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Poster Session P15 – Cariology 2

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INTERNATIONAL JOURNAL OF PAEDIATRIC DENTISTRY

Poster Session P15/Cariology 2

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Abstract withdrawn

P15–219
Characterization of enamel remineralization under sealants via polarized light microscopy
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Introduction: The objective was to characterize the remineralization behavior of enamel lesions when coated with either a resin-based sealant or an experimental resin-modified glass ionomer material.
Materials and methods: Extracted human molars with artificial caries-like lesions were sectioned longitudinally into 100 μm sections; photographed via PLM with imbibing media of water (n_D = 1.33; showing > 5% porosity), followed by an imbibing media of Thoulet's 1.41 solution (n_D = 1.41; showing > 10% porosity). Acid-resistant varnish was applied to all surfaces, leaving only the external tooth surface with lesion exposed. Three treatments were applied: untreated control group (UNTR); lesion coated with Ultraseal XT™ Plus™ Pit & Fissure Sealant (UXT); lesion coated with experimental resin-modified glass ionomer (EXM-OVR). The sections were toothbrushed with water daily for 30d. The acid-resistant varnish was removed; sections imaged via PLM again. The lesion body area was quantified via computerized imaging before and after, and percent remineralization calculated. Data were analysed via one-way ANOVA and compared with Fisher's *t*-test ($P < 0.05$).
Results: The percent remineralization (stdev,n) with water imbibition was for UNTR 10.08 (7.14,9)[a], UXT 2.96 (4.21, 9)[a] and EXM-OVR 6.87 (10.10,9)[a] as well as with Thoulet's imbibition for UNTR 1.51 (4.79,10)[b], UXT 7.08 (6.06, 10)[b], EXM-OVR 30.28 (21.84,10)[a]. Within each imbibition set, superscript letters denote groups that are not statistically different.
Conclusions: A resin-modified glass ionomer coating applied over an enamel lesion resulted in significantly enhanced remineralization compared to an untreated control; a resin-based sealant applied over the lesion did not change the remineralization behavior *versus* the control. This research was supported, in part, by 3M ESPE.

P15–220
Micro-computed tomographic evaluation of effects of CPP-ACP on demineralized dentin
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Introduction: Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been known as an additive in MI Paste® (MI; GC Co., Japan) which is one of the products for caries prevention. In our previous study the use of MI with fluoride has suggested the inhibitory effect on demineralized dentin. This study reported the validity of using a microfocus X-ray computed tomography (μCT) to evaluate the effects of CPP-ACP on demineralized dentin.
Materials and methods: Bovine dentin specimens (n = 40) were prepared and divided into four groups equally. Each specimen was stored in demineralizing solution (pH 5.3) for 23.5 h. Then each group was immersed in 4 kinds of treatment solution conditioned by the presence of MI with/without fluoride for 30 min. This process was followed by collecting μCT images of the specimens. The set of treatments was repeated four times. The μCT images were analysed by using software (TRI-BON, RATOC, Japan) to determine the effects of remineralization and to compare the previous data by microradiography and polarized light photomicrography.
Results: By using μCT it was possible to serially observe the de/remineralization process on the same site of each specimen. Compared with the previous results, μCT images were suited for quantifying the effects of CPP-ACP; however it is difficult to measure the initial remineralization.
Conclusion: Using μCT is a reliable method to evaluate the effects of CPP-ACP on demineralized dentin. Support: Grant - in - Aid for Young Scientists (#20791594).

P15–221
Selection of filling materials for conservative adhesive restorations in occlusal fissures
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Introduction: Within the framework of wide adoption of minimal invasive technologies the method of conservative adhesive restoration (CAR) takes the first place in treatment of fissure caries. Different combinations of filling materials and sealants are used in CAR successfully. The aim of our study was to investigate the clinical performance of different filling materials used for conservative adhesive restorations in occlusal fissures *in vivo* and *in vitro*.
Materials and methods: The method of CAR was applied in 53 molars in children at the age from 6 to14. GIC 'Fuji IILC' (GC) and resin sealant 'UltraSealXTplus' (Ultradent) were used in 26 teeth (1st group), flowable composite 'Charisma Flow' (Heraeus Kulzer) and resin sealant 'UltraSealXTplus' (Ultradent) were used in 27 teeth (2nd group). Method of adhesive strength determination by shift (testing machine 'Instron') was realized to determine adhesion degree between filling and sealant within limits of CAR. Samples from the same materials were made *in vitro* and two analogous groups were formed.
Results: After one year excellent states of the fillings was detected in 92% of teeth in the 1st group and in 94% of the teeth in the 2nd group. Marginal leakage was detected in 8 and 6% of the fillings, respectively. Adhesive strength amounted to 15.5 ± 2.4 MPa in 1st group and 22.0 ± 2.8 MPa in 2nd group. The findings indicate evidence of higher adhesion between a resin sealant to a flowable composite than to GIC (in 1.4 times, $P < 0.05$).
Conclusion: A combination of flowable composite and resin sealant may be advisable to use within application of CAR in fissure caries treatment.

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Use of 'atraumatic restorative treatment' by pediatric dentistry professors in Brazil

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Introduction: The aim of this study was to evaluate the use of ART by professors of Pediatric Dentistry in Brazil and the evaluation of associated factors.
Materials and methods: A structured questionnaire was made with questions relating to socio-demographic characteristics, educational level and ART.
Results: Of the 721 questionnaires sent, approximately 38% (n = 280) were returned. Descriptive statistics and logistic regression analysis were conducted to verify the association between variables and the use of ART (α = 5%). Of those interviewed, 87.9% and 85.7% have already used ART. Professionals with postgraduate course are 7 times more likely to use ART (OR = 7.42, 95% CI 2.10–26.23) compared to professionals level graduate. The amount of time that had passed since their graduation influenced the professors' experience in using ART. Professionals who had graduated over 15 years ago had twice the chance of having used this type of therapy in comparison to less experienced professionals (OR = 2.46, 95% CI 1.15–5.26). There was no difference between the Brazilian regions in relation to the use of ART.
Conclusion: It was concluded that the ART philosophy is being dissipated throughout Colleges of Dentistry, thus contributing to the training of professionals who are aware of their true role as health educators and meeting the needs of the Brazilian reality.

P15–223

Correction of artefacts and calibration of μCT for studying caries-excavation methods

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Introduction: This study presents a method to correct artefacts during reconstruction of μCT projections of carious teeth, along with pre-processing steps to segment and thresholding for 3D-evaluation of caries-excavation techniques.
Materials and methods: A beam-hardening curve was obtained from a wedge-shaped hydroxyapatite (HAp) block after scanning in a high-resolution μCT (Skyscan 1072, Skyscan, Belgium) and fitting the curve with 5th order polynomials. The correction was further applied to reconstruct the tooth slices. Calibration into HU units and mineral density was obtained by scanning, reconstructing and processing stacks of HAp phantoms with different mineral densities (0.25, 0.75, 3.14 g/cm^3). A median filter was applied to the tooth slices and a connected threshold grower was used to produce a final mask, which was used to blank-out undesired structures (background/areas of partial volume effects). A threshold for the caries lesion (8-bit gray value = 75; mineral density = 1.11g/cm^3) was obtained after correlating the μCT values with nano-hardness data obtained from carious areas (Nanoindenter, CSM Instruments, Switzerland).
Results: Excavation with carbide burs or with a new line of ceramic instruments (Ceraburs™, Komet, Germany) was performed in molars presenting small ($\pm$13 mm^3) or large caries lesions (±45 mm^3), from which a pre- and post-excavation μCT scan was obtained. The percentage of removed carious tissue was not different in the small cavities (80.5%-carbide burs, 80.3%-Ceraburs™). In large cavities, the Ceraburs™ removed less carious dentin (74.6%) than carbide burs (84%), probably due to differences in the employed excavation end-point.
Conclusion: The protocol has shown to be applicable to caries-excavation studies.

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Microhardness of dentine under glass ionomer cement after three different caries removals

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Introduction: The aim of the study was to evaluate the microhardness of the remaining dentine under glass ionomer cement (GIC) restorations in primary molars using three caries removal approaches.
Patients and methods: Forty-three primary molars from 32 schoolchildren, aged 8–12 years, were recruited. Approval was provided by Mahidol University Ethic Committee. Written informed consent was obtained from each parent and child. Participants having deep dentinal caries without sign and/or symptom of irreversible pulpitis and pathologic finding from radiographs were included. The teeth were randomly allocated into 3 study groups: group (i) partial soft caries removal by spoon excavation, group (ii) all soft caries removal by spoon excavation (ART), and group (iii) conventional caries removal by burs. All cavity preparations were restored with GIC (Fuji IX, GC Corp., Japan). After 3–6 months exfoliated teeth were processed for microhardness measurement with Knoop indenter (FM-700, Future-Tech Corp, Japan). The dentine under restoration was divided into three zones: close to restoration, intermediate, close to pulp. Six values of Knoop hardness were obtained from each zone. The mean microhardness of dentine among three groups was calculated using One-way ANOVA.
Results: After 3–6 months, 10 teeth in each group were evaluated. There were no statistically significant differences in the microhardness of the three zones among groups ($P > 0.05$). Similarly, the microhardness of the three zones were not different within groups ($P > 0.05$).
Conclusion: The microhardness of the remaining dentine after using partial soft caries removal approach followed by GIC restoration was comparable to that of ART and conventional approaches.

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The clinical performance of the occlusal minimally invasive restorations in primary molars

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Introduction: Minimally invasive procedures for small occlusal lesions in primary teeth are an important part of new restoration methods that can be successfully applied in children. The objective of this study was the evaluation of the clinical performance of the direct occlusal restorations in primary molars, applied by the minimally invasive method, using adhesive materials, in comparison with the classical method, using amalgam.
Materials and methods: A number of 258 direct occlusal restorations were placed in 144 patients with a mean age of 5 years and 5 months. The restorations were grouped in two samples: classical

amalgam fillings (n = 124) and minimally invasive restorations (n = 134).
Results: The restorations' median survival time was calculated using Kaplan-Meier statistics and survival tables methods and showed differences between the two groups. The median survival time of classical amalgam restorations (4.6 years) was higher in comparison to minimal invasive restorations (4.0 years) ($P < 0.1$ for Log-Rank test and $P < 0.05$ for Breslow and Tarone-Ware tests). The overall failure rate was 18.2%. In detail, 19.4% of the minimal invasive restorations and 16.9% of the amalgam fillings failed over the study period.
Conclusions: The survival rate of the minimal invasive restorations was slightly lower in comparison to the amalgam restorations.

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Assessment of manual restorative treatment (MRT) with amalgam: results after 5 years

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Introduction: The aim of this study was to evaluate the survival rates of amalgam restorations in manually prepared cavities in high caries Filipino children. Provision of restorative treatment was part of a 5 year comprehensive school based oral health program in the Philippines.
Materials and methods: Cavities were prepared using hand instruments and for filling an encapsulated amalgam, mixed with a manual powered triturator. The parents of the children gave written informed consent. At the final evaluation of the program a total of 1333 restorations in permanent teeth of 627 Filipino children were evaluated using ART criteria (Holmgren *et al.*, 2000).
Results: The mean observation time was 2.7 years, the overall survival rate of the amalgam-restorations was 95.3%. Related to the age of restoration a maximum failure rate of 7.3% was found for 4-year-old restorations. The overall failure rate of the class II restorations was 11.4% compared with 4.4% for the class I restorations. The survival rate of large occlusal restorations was 94.1% compared with 96.6% for small occlusal restorations. The experience and professional level of the operator did not influence the survival rate of the MRT restorations.
Conclusion: Especially for extensive occlusal lesions of permanent teeth in high caries risk populations amalgam can be considered as a suitable filling-material in ART-prepared cavities.

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Effects of lasers and fluoride on the acid resistance of human decalcified enamel

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Introduction: In order to preserve healthy enamel and increase acid resistance of incipient carious lesions, Nd: YAG laser, CO_2 laser, Er: YAG laser and APF were used to treat incipient carious lesions.
Materials and methods: Two hundred and twenty samples from human caries-free pre-molars were immersed in a 2-day pH-cycling solution for incipient caries formation. All samples were divided randomly into 11 groups and were treated respectively according to the grouping criteria. Samples were immersed in a 2-day pH-cycling solution again for acid challenge. For acid resistance evaluation, calcium concentration dissolved from the enamel surface was analysed by ISE-trol. Scanning electron microscopy was used to assay geographic changes of enamel surfaces, and polarized light microscopy was also used to observe the optical phenomenon alternation. Finally, impedance was detected by an impedance analyzer.
Results: The control group and Er: YAG laser groups showed a statistically significant ($P < 0.05$) higher calcium concentration compared with the APF group and the other two laser groups. From SEM, the melting and re-crystallization surfaces and crater-like holes of 1–20 μm in diameter were found in the CO_2 laser and Nd:YAG laser groups, and Type I, II, III etching patterns were found in Er:YAG laser groups. From PLM, the reversal of birefringence was observed in all laser groups. Finally, AC impedance and equivalent circuit analysis was useful for the evaluation of the contributor after laser irradiation on the decalcified enamel.
Conclusions: Application of Nd: YAG laser, CO_2 laser and APF on enamel incipient caries will increase acid resistance. The effects of lasers are better than APF.

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Effects of an ART restoration on dentin

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Introduction: Atraumatic restorative technique (ART) was developed to meet some particular needs of dental treatment in developing countries. A lot of ART restorations fail, especially Class II restorations. The objective of this research is to explore whether, in this context, the most appropriate solution for failed ART restorations is re-restoration or leaving the preparation further unfilled.
Materials and methods: In 2006 a group of children in Kenya has been treated with the ART technique. Out of this group 192 children who lost their Class II restoration and still had the treated molar in situ were selected. The time span for which the restoration has functioned is known and the colour, hardness and coloured dentin (with a caries detector) have been evaluated.
Results: 66% of the molars were scored with hard dentin, 78% with dark dentin and 51% with non coloured dentin. There is a positive correlation of these criteria with the time the restoration has functioned. The molars were scored with hard dentin in 59.5% of the cases when they have lost the restoration within 0–6 months, between 6–12 months 60.7% scored hard dentin and between 12–18 months 92.3%. Non coloured dentin was scored in 45.9%, 53.6% and 61.5% of the molars at the same retention times.
Conclusion: The outcomes of this research indicate that according to clinical criteria re-restoration of these molars is dependant on the retention time and the expected life time of the tooth.

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Influence of the Hall-technique on the open bite and the crown length

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Introduction: The consequence of placement of preformed metal crowns (PMCs) without preparation is that the bite will be propped open. However, it is unknown how fast the open bite disappears and whether this is caused by extrusion of the adjacent teeth or intrusion of the teeth directly involved. The aim of this study was to get more information about the open bite, created by using the Hall- technique.
Materials and methods: In 66 children, mean age 5 year 7 months, 114 PMCs were placed. The open bite was scored by measuring the distance between upper and lower cuspid just before placing the crowns, immediately thereafter and after 17–41 days. For the purpose of this study 48 crowns were used. Of another 18 crowns, its neighbors and opposing teeth the crown length was measured immediately after treatment and after 1 month. The study was approved by the Ethical Committee and informed consent was obtained from the parents.
Results: Before treatment the overbite between the cuspids was 2.4 mm (SD 1.1). Immediately after placing all crowns the bite was propped open to 0.5 mm (SD 1.8). This difference was significant. After 17 days ($n = 19$) the open bite was reduced from 2.5 to 1.8 mm, meaning that the bite was not fully closed again ($P = 0.038$). After 41 days ($n = 27$) the open bite was further reduced (from 2.2 to 1.9 mm; $P = 0.256$). The crown length of teeth with PMCs and their opposing teeth reduced significantly ($P = 0.046$ and 0.02 resp.) and that of the adjacent teeth did not change ($P = 0.739$).
Conclusion: Due to intrusion the open bite is reduced in a period of a good month.

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Validation of a prolonged filling method of dental caries treatment of children

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Introduction: Raising the efficacy of treating caries in permanent teeth with incompletely mineralized hard dental tissues.
Patients and methods: Treated for dentine caries during the first two years after eruption were 39 subjects aged 6–13 years. In the first group (29 teeth), carious cavities underwent mechanical preparation and coating of their walls and floors with Dycal calcium hydrate for 1 month's time, following which the inlays were removed and cavities permanently filled. The second group (40 teeth) was treated likewise except for the coating which was a zinc oxide-based Dentin-paste. Before doing both temporary and permanent filling the dentine mineralization was checked using the Russian-made Geosoft Dent-Est electrometer.
Results: Initially, dentine electrical conductivity in both groups was the same - 63.97 ± 0.65 mcA, 44.38 ± 0.77 mcA, and 45.29 ± 0.81 mcA in the first, and 63.87 ± 0.76 mcA, 44.39 ± 0.93 mcA, 44.27 ± 0.66 mcA in the second group, respectively. After the one-month calcium hydrate treatment, the dentine conductivity in the first group showed a significant ($P < 0.001$) decrease of 68.09% in the floors and 57.42% and 58.17% in the walls of the treated cavities. The cavities in the second group were noted to show a rise of 4% in dentine mineralization, which was as insignificant as it was unreliable.
Conclusions: The use of calcium-based materials for prolonged caries treatment of permanent teeth with incomplete mineralization enhances mineralization of dentine and helps prevent pulpitis.

P15–231

Delivery of treatment in a peadiatric dental practice – changes over a 10-year period

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Introduction: We aimed to study whether treatment needs and preferred treatment delivery has changed in a Greek private paediatric dental practice over a 10-year period.
Materials and methods: We screened the charts of all patients who shoed for an initial comprehensive examination, by K.N. only, at the part-time practice. These were 174 during 1996 and 186 during 2006. Parameters including age, gender, caries indices, reasons for visiting, source of referral, type of treatment needs (pulp treatments, extractions), treatment plan completion and response to recall, were examined and analysed with descriptive statistics.
Results: Mean age was similar in both groups, approx. 7.5 years. There was no significant difference in most parameters, i.e., DMFT: 0.7 *vs* 0.96, dmft: 2.9 *vs* 2.5, referrals from a dentist: 45% *vs* 40%, referral from a paediatrician: 12% *vs* 16%, patients who completed the treatment plan: 76% *vs* 74%, 1st recall attendance: 33% *vs* 32% for 1996 *vs* 2006, respectively. The only most notable difference was a drop from 35 to 13% in pulp treatments (not accompanied by any increase in extractions), the greatest proportion of these were vital puplotomies.
Conclusion: No significant differences were evident regarding the needs, planning and delivery of dental treatment between 1996 and 2006. The only significant decrease found in the number of pulpotomies is perhaps explained on the basis of increased experience and the shift from amalgam to resin modified glass-ionomer cements, both aiding to a more conservative approach towards indirect pulp capping *vs* pulpotomy in deep cavities.

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The influence of ozone on retention of sealing material: a clinical study

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Introduction: The aim of the present study is to clinically analyse the influence of pre-treatment of enamel with ozone prior to fissure sealing with flowable composite material, on retention of the seal. Retention of the nanohybrid flowable composite material as a material for fissure sealing was also analysed through the clinical period of 24 months.
Materials and methods: Group A consisted of 79 healthy permanent molars who were treated with ozone from KaVo Healozone device before the application of Voco Grandio Flow flowable composite. Group B consisted of 75 healthy permanent molars who were also sealed with Voco Grandio Flow but without the pre-treatment with ozone. Testing of the material's retention was conducted after 24 months of clinical use. Following values were assessed: 0-complete retention, 1-loss of 1/3, 2-loss of 2/3, 3-complete loss of seal, 4-caries. Chi square test was used for statistical analysis.

Results: After 24 months of clinical use, complete retention was found on 82.3% of the teeth in group A, and 84% in group B. Caries was found in 1.3% of the cases in group B only. No statistically significant differences between the groups ($\chi^2 = 1.976$; $P = 0.577$) were found.
Conclusion: Considering the fact that ozone had no negative effect on retention of the sealing material after 24 months, we concluded that it can be used as pre-treatment of enamel before fissure sealing. Sealing the fissures with nanohybrid flowable composite showed a high degree of retention and consequently excellent prevention of occlusal caries.

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The effect of ozone pre-treatment on the microleakage of fissure sealants

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Introduction: This study investigated the effect of ozone pre-treatment on the microleakage and marginal integrity of fissure sealants placed with or without a self-etch adhesive.
Materials and methods: Freshly-extracted, unerupted human permanent third molars were randomly assigned into two main groups ($n = 48$), in which the fissures either received ozone pre-treatment (Group A) or were left untreated (Group B). Teeth were further divided into two subgroups randomly ($n = 12$/each) so that half of teeth received conventional fissure sealants (Fissurit F, Voco, Germany), while fissures of the remaining teeth were treated with a self-etch adhesive (Clearfil Protect Bond, Kuraray, Japan) before placement of the sealant. Following thermal cycling (1000X), the specimens were sealed with varnish and immersed in 0.5% basic fuchsin for 24 h. Then, the specimens were sectioned and photographed digitally. The extent of dye penetration on the tooth-sealant interface was measured by image analysis software (ImageJ). The data were analysed statistically with Kruskal Wallis and Mann–Whitney U tests ($P = 0.05$). Two randomly-selected sections from each group were observed under SEM.
Results: Ozone-pre-treatment of fissures significantly reduced the microleakage, regardless of the application mode of the fissure sealant ($P < 0.001$). When sealants placed with or without a self-etch adhesive are compared, microleakage scores did not reveal significant differences in Group A. However, in Group B, conventional fissure sealants demonstrated lower microleakage scores ($P = 0.009$). Sections observed under the SEM demonstrated better adaptation of the sealant in ozone-pre-treated specimens.
Conclusion: Within the limitations of this *in vitro* study, it was observed that ozone pre-treatment favorably affected marginal integrity of fissure sealants.

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Effect of saliva contamination on microleakage of three different fissure sealants

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Introduction: Considering the multi-steps involved in the placement of fissure sealants, saliva contamination is inevitable. Fissure sealants with simplified application steps are in market today. This study compared the microleakage of three different fissure sealants namely, Helioseal F, Enamel Loc, Fuji VII under saliva contaminated and uncontaminated conditions.
Materials and methods: Sixty recently extracted sound third molars were randomly assigned to three groups for three different sealing materials ($n = 20$). Each sealant group was then randomly divided into two as uncontaminated ($n = 10$) and saliva contaminated ($n = 10$) prior to sealant replacement. The samples were thermocycled in water for 500 cycles between + 5 and + 55°C with a dwell time of 30 s and immersed in 1% methylene-blue for 24 h in 37°C. The samples were then sectioned and scored on a 3 point rating scale using a light microscope. Mann–Whitney-U-test was used for statistical analysis.
Results: In uncontaminated group, mean microleakage scores for Helioseal F, Enamel Loc and Fuji VII were 0.37, 0.65 and 0.90 respectively. The difference was statistically significant among three fissure sealants ($P < 0.05$). In the presence of saliva contamination, mean microleakage scores were 0.53, 0.77 and 0.90 for Helioseal F, Enamel Loc and Fuji VII respectively. Helioseal F group was given significantly the lowest score ($P < 0.05$), whereas no significant difference was noted between Enamel Loc and Fuji VII groups.
Conclusions: Under both uncontaminated and saliva contaminated conditions, the light cured resin based pit-and-fissure sealant Helioseal F yielded lower microleakage scores compared to Enamel Loc and Fuji VII.

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Risk factors for early childhood caries (ECC) in 2–5 year-old children

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Introduction: There are numerous risk factors significantly related to ECC. The most important are high-frequency intake of sugary snacks and drinks in a bottle, particularly during the night. The aim of this study was to determine the association of dietary habits and socioeconomic status for early childhood caries among 2–5 year-old children.
Patients and methods: A total of 200 children (aged 2–5 years) were examined for gender, dmft, dmfs, dietary and brushing habits, duration and contents of bottle feeding, number of individuals in the family, educational level and occupation of parents, and socioeconomic status. Ethical approval and informed consent were obtained. Statistical analysis was performed by using NCSS 2007 software and one-way ANOVA, tukey test, *t*-test, chi-square test were performed between the groups.
Results: Gender ($P = 0.031$), number of main meals ($P = 0.017$) and drinking milk before sleeping ($P = 0.038$) were positively associated with bottle feeding, and total income negatively ($P = 0.0001$). For children who were using a bottle, the mean average of dmf and dmfs scores were 9.88 and 15.5 respectively. Statistically significant differences were found between dmf, dmfs scores of bottle feeding and nonbottle-feeding groups ($P = 0.0001$). The anterior caries pattern was more pronounced for 'bottle feeding only' than for 'breast-and-bottle feeding' and 'only breast feeding' ($P = 0.0001$). Socioeconomic status was found to be significantly associated with dmf and dmfs scores ($P = 0.004$, $P = 0.036$).
Conclusion: ECC was more prevalent in pre-school children, especially those of low socioeconomic status. It was concluded that night-time breast-feeding in children, using a bottle at night and during the day correlated with the etiology of ECC.