ORCA2022

69th Congress of the European Organisation for Caries Research

Cagliari, Sardinia, Italy

June 29 - July 2

Sa Manifattura - Viale Regina Margherita, 33

Dear colleagues and attendees, my dear friends! It is a great pleasure to welcome you to the 69th ORCA Congress. After two years of pandemic restrictions that have forced us all to organize online events only, finally the ORCA family can welcome their members again and I have no words to explain you what a joy it is for me to finally meet you in Cagliari!!!

First of all I would like to express my gratitude to all the people who, over the last two years, have done their best to keep our organization alive, and I do want to thank again my colleagues and friends for their precious support in the preparation of this event.
The congress programme features more than 120 original research presentations from over 30 countries.
With warm thanks to all the authors and to the scientific committee members Carolina Ganss (Editor in Chief) and Margherita Fontana (Vice-President of ORCA), who helped to review the presentations, I am extremely happy and proud to present you the ORCA 2022 scientific overview.

Guglielmo Campus,
Congress Chair and Co-President of the 69th ORCA Congress, Cagliari

69th ORCA Congress

Cagliari, Italy, June 29 - July 2, 2022

Abstracts

Session 1

Nathan Cochrane Junior Scientist Award I

1 Investigating the bioactive properties of a fluoride containing bioactive glass composite for preventing secondary caries

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Secondary caries is the main cause of failure of resin-based composites (RBC) due to polymerization shrinkage of the material, resulting in a marginal gap. The aim of this study was to investigate the ion release and apatite formation ability of an experimental bioactive glass (BAG) containing RBC (BAG-RBC). BAG powders were incorporated into a Bis-GMA-TEGDMA based resin matrix at 45 wt% (45 BAG-RBC). Discs were prepared and immersed into artificial saliva at pH 7 (AS 7) and pH 4 (AS 4), to mimic remineralisation and demineralisation conditions in the oral environment respectively, for multiple timepoints (6 h, 1 d, 3 d, 1 week, 2 weeks, 4 weeks, 3 months and 6 months). A group with no BAG (0BAG-RBC) was used as a control. Discs were removed from the solutions and dried at each timepoint and characterized using Fourier transform infrared spectroscopy (FTIR) and X-Ray diffraction (XRD) and the supernatant solutions were characterized using inductively coupled plasma-optical emission spectrometry (ICP-OES) and an ion selective electrode to quantify the ion release; pH changes were also measured. FTIR showed vibrations (at 560, 600 and 1030 cm^{-1}) and XRD diffraction lines that correspond to apatite upon immersion for the 45 BAG-RBC in both AS 4 and AS 7, unlike the 0BAG-RBC. There was a greater magnitude of ion release (such as fluoride, calcium and phosphate) in AS 4 compared to AS 7 and the oscillations in the trend suggest release and consumption of the ions to form fluorapatite. There was a pH rise upon immersion in AS 4 (up to pH 4.4) whereas the AS7 remained constant. The studied BAG-RBCs released beneficial ions, neutralized the pH in acidic conditions and the apatite layer formed could potentially occlude the marginal gap to prevent secondary caries and promote remineralization.

2 The effect of considering marginal defects in the diagnosis of secondary caries – the CaCIA Randomized Controlled Trial

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The aim of this study was to evaluate the effect of considering marginal defects to assess secondary caries on the treatment decision and longevity of restorations. This was a randomized triple-blind (evaluator, patient and re-evaluator), controlled trial with two parallel-groups: patients who received the assessment of the restorations according to a “traditional” approach based on three FDI (International Dental Federation) criteria (marginal adaptation, marginal staining, and caries recurrence) - FDI group; patients who received the assessment of the restorations according to the CARS (Caries Associated with Restorations or Sealants) criteria from ICCMS - CARS group. The main outcome was the restoration failure and the univariate and multiple Cox regression analysis with shared frailty were conducted. A total of 185 patients were included in the study, totaling 727 teeth. A total of 55% of the restorations were assessed on the follow-up. The follow-up time ranged between 6 and 38 months, with a mean of 20.4 months. It was considered repair or replacement as failures. A total of 187 restorations randomized by the CARS criteria were reassessed, of which 12 operative treatments were performed initially and 12 failed. A total of 218 restorations by the FDI criteria were reassessed, of which 67 interventions were performed initially and 14 failed. The multivariate Cox regression did not show an association between the restoration’s failures and the diagnostic strategy. The restorations with three or more surfaces had almost eight times higher risk for failure compared to restorations with 1 restored surface. Material fracture and secondary caries were the main reasons for failures. In conclusion, considering marginal defects when assessing secondary caries initially leads to more invasive decisions without necessarily representing a medium-term health benefit.

3 Development of a novel and newly designed pH cycling model to study caries in vitro

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Funding: The authors received no specific funding for this work.

This in vitro study aimed to develop and evaluate a novel pH cycling model which accurately simulates the daily dietary cycle with respect to pH fluctuations within the oral cavity. Bovine incisors were highly polished and sectioned into 5 mm x 4 mm samples with acid resistant nail varnish on margins leaving an exposed window on the labial surface. Enamel samples were sound or had subsurface demineralisation at baseline. Enamel samples were used (n=60) in a series of in vitro studies. An automated cycling robot was programmed to run the cycling for 24 h over 14 d. The model included pH challenges mirroring fluctuations within the oral cavity including fluoride solution, demineralising solution and artificial saliva. The primary outcome measures were changes in fluorescence assessed by Quantitative Light Fluorescence (QLF) and enamel mineral loss measured using Transverse Micro Radiography (TMR). QLF and TMR data was collected and the results were analysed using single factor ANOVA with Post Hoc Tukey test to assess for statistical differences with a=0.05. Analysis confirmed the samples did not undergo mineral loss using the final cycling model described, which demonstrates an accurate representation of pH challenges/ fluctuations within the oral cavity. Analysis confirms enamel samples had similar values post-study as at baseline for mineral quantity. Despite the various pH challenges, which accurately mirrors the oral cavity environment, the cycling model developed does not contribute to a net demineralising or remineralising effect. This model has potential benefits for cariology research as various demineralisation/remineralisation agents can be investigated using this novel model. We conclude that the novel pH cycling model developed in this study is an accurate model representing the human oral cavity conditions. This model can be employed to investigate the role of various products in cariology research.

4 Comparison of clinical and digital assessment of erosive-abrasive tooth wear

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This cross-sectional clinical study investigated the feasibility of using an intraoral scanner system for assessing the degree of erosive-abrasive tooth wear in young adults, compared to the clinical examination *in vivo*. Thirty Danish volunteers (14 men and 16 women, mean age 25 ± 5 years) had at least 24 teeth, normal oral function, overjet and overbite between 1 and 5 mm. During clinical examination, the occurrence of erosive-abrasive wear was registered per sextant according to the Basic Erosive Wear Examination (BEWE) index. After 2 weeks, the BEWE index was applied on 3D models obtained from the same volunteers using an intraoral scanner system (Trios 4, 3Shape A/S, Denmark). A blind assessment of the models was performed by 2 examiners. Data was analyzed using weighted Cohen's kappa coefficient with a confidence interval of 95 %. The majority of the volunteers showed important signs of tooth wear. In the clinical assessment, approximately 50% of the young adults showed severe wear (BEWE 3) and ~30% exhibited moderate wear (BEWE 2) on their anterior teeth. Tooth wear was less severe on the posterior teeth (~35% BEWE 1, ~55% BEWE 2). A substantial agreement was observed for the sum of BEWE scores ($p < 0.001$, $k = 0.704$) given at the clinical examination and on the 3D models, while a moderate agreement was found for scores given individually per sextan ($p < 0.001$, $k = 0.585$). Inter- ($p < 0.001$, $k \geq 0.872$) and intra-examiner reliability ($p < 0.001$, $k1 = 0.844$, $k2 = 0.795$) were strong for assessments on the 3D models. Detection of erosive-abrasive tooth wear on digital models showed satisfactory agreement with the *in vivo* examination and can therefore assist diagnosis of this condition.

Session 2
Clinical Studies I

5 TiF4 varnish in the prevention and remineralization of white spot lesions during orthodontic treatment

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This study evaluated the efficacy of TiF4 varnish compared to NaF varnish in the prevention and remineralization of white spot lesions (WSLs) in patients undergoing orthodontic treatment. This clinical, randomized, longitudinal and double-blind study was carried out with 60 adolescents (11-18 years old), living in Bauru-SP, using fixed orthodontic appliances and with at least one active WSL. Participants were randomly divided into groups: G1- non-fluoridated varnish, G2- 5% NaF varnish and G3- 4% TiF4 varnish. Volunteers received weekly professional prophylaxis and application of varnish for the first 4 weeks. Clinical examinations were performed (ICDAS and Nyvad) before applications (T0) and after 6 months (T1). WSLs were evaluated using light-induced quantitative fluorescence (QLF) equipment (ΔQ- integrated fluorescence loss). Chi-square and Kruskal-Wallis were performed ($p<0.05$). In total, 1,196 teeth (G1- 398, G2- 398 and G3- 400) were evaluated. At T0, around 30 % of the teeth had Nyvad 1 and the means±SD of teeth per group were: G1 (5.9±2.6), G2 (6.1±2.8) and G3 (6.0±2.9). At T1, the percentages of teeth with Nyvad 1 were: G1 33.7 %, G2 26.4 % and G3 18.3 %. With respect to Nyvad (regression: placebo 1.2 %, NaF 8.7 %, TiF4 11.3 %; progression: placebo 7.3 %, NaF 3.5 %, TiF4 0.7 %) and to ICDAS (regression: placebo 2.2 %, NaF 11.9 %, TiF4 12.2 %; progression: placebo 11.6 %, NaF 5.6 %, TiF4 1.4 %), both NaF and TiF4 were similar and statistically different compared to placebo ($p<0.001$). Significant differences between the groups by the QLF was found ($p<0.001$). ΔQ (%.mm2) median, 25 %, 75 % percentile values were: G1 (-1.8, -4.6, 2.4), G2 (2.5, -2.8, 7.4) and G3 (5.1, 2.1, 9.1). Both fluoride varnishes were effective in controlling WSL during 6 months of orthodontic treatment, but TiF4 varnish showed the best remineralizing effect by QLF.

6 Prevalence, severity and risk indicators for erosive tooth wear in Belgian adults

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This study was undertaken to estimate prevalence and severity of erosive tooth wear (ETW), as well as to assess non-biological and biological risk indicators for ETW outcomes in Belgian adults. The hypothesis tested was that ETW would be associated with the consumption of acidic beverages. A cross-sectional study was conducted and had a convenience sample of adults ≥18 years who had at least one bilateral posterior molar contact (wisdom teeth excluded). A total of 570 participants were allocated in three age groups 18-34 years (n=232), 35-54 years (n=256) and ≥ 55 years old (n=79). Participants answered a self-applied questionnaire regarding socio-demographics, oral hygiene behavior and lifestyle habits. The questionnaire was tested-retested (ICC=0.71). Clinical examination was carried out by two examiners whose inter-examiner reliability was k=0.76. ETW was assessed according to its clinical features and using the Basic Erosive Wear Examination (BEWE) index. The prevalence of ETW, defined as the percentage of patients with at least one tooth affected was 42% (95% CI: 38.0-46.3). The severity according to BEWE highest score was mild in 35%, moderate in 32% and severe in 33% of participants. The hierarchical logistic regression model for the association between non-biological together with biological indicators and ETW showed significant associations for medical conditions with high risk for ETW ($p<0.001$), consumption of acidic beverages more than once a week ($p=0.043$) or daily ($p=0.016$), as well as for holding/sipping acidic beverages in the mouth before swallowing ($p<0.001$). In conclusion, the prevalence and severity of ETW in Belgian adults were substantial and regular consumption of acidic beverages was identified as risk indicator for this condition. These findings should contribute to improve effectiveness and sustainability of awareness in contemporary adult populations.

7 Long-term caries prevention of sealants and NaF varnish in ASD children

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Funding: The authors received no specific funding for this work.

The aim of the present study is to compare two different strategies for caries prevention of first permanent molars in children with Autism Spectrum Disorders (ASDs). A sample of 232 ASD children were retrospectively retrieved and allocated to two groups, according to whether they have undergone application of fluoride varnishes (FA group) n=92, 9.43±2.44 years; or dental sealant plus fluoride varnishes, (FA+S group) n=140, 7.77±2.57 years. Age, caries severity (as number of untreated lesions in dentine), number of systemic diseases other than ASDs and behavioral assessment at the first dental visit were collected. Logistic and Cox Proportional multivariate analysis were run to evaluate caries incidence and retention rate of sealants in first permanent molars over a period of at least 10 years. A significant higher survival rate from caries was observed overtime in FA+S group compared to FA group (LogRank test $p<0.01$). Dental sealant plus fluoride varnish revealed to be a protective factor toward the development of caries (Hazard Ratio=0.25 $_{95\%}$CI=0.00/0.55 and HR=0.34 $_{95\%}$CI=0.00/0.66 in the upper right and left first molars; HR=0.32 $_{95\%}$CI=0.00/0.66 and HR=0.26 $_{95\%}$CI=0.00/0.58 in the lower right and left first molars) and showed to be able to reduce the risk of new lesions in the lower molars in low severity caries sub-groups (HR=0.21 $_{95\%}$CI=0.07/0.66 and HR=0.29 $_{95\%}$CI=0.10/0.85, left and right respectively). Variables detected at baseline were not associated to the risk of caries development. Dental sealants retention rate was high, ranging between 58.02% and 64.29% after a period of 11-15 years. Dental sealant plus fluoride varnish were more effective than fluoride varnish alone in reducing caries risk in first permanent molars of ASDs children.

8 How entrenched are brushing movement frequencies?

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Funding: The authors received no specific funding for this work.

Toothbrushing behaviour may be based on an intrinsic spontaneous behaviour as, across time and cultures, subjects typically brush buccal surfaces for the longest, and oral surfaces for a much shorter time or not at all, and perform characteristic movements between the lateral and anterior areas. So far, however, little is known about how entrenched the intra-individual brushing behaviour is. Therefore, we observed one aspect of toothbrushing behaviour, namely brushing movement frequency (BMF), repeatedly and included various motor and visual stimuli between observations. The toothbrushing behaviour of twenty participants (24.7±2.8 years) was video-taped in a cross-over design before and after chewing gum or watching a relaxing video and after instruction on systematic brushing. Parameters studied were brushing time and BMF (back-and-forth movements per second). Statistics: t-tests for dependent data and Pearson's correlation. Values are mean ± standard deviation. Brushing time before and after gum chewing was 173±62 s and 168±52 s, and 174±53 and 184±57 s before and after video watching resp. Brushing time after instruction was 168±53 s. None of these differences reached significance. The correlation coefficients ranged from 0.726 to 0.897 ($p \leq 0.001$ each). Similar results were found for BMF with 7.5±1.2/s and 7.7±1.3/s before and after gum chewing and 7.5±1.4/s and 7.7±1.3/s before and after video watching resp. After instruction the value was 7.5±1.4/s. These differences also did not reach significance. The corresponding correlation coefficients ranged from 0.771 to 0.966 ($p \leq 0.001$ each). The results show that the BMF appears to be deeply entrenched and is neither affected by motor or visual stimuli nor by an intervention involving the acquisition of a new brushing sequence. This finding could be important for the development of strategies to train effective brushing techniques.

9 Caries-OUT study in children with CariesCare International adapted for the pandemic: Uruguay Centre - 1-year follow-up

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Funding: Caries OUT Uruguay centre is being funded by Universidad Católica del Uruguay, Universidad El Bosque and IADR RDP LAR.

Aim was to evaluate at 12 months, in 6-8 years old children from Montevideo-Uruguay, the effectiveness of CariesCare International (CCI) adapted for the pandemic (no aerosol-generating procedures, reduced in-office-time), in terms of caries-risk and tooth-surface caries progression control, and oral health behaviours' improvement. Ethics approval: Universidad El Bosque, Universidad Católica del Uruguay. Six-to-eight years old children were invited from the Uruguayan university community-practice primary school, to participate in this single-interventional study. The sample size corresponded to n=27. An ICDAS calibrated examiner and a practitioner were trained online by the Caries OUT steering committee in the study assessments and procedures. The examiner conducted at baseline (T0) and after 5 (T1), 8 (T2) and 12 months (T3) the following assessments: CCI-caries risk, toothbrushing/diet behaviours (COM-B model), tooth-surface caries (visual ICDAS-merged Epi: Sound, Initial-I, Moderate-M, Extensive-E, and Active/Inactive). The dmfs/DMFS caries experience, including ICDAS-merged Epi criteria for caries. The practitioner performed individual/tooth-surface level interventions. Descriptive statistics and Cochrane Q test with Bonferroni post hoc analysis. Twelve-month sample: n=24 (80 %), mean age: 7.24±0.72 years, 58.3 % girls. T0: Most children were at high risk (100 %), presented inadequate to very-inadequate oral-health behaviours (95.8 %), and presented dmfs/DMFS (100 %), with a mean of 10.3±18.0 (d_I/D_I: 4.4±4.0; d_M/D_M: 2.0±3.9; d_E/D_E: 3.3±7.6; m/M: 0.2±0.9; F: 0.5±1.6). Percentage of high-caries risk children decreased from T0 to T1 (41.6 %) and to T2/T3 (8.3 %) ($p<0.001$). Percentage of inadequate oral-health behaviours decreased from T0 to T1 (79.2 %), and to T2 (29.2 %), increasing at T3 (45.8 %)($p<0.05$). At T3 tooth-surface caries progression was controlled in 82.9 % of children. CariesCare International adapted for the pandemic (Caries OUT) was effective for controlling caries after one year.

10 CariesOUT single-group interventional multicentre study: 5-month caries control follow-up

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Aim was to evaluate at 12 months, in 6-8 years old children from Montevideo-Uruguay, the effectiveness of CariesCare International (CCI) adapted for the pandemic (no aerosol-generating procedures, reduced in-office-time), in terms of caries-risk and tooth-surface caries progression control, and oral health behaviours' improvement. Ethics approval: Universidad El Bosque, Universidad Católica del Uruguay. Six-to-eight years old children were invited from the Uruguayan university community-practice primary school, to participate in this single-interventional study. The sample size corresponded to n=27. An ICDAS calibrated examiner and a practitioner were trained online by the Caries OUT steering committee in the study assessments and procedures. The examiner conducted at baseline (T0) and after 5 (T1), 8 (T2) and 12 months (T3) the following assessments: CCI-caries risk, toothbrushing/diet behaviours (COM-B model), tooth-surface caries (visual ICDAS-merged Epi: Sound, Initial-I, Moderate-M, Extensive-E, and Active/Inactive). The dmfs/DMFS caries experience, including ICDAS-merged Epi criteria for caries. The practitioner performed individual/tooth-surface level interventions. Descriptive statistics and Cochrane Q test with Bonferroni post hoc analysis. Twelve-month sample: n=24 (80 %), mean age: 7.24±0.72 years, 58.3 % girls. T0: Most children were at high risk (100 %), presented inadequate to very-inadequate oral-health behaviours (95.8 %), and presented dmfs/DMFS (100 %), with a mean of 10.3±18.0 (dI/DI: 4.4±4.0; dM/DM: 2.0±3.9; dE/DE: 3.3±7.6; m/M: 0.2±0.9; F: 0.5±1.6). Percentage of high-caries risk children decreased from T0 to T1 (41.6 %) and to T2/T3 (8.3 %) ($p<0.001$). Percentage of inadequate oral-health behaviours decreased from T0 to T1 (79.2 %), and to T2 (29.2 %), increasing at T3 (45.8 %) ($p<0.05$). At T3 tooth-surface caries progression was controlled in 82.9 % of children. CariesCare International adapted for the pandemic (Caries OUT) was effective for controlling caries after one year.

11 Does vaping influence oral bacteria growth and cariogenic promoting factors? An in vitro review

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Electronic Nicotine Delivery Systems (ENDS) are becoming common worldwide particularly in young people and former cigarette smokers. Vaping ENDS is perceived as safer than tobacco smoking, even if several studies suggest the aerosol compounds can be dangerous for general and oral health. The aim of this study is to review the *in vitro* papers that investigate ENDS effects on common commensal streptococci (*Streptococcus gordonii*, *intermedius*, *mitis* and *oralis*) and *Streptococcus mutans* (Sm). This review was conducted using the Arksey and O'Malley framework. Several electronic database searches (Web of Science, PubMed, Scopus, Embase and Google Scholar) were conducted from September 1st to November 30th 2021, using an *ad hoc* research string for each database. To compare the studies a random-effect model was performed using Prometa® with a Hedges'g effect size type. After the preliminary search 23 full-text papers were evaluated and five studies were selected. Four studies had the same outcome (bacterial growth) while one evaluated *Sm* adhesion on enamel. The overall significance was 0.33 and the variance 0.29 (not-statistically significant). All the studies concluded that ENDS cause a significant arrest in oral commensal streptococci growth even after few puffs, while e-liquid compounds promote *Sm* adhesion. These effects are more significant for flavoured e-liquids and with the highest nicotine concentrations which resulted in an inhibition on common oral commensal metabolism, while furthering Sm growth. The scarce to date evidence shouldn't let this topic unfollowed. As ENDS users are rising, dental researchers will have to focus not only on vaping effects on periodontium but also on caries effects which could change depending on the type of ENDS used.

Session 3

Nathan Cochrane Junior Scientist Award II

12 What does a plaque index say about the true plaque coverage of a tooth?

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Although plaque indices are widely used, the extent to which they reflect the true plaque-coverage of a tooth has been little studied. Aim of the study was therefore to compare the results from the Rustogy-modified-Navy-Plaque-Index (RMNPI) with planimetrically measured plaque levels. Twenty participants (27.5±1.2 years) were included. Plaque was disclosed (Mira-2-Tone) and the RMNPI was clinically determined (Ramfjord teeth) followed by an intraoral-scan (Carestream 3600), which was used for planimetry (percentage of plaque-covered surface of the entire surface; P%). Time points: T1 (habitual oral hygiene), T2 (after 72 hours without oral hygiene) and T3 (after habitual toothbrushing). Statistics: t-tests for dependent samples compared results at T1 to T3 (subject level), a regression analysis with curve fitting analysed the relationship between plaque-positive RMNPI areas and P% (tooth level). Values are given as mean (range). RMNPI values (% of plaque positive areas of all areas) for T1, T2 and T3 were 62.1 (37.0), 76.9 (29.6) and 56.3 (40.7), P% values were 10.2 (14.7), 15.5 (20.5) and 9.1 (12.7). Both parameters revealed significant differences between T1 and T2 and between T2 and T3 ($p \leq 0.001$ each). The association between P% and RMNPI areas was best represented by an exponential function (T1: $r^2=0.869$, T2: $r^2=0.891$, T3: $r^2=0.855$; $p \leq 0.001$ each). Except for cases with few (0-3) plaque-positive RMNPI areas, there was a considerable range of P% values, especially at T2. This is explained by the dichotomous evaluation of the areas, which makes no difference between small plaque patches and complete plaque coverage within an area. RMNPI scores reflect low plaque levels quite well, while higher RNMPI scores do not necessarily indicate extensive plaque coverage. Planimetry data may be better suited to measure the effectiveness of oral hygiene measures.

13 Study protocol for a randomized controlled clinical trial on the efficacy and reliability of non-operative treatment amongst toddlers

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In Russia, caries prevalence in 3 years old children is extremely high. Considering that the most affected children are those having the least access to professional dental treatment, it is of a paramount importance to verify the efficacy of the non-invasive treatment methods, which can be self-applied *versus* most cost/time-consuming in-office strategies. Therefore, the aim of this study is to evaluate the efficacy of "self-applied" *versus* in-office preventive strategies *in-vivo*. A phase II single-center, randomized clinical trial will be conducted. At *priori* sample size was performed taking into account previous caries data on Russian pre-school children, and 386 children was considered as necessary to achieve an effect size of 80%. Efficacy and reliability of two different "self-applied" and two in-office treatments will be assessed. The first "self-applied" treatment group will receive oral health education plus fluoridated toothpaste (NaF, 1.000 ppm F^-) as basic treatment; the second, the same basic treatment plus daily fluoridated tooth mousse (NaF, 1.450 ppm F^-) self-applied use; the third and the fourth groups will receive both the basic treatment and professional fluoride application, one with NaF varnish (22.600 ppm F^-), and the other using silver diamine fluoride solution (44.800 ppm F^-). The primary outcome will be caries increment (dmft) and the subjects will be clinically examined at baseline, 6, 12, and 24 months follow-up. Ancillary outcomes will be bleeding-on-probing, plaque-pH, saliva and/or plaque microbiological analysis. This trial will be the first in Russia assessing the efficacy of non-operative caries treatments in toddlers. The findings could help strengthen the evidence for the efficacy of self-applied self-applied caries prevention methods.

14 Effectiveness of adhesive containing MDPB: a practice-based clinical trial

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Aim was to prospectively assess whether composite restorations made with and adhesive containing MDPB are less likely to fail due to secondary caries in general dental practice. Nine general practices in the Netherlands were provided with two adhesives for their composite resin restorations, each for a period of 9 months. One contained the quaternary ammonium salt MDPB (Adhesive P), and the other one was used as a control (Adhesive S). Patient's age, SES, and caries risk, as well as tooth type/number, reason for restoration placement, used restorative material and bonding agent, and affected surfaces were recorded. All interventions carried out on these teeth in the next 6 years were extracted from the patient records, along with their date, type, reason, and surfaces. Two dependent variables were defined: failure due to secondary caries, and general survival. Data handling and multiple Cox regression analysis were carried out in R 4.0.5. Eleven dentists from 7 practices made 10,151 restorations over a period of two years in 5,102 patients. 4,591 restorations were made with adhesive P, whereas 5,560 were made with adhesive S. The observation period was up to 6.29 years, median observation time was 3.74 years. Cox regression showed no significant difference between the two adhesive materials when corrected for age, tooth type and caries risk, for neither general failure nor failure due to caries. The hazard ratio for failure due to caries for adhesive P compared to adhesive S was 0.948 (95% CI 0.797-1.128). The restorations made with the adhesive containing MDPB did not fail less frequently due to secondary caries. There was also no difference with regards to general survival, both adhesives performed equally well in general dental practice.

15 Caries assessments on young adults using conventional techniques, 3D-intraoral scanner and near-infrared transillumination - second report

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Aim was to assess the agreement between clinical and radiographical caries scores with scores obtained by a 3D intraoral scanner (TRIOS 4, 3Shape TRIOS A/S Denmark) (3DIOS) and near-infrared transillumination camera (DIAGNOcam, KaVo, Germany) (NIR). Thirty-four young adults (18-30 years old) living in Denmark were recruited and examined using conventional caries detection methods, 3DIOS and NIR. Clinically and radiographically, the caries lesions were assessed by ICCMS as sound, initial, moderate or extensive. For the assessment with 3DIOS, the TRIOS Patient Monitoring software (3Shape TRIOS A/S, Denmark) was used to score the occlusal sites into no-, initial- or moderate/extensive caries lesions. The NIR images were scored as sound (no caries); initial (caries limited to enamel without or with single-point/extensive contact to the dentine-enamel-junction), and moderate or extensive (caries visible in the dentine). For the 3DIOS registration, three occlusal sites on each upper/lower jaw were randomly assigned (n=186). A balanced number of approximal surfaces (n=98), with/without radiographically detected caries were selected for the NIR examination. The intraexaminer agreement was expressed as weighted kappa, while absolute agreement (%) was used to express agreement between methods. Intraexaminer agreement was 100 % for 3DIOS and 85 % for NIR. The distribution of clinical ICCMS scores (n=186) was: 129 sound, 54 initial, and 3 moderate. Corresponding 3DIOS scores were: 127, 55 and 4. The absolute agreement was 78 %. The distribution of ICCMS radiographic scores (approximal surfaces) were: 29 sound surfaces, 67 initial- and 2 moderate lesions. Corresponding NIR scores were: 27 sound, 63 initial, and 8 moderate. The absolute agreement was 64 %. 3DIOS and NIR showed high reproducibility and substantially agreed with visual and radiographical ICCMS when assessing occlusal- and approximal caries lesions, respectively.

16 Assessment of different toothpaste applications without rinsing on artificial root caries

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The aim of this study was to investigate the use of toothpastes either containing low fluoride (CaF) with bioactive glass (BG) or high sodium fluoride (NaF) alone without rinsing on artificial root caries (ARC). The crowns of seven extracted sound teeth were cut by leaving root surfaces and divided into four (n=28). There were four study groups: group 1: BG with 540 ppm CaF; group 2: 5,000 ppm NaF; group 3: 1,450 ppm NaF and group 4: deionised water. The ARC for each sample was developed using demineralisation solution (pH 4.8) for 5 d. The 13 d pH cycling process included using demineralisation solution for 6 h, then placing the samples into remineralisation solution (pH 7) for 16 h. The ARC samples were not rinsed following the application of each toothpaste twice a day during pH-cycling. The hardness and chemical changes for each sample with ARC were evaluated using Knoop micro-hardness surface measurements (HK) and X-ray diffraction (XRD). ^{19}F MAS-NMR was performed to evaluate fluorapatite formation. HK data was analysed using paired *t*-test and one-way ANOVA. HK results showed statistically significant differences between test groups for hardness ($p<0.01$). The HK values [mean±SD (MPa)] for groups 1-4 were 198.68±54.25, 154.31±34.45, 163.18±54.82, 127.83±33.32 respectively. In XRD analysis, the sharp diffraction lines were seen for groups 1 and 3 which suggests apatite formation. NVF MAS- MR confirmed fluorapatite presence in groups 1-3, whilst pure fluorapatite formations were observed in groups 2 and 3 at -103 ppm. Within limitations of this study, all toothpaste treatments without rinsing contribute to fluorapatite formation on the ARC samples. However, a laboratory-based study on natural root caries with large sample size is required.

Session 4
De- and Remineralisation I

17 The effects of different surface treatment methods for orthodontic interproximal reduction on the development of caries

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Interproximal enamel reduction (IPR) is a common clinical approach in orthodontics. The aim of this investigation was to determine the acid resistance of enamel after IPR and after polishing using TMR technique. Seventy-five freshly extracted sound upper premolars were collected and were randomly divided into five groups: group 1, intact enamel surfaces (control group); group 2, manual IPR (New Metal Strips®, GC, Tokyo, Japan); group 3, rotary IPR (IPR File system®, Ortho Direct, St Ann, MO, USA); group 4, manual IPR and subsequent polishing using finishing strips (Sof-Lex® Finishing Strips, 3M, USA); Group 5, rotary IPR and subsequent polishing using the polishing tips included in the kit. After IPR and acid challenge (2.2 mM $CaCl_2$, 10 mM NaH_2PO_4, 50 mM acetic acid, 100 mM NaCl, 1 ppm NaF, 0.02% NaN_3; pH 4.5) for 4 d simulating an cariogenic attack, enamel slices of 100 µm were prepared. TMR images were obtained to observe the sub-surface enamel lesion depth and mineral density. One-way (ANOVA) was used to compare the obtained results. Statistically significant differences ($P<0.05$) were observed in the mineral loss and lesion depth among the manual and rotary IPR groups compared to the control group while differences were statistically insignificant between the manual and rotary IPR and subsequent polishing compared to the control group. In conclusion, polishing of enamel surfaces after IPR procedure is a mandatory step that may affect the acid resistance of the enamel surface.

18 Ion release evaluation of experimental resin composites containing dicalcium phosphate dihydrate (DCPD)

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This study aimed to evaluate the ionic concentrations in demineralizing solution of enamel blocks containing a cavity preparation filled with an experimental composite containing dicalcium phosphate dihydrate (DCPD) particles (G1). For comparison purposes, a commercial composite containing ion-relasing glass particles (Beautiful Bulk Restorative Universal®, Shofu) (G2) and a conventional composite (Z250®, 3M ESPE) (G3) were also tested. Fourty-five bovine tooth enamel blocks were cut and polished (6 x 6 mm). A box-shaped cavity (2 mm width and 1.5 mm depth) was prepared in the central region of the block. The blocks were randomly distributed into 3 groups, and restored with one of the composites (n=15). Specimens were submitted to pH cycling for 8 d (20 h - remineralizing solution - pH 7,0 and 4 h - demineralizing solution – pH 4,7). Calcium and phosphorous, as well as aluminum, boron, sodium, silicon, strontium and zinc concentrations in the demineralizing solution were determined using inductively coupled plasma optical emission spectrometry (ICP-OES). Fluoride release was accessed using a fluoride ion-specific electrode. Data were analyzed by ANOVA/Kruskal-Wallis and Tukey/Dunn' tests (p˂0.05) (Mean±SD). Ca2+(17.11±0.7 mg/L) and PO43-(40.3±5.7 mg/L) in G1 was found in significantly higher amounts when compared to G2 (Ca2+: 14.9±1.19 mg/L; PO43-: 28.3±3.62 mg/L) and G3 (Ca2+: 13.2±1.5 mg/L; PO43-: 23.5±5.5 mg/L). The demineralizing solution from G2 presented significantly more Al, B, Na, Si, Sr and Zn when compared to the solution from G1 and G3. All three groups presented similar concentrations of fluoride, between 15,20 and 16,53 µg/mL. Calcium and phosphorous concentrations were significantly increased through the de-remineralizing process in specimens restored with ion-releasing composites (G1 and G2), which could increase mineral redeposition around restorations facing a cariogenic challenge.

19 Influence of radiation and different artificial saliva on root caries development in vitro

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This study verified the influence of radiation on the development of root dentin caries and tested new formulations of artificial saliva to prevent root caries. Bovine root samples (n=120) were divided according to: irradiated (70 Gy) dentin or not; the type of human biofilm (biofilm pool from 2 irradiated and 2 non-irradiated patients) and the type of artificial saliva (only for the condition irradiated dentin and biofilm from irradiated patient): saliva A (inorganic); saliva A + 1 mg/ml hemoglobin; saliva A + 0.1 mg/ml sugarcane cystatin; saliva A + hemoglobin + cystatin; Bioextra (commercial) and water (negative control) (n=12/group). Microcosm biofilm was produced (5 d), using human biofilm and McBain saliva (0.2% of sucrose, 37° C and 5% CO_2) and the treatments were done 1x/d. Colony-forming units (CFU) counting was performed; demineralization was quantified by transversal microradiography. For the comparison between biofilm x dentin, we applied 2-way ANOVA/Bonferroni or Sidak test. ANOVA/Tukey or Kruskal-Wallis/Dunn was done for artificial saliva ($p<0.05$). No differences between the types of dentin and of biofilm were found for total microorganisms and none of the artificial saliva was able to reduce the CFU. The type of biofilm had no influence on demineralization. Sound dentin with control biofilm presented the highest *Lactobacillus* ssp. and *Streptococcus mutans* CFU and the greatest demineralization (ΔZ: 3466±1199; 2743±733 vol%.µm) compared to irradiated dentin (2484±591; 2173±760, $p<0.0001$) for both types of biofilm (irradiated and no irradiated, respectively). Bioextra was the only one that reduced ΔZ (475±288) compared to water (1567±2567, $p<0.0001$). Irradiated biofilm has not influence on the formation of dentin caries, while the type of dentin has; none of the experimental saliva was able to reduce the development of root caries.

20 Fluoride uptake by enamel by APF (1.23 % F) and change after different pH-cycling De-Re regimens

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In this in vitro pilot study, we evaluated fluoride uptake by carious enamel by acidulated phosphate fluoride (APF) solution and the changes in concentration after different pH-cycling regimens. Human deciduous enamel slabs (n=6/group) with induced caries-like lesions were randomized in two groups: AP (0.1 M H3PO4 solution, pH=4.6) and APF (1.23 % F, in 0.1 M H3PO4 solution, pH=4.6). After 4 min treatment, the slabs were subjected during 20 d to three standardized levels of cariogenic challenges, simulating low (L), medium (M) and high (H) risk of progression of pre-existing caries lesion. Daily cycling times in demineralizing (De-) solution (1.71 mM Ca, 0.81 mM P, 0.038 µg F/mL, pH 5.0) were 3 (L), 6 (M) and 9 (H) h; during the difference time for 24 h, the slabs were kept in remineralizing (Re-) solution (1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 µg F/mL, pH 7.4). Fluoride in enamel, after the treatment and after the pH-cycling, was extracted by acid-etching and quantified with fluoride ion-specific electrode (F-ISE). Fluoride released in De-Re solutions was determined with F-ISE. The results of pH-cycling were analyzed by two-way ANOVA, followed by Tukey's test (α=5%). The effect of treatments and cariogenic challenge level were statistically significant (p=0.0041). Fluoride in enamel after pH-cycling for groups AP-L, AP-M, AP-H, APF-L, APF-M and APF-H were, respectively (means±SD; n=6): 3887.1±1666.6; 3746.8±1353.6; 2023.5±782.0; 4524.1±2883.4; 7311.3±2850.7; 10851.4±4357.0 µg F/cm^3; the baseline concentrations for AP and APF were 7228.9 ±3563.8 and 39936.5±13234.1, respectively. The data of fluoride concentration in enamel were supported by fluoride released (µg F/mL) in De-Re solutions used. In conclusion, the level of cariogenic challenge affects both, the loss of fluoride products formed in enamel by APF application and its subsequent gain.

21 Effects of poly-gamma-glutamic acid of different concentrations and molecular weights on demineralisation inhibition of hydroxyapatite

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The non-toxic, eco-friendly, biocompatible biopolymer poly-gamma-glutamic acid (ɣ-PGA) reduces demineralisation in caries-simulating model systems. The aetiology may be similar to the protective function of statherin which contains glutamic acid. The aim was to measure the effect varying concentrations, and molecular weight of ɣ-PGA, on demineralisation inhibition of hydroxyapatite (HAP) exposed to caries simulating conditions, and, its binding to HAP. HAP discs (20 % porosity) without biological variability were immersed in deionised water. ɣ-PGA solutions were prepared at different concentrations. Also, ɣ-PGA samples with MW of 66 or 200-400 kDa were prepared at 0.1 %, 0.25 % & 1 %. Each disc was treated with any one ɣ-PGA formulation for 2 min, then exposed to acetic acid (pH 4.0) for 1 h. Real-Time Ion Selective Electrodes (RTISEs) were used to measure calcium ion release every 60 s for 1 h, and percentage demineralisation inhibition calculated for each ɣ-PGA concentration. Fourier Transform Infra-Red (FTIR) was used to investigate ɣ-PGA binding to HAP. Spectra were obtained of untreated discs, and those treated with 5ml ɣ-PGA, at each concentration after 1h demineralisation. RTISE: The demineralisation inhibition by ɣ-PGA at 0.5 %, 0.75 %, 1.0 %, 1.5 %, 2.0 %, 3.0 % & 4.0 % was 46.4 %, 37.3 %, 42.2 %, 45.1 %, 75.0 %, 54.2 %, 33.8 % respectively. Inhibition was optimal at 2 %. R^2 for each rate was over 0.95. ɣ-PGAs of 66 kDa had greatest inhibitory effect. FTIR demonstrated that ɣ-PGA remained bound to HAP surfaces after 60 min demineralisation. Peaks at 1580 cm^{-1} and 1620 cm^{-1} were only not present in untreated HAP. ɣ-PGA reduces demineralisation by irreversible binding to HAP surfaces. Binding of ɣ-PGA to HAP may result from the high affinity of carboxyl side chains for calcium, forming stable ionic complexes, similar to statherin.

22 Effect of peroxide-based products containing fluoride on white spot lesion

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This study aimed to investigate the effect of peroxide-based products containing fluoride on white spot lesions (WSL) remineralization. Bovine enamel specimens (n=120) with WSL were randomly assigned into four groups: whitening mouthrinse (WM: 2.5 % hydrogen peroxide - 100ppm F^-); placebo mouthrinse (positive control; PM: 0 % hydrogen peroxide - 100ppm F^-); whitening gel (WG: 10% carbamide peroxide - 1130 ppm F^-); and deionized water (negative control; NC). The treatments (2 min for WM, PM and NC – 2x/d, and 2 h for WG – 1x/d) were performed during a 28 d cycling model (4x60 min demineralization and 18 h remineralization). Relative surface reflection intensity (rSRI) and mineral loss (ΔZ-transversal microradiography) were assessed. Additional specimens with WSL (n=60) were cut in half: one half was kept as control (no treatment) and the other half was submitted to the pH cycling and treatments. The amount of fluoride ([F]) was analyzed in both halves (on surface, at 75 µm and 125 µm depths). Kruskal-Wallis/Dunn's and Wilcoxon's tests were applied. After pH cycling, all groups showed significantly decreased rSRI values (WM=15.68%, PM=9.62%, WG=28.70%, and NC=24.01%) ($p<0.05$). The results for mineral loss were: NC=2746.00±1228.00^{a}, WM=1563.00±482.00^{ab}, WG=1935.00±1157.00^{ab}, PM=1476.50±519.50^{b} ($p<0.05$). No fluoride was detected on the surface of specimens kept as control, but higher fluoride concentration was found on all groups submitted to the pH cycling ($p<0.01$), except for NC ($p=0.12$). Higher [F] was detected for WG at the two analyzed depths, while fluoride was detected only at 75 µm for WM and PM ($p<0.05$). In conclusion, although fluoride from the whitening products could be detected within the WSL, this did not improved remineralization. Treatment with whitening mouthrinse induced less enamel surface alteration than with the whitening gel.

23 Chemical stability of CaF_2-like products formed in sound and carious enamel

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Acidulated phosphate fluoride (APF) forms greater concentration of CaF2-like products in carious than in sound enamel. However, the chemical stability of the products formed in sound or carious enamel has not been investigated. We evaluated the concentration of CaF2-like products formed in sound (S) and with caries-like lesions (C) enamel slabs after APF application and tested their retention after immersion in saliva from 6 to 48 h. Twenty S and 20 C enamel slabs were randomized and evaluated in 4 time-points (n=5/each substrate). APF (1.23 % F in 0.1 M H3PO4, pH=4.5) was applied in all enamel slabs. CaF2-like was formed (time 0) and determined in one group of S and C enamel. The remaining slabs were immersed in a continuous flow of artificial saliva (0.5 mL/min). After 6, 24, and 48 h, groups of five enamel slabs were removed and CaF2-like retained was determined. CaF2-like products formed and retained on enamel were extracted in alkali for 24 h. The extracts were buffered and analyzed with F-ISE. Data were analyzed by two-way ANOVA (enamel substrate condition and time as factors) and Tukey's test (α=5%). The effect of substrate condition and time was statistically significant ($p<0.05$), with greater values for C enamel. The effect of interaction (substrate x time) was not significant ($p>0.05$). Means±SD (n=5) of CaF2-like concentrations (µg F/cm2) at the times 0, 6, 24, and 48 h for the S group were 6.3±3.9a; 4.0±2.6a; 1.4±0.4b and 1.2±0.7b, respectively; For C group, were: 124.3 ±64.5a; 41.8±33.4b; 30.1±15.0bc, and 12.3±1.7c. Within each substrate condition, means followed by distinct letters differ statistically ($p<0.05$). In conclusion, CaF2-like formed in carious enamel by APF application presents similar pattern of stability that in sound one.

24 A pH-cycling model to evaluate the potential of fluoride varnishes to reduce enamel demineralization

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Very few commercially available fluoride varnishes (FV) have been clinically tested and there is no validated model to evaluate the anticaries potential of FV before launching these products on the market. Thus, we developed and tested a pH-cycling model to evaluate fluoride varnishes. Sound bovine enamel blocks, selected by surface hardness (SH), were treated with a clinically proved FV (Duraphat, 2.26 % fluoride w/w) or untreated (control-C). After application of FV, all blocks remained in artificial saliva under agitation at 37°C, and the varnish was removed with acetone after 24 h. All blocks also had half of enamel surface isolated to avoid contact with the demineralizing and remineralizing solutions. Blocks, treated or not with FV, were then randomly allocated (n=5/group) to pH-cycling regimens of three lengths: 4 (4D), 8 (8D), or 12 (12D) days. Each cycle comprised 4 h in demineralizing and 20 h in remineralizing solution at 37°C. The solutions were replaced for fresh ones after the 4th and 6th cycle for 8D and 12D, respectively. Enamel demineralization was evaluated by SH loss (%SHL) and cross-sectional microhardness (ΔS=Area of caries lesion). Data were submitted to one-way ANOVA and Tukey Test (α=5%). %SHL (mean±SD; n=5/group) was: C4D: 92.4±6.6a; FV4D: 55.4±5.1b; C8D: 91.7 ±7.7a; FV8D: 61.4±12.8b; C12D: 93.0±3.9a; FV12D: 79.9±10.3b; respectively. ΔS (kg/ mm^2xµm) was: C4D: 9774.3±3442.6ac; FV4D: 415.8±1172.1b; C8D: 10528.3±2002.8ac; FV8D: 1423.9±1197.9b; C12D: 12316.0±6048.6c; FV12D: 4775.7±5542.5ab. Distinct lowercase letters show statistically different means ($p<0.05$) between C vs. FV for each time. The fluoride-containing varnish significantly inhibited demineralization when compared with the control group. In conclusion, all pH-cycling regimens tested seem to be adequate to evaluate fluoridated varnishes, but it is still necessary to check its dose-response effect with varnishes containing different fluoride concentrations.

25 Energy dispersive X-ray spectroscopy to detect and analyze fluorides, peptides and cerium-salt on dental enamel

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Aim was to investigate the elemental composition of demineralized and intact enamel after application of cariostatic agents containing sodiumfluoride or aminefluoride, oligopeptide p11-4 or ceriumchloride using energy dispersive X-ray spectroscopy (EDX) *in vitro*. Bovine enamel specimens (n=60) were ground flat (FEPA-P4000) and superficially demineralized (200 µm deep caries-like lesions). Half of the specimens were demineralized within buffer-solution (DL; pH=4.5), the other with hydroxyethylcellulose-gel (DG; pH=4.0). Each specimen included a non-demineralized area (intact). Group allocation (n=6): NaF (10,000 ppm, pH=4), AmF (aminefluoride, Elmex Fluid, CP-GABA, 10,000 ppm, pH=4), Ce (cerium(III)chloride 25%, pH=4), p11-4 (Curodont Repair, Credentis, pH=6.2), and Aqua demin. These substances were applied on demineralized and intact areas (p11-4 300 s, other substances 60 s) and rinsed off (aqua demin, 30 s). Elemental composition (F, Ce, N, Ca, P, O, Na, Mg) was measured [At%] on 3 fields (366x291 µm) each on the demineralized and intact area using EDX (FEI Quanta 400 FEG, 10 kV, 1.5 Torr, EDAX Octane-Elect-detector, sw APEX V2.0). Medians (25-75% Quantiles) were calculated. Surface elemental composition was determined and analyzed non-parametrically (Mann-Whitney-U-Tests, $\alpha=0.05$). Fluorine could only be detected on fluoridated surfaces. At%F (median; 25-75%) for NaF: DL=16.6 (9.4-26.1), intact=15.5 (13.8-17.6); DG=30.7 (28-32.5), intact=15.0 (10.8-18.6). At%F for AmF: DL=0.4 (0.3-0.7), intact=15.3 (10.3-23.8); DG=0.6 (0.5-2.2), intact=17 (7.1-22.9). Cerium (At%Ce) was found only after cerium(III)chloride application (group Ce: DL=0.3 (0.3-0.8), intact=1.3(0.7-1.8) DG=0.5 (0.4-0.6), intact=0.7 (0.5-0.8)). Nitrogen (At%N) could only be detected for DG in group p11-4 (DG=1.2 (0.1-7)). A significant increase in Ca:P ratio compared to the corresponding aqua demin specimens was found for NaF (DG, DL and intact; $p \leq 0.004$) and AmF (only intact areas; $p=0.002$). EDX is suitable for non-destructive detection and semi-quantitative analysis of cariostatics containing fluorides, oligopeptide p11-4 and cerium(III)chloride applied to demineralized and intact enamel.

Session 5
Fluoride

26 Silver diammine fluoride efficacy in arresting caries in primary molars: a systematic review and metanalysis.

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A systematic review was performed to evaluate the efficacy of silver diammine fluoride (SDF) in controlling caries progression of cavitated lesions in primary molars compared to no-treatment or any other treatment. Randomized and non-randomized clinical trials with follow-up longer than 6 months were searched using PubMed, Scopus and Embase. The Cochrane Risk of Bias tools for randomized and non-randomized studies were used for quality assessment. The effect size was calculated within each group and across all studies using an inverse-variance model. The success rate and odds ratio were chosen to calculate the effect size. A total of 792 articles were identified, eleven studies were included; ten remained in the qualitative synthesis and one was excluded due to bias in reported results. One study compared SDF to sodium fluoride varnish; 4 compared SDF to high viscosity glass-ionomer cement; 1 study compared 38 % SDF to 12 % SDF; 3 studies compared 38 % SDF to different Ag based varnish; one compared SDF to placebo; 1 study compared SDF different application protocols. Data from five studies were aggregated for meta-analysis. Heterogeneity was found moderate (I2=35.69 % p=0.18). SDF application was found effective in preventing caries progression ($p<0.01$). With a total of 622 arrested lesions on a total of 1205 considered, the caries arrest total rate using SDF>30 % applied annually or biannually evaluated at follow up major or equal to 12 months was 51.62 %±27.40 %(95% C=1.55). When applied to cavitated caries lesions in primary posterior teeth, SDF compared to active treatments or no-treatment appears to effectively prevent caries progression, even if heterogeneity among studies was detected and the evidence was graded as high or medium in eight over eleven studies.

27 Mineral release from toothpaste containing S-PRG fillers during toothbrushing and subsequent oral retention

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We carried out a clinical trial to estimate the amount and release rates of minerals from toothpaste containing S-PRG fillers during toothbrushing and also measured changes in the salivary mineral concentrations over time. Nineteen subjects (18-26 years) brushed their teeth six times according to a controlled schedule with a total of 6 groups as follows; 2 levels of dentifrice (0.5 and 1.0 g) and 3 brushing times (1, 3 and 5 min). After brushing, their saliva-toothpaste-mixtures and then two sets of water (10 mL each) rinsed from their mouths were collected. Saliva samples were also collected 10, 20, 30, 60 and 90 min after the toothbrushing. Each sample was immediately weighed, and a pair of samples for analysis was taken from the supernatant after centrifuge, lyophilized, and stored. One freeze-dried sample was used to determine F electrometrically and another to analyze Al, B, Si and Sr by ICP-AES. The amounts of minerals derived from the toothpaste by toothbrushing were calculated from the collected sample volumes and the mineral concentrations, and the release rates were estimated from the mineral concentrations in the filtrate of toothpaste slurry. The results were analyzed non-parametrically. Micrograms (mean ± SD) of Al, B, F, Si and Sr derived from the toothpaste per gram were 20.7±8.6, 21.8±5.2, 200.9±26.2, 8.2±5.4 and 57.6±17.9, respectively. The release rates of F (30-55%) and Si (20-38%) from the toothpaste were higher ($p<0.05$) than other minerals (about 2%). Si diminished more slowly ($p<0.01$) and B faster ($p<0.001$) from the mouth than the other elements did. The results showed that the S-PRG filler-containing dentifrice released minerals into the oral cavity during toothbrushing and also indicated that different elements had different release and retention rates.

28 Fluoride varnish reduces the lower resistance of fluorotic enamel to demineralization

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We have shown that daily fluoride use from dentifrice (FD) is able to overcome the higher susceptibility of fluorotic enamel to demineralization mimicking the caries-process. Thus, the aim of this study was to assess if professional application of fluoride varnish (FV) would have the same potential. Human teeth presenting Thylstrup and Fejerskov (TF) fluorosis scores from 0 to 4 were obtained and separated in TF0, TF1-2 and TF3-4. Three paired enamel slabs (n=10/group) were obtained from each tooth and allocated in groups: Control (CG), FV and FD. The FV group was pre-treated with Duraphat® (22600 µg F/g as NaF) and subjected to artificial saliva (continuous flow; 0.3 mL/min) for 24 h. All groups were submitted to a pH-cycling regimen simulating an high cariogenic challenge. The CG group received no further treatments and the FD group was treated with FD (1100 µg F/g, as NaF, Tandy®) 2x/d during the pH-cycling. After 10 d, the area of enamel demineralization (ΔS) was evaluated by cross sectional microhardness, and the area of net demineralization (ΔΔS; kg/mm^{2}xµm) was calculated. Values are given as mean±SD, the data was analyzed by two-way ANOVA, followed by Tukey's test (α=5%). FD and VF were effective in reducing demineralization ($p<0.05$). For the CG group, the order of demineralization was TF3-4>TF1-2>TF0 ($p<0.05$): TF3-4=32289.3±3642.8, TF1-2=21223.2±12241.3, TF0=20706.2±11324.2. There was no difference among the TF scores for FD (TF0=6353.6±4777, TF1-2=6325.5±4828.6, TF3-4=9168.1±6219.9) or FV (TF0=16383.1±9236.7, TF1-2=15990.8±10560.2, TF3-4=17475.2±13499.6). However, FD was more effective in reducing demineralization than FV ($p<0.05$). The findings suggest that the higher susceptibility of fluorotic enamel to demineralization can be modified by FD and FV, however the daily use of FD seems to be more effective.

29 Fluoride and silver ion concentrations and pH in silver diammine fluoride solutions from Argentina

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The objective of this study was to measure the concentrations of fluoride (F) and silver (Ag) ions and the pH over time of 2 solutions of silver diammine fluoride (SDF) at 38% produced in Argentina. Group 1 (G1): Fluorsilver® (Densell), group 2 (G2): FAgamin® (TedequimFA), each with two different lots. The fluoride concentration (ppm; a) was determined by visible spectrophotometry and the Ag content (ppm; b) by atomic absorption spectrophotometry. The pH (c) was measured at baseline (t0) and 30 d after opening (t30). Means±standard deviations and t-tests for related samples were used. The data from the freshly opened bottles (t0) were for G1 a) 9,800 ± 310, b) 8,050 ± 350, c) 11.4±0.1 and for G2 a) 107,100 ± 11,320, b) 39,000 ± 1,510, c) 6.95 ± 0.07. The respective values after 30 days (t30) were for G1 a) 8,300 ± 320, b) 77,000 ± 7,100, c) 11.45 ± 21 and for G2 a) 88,900 ± 6.510, b) 38,000 ± 1.430, c) 7.6 ± 0.07. Significant differences were found between t0 and t30 for the pH for G2 (p=0.00). The average concentration of fluoride and silver ions for G1 was lower in relation to the expected values (44800/246000 ppm), however G2 obtained higher values. G2 shows neutral pH values and G1 alkaline. Throughout the 30 days, the content of fluoride and silver tends to decrease.

30 Effect of pH of fluoride mouthrinse on reduction of enamel demineralization

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As the relevance of pH of fluoride mouthrinses on reduction of enamel demineralization has not been studied, we evaluated the effect of pH of commercial mouthrinses on fluoride enamel reactivity and demineralization. Five commercial mouthrinses, presenting 226 ppm F as NaF and pH values from 4.5 to 7.7 were tested and compared with four controls (standard NaF solutions with 226 ppm F, pH from 4.5 to 7.5). The fluoride concentration and pH was determined with a fluoride electrode and pH-electrode, respectively. Sound enamel slabs (n=10/treatment) with known surface hardness (SH) were divided in three parts: one part was used as control and the others to evaluate enamel fluoride formed and reduction of demineralization. To evaluate the total fluoride (TF) formed on the enamel, the slabs were treated for 10 min with the assigned solutions and mouthrinses, and the TF was extracted with acid. To evaluate enamel demineralization, the slabs were subjected to an 8 d cariogenic pH-cycling, being exposed to the treatment 2x/d. Enamel demineralization was evaluated by the percentage of SH loss (%SHL) and by the area of mineral loss (ΔΔS). Data were analyzed by ANOVA and Tukey's test (α=0.05). TF formed in enamel ranged from 6.6-51.7 µg F/cm^3. After pH-cycling, %SHL ranged from 46.1-87.8%, ΔΔS from 2614.7-7254.7 kg/mm^2 x µm, and TF formed from 9.6-49.3 µg F/cm^3. The mouthrinses with acid pH, either control or commercial, were more effective to increase the uptake of fluoride in enamel and to reduce demineralization. In conclusion, pH can be an important factor on the anticaries effect of fluoride mouthrinses.

31 Effect of 5,000 ppm fluoride toothpaste on salivary fluoride: a randomized, controlled clinical trial

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The aim of this study was to explore how much regular brushing with 5,000 ppm fluoride toothpaste (FT) would elevate the salivary fluoride concentration (F) compared to brushing with 1,450 ppm FT, and to study how fast the salivary F would return to baseline levels following resumption of brushing with 1,450 ppm FT. The study was designed as a randomized, controlled double-blind parallel group clinical trial (n=24+24) assessing salivary F during a three-week trial phase with twice-daily use of 5,000 ppm or 1,450 ppm FT, respectively. A two-week wash-out phase, during which all participants used 1,450 ppm FT, followed the trial phase. Saliva samples were collected on 10 different days, on average 7.2 h after the last brushing. Samples were analyzed using a fluoride electrode and two-way repeated measures ANOVA. The salivary F geometric mean for the different sampling days (0 (baseline), 7, 10, 14, 21, 22, 23, 24, 28 and 35) was 0.014, 0.020, 0.021, 0.017, 0.023, 0.015, 0.019, 0.018, 0.025 and 0.016 ppm for the $1{,}450_{ppm}$group and 0.016, 0.035, 0.036, 0.032, 0.044, 0.025, 0.023, 0.024, 0.023 and 0.025 ppm F for the $5{,}000_{ppm}$group, respectively. During the trial phase, the difference between groups was statistically significant apart from the baseline. Salivary F levels remained elevated for one day after recommencement of 1,450 ppm FT, after which the difference between groups was statistically non-significant. The results revealed large interindividual variation in salivary fluoride levels. The higher salivary fluoride concentration in the $5{,}000_{ppm}$group during the trial phase decreased fast when participants returned to 1,450 ppm FT, indicating no notable salivary fluoride reservoir. However, participants were healthy young individuals and results might differ for caries-active individuals.

32 Do toothpastes in Santo Domingo have the recommended minimum of 1000 ppm total soluble fluoride?

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Aim was to determine if the commercially available toothpastes in Santo Domingo, Dominican Republic, have the minimum of 1000 ppm total soluble available fluoride (TSAF) recommended for dental caries prevention. Twenty-two types of toothpastes for children and adults were included in the study, with six samples of each type for a total of 132 toothpastes. The toothpastes were acquired from seven different types of stores, two large direct-to-consumer stores, two small direct-to-consumer stores, one wholesale distributor, one pharmacy, and one independent supermarket, to ensure the inclusion of the largest number of brands commercially available in Santo Domingo. The toothpastes had declared concentrations between 500 and 2500 ppm of fluoride in their labels, and included sodium fluoride (NaF), stannous fluoride (SnF_2) and sodium monofluorophosphate (SMFP). The analyses were conducted using USFDA Test #29 for NaF and SnF_2, and #16 for SMFP. Tests were performed using an Ion Specific Electrode (ISE) and a pH/ISE meter. Standard curves were prepared and analyzed at the time of toothpaste slurries' analyses. 95% confidence intervals were constructed for the non-inferiority limit of 1000 ppm. The results show that 22.7% of the examined toothpastes did not contain the minimum recommended fluoride (1000 ppm), and in some cases the TSAF was much lower (e.g., one toothpaste had 95% C.I.: 182.69-189.39). One toothpaste had 500 ppm declared in its label. On average, the toothpastes contained approximately 13% of non-soluble fluoride. In conclusion, 77.3% of the toothpastes used in Santo Domingo contained a TSAF concentration equal or greater than 1000 ppm F, an amount recommended to achieve the prevention and control of dental caries. Regulations and quality control processes are needed to ensure that all commercially available toothpastes meet the recommendations.

Session 6
Epidemiology I

33 The impact of DMF-T and PUFA on quality of life

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The aim of this cross-sectional study was to evaluate the impact of caries prevalence and severity on quality of life in 12-year-old children. The study was approved by the ethics committee and participation was voluntary consented by parents. The sample consisted of 215 students enrolled in public schools in Petropolis, Rio de Janeiro, Brazil. DMF-T and PUFA indices were used to assess caries and pulpal involvement, respectively. Impact on quality of life was assessed using Oral Impacts on Daily Performances (Child OIDP). The questionnaire consists of 10 questions about pain, discomfort when eating, brushing, sleeping and other daily activities. Impact on quality of life ranges from zero (no impact) to 10 (maximum negative impact). Data were analyzed using SPSS software. Descriptive and association analyses were done. Kruskal-Wallis test and Spearman correlation were used. Caries prevalence was 61.4% and DMF-T was 1.91 (±2.08). Among 132 children with caries, 34 (25.8%) had at least one tooth with pulpal involvement. The questionnaire score was: a) 1.71 (±2.04) for the whole sample; b) 1.06 (±1.43) when DMF-T = 0; c) 1.64 (±2.01) when DMF-T ≥ 1 and PUFA = 0; and 3.47 (±2.38) when deft ≥ 1 and PUFA ≥ 1. Correlation between the questionnaire score and DMF-T score ($p < 0.001$) and pufa score ($p < 0.001$) was significant. The main complaints were related to tooth pain (31.2%) and discomfort when eating (36.7%), sleeping (245.1%) and brushing (18.6%). The presence of cavitated caries in permanent teeth impacted negatively on quality of life of the children. When pulpal involvement was present, the negative impact was significantly increased.

34 Standardizing the DMFT of 12 year-old 6th-graders in the federal states of Germany in National Oral Health Survey in Children 2016 by weighting with national distribution of school types

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The objective was to standardize DMFTs of 12-year-old 6th-graders in the federal states of Germany during the National Oral Health Survey in Children (NOHSC) 2016. Former studies have shown that in Germany pupils in Grammar Schools (GS) have consistently and markedly lower DMFTs than pupils in Other Schools (OS) and that this difference is mainly due to differences in the pupils' socio-economic background. Since in the NOHSC 2016 the relative sample sizes in GS and OS differed considerably between the 16 states, a method was sought to "standardize" federal DMFTs with view to school types / socio-economic background. Therefore, the DMFTs in GS and OS in each federal state were weighted with the national ercentages of u ils attending these two categories of school ty es in the school term 2015/16 (GS: 36.7 % vs. OS: 63.3 %) and resented descri tively. The total (GS+OS) raw DMFT of 12-year-old 6th-graders in the 16 German federal states ranged from 0.24 to 0.74. In all states the DMFT in GS was lower than in OS (~ twofold on mean). After school ty e-related standardisation the DMFTs of the federal states changed by +12.1 % to -32.2 % thus reducing the differences in DMFT between federal states. The national DMFT re orted in NOHSC 2016 (0.444) remained virtually unchanged after standardisation (0.4438), roving that the national sam le (n=54,965) was re resentative of the national distribution of 6th-graders to GS and OS. Federal DMFTs of 12-year-old 6th-graders in NOHSC may be standardized in order to com are oral health in federal states regardless of relative sam le sizes in different school ty es.

35 Oral health behaviour among the population in Russia

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Aim was to identify oral hygiene habits among the Russian population. In the course of the 3rd Russian National Oral Health Survey, 27284 children and adults (4620 6-year-olds, 6004 12-year-olds, 5746 15-year-olds, 6006 35-44-year-olds, 4908 65-year-olds and older) were interviewed using a standardized validated questionnaire (WHO, 2013). The ratio of male and female was 44 % and 56 %; urban and rural 83% and 17% respectively. The study was approved by the ethical committee of MSUMD. In the survey, 10 % of the 6-year-olds, 12 % of the 12-year-olds, 11 % of the 15-year-olds, 9 % of the 35-44-year-olds and 25 % of the 65-year-olds clean their teeth less than once a day. Around 35 % to 43 % of all age groups clean their teeth once a day. Twice-a-day toothbrushing is performed by 52 %, 50 %, 54 %, 54 % and 31 % of the corresponding aforementioned age groups. A toothbrush is used by 99 % of children and 35-44-year-olds, and by 92 % of 65-year-olds. Toothpicks are used by around 13 %, 28 %, 33 %, 40 % and 35 % of the corresponding aforementioned age groups and dental floss is used by 8 %, 18 %, 23 %, 29 % and 8 %. Mouthwash, oral irrigator or interdental brushes are used by less than 5 % of surveyed population. Toothpaste is used by 98 % of children and 35-44-year-olds and 90 % 65-year-olds, while 32 % of the 6-year-olds, 30 % of the- 12-year-olds, 30 % of the 15-year-olds, 44 % of the 35-44-year-olds and 31 % of the 65-year-olds use fluoride toothpaste. About half of the surveyed children and about a third of adults don’t know if their toothpaste contains fluoride. The results of the study showed insufficient oral health habits among the surveyed population. The data could be used to prioritize national oral health strategies.

36 Oral health behaviours, living conditions and caries experience of 6-year olds in Romania

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Funding: The Borrows Foundation for financial support and helping to implement the Romanian Oral Health Survey for children.

There have been few comprehensive oral health surveys in Romania. Out objective was to evaluate individual level predictors correlated with caries experience in 6-year-old children from Romania. Within the National Romanian Oral Health Survey for children, an estimation of the required sample size, assuming a sampling error of ±3 % at 95 % confidence level was conducted. A stratified random sample, was used based on administrative units and 45 schools distributed in rural and urban areas were selected. A total of 718 children aged 6-7 years (mean age 6.48 years) were examined. Dental caries, deciduous and permanent teeth, and teeth missing due to caries were recorded according to ICDAS criteria by 10 previously calibrated examiners. ICDAS codes higher than 3 were computed as dentinal caries, missing teeth due to complex carious lesions as MT, and restored teeth as FT. Dmft and SiC indexes were calculated, a multilevel binary model to predict prevalence, and a multilevel Poisson analysis using only non-zero values was used to analyze the dataset. A percentage of 14.76% (106 children) had a dmft index of 0. The SiC index for the whole sample was 9.83. A negative association could be seen between the dmft and the parents' level of education, being predicted by both the father's educations (β=-0.33, SE=0.07, $p<0.01$) and the mother's education (β=-0.25, SE=0.07, $p<0.01$). The Poisson analysis showed significant associations between dmft and fruit consumption (β=0.04, SE=0.01, $p<0.05$), fizzy drinks β=0.04, SE=0.01, $p<0.01$), milk (β=0.05, SE=0.01, $p<0.01$) and tea (β=0.05, SE=0.01, $p<0.01$). School level predictors were negatively associated with the dmft index (β=-0.54, SE=0.25, $p<0.01$). The caries experience is very high in the selected sample and strongly correlated to individual level predictors.

37 Dental health in a Viking population from Varnhem, Sweden

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The prevalence and distribution of dental caries, tooth wear and other clinically detectable oral pathologies were determined in complete and partial human skulls from a Viking time graveyard (900-1050) excavated in Varnhem, Sweden. Totally 171 individuals, of which 133 were adults, were studied using visual inspection and a dental probe under strong light source. Three examiners performed the examinations after calibration. All adult individuals were examined by at least two examiners. Radiographic bite-wings were taken to control the caries diagnostics in a sub-sample (18 individuals). Caries prevalence in the adult population was 62 %, while 13 % of the teeth had at least one carious lesion. Crown caries was found for 7 % of examined teeth and 7 % had root caries, while 1 % of the teeth had carious lesions in both the root and the crown. Caries experience (DMT>0) in the adult population was 71 % with a mean DMT of 4.3. The teeth most susceptible to caries were the molar teeth. In the adult population, there was a negative correlation between number of teeth with caries and age ($p<0.05$), possibly explained by the positive correlation between number of lost teeth ante mortem and age ($p<0.05$). Other findings were apical lesions, enamel pearls, aplasia, abrasive wear from tooth picking, non-erupted canines and severe amounts of calculus. One case of apical infection located in the palate was so extensive that it may have contributed to death. One individual had filed front teeth. Conclusively, this work shows that dental pathology, especially dental caries and tooth loss, was common in this Viking age community. The findings give rare insights to the life and death in this prehistoric population, and proves an extended understanding for the Vikings.

38 Oral health related dietary habits among the population in Russia

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Aim was to study the frequency of sugar-containing food-and-drink consumption in the Russian population. During 3rd Russian National Oral Heath Survey, 4620 6-year-olds, 6004 12-year-olds, 5746 15-year-olds, 6006 35-44 year-olds, 4908 65 year-olds and older were interviewed using standardized validated questionnaire (WHO, 2013). Ratio of interviewees was 44.1% male and 55.9% female, 82.6% urban and 17.4% rural. The study was approved by the ethical committee of MSUMD. The percentages consuming daily fresh fruit were 55.5 % of the 6-year-olds, 52.6 % of the 12-year-olds, 43.4 % of the 15-year-olds, 42.5 % of the 35-44-year-olds and 33.8 % of the 65-year-olds and older. Biscuits/cakes are consumed daily by 26.3 %, 28.1 %, 27.5 %, 21.1 % and 19.0 % of the corresponding aforementioned age groups. Sweet pies are consumed daily by 25 % of children, 14 % of adults. Jam/honey is consumed daily by 11.2 %, 17.6 %, 14.0 %, 16.8 % and 20.8 % of the corresponding aforementioned age groups. Sweets/candy are consumed daily 27.3 %, 30.0 %, 25.7 %, 20.5 %, 18.0 % in the following groups. Soft drinks are consumed daily by 13.7 % of the 6-year-olds, 14.3 % of the 12-year-olds, 15.6 % of the 15-year-olds, 12.5 % of the 35-44-year-olds, 9.1 % of the 65-year-olds and older. Sugared tea is consumed daily by 63.0 %, 64.2 %, 60.8 %, 56.7 % and 54.9 % of the corresponding aforementioned age groups. Sugared coffee is consumed daily by 5.9 % of the 6-year-olds, 12.4 % of the 12-year-olds, 21.0 % of the 15-year-olds, 51.0 % of the 35-44-year-olds and 35.8 % of the 65-year-olds and older. The results of the study show a high frequency of sugar-containing food-and-drink consumption among the Russian population. Oral health strategies should include recommendations on sugar intake reduction.

39 Caries trajectories decline in children 7-10 years old in the city of Greifswald in Germany

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Dental caries is still considered a major public health problem globally. In Germany, the population witnessed a decline in caries prevalence in children and adolescents. Aim was to analyse caries trajectories in children 7-10 years old in schools of the city of Greifswald/Germany over 20 years. DMFT values of the aimed population were collected during the regular school examinations in the years 2000, 2006, and 2020 (Kurzweil 2009, ÖGD 2020). Since the first permanent tooth usually erupts around the age of 6, mean caries increase per year for each age group was measured after omitting the first 5 years. Thus, caries trajectories were obtained by dividing the total DMFT value by the number of living years with permanent teeth. A descriptive statistical analysis was done using SPSS. Ethical approval (Reg. No.: BB48/10a/Greifswald University). The total number of examined children was 1187 in 2000, 586 in 2006 (48.9 % females), and 391 in 2020 (50.9 % females). The prevalence of caries-free children raised from 81.4 % in 2006 to 89 % in 2020, while the range DMFT values showed a decline through the years in the study sample (0.13-0.67 in 2000, 0.14-0.58 in 2006 and 0.13-0.26 in 2020). Caries trajectories (increase of DMFT per year) dropped in all examined children through the years, mainly for the 9- and 10-year-olds (9-year-olds 0.12 DMFT/y in 2000 to 0.05 DMFT/y in 2020; 10-year-olds 0.13 DMFT/y in 2000 to 0.05 in 2020). The results confirm a caries decline of more than 50 % in 20 years and relatively constant caries trajectories for each birth cohort in primary schools in the city of Greifswald. This emphasizes the success of preventive measures and the use of regular monitoring in school dental examinations.

40 Caries trajectories decline in adolescents 14-18 years old in the city of Greifswald in Germany

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Dental caries is still considered a major public health problem globally. In Germany, the population witnessed a decline in caries prevalence in children and adolescents. Aim was to analyse the caries trajectories in adolescents 14-18 years old in schools of the city of Greifswald in Germany over 20 years. DMFT values of the aimed population were collected during the regular school examinations in the years 2000, 2006, and 2020 (Kurzweil 2009, ÖGD 2020). Since the first permanent tooth usually erupts around the age of 6, mean caries increase per year for each age group was measured after omitting the first 5 years. Thus, caries trajectories were obtained by dividing the total DMFT value by the number of living years with permanent teeth. A descriptive statistical analysis was done using SPSS. Ethical approval (Reg. No.: BB48/10a/Greifswald University). The total number of examined adolescents was 1798 in 2000, 603 in 2006 (52.6 % females), and 668 in 2020 (46.5 % females). The prevalence of caries-free adolescents raised from 38 % in 2006 to 74.8% in 2020, while the range of DMFT values showed a decline through the years in the study sample (3.13-7.50 in 2000, 1.77-2.83 in 2006 and 0.05-0.07 in 2020). The caries trajectories (increase of DMFT per year) dropped in all examined children through the years (0.35-0.58 DMFT/y in 2000 to 0.17-0.24 DMFT/y in 2006, and recently 0.05-0.07 DMFT/y in 2020). The results confirm a decline in caries prevalence of up to 80 % in some age groups and a major decline in caries trajectories in adolescents in the city of Greifswald. This emphasizes the success of preventive measures and the use of regular monitoring in school dental examinations.

Session 7
Microbiology

41 Regulation of Streptococcus mutans virulence characteristics by probiotic Lactobacillus reuteri metabolites

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With the increasing popularity of probiotics as caries-preventives, we investigated *S. mutans* (SM) response to such treatment. The aim was to (i) evaluate the influence of metabolites obtained from commercial *L. reuteri* (LR) probiotic strains and endogenous lactobacilli on SM genes involved in virulence response (*vicR, atpD, ldh*) and glucosyltransferase production (*gtfB, gtfC, gtfD*), also to (ii) investigate expression differences of abovementioned genes between wild SM isolates from caries-free and caries-active individuals. Twenty SM isolates (10 caries-free; 10 caries-active) from the existing collection at the Department of Cariology were exposed (4 h) to PBS filtrates, containing metabolites from 2 commercial LR strains (DSM_17938; ATCC PTA_5289), BioGaia Prodentis® drops, and one endogenous lactobacillus respectively. RNA extraction and cDNA synthesis were conducted using commercial kits. Expression of interest genes was quantified using RT-qPCR with 16S rRNA as a reference gene and ratioed to corresponding controls not treated with PBS filtrate. Tests were run in duplicates; REST method was used to establish significant ($p<0.05$) differences between groups. A difference in gene expressions was observed between caries-active and caries-free SM isolates for all treatments. A significant reduction of *gtf*B and *gtfC* expression (0.02-0.07), attributing to less stable biofilm formation, was obtained with metabolites from both LR strains. Glucotransferases were less affected by endogenous lactobacillus and BioGaia metabolites (0.15-0.92). Metabolite treatment enhanced stress response (*vicR*) in caries-active isolates (1.29-2.08), suggesting higher bacterial adaptability than in caries-free isolates. Acidogenicity (*ldh*) was downregulated in all cases, and acidurity (*atpD*) was upregulated in active-caries SM isolates (1.93). Caries-active isolates are more resilient to probiotic metabolite treatment and show better adaptation ability under stress conditions than caries-free isolates. Implementation of probiotics may therefore result in different treatment outcomes in different individuals.

42 Effect of different atmospheres on microcosm biofilm formation and tooth demineralization

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This study evaluated the effect of different atmospheres on the potential of microcosm biofilm to cause tooth demineralization. Ninety bovine enamel and 90 dentin specimens were distributed according to the atmospheres: 1) microaerophilia (5 d, at 5 % CO_2); 2) anaerobiosis (5 d, at jar); 3) mixed (2 d in microaerophilia and 3 d in anaerobiosis), which were subdivided into the following treatments: 0.12 % chlorhexidine (positive control-CHX) and PBS (negative control) (n=15). Biofilm was produced from human saliva and McBain's saliva with 0.2 % sucrose. From the 2nd day onwards, the specimens were treated with CHX or PBS (1x1 min/d). After 5 days, CFUs were computed and tooth demineralization was analyzed by transverse microradiography. Data were submitted to two-way ANOVA and Tukey/Sidak test ($p<0.05$). Most atmospheres were able to differ CHX and PBS (from 0.3 to 1.48 log_{10} CFU/ml), except anaerobiosis and microaerophilia ones for total microorganisms CFU in biofilm produced on enamel and dentin, respectively. In case of dentin, the effect of CHX on *Lactobacillus sp.* was not seen neither. All atmospheres were able to differentiate CHX and PBS with respect to enamel demineralization, showing lower mineral loss and lesion depth for the former one (78 % and 22 % reduction in the mineral loss; 60 % and 16 % reduction in the lesion depth for enamel and dentin, respectively). Enamel mineral loss data did not differ between the models; however, the enamel lesion depth was significantly higher under anaerobiosis ($p=0.0148$), while dentin mineral loss was significantly lower under anaerobiosis compared to the other atmospheres ($p=0.0034$). Despite some differences were found, the type of atmosphere seems not interfere with the cariogenic potential of the microcosm biofilm.

43 Development of multi-particulate systems for natural compound release and oral biofilm control

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The aim of this study was to develop a multi-particulate systems based on sodium alginate (ALG)/gellan gum (GG) polymers for morin controlled release and oral biofilm control. Three systems with different proportions of natural polymers (50:50, 25:75, 75:25; ALG:GG) were developed. Systems without morin were used as controls. Polymicrobial ("microcosm") biofilm were grown in sterile glass coverslips using artificial saliva and 1 % sucrose (6 h daily). The systems (treatment; 2 mg morin and control) were added to 24-well plates at the beginning of the experiment. The systems were characterized in terms of morin release. The acidogenicity of the biofilm was assessed by pH readings. After 24 h, the antimicrobial activity was evaluated by counting viable microorganisms (CFU/mL) and the antibiofilm capacity was evaluated by quantifying the biomass (crystal violet staining). The data were analyzed by Two-way ANOVA analysis of variance models and Tukey post-test at 5 %significance level. After 480 min, morin release varied from 85.45 % ± 8.31 up to 99.86 %± 9.36. The pH values after 24 h for treatment and control group were, respectively [mean (±sd)]: 5.72 (±0.02) and 4.30 (±0.10) (50:50); 5.38 (±0.45) and 4.20 (±0.19) (25:75); 5.71 (±0.76) and 4.30 (±0.18) (75:25). Microbial viability reduction (Log CFU/mL) after treatment (mean) varied from 1.96-2.82 (total bacteria), 1.53-3.11 (aciduric bacteria) and 2.19-2.89 (mutans streptococci) for the 50:50, 25:75 and 75:25 systems, respectively. The mean (±sd) biomass (A_{570}) was 0.19 (±0.15), 0.17 (±0.12), 0.15 (±0.05) for the 50:50, 25:75 and 75:25 systems respectively. This difference was significant when compared to the control group ($p<0.01$ - biomass and $p<0.001$ - viability). The developed systems proved to be efficient for morin-controlled release and to control important virulence factors of polymicrobial biofilm.

44 Bacteriome analysis of biofilm from root surfaces in Colombian elderlies

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This study aimed to assess by 16S rRNA analysis, the biofilm bacteriome of root surfaces in a Colombian institutionalised elderly population. After ethical approval (012-216) and informed consent in 42-institutionalised elderlies, DNA from root biofilm samples was extracted from sound, initial and moderate/extensive root caries lesions according to ICDAS visual criteria. The V4 region of 16S rRNA gene (515R-806F) was amplified, and genomic libraries were prepared and sequenced in MiSeq (Illumina) obtaining 300-bp paired sequences. Diversity (Simpson/Shannon indices, Kruskal-Wallis-test, alpha 0.01) richness/abundance analyses and taxonomic assignment (ASVs) were conducted with HOMD database using PAST/QIIME2. Biofilm bacteriome composition of sound and carious surfaces were compared (Welch's t-test). A total of 130 biofilm samples were analyzed. Sound: n=45 and with root-caries n= 85: Initial-root caries: n=41, moderate/extensive-root caries: n=44; those which were n=60 active and n=25 inactive. A total of 5269 ASVs were found (genus: 91-taxa, species: 343-taxa). The genus present only in sound sites were mainly non-fermenting and typical of periodontal dysbiosis such as Butyrivibrio, and absent in sound sites were activity marker genus such as Bifidobacteriaceae and Bacteroides. Root-caries (regardless of severity/activity-status) showed a significantly lower species richness compared with sound surfaces ($p<0.05$). In sound surfaces there was higher relative species abundance ($p<0.05$) such as: Porfiromona pasteri, Carbiobacterium valvarum; in initial-caries: Prevotella sp. HMT 313; in moderate/extensive caries: Prevotella denticola, Fusobacterium nucleatum and Lactibacillus sp. In inactive-caries there was a greater relative abundance of Veillonella dispar, while in active-caries species similar to those found in the biofilm of moderate/severe caries predominated. This bacteriome-based study shows a complex root-caries microbial community with periodontal pathogens, participating. Lower richness and differential microorganisms' relative abundance suggest dysbiosis in biofilms from carious sites.

45 Bacterial composition of dental biofilm from adolescents with erosive tooth wear, cavitated caries or sound

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Aim was to compare dental biofilm composition from 12- to 15-year-old adolescents with erosive tooth wear (ETW), cavitated caries (CC), or sound to both conditions. Twenty-four-hour evolution coronal dental biofilm samples were obtained from 30 12- to 15-year-old adolescents with three dental conditions (ETW: n=10; CC: n=10; Sound: n=10). To avoid subgingival contamination, the biofilm from incisal/occlusal and middle thirds of vestibular surfaces were sampled. Sequences of 16S rRNA gene was obtained in Illumina-MiSeq. Taxonomic classification with Amplicon Sequence Variants (ASV,>1% abundance) and assignment according to HOMD-database were carried out. Alpha diversity analysis included richness & evenness; Simpson and Shannon indices were used. Comparisons of the biofilm composition between groups were made (Kruskal-Wallis test). Beta diversity was assessed through Welch t-test. Twenty-four ASVs at the species level were obtained. Alpha diversity analysis showed statistically significant differences in the species number between Sound and the other two groups (richness and evenness: $p<0.05$). Beta diversity comparison displayed a differential species number in the groups ($p<0.05$): -Between ETW and Sound, with a greater relative abundance of Campylobacter gracilis, Fusobacterium nucleatu subsp animalis, Neisseria perflava, Bacteroidales bacterium HMT 274, Peptidiphagasp, Treponema socanskii, Prevotella maculosa, Prevoella saccharolytica in ETW, while in Sound, of Streptococcus mitis, Gemella haemolysans, Granulicatella adiacens, Prevotella nanceiensis; between CC and Sound, higher relative abundance of Porphyromonas pasteri in Sound, while in CC of Bacteroidales bacterium HMT 274, Leptotichia sp, Treponema socanskii, Neisseria bacilliformis, Catonella sp, Precotella maculosa, Prevotella micans. Finally, between CC and ETW, Campylobacter rectus in CC and in ETW Prevotella sp HMT 300 showed higher relative abundance. Differences in the dental biofilm microbial composition among the three groups may be related to dysbiosis within these dental conditions in adolescents.

46 Antimicrobial photodynamic therapy mediated by Senna macranthera plant extract against Candida albicans suspensions and biofilm

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Natural compounds as plant extracts have large potential to act as photosensitizers in antimicrobial photodynamic therapy (aPDT). Considering their potential and the role of *Candida albicans* in caries etiology, this study aimed to evaluate the antimicrobial effect of *Senna macranthera* plant extract against *C. albicans* suspension and biofilm. *C. albicans* ATCC 90028 suspensions were incubated for 48 h and the inoculum was adjusted to a final concentration of $5x10^6$ CFU/mL. Biofilms were cultivated for 48 h and the culture medium was refreshed after 24 h. Treatment was performed in five groups: NC (negative control), PC (plant extract), LC (light), VC (vehicle control; with or without light) and PC+light (aPDT). 50 µg/ml of *S. macranthera* plant extract were applied to PC and aPDT groups and pre-incubated for 5 min (suspension) or 15 min (biofilm). Then, VC with light, LC and aPDT groups were irradiated at 450 nm and 80 J/cm^2 during 560 s (suspension) or 900 s (biofilm) in fractionated mode. Colony counting (CFU/mL) was further assessed. Two-way analysis of variance (two-way ANOVA) was performed to each suspension or biofilm. The presence and absence of light and plant extract were independent factors. Data were comparatively analyzed at 95% confidence interval using IBM SPSS 20.0. The analysis demonstrated that either suspension or biofilms reduction is dependent on light-plant extract absorption ($p<0.0001$). PC, LC and VC (with or without light) groups showed no significant log reduction whereas, the plant extract showed significant total log-reduction of both suspension (5.93 log CFU/mL) and biofilms (7.7 log CFU/mL) forms ($p<0.0001$). The present findings suggest that aPDT mediated by *S. macranthera* plant extract is an effective alternative to conventional antifungal therapies to eliminate *C. albicans.*

47 Antimicrobial effects of photodynamic therapy in artificial carious lesions in human teeth in vitro

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The aim of the present study was to evaluate the antimicrobial effect of photodynamic therapy in artificially created and infected cavities of human wisdom teeth. Defined cavities were prepared into the lateral surfaces of extracted human wisdom teeth using a round diamond bur. The teeth were incubated in pooled human saliva collected from four volunteers. The cavities were incubated in the saliva solution for 6 d and then sampled using a cut sterile paper point. The teeth were randomly distributed into three groups with 20 cavities in each group. In the first treatment group, antimicrobial photodynamic therapy (aPDT) was used. The photosensitizer, a tolonium chloride solution, was brought into the cavity and incubated for 60 s and activated using light-emitting diode (LED) at a wavelength of 635-645 nm for 60 s. In the second treatment group, antimicrobial treatment was performed using 0.2% chlorhexidine (CHX) solution. In the control group, no antimicrobial treatment was performed. A final sample was taken using a cut sterile paper point to determine the remaining contamination of the cavities. The samples were cultured on Columbia blood agar (CBA). Statistical analysis was performed using a one-way analysis of variance (ANOVA). A *P value* of <0.05 indicates significant differences. The highest contamination was found in the untreated cavities of the control group. Statistical analysis showed a significant difference between the groups. Treatment using CHX killed 86 % of the bacteria, whereas in the aPDT group a significantly lower reduction of bacterial contamination of only 74 % was observed ($p=0.015$). APDT significantly reduced bacterial contamination in artificial dental cavities. However, CHX was significantly more effective in reducing bacterial viability.

48 Anti-caries and anti-biofilm capacity of a prebiotic in association with fluoride

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The aim of this study was to evaluate the anti-caries and antibiofilm capacity of a prebiotic in association with fluoride (F). A polymicrobial biofilm using bovine enamel blocks (n=12) placed vertically were used to simulate the oral biofilm. The groups used were: negative control (NC); fluoride (NaF, 110 ppm F; “F”); prebiotic (0.8% arginine; “PB”) and PB+F. Treatment solutions were added to the culture media during biofilm growth. After 120 h, the acidogenicity (pH of spent medium), microbial composition of the biofilm (total bacteria, mutans streptocci and aciduric bacteria) and enamel hardness loss (%SH) were analyzed. Data were analyzed using SigmaPlot 12.0 with a significance level of 5%. The assumptions of normality and homoscedasticity were tested (Shapiro-Wilk and Levene's test) and the following tests were applied: two way ANOVA (acidogenicity), Kruskall-Wallis (microbial composition) and one way ANOVA (%SH). Acidogenicity of PB and PB+F groups was significantly different from NC or F groups from 56–120 h of biofilm growth (avg pH ±sd: 7.93±0.35 and 7.77±0.36, respectively; $p<0.05$). F group showed the lowest total bacteria counts (median 6.29 log UFC/mL; IQR 0.68) and PB showed the lowest aciduric baceria counts (median 5.63 log UFC/mL; IQR 0.35) ($p<0.05$). For mutans streptococci, there were no statistically significant differences between F, PB or PB+F. PB and PB+F showed lower %SH in comparison to F (avg±sd: -21.7±2.4; -14.2 ±2.7 and -39.8±4.8, respectively; $p<0.05$). In conclusion, the prebiotic is a promising approach for the ecological management of dental caries and for the control of enamel demineralization. F association with PB can enhance its mechanism of action and control biofilm acidogenicity, aciduric species and decrease enamel demineralization in comparision to F alone.

49 Anti-biofilm activity of chlorhexidine-releasing elastomerics against dental microcosm biofilms

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This study aimed to evaluate the anti-biofilm activity of chlorhexidine-releasing elastomerics (CRE). Each of the two coating solutions, containing 0.01 g/ml chlorhexidine diacetate at different solvent ratios (ethanol and dichloromethane), was coated on elastomerics and then attached to the bovine enamel specimen to form two groups: CRE1 and CRE2. In the negative (distilled water; DW) and positive (0.1 % chlorhexidine; CHX) control groups, uncoated elastomerics were attached to the specimen. 10 specimens were used in each group. Biofilms were formed by directly inoculating the surface of the specimen with saliva obtained from a single healthy donor who refrained from oral hygiene for 24 h. The DW and CRE groups were treated with DW, and the positive control was treated with 0.1 % CHX twice a day for 5 min. After 7 d of biofilm formation, the total number of viable bacteria in the biofilms was evaluated using colony-forming units (CFU). Biofilm maturation was evaluated by the red/green (R/G) ratio of the images taken by quantitative light-induced fluorescence-digital once a day for 7 d. Statistical analysis was conducted using one-way ANOVA with Tukey post-hoc test. After 7 d of biofilm formation. The CFU decreased by 13.6 %, 13.3 %, and 7.7 % in the CRE1, CRE2, and 0.1 % CHX groups, respectively, compared to the DW group ($p < 0.05$). R/G ratios of CRE1 and CRE2 were showed significantly lower than those of the DW group from days 4 and 7 after biofilm formation, respectively ($p < 0.05$). Meanwhile, no significant differences were observed between the DW and 0.1 % CHX groups. In conclusion, CRE inhibited biofilm formation and maturation. In particular, CRE1, using only dichloromethane as a solvent, was the most effective in inhibiting microcosm biofilm maturation.

50 EPS degradation and detachment of S. mutans biofilm by α-(1→3) glucanase from Prevotella melaninogenic

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Extracellular polysaccharides (EPS) are virulent factors from cariogenic biofilm matrix because they contribute for bacteria adherence between each other and to dental surface. The degradation of EPS by α-(1→3) glucanases could be a strategy for biofilm control. Therefore, we conducted a pilot study to evaluate the enzymatic activity of α-(1→3) glucanase from Prevotella melaninogenic in EPS degradation and S. mutans biofilm detachment. Early S. mutans UA159 biofilms were formed for 24 h in 96-well plates using UTEYB medium supplemented with 1% sucrose. The biofilms were submitted to an enzymatic treatment with α-(1→3) glucanase at 37ºC for 4 h at the following concentrations (n=6): 0.2, 0.5, 1.0, 1.5 and 2.0 in PBS, and only PBS (negative control). EPS was extracted with 1 M NaOH under microplate agitation for 15 min, transferred to microtubes, centrifuged, and the supernatant was precipitated with ethanol (1:3, v/v). EPS were quantified colorimetrically by the phenol-sulfuric method, using glucose as standard , being measured at 490 nm. The % of biomass and EPS reduction was calculated for each treatment group in relation to the control. Data were analyzed by one-way Anova, Tukey's Test (α=5%). The % of reduction (mean ± SD; n=6; different letters represent statistically significant differences, $p<0.05$) at 0.2, 0.5, 1.0, 1.5, and 2.0 mg/ml of enzyme was, respectively, for biomass: 60.1 ± 4.8A, 77.3 ± 3.3A, 85.9 ± 1.5A, 85.7 ± 0.3B, 86.1 ± 0.8C; and for EPS: 94.2 ± 0.4A, 95.4 ± 1.3A, 96.9 ± 0.8A, 96.7 ± 0.1AB, 96.6 ± 0.4B. In conclusion, the data suggest that α-(1→3) glucanase from Prevotella melaninogenic is able to degrade EPS favoring early S. mutans biofilm detachment.

Session 8
Erosion I

51 Salivary pellicle modification with film-forming polymers and fluoride for the protection of enamel erosion

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The addition of film-forming polymers (FFP) to fluoride solutions has been showing promising results in controlling dental erosion. This in vitro study aimed to investigate the modification of the salivary pellicle with different FFP, associated or not to sodium fluoride (NaF), in relation to their protection against dental erosion. Ninety bovine enamel specimens were submitted to a mild erosive cycling model of 5 cycles. Each cycle consisted in salivary pellicle formation for 1 min, followed by pellicle modification with one of the nine experimental solutions (n=10): water (negative control); NaF/Sn (225 ppm F^-; 800 ppm Sn^{2+}); NaF (225 ppm F^-); and FFP (Carbopol or Chitosan or Linear Sodium Polyphosphate) with or without NaF (225 ppm F^-). The specimens were incubated in saliva once again for 28 min, and thereafter, they underwent an erosive challenge (1 min; 1 % citric acid; pH 3.6). Surface hardness (SH) was measured, and the relative surface hardness (rSH = [Final SH /Initial SH] x 100) calculated. Data were analyzed with one-way ANOVA and Tukey tests (α=0.05). None of the FFP nor their association with NaF could enhance the protection of the pellicle against erosion. The groups presented rSH in the range of 52.2 – 75.9 %, but none of them presented significantly higher rSH than the control (70.0 ± 15.4 %). Under these in vitro conditions, it is concluded that the modification of the salivary pellicle with FFP, associated or not to sodium fluoride, does not improve the resistance against mild erosion.

52 Protease-inhibitors added to saliva increase the erosion protective effect of in vitro pellicles

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In vitro pellicles provide little to no erosion protection for enamel. We aimed at investigating the effect of protease inhibitors (PI) added to saliva on the erosion-protective effect of in vitro pellicles. The overall objective was achieving a similar effect as observed for in vivo pellicles. We prepared 75 human enamel specimens and randomly assigned them to five groups. Initial surface microhardness (SMH) was measured, and each specimen subjected to five cycles consisting of pellicle formation for 2 h (37°C) followed by erosion in 6 ml of citric acid for 1 min (1%, pH 3.6, 70 rpm). After each cycle, SMH was remeasured. The groups differed in the pellicle formation, which consisted either of an incubation with water (ctrl), saliva (saliva), saliva with added PI (saliva+PI), saliva that was exchanged every 30 min (saliva_exch), or saliva with added PI that was exchanged every 30 min (saliva+PI_exch). The SMH was converted to relative SMH (rSMH), and differences were analyzed with Kruskal-Wallis and Wilcoxon tests. SMH decreased in all groups, but to different extents. The groups containing PI decreased significantly less than the other groups ($p<0.001$), and between those two, saliva+PI_exch decreased significantly less than saliva+PI ($p<0.001$). Final rSMH (edian, IQR) was 90.5 %, 89.2-92.3 (saliva+PI_exch); 80.8 %, 79.2-83.8 (saliva +PI); 68.2 %, 66.2-71.0 (saliva_exch); 65.7 %, 62.9-68.0 (ctrl), 62.6 %, 59.4-64.6 (saliva). The rate of rSMH decrease relative to the ctrl over the five cycles was 0.26 (saliva +PI_exch), 0.56 (saliva+PI), 0.9 (saliva_exch), and 1.1 (saliva). We conclude that adding protease inhibitors to saliva for in vitro pellicle for ation leads to an erosion protective effect, which is further increased by repeatedly exchanging the saliva. Whether the pellicle itself ore closely rese bles in vivo pellicles re ains to be investigated.

53 Phosphoric acid modified with polyphenol-rich plant extracts: effect on bond strength to non-eroded/eroded dentine

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The aim was to modify phosphoric acid (PA) with polyphenol-rich plant extracts and to verify their effect on the 24h shear bond strength (SBS) of an etch-and-rinse adhesive system to non-eroded and eroded dentine. One hundred and twenty-eight dentine specimens were prepared from sound human molars and divided into 4 main experimental groups, according to the PA (n=32): PA-Exp (experimental control); PA- GSE (PA-Exp modified with grape seed extract); PA-GT (PA-Exp modified with green tea extract); PA-Comm (commercial control). Each group was further divided into 2 subgroups, according to the substrate (n=16): non-eroded and eroded (10 cycles of 1h in human saliva and 5 min immersion in 1% citric acid) dentine. All specimens were etched with the PAs (15s) and restored with an etch-and-rinse adhesive system and resin composite. After 24 h (humid chamber, 37°C), the specimens were subjected to SBS measurement. The results were analyzed with two-way ANOVA and Tukey posthoc tests ($\alpha=0.05$). Both factors (PA and substrate) individually influenced the outcome ($p<0.0001$), but the interaction was not significant ($p=0.6132$). The SBS values (MPa, mean±SD) for eroded dentine (PA-Exp: 12.1±4.6; PA-GSE: 13.5±5.5; PA-GT: 11.8±5.3; PA-Comm: 7.4±3.6) were significantly lower than for non-eroded dentine (PA-Exp: 19.4±5.5; PA-GSE: 19.6±3.9; PA-GT: 18.6±5.0; PA-Comm: 11.7±3.1). Whereas the modified PAs did not improve the 24 h SBS when compared to the PA-Exp, all experimental groups showed significantly higher SBS values than did the PA-Comm ($p<0.05$). In conclusion, all experimental phosphoric acids presented higher bond strength than the commercial etchant, but modification with green tea or grape seed extracts neither improved nor worsened the results. Additionally, eroded dentine consistently presented lower SBS than non-eroded dentine.

54 Impact of sodium-hexametaphosphate on the anti-erosive/anti-abrasive effect of fluoride or fluoride-stannous-combinations in enamel in vitro

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Sodium-hexametaphosphate (HMP) as toothpaste additive is claimed to enhance cleaning effects and to stabilize stannous ions. However, little is known about impacts of different HMP concentrations on anti-erosive or anti-erosive/anti-abrasive properties of preparations containing fluoride or stannous and fluoride (F/Sn), which was investigated in the present study. In a ten day, cyclic erosion-abrasion model 320 ground flat human enamel specimens were subjected daily to six erosive challenges (0.5 % citric acid, 2 min) and two applications of suspensions (2 min, 1:3 F-free toothpaste, mineral-salt-solution containing 0.23% sodium-gluconate). Ten groups (n=32 each) were included in which half of specimens were additionally brushed twice daily (200 N, 15 s) during suspension immersion time. In nine groups HMP was added (0.25 %, 1.75 % or 3.25 % final concentration in suspension) either on its own or combined with fluoride (373 ppm F-) or F/Sn (800 ppm Sn2+, 373 ppm F-). One suspension contained sodium-gluconate only (control). Tissue loss was measured profilometrically (mean±SD, µm; One-way-ANOVA, Tamhane's posthoc). Tissue loss in control was 10.9±2.0 (erosion), 22.2±1.6 (erosion-abrasion). Under both erosive and erosive-abrasive conditions loss values depended on HMP concentration. Loss increased in most cases with increasing concentration within each combination with other active agents. Erosion: Loss was only reduced by 0.25 % HMP (-28 % to -54 %; p<0.01), with the highest reduction in combination with F/Sn (p<0.001). No combination with 1.75 % HMP differed from control, 3.25 % HMP without other active agents increased loss significantly (+35 %; p=0.002). Abrasion: No suspension reduced loss significantly compared to control, instead, those without F and Sn increased loss (+23 % to +30 %; p<0.001). Conclusively, with higher concentrations, HMP exhibited erosion-increasing effects and reduced anti-erosive properties of F and F/Sn. These effects should be kept in mind when investigating anti-erosive effects of F and F/Sn containing preparations.

55 Effect of the experimental TiF4 and chitosan toothpastes on erosive-abrasive dentin wear in vitro

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This study evaluated the protective effect of the experimental TiF_4 and chitosan toothpastes on erosive-abrasive dentin wear (EDW) *in vitro*. Bovine dentin samples were randomly assigned to 5 toothpastes (n=12): 1) TiF_4 (1400 ppm F-, pH 3.08), 2) 0.5 %. chitosan (75 % deacetylation, 500 mPas, pH 7.14), 3) TiF_4 (1400 ppm F^-) plus 0.5 %. chitosan (75 % deacetylation, 500 mPas, pH 4.22), 4) placebo (no F and no chitosan, pH 6.97), 5) Erosion Protection (Elmex® - GABA, Suíça, 1400 ppm F^-, NaF, AmF and $SnCl_2$, chitosan, pH 4.55). Twelve samples were only eroded (control). All samples were submitted to erosive pH cycles (4x90 s/d in 0.1 % citric acid, pH 2.5) and the groups 1-5 to abrasive challenges using toothpastes' slurries (1.5 N, 2x15 s/d) plus 45 s of treatment, for 7 d. The final profile was overlaid to the baseline one for the EDW calculation (µm). The data were subjected to Kruskal-Wallis/Dunn tests ($p<0.05$). TiF_4 toothpastes, regardless of the presence of chitosan, were able to significantly reduce EDW (TiF_4: median: 0.40 and interquartile interval: 0.46 µm, TiF_4 + chitosan: 0.97 and 0.46 µm) compared to placebo (4.96 and 1.44 µm) and control (4.20 and 1.26 µm) (Kruskal-Wallis $p<0.0001$), which were in turn similar to chitosan only (5.17 and 1.63 µm). TiF_4 toothpastes behaved similarly to the commercial Elmex® toothpaste (1.33 and 0.86 µm). Therefore, TiF_4 toothpastes, regardless the presence of chitosan, showed to be effective in minimizing EDW *in vitro*.

56 Effect of the experimental TiF4 and chitosan toothpastes on enamel tooth wear in vitro

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This study evaluated the protective effect of the experimental TiF4 and chitosan toothpastes on erosive enamel wear (EEW) in vitro. Bovine enamel samples were randomly assigned to 5 toothpastes (n=12): 1) TiF4 (1400 ppm F-, pH 3.08), 2) 0.5 %. chitosan (75 % deacetylation, 500 mPas, pH 7.14), 3) TiF4 (1400 ppm F-) plus 0.5 %. chitosan (75 % deacetylation, 500 mPas, pH 4.22), 4) placebo (no F and no chitosan, pH 6.97), 5) Erosion Protection (Elmex® - GABA, Suíça, 1400 ppm F-, NaF, AmF and SnCl2, chitosan, pH 4.55). Twelve samples were only eroded (control, without brushing). All samples were submitted to erosive pH cycles (4x90 s/d in 0.1 % citric acid, pH 2.5) and the groups 1-5 to abrasive challenges using toothpastes' slurries (1.5 N, 2x15 s/d) plus 45 s of treatment, for 7 d. The final profile was overlaid to the baseline one for the EEW calculation (µm). The data were subjected to Kruskal-Wallis/Dunn tests ($p<0.05$). Three samples per group were analyzed by using SEM/EDX. TiF4 toothpastes, regardless of the presence of chitosan, were able to significantly reduce EEW (TiF4: median: 0.60 and interquartile interval- II: 0.31 µm, TiF4 + chitosan: 0.62 and 0.57 µm) compared to placebo (6.65 and 1.31 µm), while chitosan only was similar to placebo. TiF4 toothpastes were even superior to the commercial Elmex® toothpaste (3.75 and 1.10 µm), which significantly differed from placebo ($p<0.0001$). Both EEW from F toothpastes' groups were similar to the EEW provoked by erosion only (control). SEM images confirmed the quantitative analysis and the EDX showed titanium deposition on enamel treated with the experimental toothpastes. TiF4 toothpastes, regardless the presence of chitosan, showed to be effective in minimizing EEW in vitro.

57 Control of erosive/abrasive dentin wear using aminomethacrylate copolymer/fluoride solutions

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Film forming polymers have been used as alternatives to control erosive tooth wear. The aminomethacrylate copolymer has shown promising protective effect against enamel erosion, but its efficacy against dentin loss had not been demonstrated so far. The aim of this study was to investigate if the addition of an aminomethacrylate copolymer (AMC) on sodium fluoride (F) associated or not to stannous chloride (FS) solutions would control dentin loss progression under erosive and abrasive challenges. Ninety polished bovine dentin specimens were randomly allocated into the experimental groups (n=15/group): C (deionized water-control); F (225 ppm F-); FS (225 ppm F- + 800 ppm Sn2+); AMC (2%); AMC+F; AMC+FS. After exposition to human saliva (2 h) for pellicle formation, specimens were immersed in citric acid 0.3 % (pH 2.6 - 5 min) and saliva (1 h), 4x/d. Abrasion was performed with a toothbrushing machine (200 g/15 s), followed by exposition to the treatment solutions (2 min). Surface loss (SL) was measured by stylus profilometry after 5 d. Data were analyzed with ANOVA and Tukey tests (5 %). The dentin surface loss mean ± SD values (in µm) were: C (6.48±0.97)a; F (4.62±0.49)b; FS (3.29±0.61)c; AMC (2.85±0.43)cd; AMC+F (2.47±0.50)d; AMC+FS (2.31±0.80)d. Different letters show significant differences among the groups. It can be concluded that the aminomethacrylate copolymer tested was able to increase the protective potential of both fluoride solutions (F and FS) against erosive-abrasive challenges on dentin, being a promising agent to be added to mouth rinses to control erosive tooth wear.

58 Cellular proteins immobilized within the acquired pellicle protect gastroesophageal reflux disease patients against erosive wear

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This study compared the protein profile of the acquired enamel pellicle (AEP) formed in vivo on the lingual/palatal surfaces, in patients with gastroesophageal reflux disease (GERD) with erosive tooth wear (ETW), patients with GERD without ETW, and control patients (without GERD and ETW). Twenty-four volunteers were divided into 3 groups: 1) with GERD and ETW (BEWE≥9; GE); 2) with GERD and without ETW (BEWE=0; GNE) and 3) control (without GERD and BEWE=0; C). The AEP was formed for 120 min after prophylaxis. It was collected from the lingual/palatal surfaces of the upper and lower teeth, with filter paper previously soaked in 3% citric acid. After protein extraction, the samples were subjected to nano reverse-phase liquid chromatography coupled to mass spectrometry (nLC-ESI-MS/MS). Label-free quantification was performed using Protein Lynx Global Service software. In total, 213 proteins were identified. The number of identified proteins in each group was 106, 119, and 92 for C, GE, and GNE groups, respectively. The GNE presented a high number of phosphorylated and calcium-binding proteins. Spectrin beta-chain isoforms, which interact with actin and might be able to form protein complexes, were found exclusively in this group, as well as several intracellular proteins that come to saliva after the exfoliation of the cells of the oral mucosa. These proteins might bind hydroxyapatite or participate in the formation of supramolecular aggregates that bind to precursor proteins in the AEP. Our results suggest that cellular proteins immobilized within the AEP could play a central role in the protection of the dental surface against acid dissolution, since these proteins were increased in GNE patients.

59 Addition of plant extract to acids can reduce their erosive potential

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Additives, like calcium and fluoride, have been added to acidic beverages to reduce their erosive effect. Polyphenols could have a similar outcome in case of dentine erosion because they can cross-link the collagen and reduce the mineral loss. We therefore added a polyphenol-rich plant extract (grape seed extract – GSE) to erosive substances to verify if it reduces their erosive effect on dentine. We prepared 60 human dentine specimens and randomly assigned them to three solutions with or without GSE. The specimens were subjected to ten cycles, each consisting of immersing (6 min, 25°C) the specimens in one of the test solutions: deionized water (DW or DW/GSE), RedBull (RB or RB/GSE), or citric acid (CA or CA/GSE), then incubating the specimens in remineralizing solution (60 min, pH 7.4). Surface loss was measured by profilometry and analyzed with Kruskal-Wallis and Mann-Whitney tests with Bonferroni corrections. Significantly greater surface loss (median, IQR) was observed in the acidic solutions RB (4.61 µm, 2.50–7.38) and CA (1.51 µm, 1.04–1.91) in comparison to DW (0.17 µm, -0.03–0.37; $p=0.022$ and $p=0.087$, respectively). The addition of GSE significantly reduced ($p<0.001$) the erosive potential of these acidic solutions: RB/GSE (0.51 µm, 0.35–0.75) and CA/GSE (0.02 µm, -0.09–0.39). When added to water, the DW/GSE solution caused a sedimentation on the dentine surface (-0.30 µm, -0.41– -0.19), which was significantly different to DW alone ($p=0.045$). We conclude that the addition of grape seed extract to water can form a layer on the dentine surface, which might help explain why the addition of this extract to acidic substances significantly lowers their erosive potential on dentine.

60 May vegan or vegetarian diets increase the risk for erosive tooth wear?

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This study explored vegan and vegetarian diets in relation to symptoms associated with erosive tooth wear. In total 339 participants responded to an online questionnaire (18-60 y, 77 % female), including 101 vegans, 75 vegetarians and 145 persons who did not follow any special diet (controls). Vegans (78 %) and 65 % of vegetarians had followed their diet ≥ 3 y. Pearson's chi-squared or Fisher's exact test were used to compare frequencies between groups ($p<0.05$). Daily intake of non-citrus fruit was significantly more common among vegans (47 %) and vegetarians (33 %), than controls (17 %). Daily intake of citrus fruits, berries and acidic dressings was significantly more common among vegans than controls (19 % vs 6 %, 28 % vs 8 %, and 16 % vs 4 %, respectively). However, daily soft drink intake was significantly more common among controls (13 %) than vegans (5 %), and juice between meals was significantly more common among vegans (14 %) than controls (5 %). Furthermore, a significantly higher proportion of vegans (86 %) than controls (74 %) seldom/never consumed acidic candy, and daily intake of milk/yoghurt and smoothies was significantly more common among vegans than controls (48 % vs 28 % and 13 % vs 4 %, respectively). Cold drinks were the most common cause of mild dental pain (range 28-34 %). There were no significant differences between vegans (52 %), vegetarians (53 %) and controls (55 %) regarding dental visits 1-2 times/year. Among participants informed by dentists to have dental erosions, there were no significant differences between vegans (13 %), vegetarians (13 %) and controls (17 %). The results suggest that vegan and vegetarian diets may not lead to more signs and symptoms of erosive tooth wear, however clinical examination is necessary to accurately determine possible differences in erosive tooth wear related to specific diets.

Session 9
De- and Remineralisation II

61 Remineralization by experimental toothpastes containing S-PRG fillers

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Surface-reaction type Pre-Reacted Glass-ionomer (S-PRG) filler is known to inhibit demineralization of enamel remarkably. The aim of this study was to examine effects of experimental toothpastes containing S-PRG fillers on remineralization of enamel preliminarily. Bovine enamel specimens were demineralized by 0.1 M lactic acid solution (pH 4.5) for 48 h. After assessment of ΔF values by the quantitative light-induced fluorescence (QLFTM, Inspektor Research Systems, Netherlands) at baseline, the samples were divided into 6 groups (n = 10 per group) randomly and treated by one of the following tooth pastes as A: untreated control, B: NaF toothpaste (0.05 M F, Check-up®, Lion Corp., Japan), experimental tooth paste containing S-PRG filler by 0 wt% (C), 1 wt% (D), 5 wt% (E) or 20 wt% (F). To simulate daily brushing with the toothpastes, the specimens were treated by combined exposures to a toothpaste slurry (weight ratio of 1:3; 5 ml per sample) for 5 min and artificial saliva (200 mM Hepes, 1.5 mM CaCl2, 0.9 mM KH2PO4, pH 7.0) for 24 h. After 7 d cycling, the samples were assessed by QLFTM again. For statistical analysis, one-way ANOVA combined with the Tukey-Kramer multiple comparison were employed. The ΔF values in the groups D-F (mean ± SD, %; D: 5.3±2.9 %, E: 6.1±2.8 %, F: 2.8±2.9 %) were significantly lower compared to the untreated group A (11.2±3.5 %), and showed no statistical difference with group B (5.7±3.9 %). In comparison with the baseline ΔF value (12.4±1.9 %), the groups D-F had remineralization rates (ΔΔF) by 51-77 %. In conclusion, it was suggested that the experimental toothpastes containing 1-20 wt% S-PRG filler would provide remineralization effects to be comparable with a NaF dentifrice.

62 Effect of surface polishing protocols on roughness and in situ bacterial growth of resin infiltrated lesions

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The aim of this placebo-controlled randomized in situ study was to evaluate the effect of different surface polishing protocols on enamel roughness, bacterial adhesion and caries-protective effect of a resin infiltrant. This was single-center, placebo-controlled, blinded, randomized in situ study composed of one single- factor (polishing) at 5 levels. Eighty-four bovine enamel samples were submited to pH-cycling (8 d) to create artificial caries lesions. All lesions were treated with a resinous infiltrant and afterwards randomly dividided into five groups polished as follows: with aluminum oxide flexible disks (Al_2O_3-Disks), with silicon carbide tips (SIC-Tips), with silicon carbide brush (SIC-Brush), with silicon carbide polyester strips (SIC-Strips) or no polishing, as a negative control (NC). Average surface roughness (Ra) was assessed by profilometry. Samples were subsequently mounted in palatal appliances under a mesh for biofilm accumulation. Fifteen volunteers wore the intraoral appliances(14 d/phase) and cariogenic challenge was triggered by dripping a 10% sucrose solution over samples (8-times/d)**.** Biofilm formed on enamel was collected for microbiological analysis of caries-related bacteria (*S. mutans, Lactobacillus acidophilus*) and demineralization was assessed by cross-sectional microhardness. Data were analyzed by ANOVA and Tukey's post-hoc comparisons ($\alpha=0.05$). No polishing (mean±SD: 0.64±0.39 µm) resulted in significantly higher Ra means than Al_2O_3-Disks (0.35±0.16 µm; $p<0.001$) and SIC-Strips (0.37±0.09 µm; $p=0.040$). *S. mutans* and *Lactobacillus acidophilus counts* were not significantly different between the groups ($p>0.05$). The highest cross-sectional microhardness means were observed for Al_2O_3 – disks (283.8±45.6 kg/mm^2) and SIC -strips (283±65.8 kg/mm^2), however none of the groups were significantly different to NC (271.3±52.9 kg/mm^2, $p>0.05$). Polishing protocols Al_2O_3-Disks and SIC-Strips significantly decreseased roughness of infiltrated enamel, however none of the polishing protocols could signicantly decrease bacterial counts nor resulted in significant less demineralization.

63 Monitoring enamel and root surface modifications using 3D surface texture analysis (3DST) – a new approach in cariology

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Enamel and root surfaces of human teeth were measured three-dimensionally, and the microtopography was quantified using standardized surface texture parameters according to ISO 25178. Additionally, it was tested to what extent enamel surfaces change on a temporal scale by applying SiO_2 for surface modification (dentcoat P28®). The microtopography was measured with a confocal disc-scanning instrument (MahrSurf CM expert, 50x, Mahr) and analyzed with the MountainsMap Premium software (v8.1.9369, Digital Surf). Sixteen root surfaces (healthy, carious: hard, leathery, cavitated; each group with n = 4) were examined. In addition, eight enamel surfaces were measured at three points in time (t1 = before, t2 = after SiO_2 application, t3 = after brushing with Sensodyne® toothpaste for 30 s). The descriptive statistical evaluation was carried out with R (v4.05). With more pronounced carious lesions (healthy<hard<leathery<cavitated), the surface of the root dentine became deeper, more porous and more rounded (ΔSq = 10.76 µm; ΔVvc core void volume = 12.9 $\mu m^3/\mu m^2$; ΔSpc = 0.41 1/µm). Temporal changes in enamel porosity could be observed from the decreasing pore volume ($\Delta Vvc_{t1\text{-}t2}$ = 0.38 $\mu m^3/\mu m^2$) after coating the enamel surfaces with SiO_2. The porosities increased again after toothpaste cleaning ($\Delta Vvc_{t2\text{-}t3}$ = -0.35 $\mu m^3/\mu m^2$). The 3DST can be recommended to analyze carious dentine surfaces and modification measures of the tooth enamel. It offers new possibilities for quantitative monitoring in cariology.

64 Kinetics of Silver Diammine Fluoride (SDF) in demineralising and remineralising solutions

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SDF is used as a cariostatic treatment. The aim was to investigate the inorganic chemical changes when SDF is added to demineralising and remineralising solutions. Demineralisation: 0.01 mL SDF (3.16 M, Riva Star, SDI, Australia) was added to 50 ml 0.1 M acetic acid (pH 4) to mimic demineralisation, at 37°C. Remineralisation: 0.01 mL SDF was added to 50 mL of remineralisation solution (0.222 g/L $CaCl_2$, 0.163 g/L KH_2PO_4, 8.7 g/L NaCl) at 37°C. For both, the concentrations of NH_4^+, F^-, and Ag^+ released was measured using ion-selective electrodes (ISEs) every 120 s for 4 h and 24 h respectively. Any precipitate was identified using solid-state MAS-NMR. Each experiment was repeated. Chemical kinetics and appropriate statistical analyses were carried out using Excel. Demineralisation: concentration of Ag^+ and NH_4^+ decreased by 0.75 mM/h (R^2=0.8) and 1.55 mM/h (R^2=0.82) respectively. F^- concentration increased by 1.12 mM/h (R^2=0.5). pH changed from 4.0 to 4.4. No change in colour of solution was observed, nor any precipitate formed. Remineralisation: concentration of NH_4^+ decreased slightly by 0.00097 mM/h (R^2=0.9) for 4 h then stabilised. No change in F^- concentration was detected. Ag^+ concentration was below the detection limit of silver ISE. On addition of SDF, the colour of remineralisation solution became white. Later, the solution became clear and a white precipitate formed which turned greyish-silver on light exposure. ^{31}PssMAS-NMR confirmed apatite formation (peak: 2.7 ppm). ^{19}FssMAS-NMR indicated presence of fluorapatite (peak: -102 ppm). ^{35}ClssMAS-NMR indicated presence of silver chloride (peak: 0.8 ppm). SDF dissociates into ions in solutions. Unlike demineralisation, in remineralisation solutions, silver and fluoride ions react immediately with ions in the remineralising solution to form an insoluble precipitate composed of silver chloride and fluorapatite. This will impact its cariostatic function.

65 Release of bioactive ions by fluoride containing dental composite to enhance remineralization and prevent secondary caries

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Secondary caries is one of the common reasons for restoration failure. Dental restorative materials that release calcium, phosphate, and fluoride ions help in remineralisation and can contribute to the prevention of secondary caries The aim of this study is to develop a novel bioactive glass-based dental composite with better remineralisation properties. Bioactive glasses (BAG) containing SiO2-P2O5-CaO-CaF2-Na2O-K2O-MgO-SrO-ZnO were prepared via the melt quench route at 1400 ^{o}C. The Network Connectivity and Refractive Index were calculated as 2.43 and 1.56 respectively. Glass powder 80 %w/w was added to the resin mixture composed of BisEMA, UDMA, and TEGDMA and light-cured for 40 s. Inert glass composites were used as a control. Samples stored at 37 ^{o}C in different artificial saliva (AS; CaCl2.2H2O, KH2PO4, HEPES, and KCL or acetic acid in AS4) pH=7 and pH=4 (AS7 and AS4) for different times i.e 1, 3, 7, 30, 90, 180 d. Each sample (n=3) was characterized using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), pH measurements, the release of bioactive ions recorded by ICP-OES technique, and fluoride release evaluated by ISE studies. Mean values and standard deviations were evaluated in excel. All samples increased pH suitable for apatite precipitation i-e >4.96. FTIR spectra (560, 605, 958, and 1038 cm-1) and XRD (25° and 32°) patterns of composites showed characteristic peaks of apatite both in AS4 and AS7 which sharpens with time. It has been observed that BAG composites release more fluoride in AS7 (14ppm) than in AS4 (2.5ppm). ICP results showed the release of bioactive ions i.e Ca and P within 24 h for the formation of apatite which relates with XRD and FTIR results. This novel BAG composite has been shown to release bioactive ions and precipitate hydroxyapatite successfully. However, further investigations are required for clinical application.

66 Microleakage of self-adhesive resin-based sealant material – in vitro study

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The aim of this study was to investigate the microleakage of self-adhesive composite resin (SACR) compared to conventional resin-based material as fissure sealing. Tight sealing is a key factor for successful caries protection. The effect of enamel etching on the marginal leakage of self-adhesive material was investigated. The study was conducted on 30 intact molars with well delineated pits and fissures. The first group of molars was sealed with SACR (Constic, DMG, Germany) without pre-treatment of enamel and in the second group the enamel was etched with 37% phosphoric acid. The control group was sealed with a conventional resin-based material (3M Clinpro Sealant, 3M Espe, Germany) and etched with 37 % phosphoric acid for 30 s. After thermocycling (1800 cycles, 10 s), the teeth were immersed in 50 % silver nitrate solution for 45 min and then placed in a developer (Kodak) under lamp (120W) for 6 h and then cut into four slices. The marginal leakage is assessed under a light microscope (scores 0 to 3). Statistical analysis was performed by Kruskal-Wallis and Mann-Whitney U test. Looking at the worst marginal leakage score for each sealant, there was no statistically significant difference between the groups ($\chi 2 = 5.16$, $df = 2$, $p > 0.05$). Considering the depth of dye penetration, there was a significant difference between Clinpro Sealant (2±0.4) and SACR ($U = 24.00$, $Z = 1.96$, $p < 0.05$). Teeth sealed with SACR with etching (1±0.4) and without etching (1.8±0.4) did not differ significantly ($U = 41.00$, $Z = -0.68$; $p > 0.05$). It is concluded that SACR could be suitable for clinical use with and without enamel etching. Further study is needed to evaluate clinical retention rate.

67 Enamel pretreatment with Er:YAG laser: effects on bond strength of fissure sealant

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The application of fissure sealant is an effective method for preventing occlusal caries. The success rate depends on the adhesion quality between the sealant material and the enamel. Aim of the study was to evaluate the micro tensile bond strength of a resin-based fissure sealant to enamel after pretreated with different laser pulse lengths of an Er: YAG laser (2940 nm) with or without additional acid etching and determine the failure mode. Fourty-two extracted molars were randomized into the following six groups based on pretreatment choice: group1: Super Short Pulse Mode (SSP) 50 µs, group 2: Quantum Square Pulse Mode (QSP), group 3: Medium Short Pulse Mode (MSP) 140 µs, group 4: SSP+ acid etch, group 5: QSP+ acid etch, group 6: MSP+ acid etch (Fotona Lightwalker AT-S Fotona, Stegne 7, Ljubljana. Slovenia). After applying sealant (Helioseal F Ivoclar Vivadent Schaan Lichtenstein), teeth were prepared for the micro tensile bond strength test. SSP (24.8 MPa) had the lowest bond strength, followed by QSP + ECTH (27.9 MPa) and SSP + ECTH (28.2 MPa). The highest bond strength was achieved in the group MSP + ECTH (36.1 MPa), while the same bond strength was achieved in the MSP and QSP group (32.4 and 32.0 MPa). Different pulse lengths show to have statistically significant effects on microtensile bond strength ($p < 0.001$), while etching treatment did not result in a statistically significant effect ($p = 0.249$). There were statistically significant differences between SSP, MSP, and QSP groups; despite the fact that etching is not a statistically significant factor there is its statistically significant influence in the interaction with the studied groups.

68 45S5 bioglass remineralizes and protects enamel against simulated cariogenic attack

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This study aimed at comparing the efficiency of applying 45S5 Bioglass to an orthodontic-enamel-sealer in remineralizing and protecting enamel next to orthodontic brackets. 60 enamel samples were distributed into 3 "Treatment" and 3 "Progression" groups. "Treatment" groups were exposed to 2.2 mM CaCl2, 10 mM NaH2PO4, 50 mM acetic acid, 100 mM NaCl, 1 ppm NaF, 0.02% NaN3; pH 4.5; group I: Opalseal-Ultradent, USA, group III: bioglass, group V: control. "Progression" groups were exposed to a second cycle of demineralization; group II: Opalseal, group IV: bioglass, group VI: control. TMR images were obtained for specimens. Mean and standard deviation of lesion depth (LD) and mineral loss (ΔZ) were obtained. Group I: (LD) 123.6 ± 21.2 µm, (ΔZ) 2878.9 ± 681.2 vol% µm; Group II: (LD) 148.6 ± 32.0 µm, (ΔZ) 4121.8 ± 591.2 vol% µm; group III: (LD) 70.2 ± 29.2 µm, (ΔZ) 732.2± 210.2 vol% µm; Group IV: (LD) 156.5 ± 37.0 vol% µm, (ΔZ) 2607.8 ± 229.3 vol% µm; group V: (LD) 115.8 ± 20.0 µm, (ΔZ) 3472.7 ± 738.4 vol%µm; group VI: (LD) 160.7 ± 26.3 µm, (ΔZ) 554.9 ± 263.9 vol% µm. Analysis of variance showed that bioglass groups III and VI revealed significant lower results regarding lesion depth and mineral loss compared to their corresponding groups ($p<0.05$). 45S5 bioglass can aid as a remineralizing and a protective agent for enamel.

Session 10
Clinical Studies II

69 Salivary caries risk factors in patients with chronic graft versus host disease

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Chronic graft versus host disease (cGVHD) affects patients after allogeneic hematopoietic stem cell transplantation. Many organ systems are affected, including the oral cavity and salivary glands. Systemic cyclosporine, an immunosuppressant, is used in combination with corticosteroids and sirolimus to treat cGVHD. This study aimed to determine the effects of cGVHD and its treatment with cyclosporine on salivary caries risk factors. This cross-sectional study included 23 patients (10 males and 13 females), aged 42.3 ± 16.7 years, diagnosed with cGVHD after 24 ± 26.8 months of allogeneic hematopoietic stem cell transplantation. Twelve patients were treated systemically with cyclosporine doses ranging from 34 to 200 mcg/L (115.92 ± 59.67 mcg/L) for 8.1 ± 8.2 months. As caries risk factors, the salivary flow rate and the pH of unstimulated and stimulated saliva were evaluated. All parameters were compared using linear regression (Pearson correlation coefficient). The results are presented as mean values and standard deviation of means. The criterion of significance was $p<0.05$. The measured unstimulated salivary flow rate was 0.27 ± 0.28 mL/min, the stimulated salivary flow rate was 6.6 ± 1.94 mL/min, the pH of unstimulated saliva was 6.6 ± 1.94, the pH of stimulated saliva was 6.13 ± 1.8, and the caries risk according to Cariogram was 68.32 ± 23.51. A lower stimulated salivary flow rate was correlated with a higher dose of cyclosporine ($R = -0.672$, $p = 0.0486$) and with a higher caries risk ($R = -0.640$, $p = 0.0461$). The study found that a higher dose of cyclosporine corresponded to a reduced salivary flow rate and an increased caries risk. These preliminary findings contribute to the recognition of the dentist's role in the complex treatment of cGVHD.

70 Modification of the plaque pH and cariogenic bacteria in a 2-year RCT using HAF toothpastes

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The effect on plaque pH and cariogenic microorganisms was evaluated in children over a 2-years period using a fluoridated toothpaste containing biomimetic hydroxyapatite (HAF) *versus* sodium monofluorophosphate toothpaste (MFP). Two HAF toothpastes (1000 and 1450 ppm F^-) and two MFP toothpastes (1000 and 1450 ppm F^-) were randomly administered to 610 school-children (4-5 and 6-7 years), divided in four groups with younger children using 1000 ppm F^-toothpastes and older children the 1450 ppm F^- toothpastes. Plaque-pH was measured after a one-minute sucrose challenge using pH-strips (Merck. DE). Microbiological plaque evaluation of nine caries-related bacteria strains (*Streptococcus mutans, sobrinus, sanguinis, salivarius, mitis, gordonii* and *Lactobacillus casei, fermentum, salivarius*) was also performed using the DNA-DNA checker-board analysis. The minimum pH remains under the threshold value of 5.7 in all groups during the entire experimental period. After 2 years a statistically significant difference in minimum pH was recorded among groups ($p=0.02$). Intra-group pH changes were always statistically significant ($p<0.01$ for HAF toothpastes and 0.01 MFP toothpastes). Maximum pH fall did not show significant differences among groups, but a statistically significant intra-group decrease was measured for HAF toothpastes ($p=0.01$ for 1000 ppm $F^-$and $p<0.01$ for 1450 ppm F^-). Between the baseline and the 2-year examination, a statistically significant reduction of cariogenic bacteria was observed in all experimental groups for *S. mutans* ($p<0.01$ in all groups), *S. sobrinus* ($p<0.01$ for 1000 ppm F^- and 0.01 for 1450 ppm F^-), *S. sanguinis* ($p=0.03$ for 1000 ppm F^- HAF group, $p=0.04$ for 1000 ppm F^- MFP group and $p<0.01$ for 1450 ppm F^- groups). HAF fluoridated toothpastes performed better than for MFP toothpastes controlling plaque pH and cariogenic bacteria counts.

71 Effectiveness of HAF toothpastes on modifying caries activity lesions in a 2-years triple-blind Randomized Clinical Trial

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The present work was aimed to analyze the effect on the caries activity of toothpastes containing fluoride biomimetic hydroxyapatite (HA) complex was compared to sodium monofluorophosphate fluoridated toothpastes in Italian schoolchildren. The RCT was conducted on 610 school-children (4-5 and 6-7 years) using four toothpastes, two containing fluoride-substituted hydroxyapatite (HAF) (1000 and 1450 ppm F) and magnesium-, strontium-, carbonate-substituted hydroxyapatite, in a chitosan matrix and two mono fluoridated toothpastes (1000 and 1450 ppm F) were randomly administered during 24 months to two groups with younger children n=268 (Gyoung) and to two groups with older children n=250 (Gold), those containing 1450 ppm F. The Nyvad's criteria were used to evaluate the lesions status. Only the lesion registered at baseline were re-examined at 12 and 24 months and consider in the analysis. The difference in proportion was assessed using chi-square test. Cox regression models were performed to identify factors associated with the activity status. Overall, the activity of 933 caries lesions (Gyoung=340; Gold=543) were recorded at baseline: 275 inactive lesions (Gyoung=103; Gold=172), 243 leathery (Gyoung=74; Gold=169), and 300 active lesions (Gyoung=163; Gold=237). Overall, 483 (51.77 %) lesions were filled at end of the trial. The activity of lesions recorded at the end of trial was statistically lower in both Gyoung and Gold groups the HAF arms compared to the traditional fluoridated arms (chi-square=7.38 p=0.03 and chi-square=11.05 p<0.01, respectively). The adjusted model showed that children treated with HAF toothpastes had a higher Hazard Ratio (HR) of arresting active carious lesions (HR 0.48, 95%CI 0.28–0.67 in Gyoung group and HR 0.41 95%CI 0.27–0.78 in Gold; group). HAF fluoridated toothpastes performed better than for mono fluoridated toothpastes arresting carious lesions, after a 12-month follow-up period.

72 A 2-years triple-blind Randomized Clinical Trial on the efficacy of HAF toothpastes in primary and permanent dentitions

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This RCT aimed to compare the caries preventive efficacy of toothpastes containing fluoride biomimetic hydroxyapatite (HA) complex compared to sodium monofluorophosphate fluoridated toothpastes in schoolchildren. To validate this hypothesis a triple-blind randomized clinical trial was designed. 610 children (4-5 and 6-7 years) were enrolled. Four toothpastes, two containing fluoride-substituted hydroxyapatite (HAF) (1000 and 1450 ppm F) and two mono fluoridated toothpastes (1000 and 1450 ppm F) were randomly administered during 24 months to two younger children groups (Gyoung) and to two older children groups (Gold). ICDAS was used to score lesions as initial, moderate and severe. Caries examination was repeated at 12 and 24 months. The efficacy of the treatment was assessed as the reduction in risk ratio (RR) and the number needed to treat (NNT). 518 patients (Gyoung=268; Gold=250) concluded the trial. The caries increment at 24-month evaluation was statistically lower in the primary dentition in the HAF arms versus the traditional fluoridated arms (0.18 vs 0.27 p=0.04 in Gyoung and 0.16 vs 0.30 p=0.01 in Gold for severe lesions). In the permanent dentition, caries increase was statistically lower in the HAF arm, both for initial and severe lesions (0.09 vs 0.17 p=0.02 and 0.18 vs 0.28 p=0.01, respectively). In primary dentition, children receiving HAF toothpaste had a RR of 39% (Gyoung), a NNT of 3.97 and a RR of 38% and NNT of 4.43 (Gold), compared to children receiving traditional toothpastes. The RR in the permanent dentition was 29% in children treated with HAF toothpaste; while NNT was 5.68. Toothpastes with new compounds with remineralizing and antibacterial properties might contribute together with fluoride to reduce dental caries.

73 Is detecting initial caries lesions and activity assessment mandatory to avoid new operative treatment in primary teeth? Cardec-02 RCT

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This study aims to assess the impact of detecting and managing active initial caries lesions in primary teeth to avoid new operative treatment at a 2-year follow-up. This two-arm, randomized, parallel design, controlled trial (CARDEC-02 trial/NCT02473107), with 3-6-year-old participants, was designed for such a time horizon. Participants received the intervention as randomly allocated: detecting and managing all lesions (ICDAS 1-6), activity status assessment, or more advanced lesions (ICDAS 3-6) with no activity assessment. A standard topical fluoride protocol was adopted according to caries severity for both groups. Patients with only initial lesions would then receive fluoride application only in G1-6. The primary outcome was the number of surfaces needing new operative interventions during two years. Intention-to-treat analyses were conducted and comparisons between groups were performed using negative binomial regression. Secondary analyses were performed to investigate initial caries lesions progression patterns. Of the 260 participants, 222 were followed up until 24 months (14.6 % attrition rate). The G1-6 had more surfaces scheduled for non-operative treatment (6.3, 95%IC: 5.3-7.4) than G3-6 (1.3, 95%IC: 1.0-1.7), p<0,001. However, there was no additional difference in the number of surfaces needed to be operatively treated after 2 years (G1-6: 6.93, 95%IC: 4.90-8.96 / G3-6: 6.61, 95%IC: 4.87-8.36; p=0.70). In 2 years, approximately 8% of initial lesions progressed (G1-6:0.06, 95%IC: 0.045-0.09 / G3-6: 0.09, 95%IC: 0.06-0.13). The benefit of G1-6 for controlling initial caries was less pronounced in children who only had initial lesions (number of surfaces needed to be treated (NNT) =65) compared to those who had also other types of lesions (NNT=40). Detecting and managing specifically the initial caries lesions in primary teeth does not lead to less new operative treatment, probably because mostly lesions that progressed are in children with other severer needs and are inevitably treated.

74 Dental practitioners treatment choices for replacement of restorations

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Diagnosis and treatment planning incorporating non or minimally invasive treatment of primary dental caries is well established for primary lesions. For caries adjoining to sealants and restorations (CARS), no clear guidelines are available. There is a lack of evidence regarding non-invasive options although they may be applied by means of ‘clinical judgement’. In the present study, treatment options for CARS and defects by general dental practitioners (GDP) were compared to the choice of a clinical reference standard in a series of ‘paper cases’. A senior clinician experienced in cariology selected 22 cases, clinical and simulated in extracted teeth and documented them with photographs and radiographs. These cases encompassed sound restored teeth (n=9), restorations presenting marginal defects (n=4) and recurrent caries (n=9). Via an online survey including the images the cases were presented to a number of GDP. They could choose from six options in order of increasing invasiveness: 1-no action, 2-mark for follow-up in patient file, 3- follow-up with targeted preventive measures, 4- refurbish/reseal, 5- repair and 6- replacement of the whole restoration. Inter-rater agreement and agreement or difference with respect to the senior clinician’s opinion were calculated and further graphed and analyzed statistically (Bland-Altman (BA) plots, kappa, Inter-class correlation coefficient, ICC). Treatment choices varied widely per case with the exception of clear dentinal involvement (agreement 50% of participants), however in some of these a non-invasive approach was chosen. BA-plots showed a symmetrical distribution. Agreement was poor between GDP and senior clinician (kappa ranging from 0 to 0.53). Inter-rater agreement as estimated by ICC of all GDP amounted to 0.51 ($p<0.001$). Practitioner's treatment choices for caries or defects on the tooth-restoration interface widely disagree.

75 Dental caries lesion distribution in a US population from age 1 to age 8

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The objective was to assess the distribution of dental caries over time in a sample of U.S. children. Three distinct caries patterns in the primary dentition were hypothesized: 1) Early Childhood Caries (ages 1-4 years), 2) Late Childhood Caries (ages 4-12 years), and 3) Primary Second Molar Hypoplasia with Dental Caries. A cohort of children received periodic caries examinations using the International Caries Detection and Assessment System (ICDAS) criteria from age 1 to 8 years, and bitewing radiographs at ages 6.5 and 8. Distributions of caries experience ($d_3$mft+ D_3MFT) over time were calculated for each tooth at each visit (age) = V1(1), V2(2.5), V3(4), V4(6.5), and V5(8). Children's characteristics included: female=51 %, Medicaid-enrolled=52 %, white=45 %, and Hispanic=14 %. Percentages of children with cavitated-level lesions ($d_3$mft+D_3MFT) per visit were V1=<1 %, V2=5 %, V3=24 %, V4=76 % and V5=85 %. From V1 to V3, the percentage of caries on anterior teeth decreased from 100 % to 26 %. The least affected teeth were primary lower incisors (all visits). After age 4, the most common carious teeth were primary molars. By age 8, 39 % of all teeth affected were upper molars and 46 % were lower molars. The dominant posterior surface affected from ages 1-4 was the occlusal; however, the distal surface of the first primary molar dominated for ages 6.5-8. Primary second molar hypoplasia with associated cavitated caries experience ($d_3$mft) diagnosed at V2 and V3 was predominantly on the occlusal surface, with prevalence of <1 % and 6 %, respectively. The percentage of caries involving anterior teeth decreased by age 4, with posterior molar lesions dominating afterwards, with occlusal surfaces affected (in early childhood) and interproximal surfaces (in late childhood). Primary second molar hypoplasia with dental caries was the least common pattern observed.

76 Caries preventive measures during orthodontic treatment with fixed appliances

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The aim of this clinical study was to detect the effectiveness of chlorhexidine mouthwash on dental plaque index and incidence of WSLs in adolescents wearing fixed orthodontic appliance. Fifty subjects (aged 12 to 18 years) were included in this study, who are undergoing treatment at the University Dental Clinical Centre “St. Pantelejmon” in Skopje, Macedonia. Prior to the beginning of orthodontic treatment, the patients were given oral hygiene instructions. After the placement of fixed orthodontic appliance, the subjects were provided with CHX mouth rinse for use during the next weeks. The following parameters were measured: dental plaque index of Silness - Löe (DPI) and the incidence of WSLs - by using the WSL index. The mean DPI index and WSL index scores among the groups of adolescents with orthodontic brackets and used CHX mouth rinse were compared at baseline, 1 month, and 3 months after the placement of the appliance. The WSL values (mean±SD) between both groups were comparable at baseline (test: 1.70±0.65; control: 1.70±0.57); after 1 month (test: 1.20±0.41; control: 1.45±0.51) and after 3 months the values of WSL were significantly lower in the test group vs. control group (0.21±0.57 vs. 1.40±0.65). The DPI values (mean±SD) between both groups were comparable at baseline (test: 1.80±0.33; control: 1.19±0.22); after 1 month (test: 1.07±0.28; control: 1.42±0.32) and 3 months after the placement of the appliance (1.21±0.28 vs. 1.32±0.33), the values of DPI were significantly lower in the test group vs. control group. Usage of CHX-resulted in better dental plaque index and reduced the incidence of white spot lesions in the test group.

77 Evaluation of the masking efficacy of post-orthodontic initial caries lesions after caries infiltration: 1-year follow-up

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The aim of this study was to evaluate the masking efficacy on initial caries lesions (ICL) after fixed orthodontic treatment one year after caries infiltration. In 17 adolescents 112 ICL (ICDAS 2) located from first premolar to first premolar were treated by resin infiltration. Prior to treatment (T0), digital pictures of the anterior teeth were taken. Following etching (maximum repetition 3x) with 15%HCl (Icon etch, DMG) the teeth were dried with alcohol (Icon dry). Finally, the infiltrant (Icon infiltrant) was applied and light-cured followed by polishing. Digital pictures of the final result were taken one week (T7d) and one year after (T12m) treatment. Using a Likert-scale from zero (no lesions visible) to ten (all teeth involved, more than half of surface, high contrast), four experienced dentists evaluated the pre- and post-treatment severity of lesions on the teeth portraits. Furthermore, the success of treatment was assessed by five categories: deteriorated (1), unchanged (2), improved, but not satisfying (3), improved and no further treatment required (4), completely masked (5). Inter-observer reliability was analyzed using Fleiss-Kappa. The severity of aesthetic impairment due to white spot lesions was rated with a mean of 3.7 points (SD 1) before and 0.9 points (SD 0.9) one year after treatment (interobserver reliability; Fleiss kappa: T0: 0.440 (moderate); T12m: 0.769 (substantial)). At T12m the results of only one tooth was classified as unchanged, whereas in 55% and 39% the results were classified as “improved and no further treatment required” and “completely masked”, respectively (Fleiss kappa: T12m: 0.851 (almost perfect)). One year after infiltration resin infiltration of post-orthodontic initial caries lesions provides satisfying to excellent results in the majority of patients.

Session 11
Diagnosis

78 Validation of visual and radiographic criteria of caries associated to restorations with ICDAS-merged CARS

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Secondary caries is a caries lesion develops near margins of a restoration/sealant. Aim was to validate visual and radiographic criteria ICDAS-merged CARS, against histology ICDAS in 228 sites of examination in permanent molars and premolars, restored with amalgam/resin from UNICA human tooth bank. Three examiners were trained and calibrated in visual, radiographic, and histological ICDAS-merged CARS criteria from 76 samples pre-selected and visually pre-categorized by an external examiner (re-evaluations one week later). Afterwards, an examiner selected 228 examination sites (161 molars, Chloramine-T at 0.5% at 4°C). The other two trained examiners classified independently previously selected sites, randomly assigned, visually (ICDAS-merged CARS), and radiographically (ICDAS-merged CARS). Reference standard used was ICDAS-merged CARS, histological assessment of images with a stereomicroscope (8x) of tooth hemi-section. In cases of disagreement, an agreement session with discussion was conducted. To assess visual and radiographic reproducibility and agreement with histology weighted Kappa were used. Sensitivity, specificity and accuracy, of each method were calculated according to thresholds of 0 vs. I/M/S; 0/I vs. M/S and 0/I/M vs. S; criterion for significance was set at 5 %. Good inter-examiner reproducibility for visual (0.74±0.1), radiographic (0.97±0.1), and histological (0.90±0.2) criteria was found. There was an almost perfect intra-examiner reproducibility (0.81±0.11; 0.98 ±0.09 and 0.94±0.10, respectively). For validation, concordance against histology was found, almost perfect for visual criteria (0.90) and good for radiographic criteria (0.78). Respective sensitivity and specificity of visual criteria were: according to the threshold of -0 vs. I/M/S: 0.76 and 0.88; -0/I vs./S: 0.89 and 0.99, and -0/I/M vs. S: 0.88 and 0.99. Corresponding data for radiographic criteria were: 0.38 and 0.96; 0.89 and 0.99, and 0.88 and 0.99. This study allows us to recommend the use of visual and radiographic criteria ICDAS-merged CARS.

79 Staging non-cavitated caries in fissures of varying width with optical coherence tomography

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Objective was to assess the ability of Optical Coherence Tomography (OCT) in staging non-cavitated caries (NCC) in fissures of varying width, using depth-resolved intensity profile (A-scan). Thirty-one ICDAS code 1 and 2 NCCs on 21 extracted human premolars were identified. 3D OCT scans (5 mm x 1.5 mm x 3 mm in x-y-z axis) of the NCCs were performed. 3D Micro-CT scans of the teeth were also done, reconstructed and a representative bucco-lingual cross-section of the NCCs were chosen. Grey value thresholding of the NCCs were done and subsequently classified based upon its depth, into less (E1) (n=21) or more than (E2) (n=10) half thickness of enamel. Corresponding OCT B-scans of the NCCs were identified, mean A-scans computed from 50 A-scans of each NCCs and integrated reflectivity (IR) of up to 150 µm subsurface was computed. The fissures were subsequently divided following Ekstrand et al 1991's methodology, into structural angle (SA) ≤ 25 and > 25. The accuracy of IR in differentiating E1 and E2 NCCs were validated against Micro-CT scans using receiver operating curve analysis by considering all fissures together irrespective of width followed by considering fissures with SA ≤ 25 and > 25 separately. IR was able to differentiate E1 from E2 NCCs with high sensitivity (0.80). However, specificity and area-under-receiver-operating-curve of IR were highest for fissures with SA ≤ 25 (0.75, 0.72), followed by all fissures (0.62, 0.71), and fissures with SA > 25 (0.54, 0.67) respectively. Sensitivity of IR in differentiating E1 from E2 NCC was not affected by the width of fissures. However, specificity was affected by the width of fissures with IR demonstrating higher specificity for NCC in fissures with SA ≤ 25.

80 Saliva proteomics from caries-free and caries-affected children: longitudinal study in early childhood.

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The study aimed to perform a 2-year longitudinal comparative analysis of the saliva protein profile of caries-free and caries-affected children. At baseline, 126 children (2-6 years old) were divided into 3 groups: caries-free group (CF, n=42), enamel caries group (EC, n=42), and dentin caries group (DG, n=42). After two years, 80 children were re-evaluated. Clinical examinations were conducted by calibrated examiners using the ICDAS criteria. Saliva samples were collected at baseline and follow-up, processed, and stored at -80ºC for later analysis. The samples were digested and analyzed by nanoUPLC coupled to mass spectrometry with an ion-mobility multiplexed acquisition ($HDMS^E$). Data analyses were performed with Progenesis QI for Proteomics Software v.1.0. Protein profiles of children with caries and caries-free children have been obtained by 1D-HDMSE analysis. In the 2-year follow-up, 23 children remained caries-free, 19 with the same initial enamel or dentin caries status, and 38 had disease progression. Protein profiles from children who remained caries-free (CF), participants with caries stagnation (CS), and those that presented caries progression (CP) were compared. Thirteen unique peptides were found for CF children, 1 for CS (Q9HC38), and 12 for CP group. The 5 most prevalent peptides in children who remained CF were related to calcium biding proteins, important ions in the demineralization and remineralization processes. The highest expressed peptides in CP group were P00491, P29373, P63104, P02675, and Q9H0U4. The identification of these proteins may contribute to understanding the mechanisms for better control of dental caries.

81 Lesion activity assessment of early enamel caries using dye-enhanced quantitative light-induced fluorescence (DEQLF)

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This study aimed to evaluate whether dye-enhanced quantitative light-induced fluorescence (DEQLF) can distinguish between active and inactive enamel caries in human teeth. Seventy permanent teeth with enamel caries lesions (ICDAS code 1 or 2) on smooth surface were stored at 4°C in 100 % relative humidity. Quantitative light-induced fluorescence (QLF) defined as the process that drying teeth with compressed air for 10 s and capturing fluorescence image of the teeth with QLF-Digital (Inspektor Research Systems BV, The Netherlands). DEQLF defined as the process that dyeing the teeth with 100 μM sodium fluorescein (dissolved in 50% EtOH solution) for 10 s and drying the teeth for 10 s and then capturing fluorescence image. Caries activity was determined according to presence (active) or absence (inactive) of autofluorescence of sodium fluorescein in the caries lesion. ΔG value, defined as mean gray level of caries lesion with respect to sound enamel, was measured in fluorescence images using Image Pro (Media Cybernetics, USA). ΔΔG is the difference in ΔG between before and after conducing each process. All data are presented as mean ± standard deviation. Independent *t*-test was used to evaluate the difference in ΔΔG between active and inactive (α=0.05). Validity of ΔΔG to distinguish between active and inactive was evaluated using a receiver operating characteristics (ROC) curve. There was no difference in $\Delta\Delta G_{QLF}$ between active (-1.1 ± 1.7) and inactive (-1.3 ± 1.8). When comparing $\Delta\Delta G_{DEQLF}$, active (4.1 ± 6.3) showed a value 3.4 times higher than inactive (1.2 ± 2.5, P=0.029). $\Delta\Delta G_{DEQLF}$ value showed area under the ROC curve of 0.68, sensitivity of 0.58, and specificity of 0.80. In conclusion, the DEQLF method can be used to objectively assess lesion activity of enamel caries.

82 Investigating the ability of a blue dye to selectively stain early enamel caries lesions

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Funding: Incisive Technologies Pty Ltd.

A novel blue dye has been developed with the goal to stain areas of demineralized enamel selectively and reversibly and therefore aid clinicians in the detection of early, subclinical white spot lesions. Specific targeting of the dye amido black is conferred by hemoglobin based on hemoglobin's strong affinity for hydroxyapatite. Amido black is covalently linked to hemoglobin to enable unaided visualization of hemoglobin binding to porous enamel. The aim of the present laboratory study was to investigate whether the intensity of the staining is related to lesion severity and mineral distribution. Caries lesions were created in polished human enamel specimens (n=8) using three protocols (all lactic acid): methylcellulose gel (MeC), hydroxyethylcellulose gel (HEC), polyacrylic acid solution (C907). One group remained sound (S). Lesions were treated with the dye for 2 min. Color changes were determined spectrophotometrically and with digital photography. Lesions were analyzed using quantitative light-induced fluorescence (QLF) and transverse microradiography (TMR). Data were analyzed using ANOVA. QLF data (ΔF; all mean±standard deviation) were: -23.7±4.0%/MeC; -51.7 ±5.1%/HEC; -33.6±6.4%/C907; 0±0%/S. TMR data (ΔZ[vol.%min×µm]/L[µm]/ SZmax[vol.%min]) were: MeC (2468±340/70.4±6.9/45.3±11.1), HEC (4678±755/164.3 ±26.2/60.0±24.5), C907 (2415±360/106.2±7.6/73.2±4.3); S (not analyzed). Lesions exhibited three distinct mineral distributions. Color data (Δb*) were: 3.9±0.3/MeC; 6.5 ±0.5/HEC; 0.7±0.4/C907; 0.3±0.1/S. Blue stain intensity was uniform in the MeC and HEC lesions, with the HEC being visibly darker, and decreased in the order of HEC>MeC>C907=S. Sound specimens did not change color. The lack of noticeable staining of the C907 lesions requires further investigation. In conclusion, the novel blue dye selectively stained two of the studied lesions. Further studies will be needed to determine whether lesion depth, mineral distribution, porosity, or integrated mineral loss are determining factors influencing stain intensity.

83 In-vitro performance of BlueCheck liquid as a novel technology for detection of initial enamel demineralization

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Funding: The BlueCheck was provided by Incisive Technologies Pty Ltd, Australia. The brackets were provided by Dentaurum, Germany. The Qraycam was provided by grant GIFT-153 by American Orthodontics.

Clinical detection of demineralization is routinely performed visually. However, visual assessment is often subjective; thus, sensitive diagnostic tools may provide additional benefit to improve objectivity of caries detection. A new approach to assess early demineralization is a blue liquid (BlueCheck, Incisive Technologies, Australia) which has an affinity for porous enamel. This study aimed to evaluate the ability of the BlueCheck (BC) to detect artificial demineralization in human enamel. Sixty extracted permanent human teeth were included in the study. On the buccal sites of 30 samples orthodontic metal brackets (discovery smart, Dentaurum, Germany) were bonded. On each surface BC liquid was applied and rinsed with water after three minutes (baseline). The surfaces were checked for presence of blue areas under a microscope. The samples were demineralized with lactic acid (pH 4.6). BC was applied afterwards and the surfaces were re-examined for presence of discoloration. Fluorescence behavior (ΔF) of the demineralized areas was determined using QLF (Qraycam, Inspektor Research Systems B.V., The Netherlands). Histological sections were prepared from representative samples and lesion depth was measured (10 measurments per group). Kruskal-Wallis test was used for group comparison ($\alpha=0.05$). After demineralization, median ΔF value for all samples was: -8.25 % (min. -14.80 %, max. -5.30 %) indicating the presence of initial lesions. No significant differences were found between samples with and without brackets ($p=0.13$). At baseline no sample surfaces showed discoloration, whereas a distinctive blue color was visible after demineralization in all samples, corresponding to 100 % agreement. Mean depth of the surface lesion was 159.7 µm (105.2 µm - 265.7 µm) adjacent to orthodontic brackets and 148.0 µm (116.9 µm - 190.5 µm) in surfaces without brackets. BlueCheck is an accurate tool to demonstrate early demineralization on plain smooth surfaces and adjacent to orthodontic brackets.

84 Correlation between MMPs expression and caries surface appearance

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It is known that cariogenic bacterial acids dissolve the inorganic elements in dentine caries, leaving the dentine matrix exposed. Host-derived matrix metalloproteinases (MMPs) play an essential role in caries progression as they are significant regulators of extracellular matrix turnover and can degrade exposed collagen. This study aims to investigate the expression of gelatinolytic MMPs(MMP2 and MMP9) across various stages of caries in primary human teeth and relate expression with the diagnosis recorded by The International Caries Detection and Assessment System (ICDAS). Twenty-four extracted teeth as part of dental treatment with a clinical diagnosis of caries recorded by ICDAS were used to obtain 24 sections (150 µm in thickness) subjected to immunohistochemistry using monoclonal anti-MMP2 and anti-MMP9 antibodies. Positive staining was visualised by immunofluorescence using a VectorFluor Duet Double Labeling Kit. Images from triplicate samples for each ICDAS score were analysed using ImageJ software. Collagen degradation in caries lesions was detected using a hydroxyproline assay. Data were analysed using two-way ANOVA followed by Tukey multiple comparison test (*$p < 0.05$). A Pearson Correlation Coefficient was computed to assess the linear relationship between MMP expression and ICDAS. Our results show that MMPs were not detected in caries with ICDAS 0-2, and an insignificant increase was detected in ICDAS 3. However, we observed a significant increase in MMP expression in caries with an ICDAS score of 4-6. There was a strong positive correlation between the ICDAS score and MMP2 [$r(6)=.86$, $p=.002$], and also between ICDAS and MMP9 [$r(6)=.82$, $p=.004$]. In conclusion, MMP expression varies across different stages of decay in primary human teeth, with no expression detected in ICDAS 0-2 and significantly higher expression in caries with ICDAS 4-6 than ICDAS 3

85 Assessment of discolored caries lesion using QLF technology with stain removal

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Stain removal is needed to evaluate discolored pit and fissure caries, which make it difficult to assess lesion severity. This study aimed to evaluate changes in color and fluorescence of discolored carious lesions and to determine the severity of discolored carious lesions using quantitative light-induced fluorescence (QLF) technology with stain removal. Forty bovine incisors were demineralized and divided into two groups: white (control) and brown (experimental) spot lesions. The specimens were remineralized for 10 d. On the 6th day of remineralization, the experimental group were stained using a coffee solution for 24 h. All specimens were treated with a dental bleaching agent (15 % hydrogen peroxide) at 5 min intervals until 20 min. The differences in color (ΔE) and fluorescence ($|\Delta F|$) of the lesions compared to sound enamel at each bleaching time point were calculated. Statistical analysis was conducted using an independent t-test and one-way analysis of variance with Bonferroni *post-hoc* correction. In the control group, there were no differences in ΔE values of all bleaching time points (5.51 ± 1.25 at baseline). ΔE values of brown spot lesons (19.61 ± 1.89 at baseline) also showed similar except for 5 min. The $|\Delta F|$ values in the control group (14.00 ± 2.03 at baseline) were similar across many bleaching time points except for 5 min. However, in the experimental group, $|\Delta F|$ values (38.75 ± 3.09 at baseline) increased significantly ($p < 0.001$). In addition, after 20 min of treatment, the $|\Delta F|$ of brown spot lesions (9.39 ± 2.00) did not differ from that of the white spot lesions (8.52 ± 2.43). QLF technology after stain removal was effectively used to assess the severity of carious lesions, regardless of the presence or absence of discoloration.

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and Dental Education

86 Comparison of dental students' perception on preclinical coursework in India and Republic of North Macedonia

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Preclinical coursework is the key foundation for preparing the dental students for the formal clinics especially in field of restorative dentistry, cariology and prosthodontics. Aim was to assess the knowledge about dental students' perception on preclinical coursework of prosthodontics, conservative dentistry, cariology and endodontics in India and North Macedonia. The present cross sectional comparative research was carried out among the undergraduate 3rd year, final year students and interns of the dental institutes of two respective countries. The data was collected in July 2021 with the help of Google Form, link of which was sent via available social media platform in the respective country. Chi square test was applied for comparing the responses. The authors have not received any funding for this research. The response rate for undergraduate dental students from India and North Macedonia was 68.1 % and 55.7 % respectively. The responses related to stress involved during pre-clinical exercises showed that Indian students felt more stress (88.4 %) than the North Macedonia students (11.6 %). The majority Indian students stated that time duration of pre-clinical exercise is too long (90.9 %) whereas in contrast the students from North Macedonia reported it as too short (72.0 %). The Indian students stated (74.1 %) preclinical exercises are good to get knowledge about the treating patients whereas only 25.9 % the north Macedonia students felt the same. On comparing the responses country wise all the results were found to be statistically significant ($p<0.05$). The current study has illustrated the difference in viewpoints of dental students of the two dental teaching settings on the preclinical coursework. There is a need for a standard preclinical curriculum for strengthening the clinical skills of the dental students.

87 The application of video-presentation teaching method in cariology education

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Cariology education based on the traditional teaching method faces many challenges. Therefore, innovation in its teaching model of cariology is required. This study aimed to evaluate the impact of a teaching modality by creating a video presentation on cariology issues by the students on their exam performance. Dental students of the 3rd semester of class 2019 were enrolled (n=120). 30 volunteers participated in this study. The students were divided into two groups of 15 students. Each group had an equal number of male and female students. Group A participated in the traditional teaching method, which consisted of conventional preparation before class, traditional lectures followed by students practice in class, and questions and answers step after class. Group B followed the same learning procedure and the students had to create a video presentation on a cariology subject. The duration of the video was 8-10 min, and the presentation was carried out at the end of the semester. The reading materials were the same for both groups. At the end of the semester, the 30 students took exams on the same test. The exams consisted of 20 multiple choice questions on cariology context. The score of the grades between the two groups was compared by t-test. The data were analyzed using SPSS statistical software (version 26.0, IBM Corp). The exam scores were (mean±SD): Group A: 7.74±1.046 (95% CI 7.23-8.24) and Group B: 7.89±1.046 (95% CI 7.19-8.59). Group B presented a higher exam score than A (0.005). The video presentation created by the students was proved beneficial in learning achievement, as demonstrated by the statistically significant higher exam scores caused. This educational tool should be used to supplement the teaching of cariology.

88 Self-reported oral health behaviors and mask use during the COVID-19 pandemic in Chile

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The COVID-19 pandemic has impacted the lives of many around the globe. Changes related to caries risk and oral health practices might be expected. The present study evaluated the self-reported effects of the pandemic and mask use in preventive oral health care behaviors in Chileans. Participants were virtually invited to complete an anonymous and voluntary self-administered validated online questionnaire. Data collection commenced after 18 months of the pandemic. Data was analyzed by using chi-square test ($p < 0.05$). A total of 1,646 valid responses were obtained. Responders were mainly female (82.1 %) and aged between 18-29 years-old (42.4 %). Participants perceived that the pandemic negatively affected general (47.7 %) and oral health (32.4 %). Although the toothbrushing frequency did not change during the pandemic, 44.3 % of the sample responded that they had worsened their diet. The frequency of sugar consumption increased mainly in females (55.5 % females vs. 37.9 % males; $p < 0.05$). Regarding mask usage, 40.6 % mentioned always wear a mask when interacting with others. The use of a mask reportedly increased confidence when interacting with others ($p<0.05$). Participants feel comfortable using masks, especially those with a higher educational level ($p<0.05$). However, using the masks increased the self- perception of dry mouth and suffocation, especially among those with less educational level ($p<0.05$). In conclusion, the COVID-19 pandemic caused important changes in oral health behaviors particularly in dietary patterns, increasing the cariogenic risk of patients. It seems necessary to develop educational strategies to limit the possible irreversible effects of the pandemic on the oral health of the population, as the pandemic is still far to be controlled.

89 Performance of dental students in the detection of caries lesions - a 7-year experience at IUSD

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Aim was to assess the ability of IUSD first-year dental students to detect caries lesions after the exposition to didactic-laboratorial approaches after 7 years of implementation. First-year dental students from 7 classes (2015 to 2021) were included. Lectures on caries detection and laboratorial experiences with extracted teeth were given to students as part of the cariology program. Student performance was appraised by assessing 26 extracted teeth/surfaces with ICDAS merged-scores (sound-0; initial-1,2; moderate-3-4; and extensive-5-6). Correct answers were determined by expert consensus. Multilevel Poisson regression was conducted to verify the association between correct answers and independent variables, and the prevalence ratio was calculated. A total of 737 students participated. The overall mean of correct answers was 68% (95% CI:0.67 to 0.69). Students were more likely to misdiagnose lesions on proximal surfaces compared to occlusal (0.94; 95%CI: 0.9 to 0.98). In relation to lesion severity, students presented a higher difficulty to score moderate lesions (0.52; 95%CI: 0.51 to 0.54), followed by initial (0.68; 95%CI: 0.67 to 0.70), and sound surfaces (0.71; 95%CI: 0.70 to 0.73). Extensive lesions presented the highest percentage of correct answers (0.96; 95%CI: 0.95 to 0.97). The frequency of correct answers increased significantly in the 2020 and 2021 classes (2020:0.72; 95%CI:0.69 to 0.73, 202:0.73; 95%CI:0.72 to 0.75) in comparison to previous ones (2015-2019:0.66; 95%CI:0.65-0.67). A better performance in detecting moderate lesions was observed in the 2020/2021 students. The student’s ability to detect caries lesions was adequate. However, and given that the detection of moderate lesions represents a challenge, pedagogical approaches like the ones implemented by IUSD in the last years, should stimulate the development of skills required to detect this type of lesions in a clinical scenario.

90 Determination of caries outcomes (DMFT/DMFS) within two-years of Sjögren's Syndrome diagnosis

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Sjögren's syndrome (SS) is an autoimmune disease that affects salivary and lacrimal glands, resulting in a high incidence of caries and tooth loss despite good oral hygiene and increased awareness of the disease. This retrospective cohort study determined the presence of decay, missing, and filled teeth or surfaces (DMFT/DMFS) among SS patients and controls within two years of the diagnosis. Records of patients ≥18 years with at least one completed dental treatment at Indiana University School of Dentistry from 1/1/2005 to 31/12/2021 and a confirmed SS diagnosis was retrieved from the matched electronic dental record (EDR) and health record (EHR) data. The EDR-EHR data of 21 cases and 86 controls (n=107) were reviewed. Two calibrated dentists blinded to patients' status reviewed EDR data and recorded DMFT/DMFS using guidelines developed by cariology experts. Mann-Whitney U test was performed to compare DMFT/DMFS between SS cases and controls. Chi-square determined the association of DMFT ≥ 20 and DMFS ≥ 20, 25, 30, 35, 40 and 60 with SS. The mean ± standard deviation of DMFT score was 19.19±5.1 for cases and 16.31±5.8 for controls and DMFS score was 43.76±17.9 and 35.59±19.3 for cases and controls respectively. Mean DMFT scores were significant ($p=0.04$) and the DMFS scores trended toward significance among the SS group ($p=0.052$). A cut-off level of DMFT ≥ 20 was significantly (Chi-square = 3.91; $p<0.05$) associated with SS patients with no significant association to DMFS at various cut-off levels. An increased association in mean DMFT scores and specifically at a cut-off DMFT ≥ 20 during the diagnostic time found in SS patients was not previously reported. Further evaluation of SS patients with higher DMFT ≥ 20 for dental interventions and interdisciplinary care is needed.

91 Dental care services among Parkinson's patients in Denmark

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Funding: The Danish Parkinson's Association.

Patients with Parkinson's disease (PD) have a higher prevalence of oral diseases and orofacial dysfunction, but knowledge about the actual use of dental care is sparse. We aimed to investigate the dental attendance and usage of dental care of the total PD population in Denmark and compare it with a control group. This is a national register-based study, using the unique personal identification number system assigned to every citizen in Denmark, to merge data from several national registries. This allowed us to identify the total PD population in Denmark (n=6874) and to obtain data on their dental visits and treatments during a five-year period from 2015 through 2019. These data were compared with a five-fold age-, gender- and geographically matched control group without PD (n=34285). The dental attendance was analysed using χ^2-test with Bonferroni correction, and the type of dental care services was analysed using negative binomial regression analysis and adjusted for socioeconomic background, living situation and family support. Patients with PD had a significantly higher proportion of irregular attenders of the dental care system (21.0 %), than the control group (16.9 %) ($p<.000$). PD patients had a 1.5 higher incidence rate of tooth extractions and a 1.7 higher incidence rate of tooth fillings in the five years compared to the control group ($p<.000$). This study provides new data about the receipt pattern of dental care services for patients with PD. This knowledge can be used to raise awareness among dental health professionals of PD patients´ need for more and high-quality prophylactic initiatives to prevent and avoid high filling and tooth extraction rates.

92 Association between underlying dentin lesion shadows (ICDAS 4) and oral health-related quality of life among adolescents from southern Brazil

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This study aimed to assess the association between underlying dentin shadows (UDS) and oral health-related quality of life (OHRQoL) among 15-19-year-old adolescents from southern Brazil. A population-based cross-sectional study included a representative sample of 15-19-year-old adolescents attending 31 public and private schools from Santa Maria, Brazil. The Oral Health Impact Profile-14 (OHIP-14) was used to evaluate OHRQoL and clinical examinations were performed to detect UDS (ICDAS code 4 caries lesions). Socio-demographic information and clinical characteristics (overall caries experience, traumatic dental injury, malocclusion and gingivitis) were also collected as adjusting variables. Multilevel Poisson regression models were used to assess the association between UDS and OHRQoL. Rate ratios (RR) and 95% confidence intervals (CI) were estimated. A total of 1,197 adolescents were included in the study. The mean OHIP-14 score was 8.25 (95%CI=7.75-8.75), ranging from 0 to 49. In the adjusted models, adolescents with UDS had poorer OHRQoL than those without UDS, and the strength of the association was dependent on the number of lesions per individual. Individuals with 1-2 UDS had a mean OHIP-14 score 8% higher (RR=1.08; 95%CI=1.01-1.17) while those with 3-4 UDS had a mean score 35% higher (RR=1.35; 95%CI=1.12-1.63) than adolescents without UDS. This negative association was related to the physical disability, psychological disability, social disability, and handicap domains. In conclusion, this study showed that UDS was negatively associated with OHRQoL among 15-19-year-old adolescents from southern Brazil.

93 The novel calibration method for the oral health survey in preschool children

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Although WHO method was generally the most often used method, poor adherence to these guidelines was observed regarding the reliability of measurement. The novelty of the presented calibration procedure involves the adaptation to local circumstances so calibration could be more feasible when preschoolers are subjects in survey with 21 locations and 36 examiners. Moreover, no calibration procedure has been described before in primary teeth. The calibration procedure was divided in two stages: 1. level - agreement assessment between three main investigators; 2. level - dividing all examiners into three main groups of 11 dentists and agreement assessment between these dentists and one of main examiners. Finally, agreement between all investigators and the benchmark investigator was performed. Independent variables were number of observed healthy teeth, number of primary teeth with untreated caries lesions, and the simplified plaque index score. The calculated and analyzed dependent variables involved percentage of full agreement and median percentage of agreement between examiners on both calibration levels. Kappa statistics were tested using z test at a significance level of 0.01. Each of main investigators in Level 1 examined 180 primary teeth surfaces, and examiners in Level 2 totally examined 470 primary teeth surfaces. Dental status Kappa ranges showed lower variability on Level 1 (0.90-0.95) compared to Level 2 (0.93-1.00). Plaque index Kappa ranges showed wider values in both Level 1 (0.79-1.00) and Level 2 (0.78-1.00). The overall agreement in Level 1 was 0.94, and in Level 2 was 0.93. The median (50th percentile) strongly suggested high agreement between Level 1 and Level 2. The proposed calibration method appeared satisfactory and feasible in multicenter epidemiological oral health survey in large population groups of preschool children, with higher number of examiners.

94 Dental caries status among subjects with type 2 Diabetes Mellitus (T2DM) in an urban Indian population.

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The aim of the study was to estimate the dental caries status using ICDAS II among T2DM patients visiting tertiary care hospitals and identify risk factors/indicators. The study was designed as cross-sectional case-control survey and conducted among T2DM patients (n=300) matched to non-DM (n=300) subjects visiting 2 outpatient diabetic centers in Chennai, India. The dental caries status was measured using modified thresholds of ICDAS II. Patient level risk factors, intra-oral risk factors, lesion activity assessment were determined. DMFT values at different thresholds of ICDAS were compared using Mann Whitney-U test between T2DM and non-DM patients. Chi-square test was used to compare proportions for risk factors/indicators. Multiple logistic regression analyses were done to calculate odds ratios. Significance level was set as 5% ($p<0.05$). The prevalence of dental caries in T2DM patients was 90% compared to 47% in controls. The mean D2-6MFT in DM and non-DM patients were 10.3 ± 3.68 and 5.5 ± 2.16 respectively. The mean D3-6MFT values were 9.4 ±3.9 and 4.6 ± 2.3 respectively with statistically significant difference at both thresholds. Root caries was also significantly higher among T2DM. The odds ratio for T2DM patients with dental caries was 10.26 (95%CI=6.6-15.9) compared to controls. In T2DM patients, all the patient level risk factors were found to be significantly associated with dental caries expect for head and neck radiation and socioeconomic status, and all the intra-oral risk factors were found to be significant among T2DM patients. The prevalence of dental caries in patients visiting tertiary care centers were higher compared to matched controls. Multiple risk factors were found significant among such T2DM patients.

95 National survey on caries prevalence in 12-year-old children in Uzbekistan

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The aim of this nationaly study was to determine caries prevalence, caries experience and significant caries index (SiC) in 12-year-old children in Uzbekistan. In all 14 regions of Uzbekistan, schools were randomly selected by taking into account rural, periurban and urban areas so that about 6.500 children aged 12 years old could be examined. Prior to the start of the dental examinations in 2021, seven dentists had been trained by a person with much experience in caries epidemiology in recording the DMFT index following the criteria of the World Health Organization (WHO 2013). The examinations took place in the schools with the aid of artificial light, dental mirrors and blunt probes and DMFT values were recorded. The data were entered into an electronic file and after exclusion of incomplete data sets, 5.844 dental records of 12-year-olds could be analyzed descirptively with MS-excel. The overall prevalence of dental caries was 70.1 % (95% CI: 65.1-75.1). The mean DMFT was found to be 1.75 (SD 1.88) and the SiC was 3.84 (SD 1.66). The mean number of carious, missing and filled teeth was as follows: 1.57 (DT), 0.07 (MT) and 0.1 (FT). The mean DMFT of boys (1.61; 95% CI: 1.54-1.66) was lower than that of girls (1.89; 95% CI: 1.82-1.96). Overall the degree of caries experience of 12- year-old children living in Uzbekistan according to WHO criteria is «low». In order to decrease prevalence of dental caries in Uzbekistan national preventive strategies and school-based programs should be developed and implemented. In addition, provision of restorative dental care should be improved.

Session 13
Erosion II

96 The effect of tea on surface loss of the dental enamel under erosive challenge in vitro

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Aim was to investigate the effect of black and green tea on surface loss in enamel under erosive challenge *in vitro*. 150 bovine enamel slabs were subjected to a 28 d pH-cycling regime, the slabs were exposed to an erosive challenge five times for 2 min using 0.3 % citric acid (pH 2.6) plus three times for 10 min in each of 5 treatment groups (black or green tea (≈ 5.0 ppm F), black tea with milk, black tea with 1.0 % citric acid (pH 3.6) and fluoride-free water (control)). Throughout the cycling period the slabs were stored at 37°C in artificial saliva. The surface loss was analysed using profilometry at days 7, 14, 21 and 28 of the pH cycling period. After 28 d, data analysis was carried out using one-way ANOVA test with Bonferroni correction to compare between groups. Loss values are given as mean diff ± SE. There was a significant difference in enamel surface loss when comparing the following groups: the fluoride free-water (control) with black tea: 3.7±1.1 µm ($p<0.01$); the fluoride free-water (control) and black tea infusion with additions of milk or citric acid: 12.9±1.1 µm ($p<0.001$) and 4.4±1.1 µm ($p<0.01$) resp.. However, green tea failed to reach significance. There was no difference in enamel surface loss between groups: black and green tea, black tea and black tea infusion with citric acid. Black tea with milk provided a significant benefit over all groups. Black tea with or without additions (citric acid or milk) was significantly beneficial with respect to enamel surface loss compared to control. Furthermore, black tea infusion with addition of milk provides the greatest protection against tooth surface loss in our model.

97 Immunohistochemical study on the immune and antigen-presenting cells of the dental pulp

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Aim was to investigate the histopathology of the dental pulp as a sequel of caries, we have studied the distribution of immune and antigen-presenting cells in association with the development of the carious lesion. In this study, we have analyzed 150 teeth from patients at the age of 9 to 14 years. The condition of the pulp was classified into five groups according to the progression of the carious lesions from stages S0 (non-carious teeth) to S4 (exposed pulp). Teeth were extracted for various therapeutic reasons (mostly for orthodontic reasons), immediately cut longitudinally; pulp tissue was extirpated and fixed in formalin for 24 h at 4 °C. The specimens were embedded in paraffin, according to standardized laboratory procedures. Sections were cut at 5 µm thicknesses and stained by the streptavidin-biotin complex immunoperoxidase method, by using the following monoclonal antibodies: CD3 for T lymphocytes, CD20 for B lymphocytes, HLA-DR for dendritic cells, and CD68 for macrophages. The main numbers of cells in each stage were statistically analyzed with ANOVA. The immune response in unaffected pulp is linked with the presence of antigen-presenting cells (pulp dendritic cells and macrophages) and rare T lymphocytes. The number of T lymphocytes showed an increase in teeth with shallow dentin caries ($p<0.05$), while B-lymphocytes increased only in teeth with deep caries ($p<0.01$). A substantial change in the infiltration of immune and antigen-presenting cells occurred between S2 and S3. Invasion of the pulp tissue by bacteria caused dense accumulation of the immune cells with the predominance of B cells ($p<0.01$). T-lymphocyte-mediated immune response has a central role in the initiation of pulp-specific immunity and in the advanced phase B-lineage cells further the humoral immune response.

98 Effect of three (bio)polymers on the protection of enamel against dental erosion

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Polymers, either chemically synthesized or biopolymers, could protect enamel against demineralization by adsorbing to hydroxyapatite and forming films over the dental surfaces. This in vitro study aimed at verifying the effect of three (bio)polymers on the protection against dental erosion. Forty human enamel specimens underwent 10 cycles, each cycle consisting of 2 min immersion in the experimental solutions (negative control – distilled water, 0.5 % Chitosan, 8 % Gantrez, and 8 % Linear Sodium Polyphosphate – LPP; n=10/group), followed by 1 min immersion in citric acid (1 %, pH=3.6, shaking), totalizing 10 min of erosion. Relative surface hardness (rSH; %) was assessed after 5 and 10 cycles. Data (mean ± SD) were analyzed with one-way ANOVA and Tukey tests (α=0.05). Gantrez was the only polymer that showed protection against erosion after 5 and 10 cycles, with rSH values reducing from 100 % to 60.5 % ± 8.4 and 35.2 % ± 11.8, respectively. These results were significantly better than the negative control, with values reduced to 53.7 % ± 9.3 (p=0.009) and 24.7 % ± 6.1 (p=0.002), respectively. By contrast, Chitosan and LPP were not able to reduce erosion after 5 (35.1 % ± 5.7 and 26.5 % ± 8, respectively; p<0.0001) nor after 10 cycles (14.9 % ± 4 and 16.1% ± 6.9; p=0.03 and p=0.07, respectively). From this preliminary in vitro study, we conclude that out of the three tested polymers, only 8 % Gantrez could protect enamel against dental erosion. In addition, considering that Chitosan and LPP had shown promising results in previous investigations under more severe erosion cycles, our results suggest that our model was too mild for these polymers to show their protective effect.

99 Analysing early erosive lesions produced by silicone impressions of unpolished enamel

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Recent in-vitro studies have shown that it is possible to assess and characterise sub 5 µm erosive toothwear lesions on unpolished human enamel with profilometry and surface metrology. However, these methods cannot easily be translated to a clinical setting. The aim of this in-vitro study was to directly analyse unpolished enamel samples that underwent erosive challenges and compare the results to light body silicone impressions of the same samples. Twenty unpolished (natural) enamel samples were randomized into 2 equal groups (erosion, erosion/ abrasion). Samples were exposed to 20 and 40 min of citric acid (pH 2.7) under constant agitation. The samples in erosion/abrasion group were exposed to a reciprocal brushing machine loaded at 295 g for 120 and 240 strokes of abrasion with a fluoride-free toothpaste slurry. Clinical light body silicone impressions were taken of all samples, and both the tooth and impression scanned with a non-contacting laser profilometer and step height analysis measured using superimposition followed by subtraction. D'Agostino-Pearson test confirmed normality and a three-way ANOVA ($P < 0.05$) considered statistically significant. Bland Altman determined the agreement and the F-test the variance between the methods. For direct tooth scans the erosion group mean (SD) step height was 1.32(0.23) µm to 3.40(0.43) µm and for silicone 1.36(0.29) µm to 3.20(0.42) µm. For direct tooth scans of the erosion/abrasion group were 3.12(0.32) µm and 6.12(1.06) µm and for silicone scans 3.20(0.46) µm to 5.79(1.06) µm. Bland-Altman showed a bias (95% limits of agreement) of 0.13(-0.31,0.57) µm for the erosion group and 0.121(-0.75,0.99) µm for the erosion/abrasion group. F-tests revealed no significant variance between the two methods. Silicone was able to replicate the lesion to a high accuracy and precision, allowing for comparable analysis to be made.

Session 14
Clinical Studies III

100 Quantification of undisclosed and disclosed dental plaque using light-induced fluorescence

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Dental plaque autofluorescence derives from the bacterial production of porphyrins and has been associated with plaque age and cariogenicity. VistaCam is a fluorescence-based caries detection system that has not yet been used for quantification of autofluorescent plaque. This study investigated the performance of VistaCam to quantify dental plaque with and without the aid of disclosing procedures. 60 healthy subjects (M_{age}=25.9 years, 29 males) were enrolled and refrained from oral hygiene measures for 14 d (Ethical Committee of Region Midtjylland: 1-10-72-259-21). After 1, 7 and 14 d, undisclosed images of the facial aspect of the central incisor, first premolar and first molar (third quadrant) were acquired in fluorescence mode. In a subset of 12 participants, teeth in the first, second and third quadrants were imaged on day 1, 7 and 14, respectively, before and after plaque disclosing with 5% erythrosine. Turesky-modification of the Quigley-and-Hein-plaque-Index (TM-QHPI) was recorded on all images. Planimetric plaque index (PPI) was determined using digital image analysis. Data were analyzed by paired t-tests. The mean PPI and TM-QHPI for autofluorescent plaque slightly increased over time (day 1: 0.70 % ± 0.63 %, TM-QHPI=0.70± 0.37; day 7: 2.24 % ± 2.22 %, TM-QHPI=1.07 ± 0.39; day 14: 2.58 % ± 2.34 %, TM-QHPI=1.15 ± 0.44; mean ± SD), but were significantly lower compared with disclosed plaque at all time points (day 1: 22.10 % ± 8.47 %, TM-QHPI=2.61 ± 0.49; day 7: 53.65 % ± 15.69 %, TM-QHPI=4.03 ± 0.57; day 14: 40.46 % ± 16.42 %, TM-QHPI=3.67 ± 0.43; mean ± SD; $p<0.05$; paired t-test). In conclusion, VistaCam can be used to detect autofluorescent plaque and disclosed plaque in fluorescence mode. Planimetric and clinical plaque indices of autofluorescent plaque increase with biofilm age, but underestimate the total plaque accumulation.

101 A simple method for semi-automated planimetric quantification of dental plaque using light-induced fluorescence

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Funding: This work was supported by the Innovation Fund Denmark (9065-00244B) and Novozymes A/S.

Planimetric analysis allows for a more detailed and less subjective quantitative assessment of dental plaque than the use of clinical indices. Current planimetric analysis procedures rely on a manual and thus time-consuming determination of plaque-covered tooth surfaces. The present work describes and validates a simple semi-automated method for planimetric plaque quantification based on digital image analysis (DIA) of fluorescence images. 30 healthy participants were enrolled and abstained from oral hygiene procedures for 7 d (Ethical Committee of Region Midtjylland: 1-10-72-259-21). On day 1 and 7, plaque was disclosed (5 % erythrosine) in the first or second quadrant, respectively, and images of the first incisors, first premolars and first molars were acquired with a fluorescence camera (VistaCam). The Turesky modification of the Quigley and Hein plaque Index (TM-QHPI) was recorded and planimetric plaque scores were determined by semi-automated DIA (DIA-planimetry). In a subset of 10 participants, the plaque-covered area was identified manually on the fluorescence images (M-planimetry). M-planimetry, DIA-planimetry and TM-QHPI were compared by Pearson or Kendal correlations, and the increase in plaque scores from day 1 to 7 was assessed by paired t-tests. An excellent linear correlation was observed between DIA-planimetry and M-planimetry ($R=0.93$; $p<0.05$). TM-QHPI and DIA-planimetric scores increased significantly from day 1 to 7 ($p<0.05$), and a strong, but non-linear correlation between both scores was observed ($tau=0.75$; $p<0.05$). The discriminative power of DIA-planimetry exceeded the one of TM-QHPI recordings, especially for TM-QHPI sores ≥3. The newly developed DIA procedure for semi-automated planimetric plaque quantification constitutes an improvement to currently employed methods for plaque quantification.

102 Photobiomodulation treatment increases salivary flow in patients with xerostomia: a systematic review

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Salivation, especially hyposalivation is one of the major factors influencing the caries risk. The research aimed to systematically review whether adjunctive treatment of low-level light therapy (LLLT) of salivary glands could affect salivary flow rate in patients with xerostomia related to hyposalivation. The systematic review was based on the PICO principle and followed the PRISMA guidelines. Risk of bias assessment according to GRADE score was performed in every included article. Qualitative analysis of data considered the cause of hyposalivation and assessed effects of LLLT during follow-ups and patient's quality of life. Papers that reported quantitative data were included in a random model meta-analysis. The weighing of studies was made using the inverse variance model. Heterogeneity was explored with subgroup analysis and meta-regression. Databases from Medline, Scopus, and Web of Science were for keywords in different combinations. Initial screening yielded 386 records, 18 records were systematically reviewed and 14 were included in a meta-analysis. Most of the included records were classified as high quality according to the GRADE score. When compared to placebo, patients treated with LLLT observed an increased unstimulated salivary flow of 0.51 standard deviations (95%CI: 0.16-0.86; I^2=55%; p=0.005). When observing the change of unstimulated salivary flow before and after LLLT an increase of 0.09 ml/min was observed (95%CI: 0.15-0.12; I^2=20%; p<0.00001). The findings of the review revealed that photobiomodulation therapy could improve salivary gland function alleviating hyposalivation as one of the main factors for caries risk. However, the effects were reported as only short-term and did not induce lasting effects of LLLT therapy on patients' quality of life.

103 Percentage of increased transitions in ICDAS scores in early childhood in a US population

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Objective was to assess transitions to more severe ICDAS scores in a population of U.S. children. Children received periodic caries examinations using the ICDAS criteria from age 1 to 8 years, and bitewing radiographs at ages 6.5 and 8. Transition percentages were calculated between ICDAS scores at ages(Visit#)= 1(V1), 2.5(V2), 4(V3), 6.5(V4), and 8(V5)
Objective was to assess transitions to more severe ICDAS scores in a population of U.S. children. Children received periodic caries examinations using the ICDAS criteria from age 1 to 8 years, and bitewing radiographs at ages 6.5 and 8. Transition percentages were calculated between ICDAS scores at ages(Visit#)= 1(V1), 2.5(V2), 4(V3), 6.5(V4), and 8(V5)
years. Risk of progression between ICDAS scores was assessed using a GEE model. Children's characteristics included: female=51%, Medicaid-enrolled=52%,white=45%, and Hispanic=14%. The percentages of children with cavitated lesions (d3mft+D3MFT; d/D>ICDAS 3 or radiographically into dentin) were: 0%(V1), 7%(V2), 24%(V3), 76%(V4), and 85%(V5). The following positive surface transitions were observed: 1) From V1-to-V2 (n=1051): For surfaces ICDAS=0 [1%=increased to ICDAS 1-2; 1%=increased to ICDAS>3; <1%=were filled/extracted], for surfaces ICDAS=1-2 [30%=increased to ICDAS>3; 5% =were filled/extracted]. 2) From V2-to-V3 (n=913): For surfaces ICDAS=0 [2%=increased to ICDAS 1-2; 1%=increased to ICDAS>3; 1%=were filled/extracted], for ICDAS=1-2 [3% =increased to ICDAS 3-4; 4%=increased to ICDAS 5-6; 11%=were filled/extracted], for ICDAS=3-4 [8%=increased to ICDAS 5-6; 64%=were filled/extracted]. 3) From V3-to-V4 (n=637): For surfaces ICDAS=0 [1%=increased to ICDAS 1-2; 3%=increased to ICDAS>3; 4%=were filled/extracted], for ICDAS=1-2 [6% increased to ICDAS>3; 22%=were filled/extracted], for ICDAS=3-4 [6%=increased to ICDAS 5-6; 44%=were filled/extracted]. 4) From V4-to-V5 (only primary teeth; n=575): For surfaces ICDAS=0 [4%=increased to ICDAS>2; 2%=were filled/extracted], for ICDAS=1-2 [8%=increased to ICDAS>3; 9% =were filled/extracted], for ICDAS=3-4 [5%=increased to ICDAS 5-6; 25%=were filled/extracted]. 5) From V4-to-V5 (only permanent teeth; n=575): For surfaces ICDAS=0 [3% =increased to ICDAS>2, 1%=were filled]; for ICDAS=1-2 [13%=increased to ICDAS>3; 11%=were filled]; for ICDAS=3-4 [15%=were filled]. Risk of progression to ICDAS 5-6/filling was higher for ICDAS 3-4 than ICDAS 1-2 [p<0.001, OR (95%CI)=1.41(1.26-1.65)]. Surfaces with more severe lesions were more likely to progress to frank cavitation/filling.

104 Influence of the circadian rhythm on pH, protein and inorganic composition of unstimulated saliva, a clinical study

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Saliva is a protective fluid with small organic and inorganic fractions. Salivary secretes against multifactorial stimuli, including circadian rhythm, with an unclear influence on salivary proteins and on important caries-related inorganic components. The aim was, therefore, to determine the influence of the circadian rhythm on the pH, concentration and electrophoretic pattern of total proteins, calcium (Ca^{2+}) and inorganic phosphate (PO_4^{3-}) of total unstimulated saliva, in young adults. Eleven healthy, caries-free young adult volunteers donated total unstimulated saliva, at 6 intervals, over a period between 7:00 am and 3:00 am the following day. A Mediterranean standardized diet was provided from 3 d before sample collection. Saliva was ultrafiltered and pH assessed by a pH-meter, before 48 h. Protein concentrations were determined by the bicinchoninic acid test and electrophoresis conducted to obtain a protein pattern. The salivary concentrations of Ca^{2+} and PO_4^{3-} were assessed using a previously calibrated reflectometer. The entire experiment was repeated 3 times. Differences were estimated using a significance level of 95% ($p<0.05$). The circadian rhythm did not induce statistically significant variations in salivary pH, nor in the concentration, electrophoretic patterns of total proteins nor on the concentration of Ca^{2+} or PO_4^{3-} in unstimulated saliva, with evident individual variations among participants. A trend towards a decrease in the total concentration of salivary proteins was observed during the night (3:00 am), with consistent electrophoretic patterns. Saliva appears to be a fairly stable fluid under various conditions. Protein concentration and selective ionic components of unstimulated total saliva do not appear to be affected by circadian variations, at least in young healthy subjects. Further studies should explore other local and systemic conditions to confirm these findings.

105 Evaluation of caries experience and background factors among 8 to 11-year-old students in Mashhad schools

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Funding: Mashhad University of Medical Sciences.

The objective of this survey (approved by the Ethical Committee of Mashhad University of Medical Sciences) was to determine the relationship between caries prevalence and background factors in Mashhad primary schoolchildren. Five-hundred-fifty-two students, 391 girls (71.9%) and 161 boys (29.1%) aged 8 to 11 years were randomly selected out of 75,127 students from public primary schools in Mashhad (a city in North East of Iran). Sampling was carried out using a random cluster method. dmft/DMFT were registered, and a questionnaire about age, sex, socioeconomic status, father education, and frequency of daily tooth brushing was filled up by parents/guardians. Data was analyzed by Mann-Whitney U, Kruskal-Wallis, and Dunn post hoc test. The mean±SD age was 9.55 ±0.87 (girls) years and 9.81 ±1.13 (boys) years. Median (interquartile range, IQR) DMFT was 1(2) in girls and 1(2) in boys. That difference was not significant (p=0.597). Regarding primary dentition, the median (IQR) dmft was 3(4) in girls and 3(3) in boys, which was also not significantly different from each other (p=0.426). In general, median (IQR) DMFT in students regardless of gender was 1(2), and dmft was 3(4). Family income and father's educational level were not statistically significant associated with DMFT/dmft. The frequency of brushings was not statistically significant associated with DMFT (p=0.067), but it was to dmft (p=0.035). Brushing frequency was significantly associated to caries (dmft). This outcome underlines the necessity of early detection of caries in primary schools of Mashhad and the implementation of programs to promote oral health in schools.

106 Caries assessments on young adults using clinical and radiographic ICDAS - first report

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Eventually, the aim was to investigate if a 3D intraoral scanner (3DIOS) and near-infrared transillumination (NIR) can identify caries progression over eight months compared to the conventional methods. This interim analysis describes the participants' (thirty-four 18-30-year-olds living in Denmark) clinically and radiographically detected caries experience at baseline. The first author assessed caries using ICDAS clinically/radiographically. Each lesion was also classified as arrested or active using the following five predictors: colour (white, brown); location in terms of plaque stagnation; texture; cavitation and if the lesion was next to the gingiva, the bleeding status of the gingiva. Intra-examiner reproducibility (weighted kappa) of the clinical ICDAS (4 particpants) and the radiographic codes (4 participants) were both substantial. The mean number of active lesions+sealants+restorations (clinically and radiographically) was 13.2(1SD=9.1). The 6 ICDAS lesion codes constitute 1/3 of the mean value, of which 68 % were ICDAS code 1, while codes 2, 3, 4, 5 and 6 represented 8 %, 5 %, 19 %, 0 % and 0,7 % of the index. The radiographic codes made up 37 % of the mean value. The mean number of arrested lesions was 5.7(1SD=4.3). The distribution of radiographical scores of the 200 radiographically detected approximal lesions were as follows: score 1; 2;3;4 and 5: 55(28 %); 93(47 %); 46(23 %); 4(2 %); 2(1 %), respectively. A total of 12(6 %) of the radiographically detected approximal lesions could also be detected clinically. These were mainly scored as 3, 4, and 5 radiographically. 157(79 %) of the approximal lesions were assessed as active due to gingival bleeding. The participants are caries prone. Most of the lesions are initial when recorded by ICDAS clinically/radiographically. Thus, the sample seems suitable for investigating if 3DIOS and NIR can assess caries progression.

107 Association study on nutrition in the first year of life and molar-incisor hypomineralization (MIH) – Results from the GINI-plus and LISA birth cohort studies

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Molar-incisor hypomineralization (MIH) is a condition with specific clinical presentation whose etiology to date still remains unknown. This study prospectively investigated the association between nutrition during the 1st year of life and the presence of MIH in the permanent dentition. Data from 1070 10-year-old children from two prospective birth cohort studies were included. Information on exclusive breastfeeding (EBF) and introduction of 48 food items into the child's diet was assessed at 4-, 6-, and 12-month time-points. Food diversity was defined according to the number of food items or food groups introduced into the child's diet and then subsequent categorization into low-, middle- and high-diversity groups was performed. MIH was scored in the permanent dentition at age of 10 years. The statistical analysis included logistic and Poisson hurdle regression models adjusted for potential confounders. EBF, food item, and food group diversity at 4-, 6-, 12- month time-points were found to be non-significant in most of the categories for the development of MIH. However, significantly higher odds for the presence of MIH were found for certain categories (OR=1.82 (1.18-2.85), P<0.01, and OR=1.81 (1.08-3.09) P=0.03 both for middle-diversity group and MIH). The results of the sensitivity analyses showed a non-significant association between single food items – 8 food groups and 48 food items – and dental outcomes after adjusting for multiple testing. Despite the limitation of this study, such as arbitrary cut-offs for the categorization of food items, the results of this study suggest the lack of an association between early nutrition in the first year of life and MIH in the permanent dentition. Further investigations are needed to confirm our results.

108 Visible Occlusal Plaque Index predicting caries lesion activity: 2-yr clinical trial

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This study was undertaken to appraise the predictive validity of the Visible Occlusal Plaque Index (VOPI) in assessing occlusal caries lesion activity. A total of 618 10-15-year-olds were examined at the beginning of the trial and 511 (82.7 %) at the 2-yr follow-up. Adolescents and parents answered questionnaires about demographics, oral health behavior and family socio-demographic variables. The VOPI has a four-point ordinal scale ranging from no plaque to heavy plaque. Molar teeth were assigned to Group VOPI 0-1 (no/thin plaque; n=2,539) and Group VOPI 2-3 (thick/heavy plaque; n=843). Risk Ratios (RR; 95% Confidence Interval (CI)) and logistic regression analyses were run. At baseline, surfaces at risk of transition (n=3,382) were either sound (55%), inactive non-cavitated lesions (21 %), inactive cavitated lesions (1 %), active non-cavitated lesions (15 %) or active cavitated lesions (7 %). Sound occlusal surfaces with no/thin plaque were significantly more likely to remain sound (RR=1.3; CI:1.1-1.4) than those harboring thick/heavy plaque. Inactive non-cavitated occlusal lesions presenting no/thin plaque were significantly less likely to progress to active non-cavitated lesion (RR=0.1; CI: 0.0-0.8) than their counterparts. Active non-cavitated lesions harboring thick/ heavy plaque had the significantly lowest chance of becoming sound (RR=0.7; CI:0.5-0.9) and the highest risk of remaining active (RR= 1.5; CI: 1.1-1.9)). Regression analyses run according to surface status at baseline, showed that non-clinical predictors were not significant for occlusal caries development or progression. The presence of thick/ heavy plaque on occlusal surfaces was predictor for caries lesion development, progression and activity (OR=1.3-7.7; $p<0.002$). In conclusion, besides being an additional clinical tool for oral hygiene assessment, the VOPI is a predictor for development, progression and activity of occlusal caries lesion. This is particularly interesting for assessment of lesions undergoing health-promoting transitions.

Session 15
Epidemiology III

109 Oral health in adults and elders a global survey on tooth loss and caries

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Aim was to identify and analyze global data on oral health in adults and elderly. Studies conducted from 2016 onwards were collected on persons aged 45 years and over living in the community. Literature searches were performed on 3 databases namely, Embase, MEDLINE via Pubmed and Scopus, with the search restricted to articles published in English from January 2016 onwards. Grey literature search in Open Grey and free-hand searches were also carried out. The eligibility criteria were studies involving community-dwellers aged 45 years and over, and the data analyzed had to have been collected not earlier than the year 2005. Study selection and data extraction were undertaken by 4 authors. Quality assessment was done using The National Heart, Lung and Blood Institute for Observational Cohort and Cross-sectional studies, Case-Control studies and Controlled-Intervention studies. The search identified 734 papers. After deduplication and screening, 90 papers were eligible for data extraction. 1 study was conducted in Africa, 55 studies in Asia-Oceania, 14 studies in Europe, 5 studies in North America and 11 studies in South America. 4 studies were conducted between 2 different countries in 2 different regions of the world. Almost all the papers were cross-sectional studies with a medium-low quality. Over 1 million persons were evaluated. The prevalence of edentulism ranged from 1% to 70%. Dental caries prevalence ranged from 5% to 98%. Untreated dental caries and the rate of tooth loss are the most common health condition, showing that a dental epidemiologic transition is underway at the global level.

110 Impact of the Covid-19 epidemic on access to e-care by patients affected by Autistic Spectrum Disorder

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The COVID-19 pandemic has spread rapidly around the world putting a strain on the healthcare systems of countries, including the dental environment. During the pandemic, the vulnerability of some groups of people, especially those affected by Autistic Spectrum disorder (ASD), was emphasized. Aim was to evaluate the perception and approval rating of parents whose children were undergoing treatments in the Pediatric Dentistry Department of Policlinico Umberto I and evaluate the impact of Covid-19 emergency related to remote oral care management. The study included the parents of children belonging to the ward, starting from January 2020; an online questionnaire was anonymously administered to parents. One hundred and twenty questionnaires were analysed. During Covid-19 period, 19.2 % declared to have had dental urgencies: 41.2 % due to the difficulty in performing daily oral hygiene on their child, 41.1 % due to pain, 11.8 % due to abscess and 5.9 % due to caries. Among them, 64.3 % turned to the referral person in the Pediatric Dentistry Department and 14.3 % to their general practitioner. Among the ones not reporting dental urgencies, half of them states that wouldn’t know who to turn to in case of need. During pandemic, 30.1 % of the parents affirmed to having seek help of the dental team through Telemedicine. The Telemedicine service offered by department continued to have difficulty establishing itself. About half of the sample said they had a hard time using it, and only a small percentage used it in emergencies. All this represents a result that is not yet satisfactory. The aim is to encourage the use of telemedicine as a means of managing and preventing pathologies of the oral cavity in ASD patients.

111 Dentists' involvement in applying early childhood preventive procedures

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Early childhood caries can be prevented by implementing caries-preventive measures from early on, but it was not included in the German national health catalog until 2019. To assess the involvement of general dental practitioners (GPs) and specialized pediatric dentists (PDs) in early dental examination, prevention and fluoride recommendations. A survey on early dental examination and preventive measures in children under 2½ years old was distributed on annual dental convention in Schleswig-Holstein/Germany including four questions about themselves and six about their involvement in ECC prevention using Likert scale. Data were collected anonymously and inserted to SPSS-version23 for descriptive and statistical analysis employing 2-tailed t-test. Out of 273 responses, 212 dentists (143 female, mean age 44.9±11.9 years, GPs n=138, PDs n=74) were included in the study. Students, retired and university dentists were excluded. 54.9% of dentists provided early dental examination regularly and 31% occasionally. 85.2% of dentists counseled parents on oral hygiene measures, but only 33% training them. Significantly more PDs than GPs delivered early examinations, advice and training (p=0.08). Reduced cooperation of the child during the examination was the main problem for dentists (62.6%). Fluoride recommendations varied among dentists: 51.8% of dentists always prescribed fluoridated toothpaste for children starting with 6 months of age, while 10% waited until the completion of primary dentition, 11% until children do not swallow the toothpaste, and 9.9% recommended fluoride-free toothpaste in small children. PDs were more polarized than GPs in prescribing highly fluoridated toothpaste (p=0.026) or fluoride-free toothpaste (p=0.019). Most PDs and GPs provided early preventive measures in children below the age of 2½ years, even before this was reimbursed by the German national health system.

112 Dental caries in young children attending WIC clinics in North Florida USA

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Untreated caries, lack of insurance and poor access to care are major public health problems for low-income populations in USA. The federal Special Supplementary Nutrition Program for Women, Infants, and Children (WIC) serves low-income women and children up to age 5 at nutritional risk by providing education and health services. WIC Oral Health Program was implemented to provide preventive dental services and screenings for children enrolled in the North Central Florida WIC Program. Aim was to assess the prevalence of dental caries in the primary dentition of young children enrolled in the local WIC Program. Methods: The study was approved by the University of Florida IRB. Visual dental screenings were performed on 295 children aged 0-5 years in the local WIC offices by a dentist/hygienist team. Caries lesions were visually scored as initial (ICDAS 1/2~d1), or dentinal (ICDAS 3–6~d3) lesions. Descriptive analysis was performed. 28.5 % of children had enamel lesions and 34.3 % had dentinal lesions. Caries prevalence in 1-year-olds was 9.3 %; 2-year-olds: 35.4 %; 3-year-olds: 39.5 %; 4-year-olds: 58.0 %; and 5-year-olds: 59.1 %. Prevalence of untreated caries in children was 34.3 %. Dental caries was mostly observed in primary maxillary anterior teeth (33.0 %), followed by lower primary molars (27.3 %), upper primary molars (24.3 %) and lower anterior teeth only with 3.4 %. Children in WIC have a high prevalence of untreated caries. Caries in the primary dentition was mainly confined to molars, followed by upper-incisors. Young children in the North Central Florida WIC Program need earlier preventive and restorative care. Collaborating with WIC and other primary care providers can help to provide early preventive dental services for at-risk populations to prevent dental diseases.

113 Best practice and guidelines on caries treatment in global oral health: a scoping review

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According to the Global Burden of Disease, caries has been steadily increasing over the past 30 years. However, dental service delivery systems do not always benefit from comprehensive and robust evidence-based approaches. This study aims to systematically survey and evaluate the guidelines on caries management and prevention available to date. The latter will be conducted using the Appraisal of Guidelines, Research and Evaluation (AGREE) II Instrument. We conducted an electronic database search on EMBASE, SCOPUS, and PubMed, from 6 January to 14 February 2022, using *ad hoc* search strings for each database. Seven relevant guidelines databases were also sifted through. A total of 1165 articles were collected, and 232 duplicates were removed. Titles and abstracts were further examined for full text retrieval. Twenty-three articles met the inclusion criteria and were reviewed. The main findings included three types of practice guidelines: oral health promotion, primary caries prevention intervention, and minimally invasive techniques for caries treatment. In agreement with the AGREE II, the mean scores for each domain were reasonably high for the Scope and Purpose and the Rigour of the Development; Stakeholder Involvement, especially with regard to the opinions and preferences of the public, was mildly considered; the Clarity of Presentation benefits in many of the practice guidelines from the use of dedicated tables and paragraphs. Applicability and Editorial Independence showed lower average scores than the other domains. Notwithstanding the existence of a comprehensive and robust scientific basis, the production of clinical practice guidelines to support caries prevention and treatment-based interventions appears limited. The overall quality of the practice guidelines was moderate.

114 Assessment of PhiX174 bacteriophage in simulated tooth-preserving-operative care aerosol-generating procedures (AGPs) in natural anterior/posterior teeth

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Aim was to describe the PhiX174 bacteriophage-laden aerosols generated when conducting tooth-preserving operative caries care in natural anterior/posterior teeth. Class IV/II cavity preparations and composite fillings were independently conducted to simulate tooth-preserving operative care of extensive-caries lesions in natural anterior/posterior teeth. A trained practitioner conducted procedures on a dummy in a previously mounted dental-office setting (anterior-teeth: n=3; posterior-teeth: n=2). Aerosols were generated using PhiX174 bacteriophage ($1x10^7$PFU-Plaque-Forming-Units) diluted in SM-buffer (pH 7.4) and conventional suction. Aerosols were collected using *Escherichia-coli* C600 cultures (EC600) -PhiX174 host- seeded on a double-layer LB soft agar on Petri dishes (PD) located at 30, 60, 90, 120 and 150 cm apart from the dummy during and 30-120 min after each AGP. Additional PD were placed on the operator's shoulder, chest, face shield, and N95 mask. Moreover, a six-stage Andersen-Impactor was used to actively capture aerosols. In the presence of bacteriophage-laden aerosols on EC600, PFUs on the LB agar indicated viable/infectious particles. Comparisons between samples were conducted (U-Mann-Whitney-test). Overall, PFUs were mainly detected over the patient, practitioner and towards operator´s opposite side. During anterior-teeth AGPs, a mean of 137+39 PFUs were detected at 30 cm, up to 45 min after the procedure in PD and to 60-90 min in the Andersen-Impactor (5th and 6th stages; 1 and 3 PFU, respectively) (distance=1.5 m). During posterior-teeth AGPs, a mean of 165+18 UFCs were detected at 30 cm, with infectious viral particles decreasing to 0 PFUs at 30 min (anterior vs. posterior-teeth viral load difference; $p<0.05$). 100 % of PD on the operator showed PhiX174 bacteriophage, even under the face shield. Under simulated tooth-preserving operative care, generated aerosols spread up to 150 cm and for up to 90 min, with differential viral load between anterior and posterior teeth restorations.

115 Management of early childhood caries: Quality and adaptability of current clinical practice guidelines

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Even though numerous guiding documents have already been published regarding the management of Early Childhood Caries (ECC), the development of updated clinical practice guidelines (CPG) is always welcome to inform the best available evidence for the decision-making of healthcare professionals and managers. In the process of developing a Brazilian CPG for the management of ECC lesions in the public health system, an extensive search on the current existing CPGs was carried out, and available guidelines were identified, selected according to inclusion criteria, and critically appraised with the Appraisal of Guidelines for Research & Evaluation Instrument II (AGREE II) tool. Searches were conducted in PubMed, Scopus, Cochrane Library, and The International Guidelines Library databases. After duplicates removal, 405 guidelines were identified and confronted with the inclusion criteria. Guidelines were not included if they were not covering the research question, did not report on the external review process, had only one author, had no auditable evidence-based background, had low scores in the AGREE II appraisal, or were based only on experts' opinions. Nine guidelines were selected for full reading, and three were considered for the adaptation process, which was guided by the ADAPTE tool and followed the GRADE method. Additional searches were carried out for systematic reviews (n= 15 included) to check and update the guideline's recommendations to be adapted. Overall, a general lack of standardization and methodological rigor was observed in many of the current available CPGs about ECC treatment. This compromises the critical appraisal of the quality of those guidelines and their suitability for adaptation or adoption by other countries and organizations.

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Clinical Studies IV

116 Streptococcus oligofermentans inhibits Streptococcus mutans metabolic activity and aciduric properties

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Our analyses of supragingival biofilm samples from 300 children ages 3 to 5 (50% with caries), using metagenomic (whole genome shotgun sequencing) and metatranscriptomic technologies revealed a negative correlation between DNA of Streptococcus mutans (Sm) and logratio (RNA/DNA) of Streptococcus oligofermentans (So). This study aimed to exam the influence of So on the growth, metabolic activity, and aciduric properties of Sm. Sm and So were incubated as mono-cultures or co-cultured at 1:1 ratio in TSB with or without 1 % sucrose in a 96-well plate. To evaluate aciduric and acidogenic properties, the single- and dual-species samples were grown at 370C at 5% CO2 in pH 5.5 or 7.1. The metabolic profile was measured by Isothermal Microcalorimetry using the instrument calScreener™ microcalorimeter (SymCel Sverige AB, Sweden). Data were collected with calView™ software (Version 1.0.33.0, SymCel Sverige AB). Each experiment was performed in triplicate. Descriptive statistics are reported. When grown in co-culture, Sm was significantly inhibited by So, exhibiting a 2-log reduction in mean CFU/ml. Acid tolerance tests revealed that Sm and So, in single and co-culture growth, were able to grow at an initial pH of 5.5, with mean optical densities (ΔOD650 nm) of 0.31+0.01 (Sm), 0.23+0.02 (So), and 0.35+0.03 (Sm-So). Sm further decreased the pH from 5.5 to 4.6 when in mono-culture, but no change in the pH was observed when in co-culture with So. The species showed different metabolic profiles under the same conditions. The metabolism (measured by total heat, J) of Sm was reduced when co-cultured with So, with and without sucrose (from 1.3+0.5 to 0.9+0.3 and from 1.3+0.6 to 1.1+0.4; respectively). In conclusion, S. oligofermentans inhibited S. mutans under neutral and acidic pH conditions and affected its metabolic activity.

117 Radiographic progression of underlying dentin shadows in the occlusal surfaces of permanent teeth: 1-2-year results

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This prospective cohort study aimed to assess the radiographic progression of underlying dentin shadows (UDS) on the occlusal surfaces of permanent posterior teeth of adolescents and young adults from southern Brazil over 1-2 years and to identify possible risk factors. Data collection included the application of a questionnaire, clinical oral examination, and bilateral bitewing radiographs, performed at baseline, after 1 year and after 2 years. The association between possible predictors and UDS progression after 1 and 2 years was assessed using negative binomial regression models with generalized estimating equations to account for data clustering. Incidence risk ratios (IRR) and their 95% confidence intervals (CI) were estimated. From a total of 101 individuals enrolled in the study (149 lesions), 77 were reexamined after 1 year (115 lesions) and 48 after 2 years (74 lesions). The overall progression rates were 7 % after 1 year and 16.2 % after 2 years. Over a 2-year period, UDS in non-white individuals were more likely to progress than in white ones (IRR=2.76; 95%CI=1.13-6.73) whereas UDS presenting radiolucency at baseline were more likely to progress than those without radiolucency (IRR=2.35; 95% CI=1.06-5.21). In conclusion, this study showed low progression rates of UDS after 1-2 years of monitoring among adolescents and young adults from southern Brazil. Sociodemographic indicators (skin colour) and the presence of radiolucency at baseline were found to predict UDS progression in this sample. Considering the low progression rates observed in this study, the longitudinal monitoring of UDS seems to be the more indicated approach for this type of lesion. UDS with radiolucency should be monitored more closely.

118 Long-term treatment of dentin hypersensitivity with Bifluorid 10 and Futurabond U: a randomized clinical trial

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Funding: VOCO (Gmbh Cuxhaven, Germany) partially founded the study.

The aim of the work was to assess the efficacy of a fluoride varnish (Bifluorid 10, VOCO GmbH) and a bonding resin (Futurabond U, VOCO GmbH) in adults with dentin hypersensitivity on long-term pain reduction. A split-mouth double blind randomized control trial was conducted. The inclusion criteria was healthy adults, with ≥1 sensitive tooth (Schiff sensitivity score ≥2) and with exposed dentin in the upper or lower dental arches. Basic erosive wear examination index (BEWE), Schiff sensitivity scale and Vas scale were assessed before treatment, at baseline, at 1 week and at 1–6 months. BEWE scores are: 0 = no surface loss; 1 = initial surface loss; 2 = distinct defect, 3 = hard tissue loss < 50 % of the surface; 4 = hard tissue loss > 50 % of the surface. The study was approved by the Local Ethical Committee and all patients signed an informed consent. The efficacy of treatment in both groups and between-group differences was assessed using Student's t-test. Regression models were used to estimate the effect of age, gender and BEWE score on pain reduction. The results were considered statistically significant at $p < 0.05$. A total of 340 teeth were treated (180 teeth by Bifluorid 10 and 160 by Futurabond U) in 28 adult patients aged 23.3–69.3 years. The number of teeth treated in a single patient varied from 6 to 18 (mean 12.2; SD 4.0). Both treatments showed to be effective on pain reduction. Bifluorid 10 resulted more efficient for long-term VAS-measured pain decrease. Bifluorid 10 and Futurabond U are effective in the treatment of dental hypersensitivity. The RCT was registered at the US National Institutes of Health (ClinicalTrials.gov) #NCT04813848.

119 Investigation of the effect of Diabetes Mellitus Type 2 on supragingival plaque microbiota

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The aim of this study was to investigate the cariogenic microbial profile for supragingival plaque among patients with Diabetes Mellitus Type 2 (DMT2) using microbial and molecular analyses. This case-control study is a hospital-based and part of a clinical-PhD research. A total of 45 participants with confirmed diagnosis of DMT2 (n=24) and non-diabetes (nonDMT2; n=21) were recruited at the Dental Hospital, Barts Health-NHS. Participants were informed not to clean their teeth overnight for 12 h and not to eat and drink between 1-2 h before sample collection. The Silness&Löe (1964) plaque index (PI) and HbA1c analyses were carried out. Plaque samples were collected from maxillary molar tooth using a sterile excavator and immediately placed into a sterile cryo-vial containing 0.5 ml phosphate-buffered saline. Microbiological culturing of aerobic and anaerobic bacteria and molecular analyses using qPCR technique were used. The PI of DMT2 and nonDMT2 groups were 0.84±0.47; 0.82±0.42 respectively. There was no significant difference in mean PI between two groups (independent t-test; p=0.890). The mean HbA1c values in DMT2 and nonDMT2 groups were 52.47 ±10.89; 37.25±2.93 mmol/mol respectively. Independent t-test showed significant differences in the mean HbA1c levels between two groups ($p<0.001$). Mann-Whitney U test failed to show any significant differences in total microbial load between two groups (p=0.750). There were no significant differences in bacterial load (Cp values), colony forming units (CFU/ml) of S. mutans, A. naeslun ii and Lactobacillus ($p>0.05$) and in hemolysis and black-pigmented colonies of bacteria between the two groups ($p>0.05$). Within limitations of this study, the microbiota profile in supragingival plaque for patients with DMT2 was similar in comparison to the non-diabetes participants. A large sample size in a prospective case-control matched study is required.

120 Extracellular polysaccharide rich biofilm reduces the effect of chlorhexidine on enamel demineralization: a pilot in situ study

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Extracellular polysaccharides (EPS) in dental biofilm matrix may interfere on the effect of chlorhexidine on microorganisms and on enamel demineralization. Therefore, we investigated the effect of a 0,12 % chlorhexidine solution on cell viability and enamel demineralization in two *in situ* formed biofilms: one rich in EPS (EPS^+) and other not (EPS^-). A double-blind, randomized, split-mouth study was conducted in 2 phases of 14 d. Four volunteers used a palatal appliance containing 4 bovine enamel blocks. Two blocks were exposed 8 x/d to a 20 % sucrose solution (EPS^+), and the other two to a 10.5 % glucose + 10.5 % fructose solution (EPS^-) during biofilm growth. On the 7th day, volunteers started to rinse 2x/d a placebo (P) or 0,12% chlorhexidine (CHX) solution. Four groups were evaluated: EPS^-/P; EPS^+/P; EPS^-/CHX, and EPS^+/CHX. Biofilm and its respective block were collected on the 7th day (baseline) and on the 14th day (after the treatment). Biofilm was resuspended in 0,9 % NaCl, plated on blood agar, incubated at 37ºC, 10 % CO_2 and the CFU counted. EPS was extracted and analyzed with phenol-sulfuric method. Enamel cross-sectional hardness was determined and lesion area (ΔS) calculated. Three-way ANOVA was performed considering biofilm, treatment and time as factors (α=5 %). After treatment, ΔS values ($x10^4$ Kg/mm^2 x µm) were similar between E+P (4.0±1.5) and E+CHX (2.7±1.6) ($p>0.05$), however E-P (2.3±0.9) showed higher ΔS than E-CHX (0.7±0.2) ($p<0.05$). CFU counts (Log_{10}/mg wet biofilm) were similar among the groups ($p<0.05$). EPS values (µg/ mg wet biofilm) were similar between E+P (35.3±31.9) and E+CHX (25.2±11.3) ($p>0.05$%), but higher than E-P (3.0±1.9) and E-CHX (7.2±2.8). An EPS-rich biofilm may reduce chlorhexidine effect on dental demineralization.

121 Chlorhexidine exposure does not change EPS concentration in the matrix of early S. mutans biofilm

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Chlorhexidine (CHX) has a proven antimicrobial effect in biofilm formation, but its action in biofilm already formed is limited. In addition, the effect of CHX on the extracellular polysaccharides (EPS) present in the biofilm matrix is not known, which was evaluated in this pilot study. Early Streptococcus mutans UA159 biofilms were formed in 96-well plates using UTEYB medium supplemented with 1 % sucrose for 24-h. Then, biofilms were submitted to antimicrobial treatment with chlorhexidine digluconate for 5 min at the concentrations (n=6): 0.12, 0.06, 0.03, 0.015 % (v/v in purified water), and NaCl 0.9 % (negative control). For biofilm viability test, resazurin in PBS (0.1 mg/mL) was added per well and the plate was incubated at 37°C for 6 h. Biomass was stained with 0.05 % crystal violet, water washed and the remaining dye was recovered with 30 % acetic acid. Samples of biofilm viability test and biomass were measured in a microplate reader at 570 nm. EPS was extracted with 1 M NaOH under microplate agitation for 15 min, transferred to microtubes, centrifuged, and the supernatant was precipitated with ethanol (1:3, v/v). EPS were quantified colorimetrically with the phenol-sulfuric method, measured at 490 nm. The % of biofilm viability was calculated considering the viability of negative control as 100 %, and only resazurin as 0 %. Data were analyzed by one-way Anova, Tukey's Test (α=5%). The % of biofilm viability (mean±SD; n=6; different letters represent statistically significant differences, $p<0.05$) at 0.12, 0.06, 0.03 and 0.015 % chlorhexidine, and 0.9 % NaCl was, respectively, -2.2±2.7A, 68.5±1.2B, 79.6±3.3C, 86.8±2.7D, and 100.0±2.8E. Biomass and EPS values were similar among the groups ($p<0.05$). In conclusion, CHX exposure has antimicrobial effect on early S. mutans biofilm, but it does not change the EPS concentration in the matrix.

122 Approximal sealing during operative treatment in the neighbor tooth - initial findings based on cases

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The aim was to investigate the efficacy of resin-sealing (Clinpro, 3M) on an approximal surface of a pre-or molar tooth with an active caries lesion ICDAS code 1 or 2 next to an approximal caries lesion in the neighbor tooth, requiring operative care. (Cartagena et al.: Acta Odontol. Scand. 2018, 76:459-465). In 2019, we began a multi-centered RCT study. In the Chilean part, we enrolled twenty-eight 20-30 years old patients. An approximal control lesion was treated with fluoride varnish. It was planned to recall the participants every sixth month, with the final evaluation after 24 months. But with the COVID-19 conditions, the reapplications of fluoride varnishes were not possible; thus, we lost the control lesions. After two years, we managed to recall nine (32 % of the participants). The sealed lesions were clinically and radiographically assessed by the first author and blindly to the study aim by one expert in dental radiography at the university in Santiago. All sealants were still in place according to the first author and both scored that none of the lesions had progressed, radiographically. The patient files indicate that 7 participants had active plaque induced gingival deseases during the 2 years of study and received hygiene control, during the period. 2 remaining participants received recommendations to go to hygiene control, and 5 of them derived to caries treatment in another teeth. Sealing of active, initial staged approximal caries lesions during the operative procedure of the neighbor approximal lesion, seems to be an effective treatment, at least 2-year period. It would be interesting to perform a new MCRCT study about this technique.