in vitro. **Methods:** Bovine enamel blocks (4×4 mm, n = 120) were randomly allocated to 4 groups (n = 30/group) and surface microhardness was measured at baseline. The blocks were then treated with the following varnishes: 20% xylitol; 20% xylitol plus F (5% NaF); Duraphat<sup>TM</sup> (5% NaF, positive control) and placebo (no-F/xylitol; negative control). The varnishes were applied in a thin layer and removed after 6 h of immersion in artificial saliva. The blocks were then subjected to pH-cycles (demineralization- 2 h/ remineralization- 22 h per day, during 8 days) and finally surface and cross sectional hardness were measured to calculate the percentage of surface hardness loss (%SH) and integrated loss of subsurface hardness ( $\Delta$ KHN). Data were statistically analyzed by Kruskal-Wallis and Tukey tests (p < 0.05). The surface hardness loss (% median / CI) was significantly decreased by 20% xylitol plus F (17.7 (CI 13.5/27.0)), Duraphat<sup>TM</sup> (21.7 (IC 16.9/30.6)), and 20% xylitol alone (26.5 (CI 21.7/30.4)) compared to placebo (35.5 (CI 30.5/39.6)). Besides, 20% xylitol plus F performed significantly better than 20% xylitol alone. Experimental and commercial varnishes also led to significantly lower subsurface demineralization when compared to placebo, and did not differ from each other. **Conclusion:** xylitol varnishes alone or combined with fluoride seem to be promising alternatives to prevent enamel demineralization, which should be confirmed by clinical studies.

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### Minimum Gap Size and Dentin Wall Lesion Development Next to Composite in a Biofilm Model

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This in vitro study investigated the dentin wall caries lesion development next to resin composite in small gap sizes and evaluated whether a relevant threshold for the gap size can be established using an in vitro biofilm model. Microcosm biofilms originated from human saliva were grown for 14 days within interfacial small gaps between dentin-resin composite discs under intermittent cariogenic challenge. The factor under study was the small gap condi-

tion: placement of composite resin without adhesive procedure with intentional gaps of 30, 60, or 90  $\mu$ m, placement without adhesive procedure (no intentional gap; NB), and placement with complete adhesive procedure (no gap; B). The secondary caries wall lesion progression was measured in lesion depth (LD) and mineral loss (ML) using transversal wavelength independent microradiography (T-WIM) at three locations: outer surface lesion (SL) and 200  $\mu$ m and 500  $\mu$ m distance from SL. Results from linear regression analysis showed that gap size influenced the secondary caries lesion progression at 200  $\mu$ m from SL (p < 0.001 – results are LD/ML; B [45.4  $\pm$  18.5/1026.6  $\pm$  288.2], NB [51.1  $\pm$  14.3 /1192.1  $\pm$  322.9], 30  $\mu$ m [81.5  $\pm$  24.5/1452.0  $\pm$  379.3], 60  $\mu$ m [118.7  $\pm$  36.0/2102.3  $\pm$  546.6] and 90  $\mu$ m [105.7  $\pm$  34.9/1879.3  $\pm$  704.4]). In general gap conditions did not affect the lesion progression at 500  $\mu$ m distances from SL (B [50.5  $\pm$  17.1/1038.4  $\pm$  333.4], NB [61.1  $\pm$  20.1/1305.8  $\pm$  307.2], 30  $\mu$ m [55.0  $\pm$  22.8/1309.8  $\pm$  457.7], 60  $\mu$ m [72.6  $\pm$  26.4/1509.1  $\pm$  539.1] and 90  $\mu$ m [60.6  $\pm$  25.1/1345.4  $\pm$  466.3]). In conclusion, considering the limitations of the present study, the results support the conclusion that the minimum gap size for secondary caries wall lesion developed in this microcosm biofilm model is around 30  $\mu$ m and secondary caries can develop in smaller gap sizes.

**Summary:** Even small gaps with width around 30  $\mu$ m can allow the development of secondary wall caries lesions under a controlled biofilm model.

### Simulated In Vitro Post-Eruptive Maturation of Sound Mature Bovine Enamel Through pH-Cycling

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This study aimed to simulate a proposed mechanism for post-eruptive maturation (PEM). A pH-cycling protocol was designed replicating the plaque/enamel interaction occurring during PEM. Polished bovine enamel blocks were randomly divided into groups (12 blocks/group) and pH-cycled (16–32 d) using sequential cycles of three 20 min acid challenges/day (50 mmol/l acetic acid, 4.1 mmol/l CaCl<sub>2</sub>·2H<sub>2</sub>O, 8.0 mmol/l KH<sub>2</sub>PO<sub>4</sub>, 130 mmol/l KCl; pH:4.90), plaque fluid proxy (20 mmol/l HEPES, 5.5 mmol/l CaCl<sub>2</sub>·2H<sub>2</sub>O, 9.4 mmol/l KH<sub>2</sub>PO<sub>4</sub>, 63 mmol/l KCl; pH:6.58) between acid challenges and overnight and two 2 min F<sup>-</sup> treatments/day (10/250 ppm F<sup>-</sup>) simulating the Freaching the enamel surface following toothbrushing at different eruption stages. Background-F<sup>-</sup> (0.2/1/2 ppm) was also added in the remineralising and demineralising solutions. Appropriate control groups incorporating all possible combinations of the experimental conditions were also studied. Three blocks and their respective solutions were analysed every 5 d for chemical, morphological and hardness changes (FAAS, ion-chromatography, FTIR, AFM and Vickers-hardness respectively). Following pH-cycling, deposits were observed on the surfaces of the enamel (AFM), in the form of acidic calcium-phosphates (e.g. OCP) and/or hydroxyapatite (HA) and fluoridat-

edhydroxyapatite (fHA) (confirmed through FTIR). Hardness increase was also observed, which plateaued towards the end of the experimental procedure. The increase ranged between  $0.30 \pm 0.03$  and  $0.49 \pm 0.02$  GPa (depending on the amount of  $F^-$  used and the experimental duration). This suggests dissolution of the upper enamel layers and subsequent reprecipitation, probably in the form of remineralised early carious lesions. Strong correlation (ANOVA,  $p < 0.05$ ) was observed between enamel's  $Ca^{2+}$ ,  $(PO_4)^{3-}$  and  $F^-$  uptake (FAAS and ion-chromatography respectively) and hardness increase. Surprisingly,  $Ca^{2+}$  and  $(PO_4)^{3-}$  uptake was observed under low-pH conditions. In conclusion, the observed  $F^-$  uptake, chemical changes and hardness increase of sound enamel, following pH-cycling under the conditions used, suggest that this model is appropriate for simulating PEM.

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155

### Antimicrobial Effect of the Incorporation of Silver Nanoparticles in Glass Ionomer Cement: An In Vitro Study

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To control secondary caries, incorporation of silver nanoparticles (NAg) has been tested in several materials, presenting antimicrobial effects. This study evaluated the effect of NAg incorporation in a glass ionomer cement (GIC) regarding the antimicrobial potential and inhibition of caries lesions adjacent to restorations. Control specimens had no NAg incorporated. Beyond, a commercial silver alloy-reinforced glass ionomer restorative material was tested. Bovine enamel slabs with cavity preparations were randomly filled by restorative materials and allocated into the groups: GIC (Riva Self Cure<sup>®</sup>-control), GICS (Riva Silver<sup>®</sup>), GICNAg0.5 (Riva Self Cure<sup>®</sup> + 0.5% NAg) and GICNAg1 (Riva Self Cure<sup>®</sup> + 1% NAg). Three slabs of each group were subjected to a 5-day *Streptococcus mutans* UA159 biofilm model and microbiological experiment was done in triplicate. Antimicrobial effect was assessed by CFU counts (colony forming units) and dry weight analyses. Enamel demineralization around restorations was determined by cross-sectional microhardness (CSMH) at 50  $\mu$ m and 100  $\mu$ m from the restoration margin. Delta S ( $\Delta$ S) parameter was calculated. Data were analyzed using one-way ANOVA for CFU and dry weight variables and two-way ANOVA was used for  $\Delta$ S data analysis considering the study factors treatment and distance, at a pre-determined significance level of 5%. No statistically significant differences were observed among tested groups in relation to mean of CFU/ml/mm<sup>2</sup>, which ranged from 6,855 to 7,016 ( $p = 0.644$ ), dry weight (2,414–3,848 mg/ml;  $p = 0.569$ ) and mineral loss of enamel ( $\Delta$ S) at 50  $\mu$ m (4420.31–

8879.46) and at 100  $\mu$ m (5459.80–7499.98) ( $p = 0.241$ ). No significance was also found for distance factor ( $p = 0.777$ ). The NAg incorporation at tested concentrations in GIC showed no significant differences when compared to conventional GIC and to GICS in relation to the antimicrobial activity and to the effect on demineralization inhibition around restorations.

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156

### In Vitro Anticaries Potential of Chlorhexidine-Containing Pit and Fissures Sealants

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This study evaluated the effect of the incorporation of chlorhexidine digluconate (CD) on anticaries properties of a resin-based sealant (RS) and a glass ionomer sealant (GIS). Bovine enamel slabs with cavity preparations were restored using the restorative material as follows: RS (Bioseal<sup>®</sup>-control group), RSCD (Bioseal<sup>®</sup> + 0.5% CD), GIS (Riva Protect<sup>®</sup>) and GISCD (Riva Protect<sup>®</sup> + 0.5% DC). An *S. mutans* UA159 biofilm model was used for caries induction and the antimicrobial effect was determined per colony-forming units (CFU), dry weight analysis and confocal microscopy. Enamel demineralization around sealants was determined by cross-sectional microhardness (CSMH) at 50  $\mu$ m and 100  $\mu$ m from the restoration margin. In all groups, surface roughness was measured using a surface profilometer before and after being submitted to the biofilm model. Two-way ANOVA and Tukey tests were used for analyzing the data. Resin (R) groups presented lower CFU counts (6,461 CFU/ml/mm<sup>2</sup>) and dry weight (2,027 mg/ml) of biofilm compared to glass ionomer (GI) groups (6,927 CFU/ml/mm<sup>2</sup> and 2,513 mg/ml). At 50  $\mu$ m, lower demineralization (DZ = 6,679.66) was observed for GI groups compared to R groups, (DZ = 2,016.71;  $p < 0.001$ ), as well as between groups containing CD (DZ = 11,045.95) and without CD (DZ = 15,794.43;  $p = 0.026$ ). At 100- $\mu$ m distance, GI showed a lower demineralization (DZ = 6,740.26) compared to R (DZ = 18,425.75;  $p < 0.001$ ). The CD incorporation did not affect roughness regardless the tested material even before or after cariogenic challenge, however, R groups presented always less surface roughness (Ra-before = 0.214; Ra-after = 0.202) than GI groups (Ra-before = 0.348; Ra-after = 0.801). Confocal microscopy showed that mean biomass thickness and coefficient of homogeneity of dead and live cells was higher in GI groups when compared to R groups. Concluding, ionomeric sealants presented greater anticaries effect, however CD incorporation seems to be more effective when carried out in resinous materials.

### The Impact of the Stannous Ion on In Vitro Enamel Caries Lesion De/Remineralization

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The aim was to build on a previous study and investigate the impact of the stannous ion in the absence or presence of fluoride on enamel caries lesion de/remineralization under plaque fluid like (PF) conditions in vitro. The study followed a 3 ([Sn] 0/0.05/0.5 mM) × 2 ([F] 0/10.5 μM) × 4 (pH 4.0/5.0/6.0/7.0) factorial design. Early caries lesions were created in human enamel specimens (24 h; 50 mM lactic acid; 0.2% Carbopol 907; 50% saturated with respect to hydroxyapatite; pH5.0). Lesions were assigned to 24 treatment groups based on Knoop surface microhardness and exposed for 24 h to a PF solution (30 mM lactic acid; 4.1 mM CaCl<sub>2</sub>; 8.0 mM KH<sub>2</sub>PO<sub>4</sub>; 63 mM KCl; 0.2% Carbopol907; 1.5 mM Na gluconate. This solution was adjusted for [Sn], [F], and pH for each treatment group. Hardness was measured again before lesions were demineralized for 8 h (50 mM acetic acid; 2.25 mM CaCl<sub>2</sub>; 1.35 mM KH<sub>2</sub>PO<sub>4</sub>; 130 mM KCl, 0.2% Carbopol 907, pH5.0). Hardness was measured and percent surface microhardness recovery (%SMHr) and acid resistance (%AR) calculated. Data were analyzed using three way ANOVA. The [Sn]×[F]×pH interaction was significant for both %SMHr (p < 0.001) and %AR (p = 0.016). Both variables were most affected by changes in pH with increasing values resulting in greater %SMHr and %AR. Sn prevented further demineralization in a dose response manner, but retarded remineralization. Fluoride enhanced remineralization, mitigated the effect of Sn at higher pH values and provided additional protection when present with [Sn]. %AR were similar to %SMHr data at pH4.0. At higher pH values, data were less clear, but generally favored Sn and/or F treatment groups. Sn affects enamel caries lesion de/remineralization in both presence and absence of fluoride and at all tested pH values.

This study was funded by GSK Consumer Healthcare.

### Efficacy of Mineral Containing Gel for Remineralization in MIH-Affected Incisors: A 3-Months Clinical Study

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The aim was to investigate the effect of calcium, phosphate, magnesium and xylitol containing R.O.C.S.<sup>®</sup> Medical Minerals gel on remineralization of anterior teeth affected by Molar-Incisor

Hypomineralization (MIH) using Light-Induced Fluorescence-LF. Twenty-six healthy 8–12-year-old children (mean±SD: 9.31 ± 1.35) (14 boys 12 girls) were selected according to different clinically diagnosed levels (white or creamy opacities-demarcated opacity) of MIH [Ghanim et al.: Eur Arch Paediatr Dent 2015 16:235–246]. The patients were instructed to use R.O.C.S.<sup>®</sup> for two times per day for three months. The efficacy of remineralizing agent on the remineralization of MIH was evaluated by DIAGNOdent pen measures at 1 week, 1 and 3 months. Repeated Measures Analysis of Variance and Bonferroni Multiple Comparisons Test were used for descriptive statistics. All patients (n = 117 lesions) showed enamel alterations in lower and upper incisors affected by MIH (83.2%). The mean of DIAGNOdent measurement of all patients at baseline was 6.40 ± 3.18 and 5.33 ± 3.20 after 3 months. There was a significant difference in the mean of fluorescence over the studied time (p = 0.044). Twice daily use of R.O.C.S.<sup>®</sup> gel had significant effect on remineralization of lower and upper anterior teeth with MIH lesions at various intervals of observations (p < 0.05). Application of twice daily use of R.O.C.S.<sup>®</sup> Medical Minerals gel for 3 months significantly decreased the severity of white or creamy opacities of MIH lesions. (p = 0.044). In conclusion the R.O.C.S.<sup>®</sup> Medical Minerals gel is highly beneficial for the remineralisation of non-cavitated MIH lesions. We observed favorable effect on the remineralization of MIH lesions in anterior teeth after 3 months.

### Effect of Various Topical Fluoride Treatments on Dentine During pH-Cycling

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This study examined the effect of various topical fluoride treatments on dentine using a demineralizing pH-cycling model. Forty pre-demineralized bovine dentine specimens were randomly allocated to 5 groups: SDF solution (35,400 ppm F), NaF varnish (22,600 ppm F), TiF<sub>4</sub> solution (9,180 ppm F), SnF<sub>2</sub> gel (1,000 ppm F) or no treatment. The topical fluoride treatments were applied once. The specimens were pH cycled for 3 weeks (week days: six cycles of 0.5 h demineralization and 2.5 h of remineralization, followed by 6 h of remineralization; weekends: continuous remineralization). Calcium uptake and loss in the re- and demineralization solutions were assessed daily. Significant differences were found in the cumulative calcium loss, cumulative calcium uptake and cumulative net calcium values (ANOVA, p < 0.001). Cumulative calcium loss: Inhibition of NaF and SnF<sub>2</sub> was constant during the experiment resulting in a cumulative effect of 18 and 16 %. The inhibition by TiF<sub>4</sub> and SDF gradually decreased, for TiF<sub>4</sub> from 80

to 40% resulting in a cumulative effect of 60% and for SDF from 60 to 4% resulting in a cumulative effect of 30%. Cumulative calcium uptake: Remarkably, the control specimens further demineralised during the remin periods. All fluoride treatments inhibited this process but only after treatments with NaF and SDF the specimens remineralized. SDF treatment showed the highest cumulative calcium uptake (9,85  $\mu$ moles) with NaF being 72% less effective. Cumulative net calcium values: All treatments showed cumulative net calcium loss but SDF resulted in a reduction of 80%, TiF<sub>4</sub> of 60%, NaF of 40% and SnF<sub>2</sub> of 20 %. In conclusion, all topical fluoride treatments protected the dentine lesions against further demineralization although the mechanisms differed between various compounds. SDF was the most effective followed by TiF<sub>4</sub>, NaF and thereafter SnF<sub>2</sub>.

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160

### **Abrasion of Initial Carious Lesions with a Powered Toothbrush: Impact of Remineralization and Brushing Duration**

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It is widely accepted that initial carious lesions can be remineralized; however, the effect of abrasion on surface integrity was seldom investigated. The aim of this in vitro study was therefore to evaluate whether initial caries lesions, either remineralized or not, can be abraded by powered toothbrushing. 140 bovine enamel samples with three reference surfaces and two test areas were prepared and randomly divided into three groups: One group was demineralized for 5 days to form initial carious lesions (demin), one group was remineralized after demineralization for 15 days (remin), one group was neither de- nor remineralized and served as control. After de-/remineralization one of the experimental areas was covered and served as internal control. Afterwards, half the samples of each group were brushed for 15 s, the other half for 60 s with powered toothbrushes (2.5N; elmex Kariesschutz (1400 ppm fluoride as aminefluoride, CP GABA GmbH, Hamburg, Germany)). Parameters under investigation were dimension of tissue loss (optical profilometry; median, min/max; statistical method: regression analysis) and descriptive surface characterisation. Demin showed highest tissue loss both after 15 s (8.8, -0.8/29.4  $\mu$ m) and 60 s (12.9,0.0/48.8  $\mu$ m). Tissue loss after 15 s and 60 s was significantly lower in control (-0.8, -1.3/-0.4  $\mu$ m and -0.8, -1.3/-0.3  $\mu$ m;  $p < 0.0001$ ) and in remin (-0.3, -0.9/4.3  $\mu$ m and -0.2, -1.2/6.0  $\mu$ m;  $p < 0.0001$ ) with no significant differences between each other. Brushing duration made no significant difference. Microscopy

showed no morphological changes in control, demin showed cavitations with sharp edges, while cavitations in remin appeared less deep and smooth. Brushing of non-remineralized initial carious lesions with a powered toothbrush bears the risk of provoking distinct surface breakdowns; in contrast remineralizing such lesions has a marked protective effect. From a clinical perspective "fresh" initial caries lesions should be brushed cautiously to avoid surface breakdowns impeding remineralization.

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161

### **In-Vitro Comparative Analysis of the Efficacy of Fluoride Varnishes With and Without Added Calcium Phosphate**

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The aim of this study was to investigate the effect of added calcium and phosphate on the performance of fluoride varnishes and to explore the remineralization efficacy of different fluoride varnishes in vitro. Imaging was undertaken using a high resolution, desktop micro-computed tomography ( $\mu$ CT) system. A calibration equation was used to transform the grey level values of the images into true mineral density values. Lesion parameters including the mineral density and the thickness of the enamel lesion surface layer were extracted from mineral density profiles plotted in FIJI (W.S. Rasband, U. S. National Institutes of Health, Bethesda, Md, USA) and the visualized mineral maps using MATLAB software (MatLab R2012b 8.0.0.783, Mathworks, Natick, MA, USA). The nano-indentation mechanical testing was conducted using an Ultra Micro Indentation System (UMIS-2000, CSIRO, Australia), equipped with a three-sided Berkovich indenter tip calibrated on a fused silica standard sample of known properties. For the nano-indentation experiment, teeth with artificial lesions were sectioned horizontally to measure force-displacement response throughout the lesion using a 20 mN load. UMIS system software was used for the subsequent calculation of the elastic modulus and hardness of the samples. Statistical analysis was performed using statistical software GraphPad Prism (Graphpad Software, San Diego, CA) to test for differences between the means. The results of mineral density quantification and nano-indentation mechanical testing were analyzed by One-way analysis of variance (ANOVA). P-values less than 0.05 were considered to be statistically significant. The evaluation of the mineral content of demineralized specimens in both the study groups (Group 1: MI varnish with 5% Sodium fluoride and casein phosphopeptide-amorphous calcium phosphate; group 2: Clinpro<sup>TM</sup> White Varnish with 5% Sodium fluoride and tri-calcium phosphate; group 3: Duraphat with 5% Sodium fluoride; group 4: Duraphat Unidose with 5% Sodium fluoride) and the control group (No fluoride varnish) indicated that the application of the fluoride varnishes significantly decreased the progression of

the lesion and the demineralization process in enamel. Accordingly, the depth of the lesion was 18–34% lower in varnish treated specimens ( $67 \pm 7.03 \mu\text{m}$ ) compared to the control group ( $95 \pm 8.25 \mu\text{m}$ ). The mineral density of the lesion sections with no fluoride varnish treatment had an average of  $1.85 \text{ gr/cm}^3$  which was 25% lower than the corresponding value in varnish treated sections and 37% lower than sound enamel. However there was no significant difference between the preventive efficacy of varnishes containing added calcium and phosphate with varnishes containing sodium fluoride only ( $P > 0.05$ ). The results of mechanical testing and micro-CT mineral characterization of enamel lesions indicated that the addition of calcium and phosphate compositions does not enhance the remineralization activity of fluoride varnishes. Overall, the findings from this study highlight the role of fluoride varnish in the prevention of enamel demineralization. Addition of calcium and phosphate compositions does not seem to enhance or inhibit the prevention and remineralization performance of fluoride varnishes.

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162

### **Influence of Carolacton-Containing Composite Materials on the Development of Secondary Caries In Vitro**

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The present study evaluated the ability of Carolacton-containing composite [25  $\mu\text{g/ml}$ ] to inhibit caries formation adjacent to restoration margins using an artificial biofilm model. Within bovine enamel samples ( $n = 70$ ,  $8 \times 6 \times 3 \text{ mm}$ ) a standardized cavity ( $6 \times 3 \times 2 \text{ mm}$ ) was prepared. After an experimental pretreatment the samples were restored with various dental materials: Tetric Ceram (T), GrandioSo composite with (GC) and without Carolacton (G), Grandio flow with (FC) and without Carolacton (F), GrandioSo containing 30 wt% sodium fluoride (GNaF) and Ketac Fil (K). To create gaps next to restoration margins artificial aging was simulated by storing of samples in distilled water (7 d;  $37^\circ\text{C}$ ) and thermocycling (5000 cycles;  $\pm 5 / \pm 55^\circ\text{C}$ ). To initiate enamel caries lesions *Streptococcus mutans* (UA 159; approx.  $10^6$  cells/ sample) was grown on the samples for seven days. The gap size and the formation of enamel caries lesions next to restoration margins were analyzed using confocal laser scanning microscopy. Median gap size in enamel was 10.2 (interquartile range 7.2–13)  $\mu\text{m}$  and differed significantly only for K ( $p < 0.05$ ; Mann-Whitney test). FC [3408 (2318/ 4191)  $\mu\text{m}^2$ ] and F [3169 (2528/ 4126)  $\mu\text{m}^2$ ] showed

the greatest lesion areas. Compared with G [3149 (2698/ 3638)  $\mu\text{m}^2$ ] smaller lesion areas could be observed in GC [2709 (2218/ 4396)  $\mu\text{m}^2$ ] and T [2341 (1975/ 2959)  $\mu\text{m}^2$ ]. Significantly smaller lesion areas could only be observed in K [693 (502/ 769)  $\mu\text{m}^2$ ] and GNaF [103 (84/ 167)  $\mu\text{m}^2$ ] ( $p < 0.05$ ; Mann-Whitney test). It can be concluded that compared to conventional composite Carolacton-containing composite in the tested formulation could not significantly reduce the development of enamel caries lesions next to restoration margins. Only dental materials containing fluoride showed significant inhibition of secondary caries formation in the *S. mutans* biofilm model.

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163

### **Effect of TiF<sub>4</sub> Varnish on Remineralization of Enamel Caries Under Different Cariogenic Activities In Situ**

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This study aimed to compare the effect of TiF<sub>4</sub> and NaF varnishes on bovine enamel remineralization under different cariogenic activities in situ. Twenty volunteers (final  $n = 17$ ) participated of the in situ study with 3 crossover phases (14 days each), in which they wore palatal appliances containing demineralized bovine enamel samples (8 samples/appliance for each phase) treated with TiF<sub>4</sub>, NaF or placebo varnish. The samples were subjected to different cariogenic conditions (1. absence of biofilm accumulation and sucrose exposure; 2. presence of biofilm and absence of sucrose exposure; 3. presence of biofilm and 20% sucrose exposure 4x/day; 4. presence of biofilm and 20% sucrose exposure 8x/day) and exposure to fluoride dentifrice (2x/day). The mineral content and lesion depth were evaluated using transverse microradiography (TMR) and the data subjected to 2-way ANOVA/Bonferroni tests ( $p < 0.05$ ). The TiF<sub>4</sub> varnish significantly increased the remineralization of artificial carious lesions compared to placebo, regardless of the severity of the cariogenic activity in situ. On the other hand, the remineralizing effect of NaF was dependent on the cariogenic condition. For NaF, remineralization happened only in conditions 1 and 3 compared to control ( $p < 0.0001$ ). NaF was unable to prevent further demineralization under biofilm accumulation and sucrose exposure 8x/day. In the absence of fluoride treatment, demineralization occurred in all conditions, except in condition 1. Therefore, the TiF<sub>4</sub> varnish was the only treatment able to improve remineralization regardless of the cariogenic activity, while NaF failed in preventing further demineralization under high cariogenic activity in situ.

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**CaF<sub>2</sub> Acts as a Fluoride Reservoir in Test Plaques and Reduces Mineral Loss***D.F. Nóbrega\*, L.M.A. Tenuta, A.A. Del Bel Cury, J.A. Cury*

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The relevance of fluoride bound to bacteria or in the form of precipitated calcium fluoride (CaF<sub>2</sub>) in dental biofilm on enamel demineralization is unknown. In a crossover, double-blind, split-mouth, short-term in situ study we evaluated fluoride release from these two pools to biofilm fluid and the effect on enamel demineralization. Twelve volunteers wore palatal appliances containing enamel blocks with known surface hardness (SH), mounted in contact with *Streptococcus mutans* test-plaques, which have been pre-treated with PIPES buffer, pH 7, containing or not calcium and fluoride, resulting in groups containing: G1. no calcium or fluoride (negative control); G2. bacterially-bound calcium and fluoride (from 4 mM CaCl<sub>2</sub>, 0.5 mM NaF-containing PIPES); G3. particles

of CaF<sub>2</sub> (from powdered CaF<sub>2</sub>); and G4. both bacterially-bound calcium and fluoride and CaF<sub>2</sub>, at the same concentrations of G2 and G3. Volunteers used the appliance for 30 min, when half of the test-plaques were collected and baseline fluoride concentration in their fluid phase was determined using ion-specific electrode. Then, volunteers rinsed (1 min) with 20% sucrose solution (cariogenic challenge) and 45 min later, the other half of samples were used to determine plaque fluid fluoride and %SH Loss (%SHL) in the blocks. At baseline, test-plaque fluid fluoride concentration (μM) was highest in G3 and G4 (567.1 ± 83.1 and 717.0 ± 147.8, ANOVA,  $p > 0.05$ ), followed by G2 (55.2 ± 18.6) and G1 (18.4 ± 13.6) ( $p < 0.05$ ). Accordingly, G1 had the highest %SHL -(31.7 ± 9.6%), followed by G2 -(16.9 ± 7.7%), with no significant difference between G3 -(6.7 ± 3.7%) and G4 -(0.8 ± 2.3%; ANOVA,  $p > 0.05$ ). Only G3 and G4 maintained significantly higher fluid fluoride concentrations than G1 throughout the experiment (regression analysis,  $p < 0.05$ ). These results suggest that CaF<sub>2</sub> is able to maintain increased fluoride concentrations in the biofilm fluid phase, reducing enamel demineralization.

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## Session 8 Fluoride

165

### Fluoride Measurement in Toothpastes Using Acidic Phosphatase: Reproducibility of Results in a Three-Laboratory-Study

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Some years ago it was proposed to measure the fluoride content in toothpastes containing NaMFP by using Acidic Phosphatase (AcP) instead of hydrochloric acid (van Loveren et al. 2005). So far, no data are available about the reproducibility of this method when applied in different laboratories. The main aim of this study was to compare the reproducibility of F<sup>-</sup> measurements on toothpastes obtained using the AcP method in different laboratories. 19 different toothpastes were purchased in supermarkets in Manila, Philippines. All were labeled to contain fluoride (8 with NaF, 11 with NaMFP). Samples from these toothpastes were sent to the laboratories in the Food and Drugs Administration in Manila, the University of Heidelberg Dental School and ACTA Amsterdam, where the F<sup>-</sup> content was determined independently but following the same protocol. From each sample two grams were taken and diluted in two steps to a 1:60 dilution. Afterwards 4U of AcP was dissolved in NaOAc buffer (0.5 ml/trial; pH 4.8) and MFP-containing samples were digested for 24 h at room temperature. NaF samples were treated with NaOAc buffer following the same protocol. The F<sup>-</sup> concentration was measured with a F<sup>-</sup> Ion specific electrode.

In the NaF toothpastes, the mean F<sup>-</sup> concentration was 985 mg/kg (SD 355) in Manila, 1084 mg/kg (SD 400) in Heidelberg and

1068 mg/kg (SD 392) in Amsterdam. For the NaMFP toothpastes the respective values were 1002 mg/kg (SD 457), 1011 mg/kg (SD 482) and 989 mg/kg (SD 511). The results measured for NaF and NaMFP toothpastes in the three laboratories did not differ statistically significantly ( $p > 0.05$ , Kruskal-Wallis Test). This study shows that it is possible to obtain comparable results for the measurement of the F-concentration in MFP-containing toothpastes in different laboratories using the Acidic-Phosphatase Method.

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166

### A Systems Analysis of Fluoride Varnish Application in General Dental Practice in Scotland Using the Functional Resonance Analysis Method

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Under Scotland's child oral health improvement programme (Childsmile) programme dental practices delivering National Health Service (NHS) care are expected to apply sodium fluoride varnish to the teeth of all children at six-monthly intervals from the age of two years. Despite some recognised programme success this key preventive intervention is still variable. **Aim:** This organisational-level project aimed to study the work system around fluoride varnish application in the practice setting, to identify mechanisms and contextual factors likely to come into play in complex interventions to increase application rates. The methodology involved applying the Functional Resonance Analysis Method to synthesise multiple data sources, including: a longitudinal survey of GDPs ( $n = 1,090$ ; 65% follow up rate); in-depth interviews with practitioners and public health experts ( $n = 43$ ); 'World Café' (4) workshop activities with Childsmile staff ( $n = 56$ ); and NHS rou-

tine varnish claims data for 2015/16 (n = 86,726 claims at 807 Practices). Results Various crosslinked areas affecting varnish application were identified: priorities of care and preventive culture; practice management including time, space and use of the dental team; parental wishes and child compliance; risk and efficacy evidence; and issues with regards to the NHS remuneration system. Conclusion This study identified a number of interlinked organisation-level issues around fluoride varnish application in general dental practice. These have been developed into a randomised pilot and feasibility study which is now underway, involving an enhanced audit and feedback intervention.

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167

### Fluoride Concentration in Salt Marketed in Managua, Nicaragua

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The program of salt fluoridation in Nicaragua is supported by law and the minimal concentration established should be from 200 to 225 mg F/kg. Since there are not reports about fluoride concentration in salts marketed in this country, we evaluated whether the salts for direct human consumption marketed in Managua, Nicaragua are being properly fluoridated. Thirty-three packages of 11 salt brands (9 produced in Nicaragua and 2 imported) were purchased in the main supermarkets of Managua city. According to the package information: in 2 brands no fluoride addition was declared, in 8 brands a fluoride concentration ranging from 200 to 225 mg F/kg was declared and in one fluoridated brand the concentration was not declared. Six aliquots of each package were weighed ( $2.5 \text{ g} \pm 0.01$ ) and dissolved in the proportion of 0.025 g/mL of purified water. Duplicates of 1.0 mL of the salt solutions prepared were buffered with the same volume of TISAB II. Fluoride concentration in the mixture was determined with ion specific fluoride electrode, calibrated with standards solutions ranging from 0.25 to 16.0  $\mu\text{g F/mL}$ , which were mixed with TISAB II (1:1; v/v) containing 0.025 g NaCl/mL. Results were expressed in mg F/kg salt (w/w). In the two non-fluoridated brands only traces of fluoride were found. In the nine fluoridated brands, two presented only traces of fluoride (mean  $\pm$  SD; n = 3) ( $1.8 \pm 0.1$ ;  $18.6 \pm 13.1$ ), five brands showed fluoride concentration below the local regulation ( $131 \pm 34.3$ ;  $180.6 \pm 12.3$ ;  $184.6 \pm 34.8$ ;  $190 \pm 47.2$ ;  $199 \pm 18.9$ ) and two brands contained a fluoride concentration according to the Nicaraguan law ( $209.8 \pm 48$ ;  $211.4 \pm 26$ ). Considering the inconsistencies found in fluoride concentration and in labeling, the findings suggest that the surveillance system of salt fluoridation program in Nicaragua should be improved.

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168

### The Effect of Different Fluoride Containing Products on the Occlusion of Dentinal Tubules In Vitro

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The aim of this study was to assess the occlusion of dentinal tubules by different fluoride products used for hypersensitivity alleviation. **Method:** Twelve extracted single rooted human teeth were sectioned to provide 70 dentinal slabs with 10 slabs in each of seven groups to evaluate five commercial products used for treating tooth hypersensitivity: 1) fluoride slow-release device (FSRD), 2) fluoride toothpaste (1400 ppm F), 3) toothpaste) Strontium chloride hexahydrate 10% 1450 ppmF, 4) fluoride mouth wash 0.05 % NaF, 5) fluoride varnish 22,000 ppm F and two control groups: 6) artificial saliva and 7) distilled water. The total test period was 21 days and the slabs were stored in artificial saliva (except for the distilled water group) in an incubator at 37°C. Scanning Electron Microscopy (SEM) was used to visualise the precipitation layer and the occlusion of the dentinal tubules. Each slab was divided into three zones under SEM to assess different areas and the occlusion of the tubules was scored as either: no, partial or full occlusion. **Results:** SEM images showed there was obvious occlusion of the dentinal tubules for all groups that had application of fluoridated materials after 21 days. FSRD and toothpaste (1426 ppm Fluoride + 5% potassium nitrate) were significantly ( $p < 0.0001$ ) superior to the other fluoride groups. Both control groups did not show any occlusion of the dentinal tubules at all. **Conclusion:** Both the FSRD device and toothpaste 1426 ppm Fluoride + 5% potassium nitrate toothpaste resulted in the occlusion of exposed dentinal tubules, which could lead to an alleviation of hypersensitivity in vivo.

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169

### Hair: A Biomarker of Chronic/Sub-Chronic Fluoride Exposure

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Despite the suggested use of hair as a non-invasive biomarker of chronic/sub-chronic fluoride (F) exposure, it has not been fully investigated. This study aimed to investigate the correlations between F concentration in hair (FHair) and i) drinking water F (FWater) and ii) urinary F excretion (UFE) in children and adults. Healthy children aged 3–5 y and their parents aged >20 y were recruited in low-fluoride (LF) and high-fluoride (HF) areas of Nigeria. UFE was measured in voided urine collected over a 24-hr period and FHair was measured in hair swatches. Fluoride concentration of urine and water samples was measured directly using a

F-ion-selective electrode after adding TISAB and of hair samples using a standard HMDS-diffusion method. In total, 56 children and 56 adults provided valid samples: 28 children and 28 adults receiving LF water, mean (SD) = 0.04 (0.20) mg F/l, and the same number receiving HF water, 3.05 (1.11) mg F/l. Mean (SD) UFE for adults and children, respectively, was 0.140 (0.081) and 0.300 (0.143) mg/kg body weight/d in the HF area and 0.005 (0.003) and 0.004 (0.002) mg/kg body weight/d, respectively, in the LF area. Mean (SD) FHair was 5.691 (3.182) and 1.831 (1.091) µg/g for adults and children, respectively, in the HF area and 1.368 (1.062) and 0.743 (0.609) µg/g, respectively, in the LF area. A moderate statistically significant correlation (Pearson Correlation = 0.510,  $p < 0.001$ ) was found between FHair and FWater; whereas the corresponding correlation between FHair and UFE was weak (Pearson Correlation = 0.275,  $p < 0.005$ ). The statistically significant positive correlation between FHair and FWater indicates that hair can be used as a non-invasive biomarker of chronic/sub-chronic fluoride under conditions where local customs permit.

170

### Effect of Arginine-Containing Dentifrice on Plaque Composition and on Enamel Demineralization Under Distinct Cariogenic Conditions

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Clinical studies have suggested that arginine-containing fluoridated dentifrices (ArgFD) present better anti-caries effect than regular fluoridated dentifrice (FD). We aimed to test the effect of ArgFD on the inhibition of enamel demineralization and on the biochemical composition of dental plaque formed in situ under distinct cariogenic conditions. In a split-mouth design, fourteen adult subjects, who were instructed to use FD for at least 2 months prior to the beginning of this study, wore palatal appliances containing bovine enamel specimens (one pair at each side of the appliance) during 2 phases of 14 days each. During the first experimental phase, FD slurry was applied 3x/day and 20% sucrose solution was applied 4x and 8x/day by being dripped on the specimens. Subjects were then instructed to use ArgFD during 2 months wash-out period between the two experimental phases. During the second experimental phase, the same subjects applied ArgFD slurry and sucrose solution onto new subset of specimens. Percentage of surface hardness loss (%SHL), F and insoluble extracellular polysaccharide (IEPS) biofilm concentrations were determined and compared by Generalized Estimating Equations and Bonferroni test. Higher %SHL and IEPS (µg/mg) and lower F (µg/g) were found when plaque was formed in the presence of sucrose 8x/day (mean  $\pm$  se; %SHL: 40.2  $\pm$  2.5a; IEPS: 371.9  $\pm$  19.9a; F: 107.7  $\pm$  11.8a) compared to sucrose 4x/day (%SHL: 26.4  $\pm$  3.9b; IEPS: 234.8  $\pm$  39.7b; F: 199.6  $\pm$  39.4b). Lower IEPS concentration was found on plaque formed in the presence of ArgFD compared with FD (ArgFD: 243.0  $\pm$  23.7a and FD: 363.8  $\pm$  32.8b) ( $p = 0.002$ ), but

no difference on %SHL was found between ArgFD and FD (ArgFD: 37.4  $\pm$  3.3a and FD: 29.2  $\pm$  4.5a) both irrespective to sucrose concentration. Although reducing IEPS on dental plaque, ArgFD use presented similar anti-caries effect compared to regular FD use when plaque was exposed to sucrose 4x or 8x/day during 14 days.

171

### Mineral Ions Released from Experimental Toothpaste Containing S-PRG Filler Enhance Fluoride Retention in Oral Biofilm

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A three-way crossover trial using depth-specific analysis was conducted to estimate the enhancement of fluoride retention in oral biofilms when fluoride is applied at the same time as other mineral ions are released from experimental toothpaste containing surface pre-reacted glass-ionomer (S-PRG) filler. Eighteen healthy participants wore in situ plaque-generating devices (a pair of 4 mm<sup>2</sup> enamel slabs) on their upper molars, and biofilm was allowed to form for three days. A slurry of toothpaste containing S-PRG filler ( $\phi$  3 µm, 5 wt%) was filtered. This filtrate contained Al (340.5 ppm), B (447.7 ppm), Sr (985.2 ppm) and F (181.0 ppm). Devices were removed twice each day, immersed in the filtrate for one minute, and reinserted at the same location. Thirty minutes after the final immersion, devices were collected and samples were serially sectioned, with 2'2-µm sections taken first, followed by 4'4-µm sections for separation into outer, middle and inner layered fractions (300 µm thick). This procedure was repeated until the required thickness was obtained. Samples treated with filtrates containing F and Sr (PC) or F (NC) without S-PRG filler extract served as positive or negative controls. Fluoride and the three other mineral ions extracted from the thicker sections were quantified using a fluoride-selective electrode and ICP-atomic emission spectroscopy, respectively. The results were corrected for biomass volume estimated by area measurement of stained 2-µm sections. The mean uptake ratios of Al, B, Sr and F in the outer layer were 5.29, 4.56, 8.50 and 7.64 (S-PRG/NC), and 2.66, 3.15, 3.56 and 1.97 (PC/NC), respectively. These results suggest that toothpaste containing S-PRG filler promotes fluoride retention in oral biofilms, although toothpaste containing Sr and F accumulates Al and B, but not F.

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### Fluoride Content of Bottled Waters Available in South Korea

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The market for bottled water is increasing steadily in South Korea. Bottled water contains several mineral components such as calcium, magnesium, sodium, and fluoride from nature. Although fluoride is proven to be effective in preventing dental caries, the maximum permissible concentration of fluoride is 2 ppm for bottled water and 1.5 ppm for tap water in South Korea. Studies have shown that concentration exceeding the permissible amount may have detrimental effects on the human body. The aim of this study was to investigate the fluoride content of different bottled water commercially available in South Korea and compare it to the concentration written on the label. Twenty-seven of the 59 different bottled water produced in South Korea were investigated in this study. Three bottles of each brand were purchased from supermarkets, marts, and convenience stores in each region of Korea in August 2016. The fluoride content was measured three times using a fluoride-ion selective electrode (Orion ionplus Fluoride Electrode 9609, Orion Research, USA). The calibration curve was prepared using the 0.2, 2 ppm standard solution and confirmed with 1 ppm standard solution. The mean fluoride content of the bottled waters was  $0.37 \pm 0.33$  mg/L with a range from 0.04 to 1.17 mg/L. All tested bottled water indicated the fluoride content on the label. But eight brands showed different fluoride content with experimental data. The label of 10 brands showed the range of 0.3 mg/L or more from the minimum to the maximum fluoride content. This study investigated the fluoride content of bottled waters produced in South Korea and compared with the fluoride information on the label. Correct label information on fluoride content of bottled water is important to inform suitable fluoride exposure.

### Effect of Histidine and pH on Fluoride Reactivity with Carious Dentine

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We have previously shown in vitro that histidine increases the reactivity of fluoride with enamel, but it is not known whether the same would happen with dentine. Also, the effect of pH in the reactivity was not clarified. Bovine dentine blocks ( $n = 12$ /group) with artificial caries lesions were randomized and submitted to treatment with fluoridated solutions (226  $\mu$ g F/mL) presenting pHs 5.0; 5.5; 6.0 and 6.5. The solutions were buffered with 0.1 M histidine (groups 4, 6, 8 and 10) or the pH was only adjusted with HCl (groups 3, 5, 7 and 9). Purified water (group 1) and 0.1 M histidine solution

without pH adjustment (group 2) were used as controls. Loosely bound ( $\text{CaF}_2$ ) and firmly bound F (FA) formed in dentine were determined after 10 min of reaction of the blocks with the solutions. The concentrations of  $\text{CaF}_2$  and FA formed were analyzed by two-way ANOVA considering the factors histidine and pH effects. The comparisons among experimental groups and between the experimental and control groups were analyzed by Bonferroni's and Dunnett's tests, respectively. The concentration (mean $\pm$ SD;  $\mu$ g F/cm<sup>2</sup>) of  $\text{CaF}_2$  in groups 1 to 10 were:  $0.26 \pm 0.08$ ;  $0.45 \pm 0.12$ ;  $8.46 \pm 1.16$ ;  $13.87 \pm 3.61$ ;  $8.56 \pm 1.70$ ;  $14.35 \pm 4.07$ ;  $7.20 \pm 1.58$ ;  $13.61 \pm 1.67$ ;  $6.97 \pm 2.16$ ;  $12.36 \pm 3.36$  and FA:  $1.78 \pm 0.60$ ;  $1.34 \pm 0.41$ ;  $2.66 \pm 0.62$ ;  $2.90 \pm 0.73$ ;  $3.01 \pm 0.51$ ;  $3.25 \pm 0.89$ ;  $2.67 \pm 0.43$ ;  $3.38 \pm 0.67$ ;  $2.69 \pm 0.59$ ;  $2.77 \pm 0.51$ . All experimental groups differed from the controls for both  $\text{CaF}_2$  as FA. The effect of histidine was significant ( $p < 0.05$ ), with higher concentrations of  $\text{CaF}_2$  and FA in dentine, but the effect of the pH was not significant. The results suggest that the effect of histidine increasing fluoride reactivity with dentine with caries lesion is independent of the pH of the solutions tested.

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### Penetration and Clearance of Fluoride Is Higher in Polysaccharide-Rich Test Plaque

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Extracellular polysaccharides formed from sucrose increases the porosity of dental biofilm, enhancing its cariogenicity. However, besides the facilitated diffusion of sugars into this biofilm, anticaries agents such as fluoride may also easily diffuse. Therefore, the aim of this in situ study was to assess the concentration of fluoride in the fluid phase of a *Streptococcus mutans* test plaque grown in the presence of sucrose or its monosaccharides glucose and fructose (control). In a crossover, double blind study, six volunteers wore a palatal appliance containing a test plaque prepared from *S.mutans* Ingbritt 1600 grown in the presence of 0.525% glucose+0.525% fructose or 1% sucrose. After 30 min using the appliance in situ, samples of test plaque were collected for baseline fluoride determination. Then, volunteers rinsed for 1 min with purified water (control) or 0.05% NaF solution (225 ppm F) and samples of test plaque were collected after the rinse, and after 15 and 60 min. Fluoride was determined in the fluid phase of the test plaque with specific electrode adapted for microanalysis. At baseline, fluoride concentrations in the fluid phase of the test plaques grown in glucose+fructose or sucrose were  $0.17 \pm 0.04$  and  $0.20 \pm 0.09$  ppm F, respectively. Immediately after fluoride rinse, fluoride concentration was higher in the sucrose test plaque ( $28.8 \pm 6.8$  ppm F) when compared with the glucose+fructose test plaque ( $10.8 \pm 5.2$  ppm F) (ANOVA,  $p < 0.05$ ). Nevertheless, with time, fluoride concentration decreased linearly (regression analysis,  $p = 0.002$ ) in the sucrose test plaque, reaching  $12.8 \pm 10.1$  ppm F after 60 min, while in the glucose+fructose test plaque no significant linear decrease was found ( $p > 0.05$ ,  $7.0 \pm 4.4$  ppm F at 60 min). These data suggest that the penetration and clearance of fluoride are both enhanced in polysaccharide-rich plaques.

### Fluoride in Dental Biofilm and Saliva After Use of 1,500 and 5,000 ppm Fluoride (NaF) Mouth Rinses

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Toothpaste containing 5,000 ppm fluoride (F) is prescribed for individuals with abundant caries. Use of 5,000 ppm F toothpaste relies on the assumption that more fluoride is accumulated in the oral cavity than with conventional pastes ( $\leq 1,500$  ppm F). Measurements of the effects of exposure to 5,000 ppm F on fluoride accumulation in biofilm and saliva are lacking. The aim of this randomized double-blind crossover study in 12 individuals was to measure fluoride in saliva and in 7-day old biofilm fluid and solids after rinsing (10 ml, 2 min, 3x/day) for three weeks with 0, 1,500 or 5,000 ppm fluoride (NaF). Biofilm and saliva samples were collected in the morning on day 22 approximately 10 h after rinsing (background fluoride); participants then rinsed and 10, 30 and 60-minute-samples were collected. Samples were analyzed using a fluoride electrode adapted for microanalysis. Mean background fluoride concentrations were 8.1 and 22.3  $\mu\text{M}$  in saliva, 58.5 and 126.8  $\mu\text{M}$  in biofilm fluid and 4.8 and 10.9  $\mu\text{mol/g}$  in biofilm solids when using 1,500 and 5,000 ppm F, respectively. Background fluoride was statistically significantly higher for the 5,000 compared to the 1,500 ppm F group in all three compartments. Sixty minutes after rinsing, comparing 5,000 to 1,500 ppm F, accumulation of fluoride showed similar trends as the background data; saliva was approximately fourfold ( $\Delta\text{AUC}$  97.7 vs. 27.0  $\text{mM}\cdot\text{min}$ ) and biofilm fluid twofold ( $\Delta\text{AUC}$  293.9 vs. 130.9  $\text{mM}\cdot\text{min}$ ), although the fluoride concentration in biofilm solids barely changed ( $\Delta\text{AUC}$  763.0 vs. 712.6  $\mu\text{mol/g}\cdot\text{min}$ ). We conclude that increasing fluoride exposure from 1,500 to 5,000 ppm fluoride doubles the background fluoride concentrations in biofilm and saliva.

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### Fluoride Release from Toothpastes Sold in Brazil Simulating Brushing Time

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A fluoride toothpaste should release most of the fluoride from its formulation during toothbrushing, but this property has not been evaluated in toothpastes sold in Brazil. This study evaluated total soluble fluoride (TSF-R) and total fluoride (TF-R) release of 6 toothpastes ( $n = 3$ ): three  $\text{CaCO}_3/\text{MFP}$ -based (Sorriso Dentes Brancos, Colgate Anti-cáries and Close-Up Triple), and three

$\text{SiO}_2/\text{NaF}$ -based (Colgate Total 12 Clean Mint, Tandy and Close-Up Ação Profunda). Four grams of each toothpaste was weighed into plastic vials, and after adding 12 mL water (proportion 1:3), the suspensions were immediately stirred (200 rpm) for 1 minute using a mechanical agitator. After stirring, an aliquot of the suspension was collected for TF-R analysis. The remaining were centrifuged (3000 g, 10 min) and the supernatant were used for TSF-R analysis. All samples were diluted 1:10, and analyzed according to the protocol of the Laboratory of Oral Biochemistry at Piracicaba Dental School: samples were treated with 2 M HCl for 1 h at 45°C, neutralized with 1M NaOH, buffered with TISAB II, and F concentration was determined using ion specific electrode. Total soluble fluoride (TSF) concentration of each toothpaste was also determined. Data were expressed as percentage of TSF-R in relation to TSF found in the formulation (%TSF-R/TSF), and as percentage of TF-R in relation to total F declared by the manufacturer (%TF-R/TFdeclared). One way ANOVA followed by Tukey's test was used for statistical analyses ( $\alpha = 5\%$ ). The %TSF-R/TSF ranged from 24.9 ( $\pm 2.4$ ) to 62.3 ( $\pm 8.6$ ), and the %TF-R/TFdeclared from 27.9 ( $\pm 5.8$ ) to 68.0 ( $\pm 7.4$ ). The formulations  $\text{CaCO}_3/\text{MPF}$ -based showed higher values of %TSF-R/TSF and %TF-R/TFdeclared when compared to  $\text{SiO}_2/\text{NaF}$ -based ( $p < 0.05$ ). The findings suggest that Brazilian  $\text{CaCO}_3/\text{MPF}$ -based toothpastes are able to release more fluoride during toothbrushing than  $\text{SiO}_2/\text{NaF}$ -based.

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### Influence of the Amount of Dentifrice and Fluoride Concentration on Enamel Demineralization and Biofilm Fluoride Concentrations *In Situ*

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This study evaluated the effect of conventional (CD, 1100 ppm F) and low-fluoride (LFD, 550 ppm F) dentifrices, applied in different quantities, on enamel demineralization, and on fluoride (F) concentrations in the dental biofilm formed *in situ*. Five combinations of dentifrices and quantities were tested: placebo (P – F-free) applied on all bristles of the brush; LFD applied using the transversal technique (0.3 g – T1) or on all bristles (0.6 g – T2); and CD applied in a pea-sized amount (0.15 g – T3) or by the transversal technique (0.3 g – T4), in order to produce comparable intensities (F concentration in the dentifrice  $\times$  amount applied to the brush). Volunteers ( $n = 13$ , 20–36 years old) wore palatal devices containing 4 bovine enamel blocks (selected by surface hardness), and performed cariogenic challenges (30% sucrose solution) 6x/day, and brushing with assigned treatment combination 3x/day, following a double-blind, cross-over and randomized protocol. On the 8<sup>th</sup> day, biofilm was collected 5 and 60 min after brushing. The percentage of surface hardness loss

(%SH), integrated loss of subsurface hardness ( $\Delta$ KHN) and biofilm F concentrations (solid and fluid phases) were determined. Data were analyzed by repeated-measures ANOVA, Student-Newman-Keuls' test, and Pearson's correlation coefficient ( $p < 0.05$ ). The %SH of T3 was significantly higher than of T4, without significant differences for other comparisons. For  $\Delta$ KHN data, treatments with higher intensity (T2 and T4) promoted values significantly lower than those seen for the lower intensity (T1 and T3). Moreover, strong correlation was observed between  $\Delta$ KHN and F concentrations in the total biofilm ( $r = -0.71$ ) and biofilm fluid ( $r = -0.72$ ) 5 min after brushing, while a moderate correlation was observed between  $\Delta$ KHN and %SH ( $r = 0.60$ ). It was concluded that the treatment intensity has a significant influence on the development of caries lesions *in situ*.

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178

### Effect of Different Concentrations of Fluoride on Bovine Enamel Within a Controlled Biological Model

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The current study aimed to investigate the effects of different concentrations of fluoride on enamel within a controlled biological model containing plaque bacteria. Polished bovine enamel disks were placed within a dual constant depth film fermenter (CDFS) to model the conditions of the mouth. Both CDFSs were inoculated using pooled human saliva and supplemented with artificial saliva growth media (1 g/L Lab Lemco Powder, 2 g/L Yeast Extract, 5 g/L Protease Peptone, 2.5 g/L Mucin from porcine stomach, 0.2 g/L NaCl, 0.2 g/L KCl, 0.05 ppm F<sup>-</sup>) at a constant rate and sucrose (2%) pulsed in at regular intervals daily (30 minutes every 2 hours). One CDFS had 228 ppm Fluoride whilst the other used 1450 ppm Fluoride, both pulsed for 2 minutes at the start and end of the day. Enamel disks were removed on days 14 and 20 for analysis using Quantitative Light Induced Fluorescence (QLF-D), Surface Roughness and Transverse Microradiography (TMR). QLF-D results showed 228 ppm F<sup>-</sup> disks have less mineral loss than 1450 ppm at day 20 (48% vs. 56%  $\Delta$ F,  $p = 0.047$ ,  $n = 30$  (independent samples t-test)). Surface roughness values showed 1450 ppm had a greater effect than 228 ppm after 20 days with a significantly less surface roughness produced (0.2Ra vs. 2.02Ra,  $P < 0.001$ ,  $n = 30$ ). 228 ppm also had increased roughness between days 14 and 20 (0.72Ra to 1.29Ra,  $p = 0.009$ ,  $n = 26$ ) whereas 1450 ppm disks had reduced roughness (0.36Ra to 0.26Ra,  $p = 0.034$ ,  $n = 30$ ). TMR results showed 1450 ppm F<sup>-</sup> significantly reduced mineral loss compared with 228 ppm F<sup>-</sup> (166%.Vol. $\mu$ m vs. 618%.Vol. $\mu$ m,  $P < 0.001$ ,  $n = 41$ ) but is contradictory to the QLF-D results and requires further investigation. Initial results indicate that the higher concentra-

tion of fluoride is more effective at reducing mineral loss of enamel within a controlled biological environment modelling the mouth.

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179

### Knowledge of Polish School Nurses Regarding Caries Prevention by Topical Fluoride Treatment

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Polish school nurses provide NHS-funded caries prevention with topical fluoride gel for primary school children. The aim of the study was to assess the knowledge of school nurses regarding topical fluoride treatment. Materials and methods: School nurses were randomly selected from the internet database of the Central Statistical Office of Poland. They were asked to participate in a mail survey on topical fluoride treatment. Obtained data were statistically analyzed using the Kruskal Wallis test. **Results:** One hundred and sixty five school nurses responded to the survey, however, only 134 of them (study population) provided topical fluoride treatment. 96 nurses of the total number of 134 declared to have secondary education, 18 a Bachelor degree, and 15 a Master degree. 61 (45.5%) were never trained in caries prevention. 77 (57%) respondents assessed the efficiency of fluoride gel application to be sufficient and 32 (23.8%) considered it to be very effective. The general knowledge of fluoride toxicology was low with a mean of 6.29 points out of 12. The nurses with secondary education had statistically significantly lower knowledge (5.9) than the respondents with higher education: a Bachelor and Master degree, respectively: 7.29 and 7.6 ( $p < 0.01$ , Kruskal Wallis test). 41% of the study population were not familiar with the fluoride level in drinking water in their area. 35% did not know the fluoride toxic dose and 18% were not able to choose a correct fluoride concentration in fluoride gels. There were no differences in the level of knowledge depending on years of practice as school nurse, location of school and participation in training on caries prevention. **Conclusion:** Polish school nurses should be better prepared to provide the topical fluoride treatment.

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### Prevalence of Dental Caries and Level of Caries Risk Among COPD Patients

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Although chronic obstructive pulmonary disease (COPD) is a highly prevalent illness, few studies have evaluated its impact on oral health. The aim of this pilot study was to identify the dental caries status and evaluate caries risk among COPD patients. The study population consisted of 52–83-year old COPD patients ( $n = 52$ , mean age 67) stage B-D according to GOLD guidelines. Fifty-two healthy controls were matched for age, sex, and smoking status. Coronal and root surface caries was determined through clinical examination by one calibrated dentist using the ICDAS-II scoring criteria. Questionnaires and data from the patients' medical records provided information on demographics, medical history, medication usage, dietary history, oral hygiene habits, smoking status, and fluoride exposure. Saliva analyses included mutans streptococci and lactobacillus counts, buffer capacity and secretion rate. The Cariogram was used to evaluate the risk profiles. The two groups were compared statistically using Mann-Whitney-U test. When compared based on D12MFS (non-cavitated and cavitated), the coronal caries prevalence was significantly ( $p \leq 0.05$ ) higher in COPD patients ( $95.71 \pm 25.96$ ) than in their matched controls ( $84.02 \pm 26.27$ ). COPD patients had more untreated decayed surfaces (COPD:  $8.08 \pm 9.71$ ; controls:  $4.59 \pm 5.24$ ;  $p \leq 0.05$ ). Restored surfaces were found more frequently in controls (COPD:  $27.45 \pm 18.76$ ; controls:  $36.75 \pm 21.01$ ;  $p \leq 0.05$ ). The Root Caries Index values were higher in COPD patients (COPD:  $8.53 \pm 14.34\%$ ; controls:  $3.30 \pm 7.44\%$ ;  $p \leq 0.01$ ). The mean Cariogram chance of avoiding caries was significantly lower in COPD patients (COPD:  $44.52 \pm 22.96\%$ ; controls:  $57.92 \pm 17.72\%$ ;  $p \leq 0.01$ ). The results showed that COPD patients had higher prevalence of coronal and root caries and higher caries risk.

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### Drinking Habits in Nursing Homes for Elderly People in Denmark. An ACFF-Nordic Chapter Activity

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Based on a recommendation from WHO, one of the goals of the Alliance of Cavity-Free Future (ACFF) Nordic chapter is to increase the focus on sugar consumption in relation to caries in populations in the Nordic countries. This questionnaire-based study investigated the daily drinking habits in nursing homes (NH) for elderly people in Denmark. Ten municipalities (10%) across Denmark were selected. All the managers of 54 NH in the selected municipalities were approached and the purpose of the study explained. The following drinks were listed in the questionnaire: Milk; juice (apple); juice (orange); sugary fruited flavoured soft drinks (SSD, 50% sugar/litre); yellow fruit juice (YFR, sugar-free); carbonated soft drinks; protein drink; water; others. The day was divided into 7 periods: Breakfast, morning, lunch, afternoon, dinner, evening and night. For each of the 7 periods, the responders were asked to range the three most frequently consumed drinks as number 1, 2, or 3, respectively. Questionnaires were received from 29 NH (54%) covering all municipalities. 41% of the NH answered that milk was the most frequently consumed drink during breakfast, and 31% of the NH answered SSD. At night, water was the most frequently consumed drink in 59% of the NH and SSD was the most frequently consumed drink in 21% of the NH. More than 50% of NH answered that SSD was the most frequently consumed drink in the other periods. 7 NH had a restricted policy concerning serving drinks containing sugar. **Conclusion:** SSD was the drink most often consumed during the day in the majority of the selected NH, being an unnecessary caries risk.

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## Session 9 Epidemiology

182

### Sugar Consumption and Obesity Associated with “Burden of Chronic Oral Diseases” in Adolescents

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The objective of the present study was to estimate the association between added sugar intake in the latent variable “burden of chronic oral diseases” in adolescents. This cross-sectional study was carried out in a representative sample of public school students (17–18 years old) from São Luís, Maranhão, Brazil (n = 400). The study considered as hypotheses: 1) Chronic oral diseases in adolescence are correlated, that would form a latent variable “burden of chronic oral diseases”; 2) Added sugars would be linked to this latent variable. The explanatory variable was the frequency of sugar intake (soda, candies, cookies, chocolate). The outcome was “load of chronic oral diseases” analyzed as latent variable formed by: number of teeth with probing depth  $\geq 4$  mm, number of teeth with bleeding on probing, number of decayed teeth using DMF-T index and number of teeth with (PUFA index-pulpal involvement, ulceration, fistula or abscess). The proposed model were also adjusted to “familiar socioeconomic situation” – SES (maternal schooling and family income), body mass index – BMI and serum levels of interleukin-6, using structural equation modeling. The “burden of chronic oral diseases” was a latent variable that included four indicators of caries and periodontal diseases; and all had factor loadings greater than 0.5, indicating that this latent had a good fit. The highest consumption of added sugars (standardized coefficient, SC = 0.252,  $p = 0.017$ ), higher values for serum levels of interleukin-6 (SC = 0.137,  $p = 0.024$ ) and lower values for SES (SC = 0.462,  $p = 0.004$ ) were associated with increased values to “burden of chronic oral diseases”. Adolescents with higher values of body mass index also presented higher serum levels of IL-6 (FL = 0.220,  $p = 0.002$ ). Added sugars intake was associated to

“burden of chronic oral diseases”. IL-6 level was correlated to higher “burden of chronic oral diseases” suggesting chronic inflammation underlying oral diseases in adolescents. Higher systemic inflammation (IL-6 level) was also associated to obesity. The burden of oral diseases and other chronic diseases can be decreased early by addressing common risk factors as excessive added sugar consumption.

Study was funded by FAPEMA (Brazilian acronym for the Maranhão State Research and Scientific and Technological Development Foundation) CNPq (Brazilian acronym for the National Council for Scientific and Technological Development).

183

### What Are the Barriers and Enablers for Dentists in Managing Proximal Caries Lesions?

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To explore why dentists perform certain treatments (non-invasive, micro-invasive or invasive) for managing proximal caries lesions confined to enamel and at the enamel-dentine junction, and to determine the barriers and enablers influencing adoption of evidence-based strategies. **Methods:** Three trained interviewers conducted interviews in three different countries (USA, New Zealand and Germany) by telephone, with interviews audio-recorded. The Theoretical Domains Framework—comprising 12 domains (knowledge, skills, social/professional role, beliefs about capacities/consequences, motivation/goals, memory/attention/decision process, context/resources, social influence, emotion, behavioural regulation, nature of behaviours)—guided the interviews. Dentists were sampled to include a broad mix of practice characteristics.

Audio-recordings were professionally transcribed. Data analysis employed the principles of thematic or content analysis and followed the steps of familiarization/description, data coding employing the theoretical domains framework (reduction), data analysis (connecting themes similar to axial coding, displaying agreement and disagreement), and interpretation (theory building or confirmation). Re-coding of 20% of the transcripts by the last author was undertaken to enhance credibility. **Results:** 44 interviews were conducted, with 20 from the USA, 12 from New Zealand and 12 from Germany. Preliminary thematic data analysis shows support for dimensions with the Theoretical Domains Framework and, in particular, the role of environmental context and resources, patient-related factors (attendance, oral hygiene, co-operation, caries history) and financial rewards/systems. **Conclusion:** The Theoretical Domains Framework is a useful analytical tool for exploring the barriers and enablers in managing proximal caries lesions. Findings will be used to inform implementation of modern caries strategies and develop ways of positively influencing provider behaviour in general dental practice.

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184

### Current Current Cariology Education in Dentistry and Oral Health Therapy Programs in Australasia?

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The Australian and New Zealand chapter of ACFF was launched in 2013 and one of its primary aims was to conduct a survey of the local learning and teaching of cariology in dentistry and oral health therapy programs. **Methods:** A questionnaire was developed using the framework of the European Organisation for Caries Research (ORCA)/Association of Dental Education in Europe (ADEE) cariology conducted in Europe in 2009. The questionnaire was comprised of multiple choice and open-ended questions exploring both factual and attitudinal aspects of the cariology teaching. The survey was distributed to the cariology curriculum coordinator via Survey Monkey in January 2015. Simple analysis of results was carried out with frequencies and average numbers of hours collated with open-ended responses collected and compiled into tables. **Results:** 17 responses from a total of 21 programs had been received including 7 Dentistry and 10 Oral Health Programs. Key findings from the survey were – one quarter of respondents indicated that cariology was identified as a specific discipline with their course and 41% had a cariology curriculum in written format. With regard to caries detection and diagnosis, all programs indicated that visual/tactile methods and radiographic interpretation were recommended with ICDAS also being used by over half. Despite all respondents answering that early caries management, centred on

prevention and remineralisation, was taught – many taught operative intervention at an earlier stage of lesion depth than current evidence supports. In fact over 40% of respondents still teach operative intervention for lesions confined to enamel. **Conclusion:** Despite modern theoretical concepts of cariology being taught in Australia and New Zealand, they do not appear to be fully translated into clinical teaching at the present time.

185

### Multivariable Risk Models for Prediction of Coronal and Root Caries in an Adult Population

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The aim of the present study was to determine the risk indicators of coronal and root caries in adults from Porto Alegre, South Brazil. This cross-sectional study used a multistage sampling strategy to draw a representative sample of 1,023 individuals 35 years and older. Questionnaires recorded age, gender, tooth brushing frequency, proximal cleaning frequency, and access to dental services. Oral assessment included gingivitis, gingival recession, and coronal and root caries. Survey negative binomial regression model and survey Poisson regression model were used to assess the relationship of predictor variables with coronal DMFT and root caries index (RCI), respectively [incidence rate ratio (IRR)/prevalence ratio (PR)/95% confidence interval (95% CI)]. The prevalence of coronal and root caries was 99.73% (95% CI 99.31–1.00) and 41.14% (95% CI 37.57–44.72), respectively. The coronal DMFT and RCI was 18.73 (95% CI 18.29–19.17) and 10.14% (95% CI 8.46–11.82), respectively. Individuals 45–59 (IRR 1.29, 95% CI 1.19–1.40) and 60 years and older (IRR 1.39, 95% CI 1.29–1.49) and women (IRR 1.09, 95% CI 1.04–1.14) were more likely to have coronal caries. A higher educational status (IRR 0.88, 95% CI 0.78–0.99) and interproximal cleaning frequency (IRR 0.94, 95% CI 0.91–0.97) was associated with a lower probability of coronal caries. Individuals 45–59 (PR 1.51, 95% CI 1.15–1.99) and 60 years and older (PR 1.54, 95% CI 1.17–2.01) and those presenting higher coronal DMFT (PR 1.06, 95% CI 1.05–1.08) were more likely to have root caries. A lower probability of root caries was found for women (PR 0.76, 95% CI 0.68–0.86). In conclusion, age, gender, educational status, and interproximal cleaning frequency emerged as significant predictors of coronal caries among adults. Age, gender, and coronal DMFT were risk indicators for root caries.

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### Prevalence and Severity of MIH in 8 Year-Old Children from Valdivia, Chile 2016

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Molar Incisor Hypomineralization (MIH) is a developmental enamel defect that affects permanent first molars and incisors. There are few reports from Latin America about MIH prevalence. The aim of this study was to determine the prevalence and severity of MIH in 8-year-old children attending public schools in Valdivia, Chile 2016. **Material and Methods:** A cross-sectional study was conducted between March and June 2016. The study was approved by the Ethics Committee of the Health Service-Valdivia, informed consent forms were signed by parents of all participants. The population studied covered 712 children attending third grade in 15 public schools of Valdivia. To select the group study, a two-stage cluster sampling was performed. 145 children were evaluated by two calibrated examiners ( $\kappa = 0.7$ ) through a clinical examination of permanent molars and incisors, detecting the presence of MIH, according to the criteria established by Ghanim et al.: *Eur Arch Paediatr Dent* 2015;16:235–246 and the severity (Mathu-Muju & Wright: *Compend Contin Educ Dent* 2006; 27:604–610). **Results:** The MIH prevalence was 16.2%. No significant difference was found between the sexes ( $X^2 = 1.3531$ ,  $df = 1$ ,  $p = 0.24$ ). Severity of MIH ranged from mild (96.4%) to moderate (3.6%). No severely affected teeth were found. **Conclusion:** 8 year-old children of public schools of Valdivia have a MIH prevalence similar to that reported worldwide. This prevalence, 16.2%, indicates that MIH is a common condition indicating the need to improve its detection and timely implementation of treatment.

### Caries Risk of the Proximal Area According to Age Group

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There are few papers concerning the caries risk of the proximal area. Caries risk is the probability of sound sites developing dental caries. The aim of this study was to assess caries risk of proximal area according to age. The data from the 2014 Korea National Health and Nutrition Examination Survey (KNHANES) were used. Oral examinations were performed by trained dentists for dental caries experience at tooth level according to the World Health Organization methods. The analysis in this study was confined to a total of 5,118 subjects who had no missing values of the mean number of decayed or filled permanent teeth (DFT) scores. The proximal decayed or filled permanent teeth (prox\_DFT)

scores were calculated by age group (19–29, 30–39, 40–49, 50–59, 60–69 and >70 yrs). The results of the oral examinations and questionnaire surveys were analyzed using SPSS software version 19.0 (SPSS, Chicago, IL, USA). The proportion of prox\_DFT to DFT scores increased with age. The prox\_DFT and DFT values were 1.26 and 4.39 in the 19–29 yr age group, 1.98 and 4.85 in the 30–39 yr age group, 1.77 and 3.68 in the 40–49 yr age group, 2.16 and 3.33 in the 50–59 yr age group, 2.81 and 3.35 in the 60–69 yr age group and 2.60 and 2.88 in the >70 yr age group. The proportion of prox\_DFT to DFT scores was 28.7% at 19–29 age group and 90.3% at 70 over age group. In conclusion these data suggests that caries risk of proximal area increases with age.

### Dental Caries Experience Among Indigenous Children of Maranhão Indigenous Special Health District, Brazil

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**Aim:** to describe the prevalence and severity of dental caries among indigenous children from Maranhão Indigenous Special Health District (DSEI-MA). **Methods:** Data were obtained from an oral health survey conducted in all children aged 5 and 12-years from DSEI-MA, in which the CAST instrument was used to assess dental caries were analysed by means of descriptive statistics. **Results:** A total of 981 (56.8%) children aged 5-years ( $SD \pm 0.28$ ) and 745 (43.2%) aged 12-years ( $SD \pm 0.27$ ) from 9 ethnic groups in 283 and 259 indigenous villages participated, respectively. At 5-years, the mean dmft score for enamel and dentine lesions was 6.86 ( $SD \pm 4.32$ ) and for dentine lesions was 3.60 ( $SD \pm 3.69$ ). At 12-years, the mean DMFT-score for enamel and dentine lesions was 5.09 ( $SD \pm 4.24$ ) and for dentine lesions was 2.03 ( $SD \pm 2.60$ ). Only 7.5% and 9% of 5 and 12 years-olds presented with a maximum CAST score of 0 (sound) for the deciduous and permanent dentition, respectively. At the age of 5, 61% of the children presented at least one cavitated dentine lesion of which 22.8% had already pulp involvement and/or a fistula/abscess. At 12-years, 17.7% of the children presented a maximum CAST score of 3 (enamel lesion) and 45.1% a maximum CAST score of 5, 6 or 7, with CAST 5 (cavitated dentine lesion) most prevalent (24.7%). Sealants (CAST 1) and restoration (CAST 2) were rarely observed. **Conclusion:** A high caries prevalence and a low frequency of treatments were observed for both age groups, indicating that oral health policies being applied for this population need to be revised.

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189

### Caries Assessment Spectrum and Treatment Index in a Group of Turkish Children: A Pilot Study

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The aim of this pilot study to evaluate the caries prevalence and experience in primary molars and permanent molars in a group of Turkish 7- to 8-year-old children using the Caries Assessment Spectrum and Treatment index. **Methods:** This cross-sectional pilot study was carried out on a sample of 50 out of 150 examined children (7 and 8 year olds) from Department of Pediatric Dentistry, Dental School, Marmara University. Dental caries status of 7–8-year-old children was evaluated and only those children who had all four permanent molars fully erupted were selected for a further analysis. The study was conducted after informed consent was obtained from the concerned parents of children. Dental caries status was evaluated with CAST, a new caries assessment system. Before examination, each tooth was wiped with sterile cotton roll to get a dry surface for proper evaluation. The means of the individual CAST scores were assessed. The percentage of children according to the highest CAST score was calculated. None of the children showed a healthy, functional dentition (CAST codes 0-2). For permanent molars, “No visible carious lesion” was most often recorded (29%), and for primary molars “distinct cavitation into dentine” was most often recorded and “the pulp chamber intact” (20%) The means for CAST codes 3, 4 and 5 were  $0.12 \pm 4.4$ ,  $0.11 \pm 3.4$ , and  $0.14 \pm 5.3$  respectively. The means of molars with pulpal involvement (Code 6) was  $0.05 \pm 2.1$ , and with dental sepsis (Code 7)  $0.01 \pm 0.6$ . In conclusion, the Turkish children showed high caries prevalence and severe caries experience in the primary molars. The CAST index was a useful tool for the epidemiological studies of 7–8 year-old children.

190

### Caries Experience in 10–16-Year-Old Adolescents with Disabilities from the German Rhein-Erft-District

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In Germany a steady decrease of the caries prevalence rate has taken place in children and adolescents until recently, e.g. in 12-year-olds this value decreased from 67.2% in 1997 to 18.7% in 2014 (Jordan and Micheelis 2016). It remains unclear whether children and adolescents with disabilities have also benefitted from this development. It was the aim of this study to answer this question by evaluating caries epidemiological data which had been collected by the health authorities of a German region. Caries epidemiological data of children with a variety of physical or intellectual disabilities stored in the data bank of the health authorities of the German Rhein-Erft-District were exported and assessed. These data had been obtained by 3 calibrated public health dentists in the school years 2010/2011 and 2015/2016 by examining children with disabilities in the schools and by following the 1997 WHO guidelines for caries epidemiology. Mean D3MFT-values, 95% confidence intervals and caries prevalence rates (D3MFT >0) were calculated. Prior to the start of this study, consent had been obtained from the ethical committee of Witten/Herdecke University. In the school years 2010/2011 and 2015/2016 resp. the number of school children aged between 10 and 16 years and examined in schools was 611 and 529. Their mean D3MFT values were 1.07 (95% CI 1.03–1.11) in 2010/2011 and 1.00 (95% CI 0.97–1.03) in 2015/2016. The respective values for the 12-year-olds were 0.71 (95% CI 0.46–0.96) and 0.60 (95% CI 0.44–0.94). The caries prevalence rates were 36.3 % and 36.9% in the 10–16-year-olds but 31.5% and 33.5% in the 12-year-olds. Comparison of these values did not reveal statistically significant differences between the two school years. In contrast to the observations on the German national level children and adolescents with a variety of physical or intellectual disabilities have not benefitted from a decrease in caries prevalence and experience. It is recommended to elucidate the reasons for this with the aid of a scientific questionnaire presented to teachers, parents and dentists of these children.

This study was funded by the Department of Special Care Dentistry of the Witten/Herdecke University.

### A Prospective Cohort Trial Regarding Medical History, School Success and Caries Experience in First-Graders: Study Design

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Chronic diseases can have a negative impact on school adjustment, early learning and later school career. This study aims to investigate the connection between several chronic diseases, educational opportunities, school success and caries experience at the beginning of school in a prospective cohort trial. **Experimental Approach:** This study is developed to evaluate predominantly 6–7 year old first graders over a period of two years. The study includes two phases: In the pilot phase, at maximum 400 first-grade school children on 16 schools from the regions Mainz and Mainz-Bingen are included, in the main phase, 2100 children from all 69 primary schools and 9 schools for children with special needs are planned to be included. For every participating child, the results of the medical school entrance examination regarding chronic diseases are recorded. In the further course of the school year at least four questionnaires are sent to the parents, each modified depending on the presence of chronic diseases. Additionally, teachers are interviewed concerning their own situation and the children's development at the end of the first grade. Children are surveyed concerning her own school-related quality of life and their subjective learning success as well as sleep habits. At the end of the study, after two years, the level of education is recorded. For the dental results, instructed examiners record the  $d_3mft$  index according to WHO criteria. Following a catalogue of criteria, need for orthodontic treatment is also evaluated. **Conclusions:** This study will allow finding correlations between chronic diseases, quality of life, sleep habits, the children's environment, and caries experience. Additionally it will be possible to reassess an assumed impact of caries on early school success.

This study is funded by the German Federal Ministry of Education and Research.

### Effect of Tooth Age on the Presence and Severity of Dental Hard-Tissue Conditions

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Tooth retention has increased over the past decades, due to higher life expectancy and availability of preventive measures. Therefore, it is essential to understand how aging affects dental conditions. This study focused on the presence and severity of dental problems in extracted premolars ( $n = 1,500$ ), with individual age estimated using established dental forensic methods. Ages varied from 9 to 101 years old (yo;  $\pm 10$ ). A trained and calibrated examiner blindly assessed dental caries, fluorosis, erosive wear, staining and color, using ICDAS (0–5 for crown and 0–2 for root), TFI (0–9), BEWE (0–3), modified Lobene (0–3) and VITA shade (B1–C4) indices, respectively. Indices were plotted against tooth age, and relationships between the indices and age were tested using regression models. Mean ICDAS score for crown increased steadily from 0 at  $\sim 10$  yo to 2 at  $\sim 40$  yo and remained stable between scores 2 and 3 at older ages; while for root, it increased from 0 to 0.5 at  $\sim 60$  yo, remaining stable after. Mean BEWE increased from 0 to 2 (occlusal) and to 1.5 (smooth) at  $\sim 50$  yo, remaining stable after. Mean TFI stayed around 1 until  $\sim 30$  yo before decreasing to 0.5 at  $\sim 40$  yo, approaching 0 at  $\sim 80$  yo. Mean staining increased from 0 at  $\sim 20$  yo to around 2 at  $\sim 50$  yo, remaining stable after. Mean VITA shade of crown increased from A3 to B3 at  $\sim 50$  yo, and remained stable after; while of root remained around C2 until  $\sim 30$  yo, then increased to B4 at  $\sim 50$  yo, and to A4 at  $\sim 80$  yo. Considering the limitations of this study, we concluded that age affected the presence and severity of different dental diseases and conditions. Future studies will focus on how age-related biological and behavioral changes impact the observed differences.

### 193 Withdrawn

### 30 year Study on Epidemiology of Dental Caries in 12 Year Olds in Slovenia

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The aim of the study was to begin monitoring and evaluating the prevalence of dental caries in 12-year-olds in Slovenia over the extended period of time. **Methods:** The first nationwide survey of oral health, conducted in 1987 in all nine geographical regions, covered about 2% of all 12-year-olds in Slovenia and contained an equal number of females and males. Caries experience was diagnosed at the cavitation level, using artificial light, plane mirror and sharp explorer. The presence of sealants was also recorded for each child. After the initial survey, five further nationwide surveys were conducted in five-year intervals, followed by the last final survey which was completed in January 2017. For data processing the Statistical Package for Social Sciences (SPSS) was used. The level of significance was set to  $p < 0.05$ . **Results:** The mean DMFT decreased significantly from 5.10 (CI = 4.77, 5.43) in 1987 to 1.89 (CI = 1.66, 2.12) in 2013, while in the last survey in 2017 it changed to 1.53 (1.32, 1.74). While the percentage of children with one or more sealed teeth increased from 6.0% (1987) to 93.7% (2017), the percentage of caries-free children increased from 6.4% (1987) to 42% (2017). **Conclusion:** The decline in dental caries among 12-year-olds in Slovenia was substantial at first, later in 2013 survey a slight, although still, insignificant increase occurred, while in 2017 survey it again decreased significantly.

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### Risks Factors for History and Activity of Early Childhood Caries

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The aim of this study was to identify different risk factors for history or activity of early childhood caries (ECC). **Methods:** Cross-sectional study. 291 children aged 2 to 5 years from Panguipulli, Chile, were examined using Nyvad's activity criteria [Nyvad et al: Caries Res 1999;33:252–260] by four clinicians ( $\kappa$  intra/inter  $> 0.7$ ). Categories 1–3 were recorded as active caries lesions and 4–6 as history of caries. Dependent variable was categorized into four mutually exclusive groups according to caries history or activity: no history-no activity [NoH\_NoA]; activity only [NoH\_YesA]; history only (YesH\_NoA); or both [YesH\_YesA]. Sample size was estimated to detect 15 different risk factors. Parents completed a questionnaire to assess possible risks factors. A multinomial logistic regression was done to detect differences in risk factors for groups (NoH\_NoA, NoH\_YesA, YesH\_NoA, YesH\_YesA). Reference level was NoH\_NoA. Significance was set at  $p = 0.05$ . Analysis were performed using R.

The proportion of children with no caries history or activity was 34.7%, with history only 33.0%, with active caries only 7.9%, and with both 24.4%. Exclusive risk factor for activity of ECC was presence of visible plaque ( $p = 0.041$ ). Risk factors for both history and activity were bedtime-bottle ( $p = 0.03$ ), sugary-drinks ( $p = 0.03$ ), lack of dental check-ups ( $p = 0.018$ ) and lack of vegetable-patch at home ( $p = 0.024$ ). In conclusion there are different risk factors that explain early childhood caries history, activity (plaque) or both (night-bottle, sugary-drinks, lack of dental control and lack of vegetable-patch).

## The Oral Health Status of Inhabitants of Sal, Cape Verde

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The aim of this study was to describe the oral health of children and adults living on the island of Sal, Cape Verde. The study protocol was performed following the Dutch regulations as stated by the Medical Ethics Review Committee of VU University Medical Center and the local government of Sal. Signed informed consent was obtained from all participants and from the parents of the children under the age of 16 years. Clinical examinations were performed in duplicate by four final-year dental students. Oral assessments (caries assessment: dmft/DMFT and pufa/PUFA) were performed at 620 persons (303 female) with a mean age of 24.9 years (SD 19.5, range 1–87). Individual oral hygiene instructions were given after assessment of the brushing time and brushing technique. 4% of the participants reported to have no toothbrush and 8.5% never used toothpaste when brushing their teeth. Under supervision, the participants brushed their teeth with an average brushing time of 59.0 s (SD 36.2) which resulted in a mean percentage of 66.7% (SD 21.2) plaque coverage of the teeth. Good kappa values (>0.92) were obtained for all clinical parameters tested. The mean dmft/DMFT was 5.6 (SD 5.5, range 0–28); with 13.9% dmft/DMFT = 0. The need for dental treatment (DS/(DS+FS) x100%) in this group was 75.8% (SD 41.4). 37% experienced dental pain at the time of investigation and 48% had never visited a dentist. The mean pufa/PUFA was 1.3 (SD 2.2, range 0–18); with 45.2% pufa/PUFA = 0. 28.4% of the decayed teeth were progressed to odontogenic infections. This study indicates the poor oral health of the inhabitants of Sal, there is an urgent need for structural long term solutions.

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## Prevalence and Severity of Dental Caries Among Immigrant and Chilean School Children: A Cross-Sectional Study

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The phenomenon of migration is becoming increasingly important worldwide and may affect country epidemiological indices. Recently, Chile has received an increasing number of immigrants. However, there are no epidemiological studies addressing oral health status of the immigrant children population. Therefore, the objective of this study is to compare prevalence and severity of caries lesions in immigrants versus Chilean school children in representative samples. **Methodology:** The sample of this cross-sectional study consisted of 332 children aged between 6 and 8, who attended public schools in Santiago – Chile. The clinical examination was performed in a school environment by a group of previously calibrated dentists trained to detect caries lesions according to ICDAS II criteria (codes 5 and 6 used to calculate caries prevalence) and World Health Organization (WHO) detection criteria. All parents gave their informed consent for their children being enrolled in the study. **Results:** The total prevalence of caries was 72.5% (95% CI: 67.5%–77.4%). The mean DMFT index was 0.25 (CI 95%: 0.17–0.32) and of dmft was 3.06 (CI 95%: 2.71–3.41). The prevalence for immigrant population was 75.5% (CI 95%: 66.5–84.5), the mean DMFT was 0.27 (CI 95%: 0.12–0.42) and dmft was 2.85 (CI 95%: 2.21–3.49). For Chilean population, these values were 73.3% (CI 95%: 67.4–79.2) for overall prevalence, 0.24 (CI 95%: 0.15–0.33) for mean DMFT and 3.24 (CI 95%: 2.80–3.67) for dmft. No significant differences were detected in prevalence ( $p = 0.69$ ), DMFT ( $p = 0.79$ ), or dmft ( $p = 0.40$ ). **Conclusion:** In our sample, there were no significant differences between caries experience of immigrant children versus Chilean children, in terms of prevalence and caries severity for both permanent and primary teeth.

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### Incidence of Dental Caries in Children with Herpetic Stomatitis

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Comprehensive oral examination of the children with the herpetic stomatitis (HS) was conducted. Objective: To study the occurrence of herpetic stomatitis and the development of dental caries in children. Epidemiological examinations conducted in organized children's collectives (kindergartens and schools). Compared groups were randomized and social status, living conditions and nutrition to eliminate their influence on the risk of herpes virus infections. 309 children from Tashkent were examined. Dental caries was recorded according to World Health Organization guidelines at D3 level. One professor trained the three dentists. During the survey, duplicate examination was done on 5% of the participants examined daily. Inter-examiner reliability maintained at levels of Cohen's kappa value was 0.70.

156 children (50%) had herpetic stomatitis (HS). Another 153 (50%) children never had herpetic stomatitis (unaffected controls, UC). Comparative analysis of the two groups was performed by using t-student test. **Results:** the prevalence of dental caries in children aged 6 months to 3 years was 65% in HS compared to 35% in UC. In the age group from 3 to 6 years it was 91% (HS) versus 48% (UC). In the age group from 6 years to 15 years with HS showed 100.0% prevalence of dental caries; correspondingly to the prevalence of caries in the second group was 76%.

The average severity of caries – dmft in children with HS in the age group of 6 months to 3 years was equal to  $2 \pm 0.1$  compared to  $1 \pm 0.05$  (UC); In the age group from 3 years to 6 years in children with HS –  $4 \pm 0.2$ ; compared to  $3 \pm 0.15$  In UC; the age group of 6 to 10 years HS:  $7 \pm 0.35$ ; versus UC  $6 \pm 0.21$  and from 10 years to 15 years old age group – 6 vs. 4 ( $p < 0.05$ ). **Conclusion:** Herpetic lesions of the oral mucosa are concurrent with a higher caries experience. We found a significantly higher prevalence and severity of dental caries in children with HS. Future research will be studying common pathogenetic or lifestyle risk factors for dental caries in children suffering from HS, such as the immune system, oral hygiene and diet.

### Discriminative Validity of Version of the Scale of Oral Health Outcomes for Five-Year-Old Children

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The perceptions of parents and children regarding all dimension of oral health are useful to public health and clinical practice dentistry. The aim o was to evaluate the discriminative validity of the Spanish version of the Scale of Oral Health Outcomes for Five-Year-Old Children (SOHO-5). A cross-sectional study was conducted with preschool children of five years of age and their parents in the city of Santiago, Chile. The SOHO-5 consists of a child self-report and a parental report of the child's oral health history. Both versions contain 7 items. For the child version, the report refers to difficulties eating, drinking, speaking, playing, sleeping, smiling (due to pain) and smiling (due to appearance). The items in the parental version include difficulty eating, difficulty playing, difficulty speaking, difficulty sleeping, avoiding smiling due to pain, avoiding smiling due to appearance and affected self-confidence. Scores are calculated as the sum of response codes. The SOHO-5 was translated to Spanish and adapted according to standard guidelines. Children were also clinically examined for dental caries using ICDAS II criteria, by two calibrated dentists (Kappa = 0.82, 0.78. n = 20). Female were 42.3% of the total of the sample. The caries prevalence ICDAS 5–6 was 55.8%, caries prevalence ICDAS2–6 was 86.5%, the mean of D<sub>5–6</sub>MFT was 1.9 (SD 2.8) and D<sub>2–6</sub>MFT was 5.29 (SD 4.64). Spanish version of SOHO-5 shows positive correlation with D<sub>5–6</sub>MFT,  $r = 0.41$  ( $p < 0.01$ ) and with D<sub>2–6</sub>MFT,  $r = 0.34$  ( $p < 0.01$ ). In conclusion the Spanish version of SOHO-5 correlates with D<sub>5–6</sub>MFT and D<sub>2–6</sub>MFT.

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- Abrams, S.H. 141  
Abreu-Placeres, N. 125  
Adams, G.G. 95  
Adilkhanova, N.A. 5  
Aguila, J. 96  
Ágústsdóttir, I. 181  
Ahumada, N. 70  
Aimée, N.J. 77  
Aimée, N.R. 61  
Akman, S. 44  
Akyuz, S. 30, 92  
Al Hothali, M.M. 159  
Alaraudanjoki, V. 46, 126  
Albeshir, E. 33  
Aldosary, S. 168  
Alfaro, L. 52  
Algarni, A. 192  
Alison, S. 35, 113, 122  
Al-Jawad, M. 65  
Alkilzy, M. 75  
Al-Mandil, H.B. 65  
Altenburger, M.J. 160  
Alves, A.N.N. 21  
Alves, C.M.C. 124, 182  
Alves, K.S.S. 22  
Alves, L.S. 143, 144  
Alves, R.H.P. 22  
Amaechi, B.T. 141  
Amaral, C.R.A. 188  
Ambarkova, V. 137  
Ammari, M.M. 84  
Anderson, P. 65  
Ando, M. 116  
Andrejeva, K. 96  
Antonio, L.P. 101, 120  
Anttonen, V. 46, 126  
Apel, C. 162  
Aqrawi, L.A. 63, 67  
Arango-De la Cruz, M.C. 52  
Aranha, A.C.C. 47  
Araújo, J.T.C. 25  
Araújo, L.F. 60  
Araujo, M.P. 86  
Araya-Bustos, F. 70  
Arrica, M. 128  
Arteaga, O. 195  
Arthur, R.A. 11, 170  
Attin, T. 26, 38  
Augusto, M.G. 42  
Austin, R.S. 50  
Avci, H. 119  
Avila, V. 100  
Ávila, V. 125  
Baelum, V. 175  
Bågesund, M. 59  
Baginska, J. 145, 179  
Baker, S. 183  
Bakhshandeh, A. 4, 108, 110  
Bartlett, D.W. 50  
Basner, R. 132, 133, 134, 135  
Baumann, T. 37, 39, 47  
Bayram, M. 78  
Becerra, L. 186  
Becker, K. 26, 38  
Bekiroglu, N. 158  
Bekjanova, O.E. 198  
Belibasakis, G.N. 14  
Benatti, B. 182  
Bertoletti, A.V.S. 57  
Bezerra, D.S. 23  
Bierman, I. 106  
Bilandzic, M. 36, 45  
Biscaglia, L. 128  
Bisignano, S. 58  
Bonecker, M. 120  
Bonifacio, C.C. 86  
Bonifácio, C.C. 97  
Bontà, G. 138  
Borges, A.B. 42, 147  
Bottenberg, P. 5, 31, 198  
Bradshaw, D.J. 178  
Braga, M.M. 4, 28, 57, 101, 102, 105, 107, 120  
Brandt, B.W. 18, 20  
Brianezzi, L.F.F. 90  
Brinkmane, A. 96, 141  
Bronkhorst, E.M. 188  
Buchalla, W. 14  
Budde, T. 27  
Buijs, M.J. 165  
Burnside, G. 69  
Butler, C. 148  
Butler, C.A. 95  
Buzalaf, M.A. 163  
Buzalaf, M.A.R. 34, 152, 177  
Cabello, R. 80, 197, 199  
Cabral, R.N. 109, 131  
Cabrera, D.F. 100  
Cagetti, M.G. 54, 66, 73, 138  
Cai, Y.L. 3, 18  
Calixto, L.H.M. 79  
Callaway, A. 149  
Campos, P.H. 114  
Campus, G. 54, 55, 66, 73, 128, 138  
Cankar, K. 87, 88  
Cardoso, C.A.B. 152  
Carmo, C.D.S. 182  
Cartagena, A. 108  
Carvalho, J. 143  
Carvalho, J.C. 61, 77  
Carvalho, T.S. 37, 39, 47  
Castelo Branco, C.M.C. 60  
Castiblanco, G.A. 100  
Castilla, C. 186  
Cavanaugh, J.E. 139  
Cenci, M.S. 153  
Chałas, R. 72  
Chen, X. 67  
Cheng, J.X. 116  
Cho, H.J. 187  
Cieplik, F. 14  
Cifuentes-Aguirre, O.L. 52  
Cocco, F. 54, 55, 66, 73, 99, 138  
Coclete, G.E.G. 177  
Comar, L.P. 163  
Conrads, G. 1, 162  
Contador, R. 199  
Cook, N.B. 33  
Correa, A. 80  
Corridore, D. 99  
Cortes, A. 52  
Cortés, A. 100  
Côrtes, G. 188  
Cortesi Ardizzone, V. 66  
Cosma, G. 29  
Cossellu, D. 55  
Costa, E.L. 124  
Costa, R.S.A. 144, 185  
Costa-Oliveira, B. 24  
Crastechini, E. 147  
Crielaard, W. 3, 18, 20  
Crystal, Y.O. 68  
Csák, B. 2, 16  
Cunha, P.L.R. 155  
Cunha, R.F. 177  
Curtis, A.M. 139  
Cury, J. 24, 176  
Cury, J.A. 164, 167, 173, 174

- Dalla Nora, A. 143  
Dalla-Nora, Â. 123  
Danelon, M. 177  
Danielsen, B. 181  
Dantas, E.D.V. 173  
Dantas, T.C. 17  
Dashper, S. 148  
Dashper, S.G. 95  
de Andrade, C.G. 11  
de Josselin de Jong, E. 104, 115, 117  
de Lima, M.D. 120  
De Marchi, R.J. 70  
de Mey, L. 56  
de Oliveira, C.L. 170  
de Soet, J.J. 196  
Deas, L. 166  
Deery, C. 111  
Del Bel Cury, A.A. 164, 174  
Delbem, A.C.B. 177  
Demuria, L. 142  
Deng, D.M. 3, 18, 20  
Denison, P. 184  
Di Caccamo, P. 128  
Diaconu, O. 29  
Díaz-Garrido, N. 81  
Dige, I. 10  
Diniz, M. B. 140  
Diniz, M.B. 60, 114, 152  
Duarte, S. 17, 22  
Duckworth, R.M. 169  
Duggal, M.S. 168  
Durmus, B. 158
- Eckert, G.E. 192  
Eckert, G.J. 116  
Edwardson, J.M. 34  
Eidt, G. 11  
Ekstrand, K. 110  
Ekstrand, K.R. 4, 100, 105, 108, 120, 125  
Ekstrand, K.R. 142  
Ekstrand, K.R. 181  
Elkassas, D. 150, 151  
Elkawy, M. 151  
Elkawy, W. 151  
Elsenberg, C.P.M. 106  
Eren Giray, F. 30  
Escobar, K. 95  
Esteves-Oliveira, M. 1, 36, 45, 146  
Evans, W. 184  
Exterkate, R.A.M. 159
- Faleiros, S. 80, 197, 199  
Fechine, F.V. 79  
Fernandez, C. 32  
Fernando, J.R. 95  
Ferreira, F.R. 105, 120  
Fiehn, N.E. 110  
Fischer, H. 45  
Flannigan, N.L. 69
- Floriano, I. 4, 57, 101, 120  
Fonseca, S.G.C. 79  
Fontana, M. 183  
Fonteles, M.M.F. 79  
Forte, F.A. 21  
Fortich-Mesa, N. 52  
Foster Page, L. 184  
Foster Page, L.A. 183  
Franguez, I. 87, 88  
Freitas, E. 131  
Furtado Jr, J.H.C. 79
- Gabler, S. 132, 134, 135  
Gallie, A. 35  
Gamboia, L.F. 52, 100  
Ganss, C. 48, 49, 94  
Garan, A. 30  
Garen, T. 63  
Georgiev, Z. 137  
Georgiou, A.C. 3  
Gheorghita, L. 29  
Ghidán, Á. 2, 16  
Giacaman, R.A. 70, 81  
Giacomini, M.C. 90  
Gimenez, T. 102, 105  
Girard, F. 136  
Girdwood, J. 183  
Gironda, C.C. 34  
Giuliani, E. 128  
Glavina, D. 137  
Glöckler, J. 36  
Gnich, W. 166  
Gokkaya, B. 44  
Gomes, R.A. 57, 101  
Gonçalves, R.S. 90  
Gorbatova, L. 127  
Gorbatova, M. 127  
Gorseta, K. 137  
Gossweiler, A. 116  
Göstemeyer, G. 62  
Granja, G.L. 140  
Grjibovski, A. 127  
Groselj, M. 87, 88  
Gudkina, J. 141  
Guedes, S.F.F. 23  
Guerra, F. 99  
Guler, Z. 44  
Güner, Ş. 130  
Gupta, A. 58  
Gutierrez, M.T.M. 165
- Haas, A.N. 144, 185  
Halici, B. 106  
Hamilton, D.S. 68  
Hamza, H. 150, 151  
Hara, A.T. 33, 170, 192  
Hashizume, L.N. 170  
Hassanein, E.H. 151  
Hassanein, H. 150  
Hatakeyama, R. 9
- Hayran, O. 103  
Haznedaroglu, E. 30, 130, 189  
Hellwig, E. 160  
Henne, K. 1  
Henrique-Silva, F. 34  
Herczegh, A. 2, 16  
Herlofson, B.B. 63, 67  
Hesse, D. 86, 97  
Hetrodt, F. 162  
Higham, S.M. 6, 69, 178  
Hilgert, A.L.S. 188  
Hiller, K.-A. 14  
Hjortsjö, C. 43  
Höfer, M. 75  
Honório, H.M. 90  
Hopcraft, M. 184  
Hove, L.H. 43, 63, 67  
Hultquist, A.I. 59  
Huysmans, M.C.D.N.J.M. 153
- Idowu, O.S. 169  
Imparato, J.C. 120  
Imparato, J.C.P. 57  
Innes, N. 62  
Innes, N.P. 86  
Innes, N.P.T. 71  
Inukai, J. 41  
Ishikiriana, S.K. 90
- Jablonski-Momeni, A. 112  
Jácome, S. 125  
Jacquet, W. 31, 32  
Jamiolkowski, J. 145, 179  
Jan, J. 180  
Janal, M.N. 68  
Jankulovska, M. 137  
Jansen, P. 45  
Jardim, J.J. 144, 185  
Jensen, J.L. 63, 67  
Jeong, S.H. 15, 172  
Joao-Souza, S.H. 47  
Jorge, R.C. 84  
Jun, E.J. 172  
Jung, E.H. 13
- Kahharova, D. 104  
Kalaoglu, E. 91, 93  
Kamal, D. 150  
Kanberoglu, E. 189  
Kang, S.M. 12, 118  
Kang, Y.M. 83  
Kargul, B. 44, 98, 158, 189  
Karimi, F. 56  
Kato, K. 171  
Kennedy, A. 113, 122  
Khudanov, B. 104  
Khudanov, B.O. 5  
Kidd, J. 166  
Kierklo, A. 179  
Kim, B.I. 12, 13, 115, 117, 118

- Kim, B.R. 118  
Kim, C.H. 13  
Kim, J.B. 15, 172  
Kim, J.S. 15, 172  
Kim, S.K. 115, 117  
Kim, S.Y. 15, 172  
Klatka, M. 72  
Kopperud, S.E. 51  
Kosaka, T. 41  
Krapež, J. 180  
Kristensen, M. F. 19  
Kuper, N.K. 153  
Kuzmina, I. 142  
Kwon, H.K. 12, 13, 117, 118
- Laajala, A. 126  
Ladewig, N.M. 101  
Lagerweij, M. 53  
Lagerweij, M.D. 159  
Lai, S. 55  
Laitala, M-L. 46  
Laitala, M.L. 126  
Lamont, T. 62  
Lara, J.S. 102, 107, 120  
Lara-Capi, C. 138  
Lausch, J. 146, 162  
Leal, S.C. 109, 131, 140, 188  
Lee, E.S. 13, 115, 117  
Lee, H.S. 115, 117  
Lee, J.H. 15, 172  
Lee, M.A. 15  
Lee, S.Y. 82, 83  
Leegaard, M.C.B. 64  
Leite, A.L. 34  
Leme-Junior, J. 176  
Lemos, L.F.C. 123  
Lenzi, T.L. 28, 120  
León, S. 70  
Levey, C. 62  
Levy, S.M. 139  
Li, J. 18  
Li, J.Y. 20  
Liao, C.S. 116  
Liao, Y. 18, 20  
Lima, A.G.F. 79  
Lima, A.S.G.H. 17  
Lima, M.M.R. 155, 156  
Lima, L.A. 25  
Lima, R.A. 22, 25, 156  
Lind, S. 64  
Lingstrom, P. 54, 73, 138  
Lingström, P. 55, 181  
Lippert, F. 33, 78, 157, 192  
Littlejohn, C. 78  
Liu, H. 18  
Liu, H.Y. 3  
Lobo, P.L.D. 79  
Lohinai, Z. 2, 16  
Lourenço, J. 61  
Louzada, I.C. 57, 101
- Løvik, S.E. 43  
Luka, B. 160  
Lumbau, A. 55  
Lussi, A. 37, 39, 46, 47, 60, 114, 122, 140  
Lynch, R.J.M. 154, 157, 178
- Machado, G.M. 57, 101  
Machiulskiene, V. 71  
Macpherson, L. 166  
Magalhaes, A.C. 163  
Magalhães, A.C. 120  
Maisch, T. 14  
Maldupa, I. 96, 129, 195  
Malinowski, M. 168  
Maltz, M. 77, 143, 144, 170, 185  
Mann, D.B. II, 89  
Männikkö, M. 46  
Mantzourani, M. 58  
Marcenes, W. 144, 185  
Marcondes, A.P. M. 114  
Marggraf, T. 7  
Marino, R. 195  
Marks, L. 32  
Marques, L.A.R.V. 79  
Marro, F. 31, 32  
Marshall, T.A. 139  
Martens, L. 31, 32  
Martignon, S. 52, 100, 120, 125  
Martinez-Mier, E.A. 89, 192  
Martins, J. 163  
Maske, T.T. 153  
Maslak, E.E. 5  
Masoudi, S. 106  
Mastnak, M. 180  
Matkulieva, S. 5  
Matsumoto-Nakano, M. 9  
Mazur, M. 99  
Medrano, R.M.C. 165  
Megert, B. 37  
Mejía, L. 52  
Meloni, G. 55  
Mendes, F. 105  
Mendes, F.M. 57, 101, 102, 107, 120  
Mendonça, K.M. 79  
Menteş, A. 91, 93, 130  
Mesquita, R.L.P. 155  
Mestrinho, H.D. 61, 77  
Meyer, R. L. 19  
Meyer-Lueckel, H. 1, 36, 45, 146, 162  
Miguel, M.C. 34  
Mitchell, H. 148  
Mitchell, H.L. 95  
Modesto, A. 78  
Monse, B. 165  
Monteiro, D. 176  
Montenegro, R.C. 21  
Moos, J. 112  
Moraes, F.R.N. 177  
Moraes, W.A. 155  
Morais, W.A. 156
- Morarar, I. 29  
Moreno, B. 199  
Moreno, P.A. 124  
Moro, B.L.P. 102, 107  
Mota, M.R.L. 21  
Moura, A.L. 152  
Moura, M. 182  
Moura, M.S. 120, 144, 185  
Moura-Netto, C. 114  
Mühler, D. 14  
Mulic, A. 51  
Mullan, F. 50  
Murisi, P.U. 57
- Nagata, M.E. 177  
Naka, S. 9  
Nakano, K. 8, 9  
Nam, E.J. 83  
Narbutaite, J. 71  
Nardi, G.M. 99  
Negrini, T.C. 11, 170  
Nemeth, L. 87, 88  
Neu, T. 19  
Neuhaus, K. W. 122  
Neves, B.G. 23, 155, 156  
Neves, P. 85  
Newton, J.T. 125  
Niederman, R. 68  
Nizamovs, M. 129  
Nóbrega, D.F. 164, 174  
Nomura, R. 8, 9  
Novaes, T.F. 60, 102, 107  
Nugent, Z. 111  
Nyvad, B. 109, 131, 175
- Ochoa, E.M. 125  
Okutan, A.E. 130  
Olegario, I.C. 86  
Olegário, I.C. 97  
Oliveira, R.S. 123  
Ooshima, T. 8, 9  
Ortega, R. 81  
Ortiz, M.E. 186  
Otsugu, M. 8  
Ottolenghi, L. 99  
Ozbek, N. 44
- Pacheco, C.J.B. 124  
Paes, A.B. 61  
Pailahual, V. 81  
Paiva, M.F. 177  
Palm, Ø. 63, 67  
Papadiamantis, A.G. 154  
Paris, S. 7  
Park, S.W. 115, 117  
Parma, V. 64  
Partyka, M. 72  
Pastbin, M. 127  
Paul, W. 6  
Pedrotti, D. 28

- Pedroza-Uribe, I. 120  
 Pedroza-Uribe, I.M. 101  
 Pehlivan, Z.C. 103  
 Pelá, V.T. 34  
 Pereira, R.A. 101  
 Pesonen, P. 46, 126  
 Pessan, J.P. 177  
 Petrakakis, P. 190  
 Pinheiro, S.A.A. 140  
 Pinto, D.N. 22  
 Pion, L. 57  
 Pion, L.A. 4  
 Pitts, N. 125  
 Pitts, N.B. 100  
 Plett, W. 165  
 Pontes, L.R.A. 102, 107  
 Poprawe, R. 45  
 Pucci, C.R. 42  
 Pummer, A. 14
- Qvist, V. 110
- Raggio, D.P. 57, 86, 97, 101, 102, 107, 120  
 Ramírez, S. 125  
 Ramos-Martínez, K. 52  
 Raphael, S. 184  
 Raphy, P. 69  
 Rebolledo, C. 81  
 Reis, A.C.M. 23  
 Restrepo, L.F. 125  
 Reynolds, C. 40, 95, 148  
 Reynolds, E.C. 40, 95, 148  
 Ribeiro, C. 85  
 Ribeiro, C.C.C. 124, 182  
 Ricciardi, C. 73  
 Richards, A. 175  
 Ricomini-Filho, A. 24, 176  
 Rinco, L. 77  
 Rios, D. 120  
 Rios, F.S. 144, 185  
 Rizaev, E.A. 198  
 Rizaev, J.A. 198  
 Rizaeva, S.M. 5  
 Roberts, J. M. 178  
 Rocha, E.S. 57, 101, 120  
 Rocha, R.O. 28, 120  
 Rodakowska, E. 145, 179  
 Rodd, H. 111  
 Rodrigues, H. B. 140  
 Rodrigues, J.A. 123  
 Rodrigues, L.K.A. 17, 21, 22, 23, 25, 155, 156  
 Rodriguez, G. 199  
 Rodriguez, G. 80, 197  
 Ross, A. 166  
 Ruiz, B. 80, 197, 199  
 Rupf, S. 49  
 Rusthen, S. 63, 67  
 Rykke, M. 63, 67
- Said, D. 142  
 Sales, E.M.A. 155, 156  
 Salobir, B. 180  
 Sanchez, A.Y. 170  
 Sánchez, S. 197  
 Sanjuán-Acero, J.A. 52  
 Santamaria, R.M. 71, 132, 133, 134, 135  
 Santos, J.T.L. 140  
 Santos, M.G. 163  
 Santos, M.T.B.R. 60  
 Santos, P.S.S. 90  
 Santos, S.V. 25  
 Sarac, S. 44  
 Scaramucci, T. 47  
 Schlafer, S. 10, 19  
 Schlueter, N. 42, 48, 49, 94, 160  
 Schmidt, P. 190  
 Schmidt, S.-C. 94  
 Schmoeckel, J. 132, 133, 134, 135  
 Schnarr, M. 49  
 Schüler, E. 132, 133, 134, 135  
 Schulte, A.G. 165, 190  
 Schwendicke, F. 7, 62, 183  
 Sen Yavuz, B. 98  
 Senakola, E. 96, 129  
 Sencak, R.C. 78  
 Sepúlveda, S. 70  
 Seymen, F. 78  
 Sezer, B. 158  
 Sezgin, B.I. 130  
 Shahinian, H. 106  
 Shahmoradi, M. 161  
 Sharma, C.P. 6  
 Shen, P. 40, 95, 148  
 Sherriff, A. 166  
 Shierzada, F. 56  
 Shimazaki, Y. 171  
 Silva, H.S.F. 17  
 Silva, R.A. 79  
 Silveira, P.S. 28  
 Sim, C.P.C. 148  
 Simakova, A. 127  
 Singh, P.B. 63, 64  
 Smith, L. 183  
 Soares-Costa, A. 34  
 Sonmez, I. 92  
 Sopule, A. 96  
 Sotirovska-Ivkovska, A. 137  
 Soto-Rojas, A.E. 89  
 Sousa, F.B. 21  
 Sousa, F.F.O. 25  
 Souza, B.M. 163  
 Souza, D.C.B. 156  
 Souza, I.P.R. 84  
 Souza, S. 85  
 Souza, S.F.C. 124  
 Soviero, V.M. 84, 109, 131  
 Spinola, M.S. 174  
 Splieth, C. 75  
 Splieth, C.H. 71, 132, 133, 134, 135
- Srinivasan, S. 58  
 Stacey, M.A. 40  
 Staun Larsen, L. 175  
 Stausberg, S. 146  
 Stavreva, N. 137  
 Steiger-Ronay, V. 38  
 Sternitzke, V. 27  
 Stevning, K. 43  
 Stollenwerk, J. 45  
 Strafford, S.M. 168  
 Strohmenger, L. 54, 66, 73  
 Subka, S. 111  
 Sun, J.N. 58  
 Suominen, L. 181  
 Svendsen, G. 51  
 Swain, M. 161
- Tabchoury, C.P.M. 173  
 Tagtekin, D. 103, 119  
 Takashima, Y. 9  
 Takeichi, S. 41  
 Tamura, K. 171  
 Tao, D. 58  
 Tashbayev, B. 63  
 Tashbayev, B. 67  
 Tawakoli, P.N. 26  
 Tedesco, T.K. 57, 101  
 Teitge, J. 149  
 Tenuta, L. 176  
 Tenuta, L.M.A. 164, 174, 175  
 Terčelj, M. 180  
 Thomson, W.M. 183  
 Thurnheer, T. 14  
 Tikhonova, S. 136  
 Toloza, P. 96  
 Torres, C.R.G. 42, 147  
 Tóth, Z. 2, 16  
 Toumba, K.J. 168  
 Trévia, L.R.A. 79  
 Tsuruta, S. 41  
 Tuğcu, N. 158  
 Tuculina, M. 29
- Ungar, P. 192  
 Uribe, S. 96, 129, 186, 195  
 Urschitz, M. 191  
 Urzua, I. 199  
 Usuga-Vacca, M. 52  
 Utheim, O. 67  
 Utheim, T.P. 67
- Vach, K. 160  
 Valappil, S.P. 6, 178  
 Valentine, R.A. 169  
 Valsami-Jones, E. 154  
 Van Buren, J. 139  
 van der Veen, M.H. 56, 106  
 van Loveren, C. 3, 18, 20, 53, 159, 165, 196  
 van Strijp, A. 53

van Strijp, A.J.P. 159  
van Wijk, A.J. 61  
Varjão, M.M. 61  
Vasconcelos, H.L.P. 79  
Velo, M.M.A.C. 90  
Vernon, B. 35, 113, 122  
Vieira, A.R. 78  
Vieira, P.H.A.G.P. 155, 156  
Vigano, M.E. 101  
Viganó, M.E. 120  
Volgenant, C.M.C. 196  
Vrbic, M. 194  
Vrbic, V. 194

Wagner, M.B. 143  
Walker, G. 148  
Walker, G.D. 40, 95  
Walsh, K.I. 167

Walther, C. 1  
Wang, L. 90, 120  
Warren, J.J. 139  
Wei, X. 3, 18  
Wennekers, A. 48  
Weusmann, J. 191  
Widmer, R. 184  
Wierichs, R.J. 146  
Wijzenbeek, T.M. 106  
Wilde, S. 114  
Willershausen, B. 149, 191  
Willershausen, I. 149  
Williams, R.A.D. 65  
Wimmer, F. 14  
Wollgarten, S. 45

Yanagida, A. 41  
Yang, W. 58

Yanikoglu, F. 103, 119  
Yasar, D. 92  
Yazıcı, B. 91, 93  
Yeo, A.N. 82  
Yoshioka, L. 57, 101  
Young, A. 43, 63, 64, 67, 181  
Yuan, Y. 40, 95, 148

Zabeu, G.S. 90  
Zanin, I.C.J. 17, 22  
Zeng, G. 19  
Zenkner, J.E. 120  
Zenkner, J.E.A. 123, 143  
Zerosi, C. 128  
Zhang, Q. 58  
Zohoori, F.V. 169