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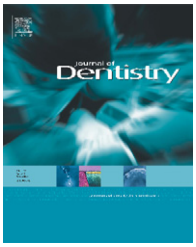

Inhibition of enamel mineral loss by fissure sealant: An *in situ* study

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ABSTRACT

Objectives: This study evaluated the effect of fluoride and non-fluoride sealants on hardness decrease (HD) and marginal adaptation (MA) on enamel substrates after cariogenic challenge.

Methods: Occlusal enamel blocks, from human third molars, were randomly divided into six groups ($n = 12$), according to occlusal fissures condition (S – sound; C – caries-like lesion; CF – caries-like lesion + topical fluoride) and sealants (F – FluroShield; H – Helioseal Clear Chroma). Lesion depths were 79.3 ± 33.9 and 61.3 ± 23.9 for C and CF groups, respectively. Sealants were placed on occlusal surface and stored at 100% humidity (37 °C; 24 h/d). HD was measured by cross-sectional microhardness analysis at the sealant margin distances: −1 (under sealant), 0 (sealant margin), 1, 2 (outer sealant). Sealant MA was observed by polarized light microscopy and scored according to: 0 – failure (no sealant MA or total sealant loss); 1 – success (sealant MA present). MA and HD were analysed by ANOVA-R and mixed model analysis, respectively.

Results: For HD (ΔS), *F* values (6900.5 ± 3686.6) were significantly lower than *H* values (8534.6 ± 5375.3) regardless of enamel substrates and sealant margin distances. Significant differences were observed among sealant margin distances: -1 $(5934.0 \pm 3282.6) < 0$ $(8701.5 \pm 6175.7) = 1$ $(8473.2 \pm 4299.4) = 2$ (7761.5 ± 4035.1), regardless of sealant and substrate. MA was similar for all groups ($p \geq 0.05$).

Conclusion: MA was not affected by sealant type or substrate condition, whereas enamel HD was favourably impacted by fluoride in the sealant. In addition, sealants were more effective as a physical barrier than as its chemical potency in reducing enamel HD.

Clinical significance: Sealing with a fluoride material is a recommended procedure to prevent caries of occlusal permanent molars in high-caries-risk patients, even though those exhibiting white spot lesions, since the enamel hardness decrease when fluoride sealant was used *in vitro*.

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1. Introduction

Even though the prevalence of dental caries has declined remarkably in most industrialized countries over recent decades, population subgroups continue to experience a high incidence of dental caries.[1,2] In primary and permanent teeth, the fissure region in the occlusal surfaces of molars is the most susceptible caries site.[1,3] Biofilm stagnation is promoted there because the morphology (shape, depth, and narrowness of fissures) prevents self-cleaning by food, tongue, cheeks and lip and makes cleaning by other measures difficult.[4]

Common non-drilling strategies to prevent caries progression include the application of fluoride as well as education in oral hygiene and proper diet. However, these approaches have limitations in non-compliant individuals.[5] Therefore, the prudent use of non-invasive fissure sealants is currently one of the most effective ways to protect against caries development on the occlusal surfaces of high-caries-risk children and adolescents.[6,7] This population frequently presents white spot lesions, however, while the diagnosis of initial caries in occlusal fissures is extremely difficult, the decision as to whether the fissure is sound or not must be made before the application of sealants.

Traditionally, the tooth surface with questionable active caries has been contraindicated for sealant treatment since a sealed demineralized area will no longer remineralize.[8] Thus, it has been accepted that it may be necessary to remineralize the caries lesion before applying the sealant. Since the early non-invasive intervention has the benefit of being suitable for all patients, a current approach suggests that operative intervention should not be a management option for the non-cavitated lesion. Thus, within the context of the minimal intervention approach, infiltrating regimens should be considered for treatment of demineralized enamel areas in non-compliant individuals. Once the material is cured, a mechanical support of the fragile enamel framework in the lesion is achieved, promoting obturation of porous and arrest of lesion progression.[9] Because the extent of demineralization cannot be estimated clinically, another contemporary protocol for the treatment of white spot lesions recommends the use of sealants, not only as a preventive treatment for sound fissures, but also to arrest caries progression by sealing over active caries lesions.[6,7] Although the proposing of infiltrants regimen is to occlude the tiny pores within the lesion body with low viscous light curing resins, white spot lesions remineralization before sealing applying is an approach to heal that area. However, whether early pit and fissure carious lesions can be sealed effectively to the levels of sound or remineralized fissures has not been sufficiently investigated. Even so, the use of fluoride-containing resin sealants on white spot lesions may be a viable approach to arrest hardness decrease in all high-caries-risk children and in adolescent patients even those who are non-compliant. Fissure sealants that provide fluoride will be important not only as passively (*via* physical barrier between the tooth and the oral environment), but also as active cariostatic agents, possibly providing increased caries inhibition (since the fluoride inhibits demineralization and favours the remineralization processes).[10,11]

The key consideration for sealant procedural success is adequate adhesion, while an important parameter for clinical success is the materials' marginal adaptation. Absence of marginal adaptation may imply that there is no occlusal surface isolation from oral microorganisms and, consequently, an increased risk for the development of dental caries.[12] Also, the presence of a marginal gap can lead to marginal staining, which can be considered the first sign of resin-based material failure.[13] Furthermore, the lack of marginal adaptation might generate interfacial stresses that potentially cause de-bonding of the sealant from the tooth.[14]

Sealant performance can be influenced by the high cariogenic challenges present in the oral environment. One of the methods that simulate this situation is the *in situ* study, which assesses the capability of dental materials to enhance remineralization and/or inhibit demineralization of tooth enamel in a controlled cariogenic environment.[15] Also, considering the structure of the different enamel conditions, such as caries-like lesions or remineralized caries-like lesions, no study has hitherto focused on sealant application on different occlusal enamel substrates in an attempt to prevent the progression of the initial lesion, particularly in high-caries-risk children.

Therefore, the aim of this *in situ* study was to evaluate the effect of fluoride and non-fluoride containing sealants on enamel hardness decrease under different enamel conditions (sound, caries-like lesions and caries-like lesions + topical fluoride application) all under different distances from the sealant margin. The first null hypothesis was that there are no statistically significant differences in the enamel hardness decrease with fluoride and non-fluoride containing sealants under those enamel conditions. The second null hypothesis was that there are no significant differences in marginal adaptation using different sealants on enamel substrates.

2. Materials and methods

This study was conducted after approval from the Ethics Committee of Piracicaba Dental School, State University of Campinas (protocol #046/2006).

2.1. *Experimental design*

Twelve healthy volunteers (22–31 years old) took part in the study after signing their informed consent form. The study involved a factorial 2 × 2 design of caries induction by biofilm accumulation and sucrose use. The three factors under evaluation were: (1) enamel substrates (S = sound; C = caries-like lesion; CF = caries-like lesion + topical fluoride application), (2) sealant materials' performance (F = FluroShield or H = Helioseal Clear Chroma) and (3) the distances from the sealant margin. During two phases of 14 days each, the volunteers wore acrylic palatal devices containing six dental occlusal enamel blocks each, to which 20% sucrose solution was applied extra-orally 8×/day (Fig. 1D and E). New enamel blocks were placed for the second 14 days phase. All volunteers and blocks sites were held constant for the two phases. The blocks were placed as close as possible to the

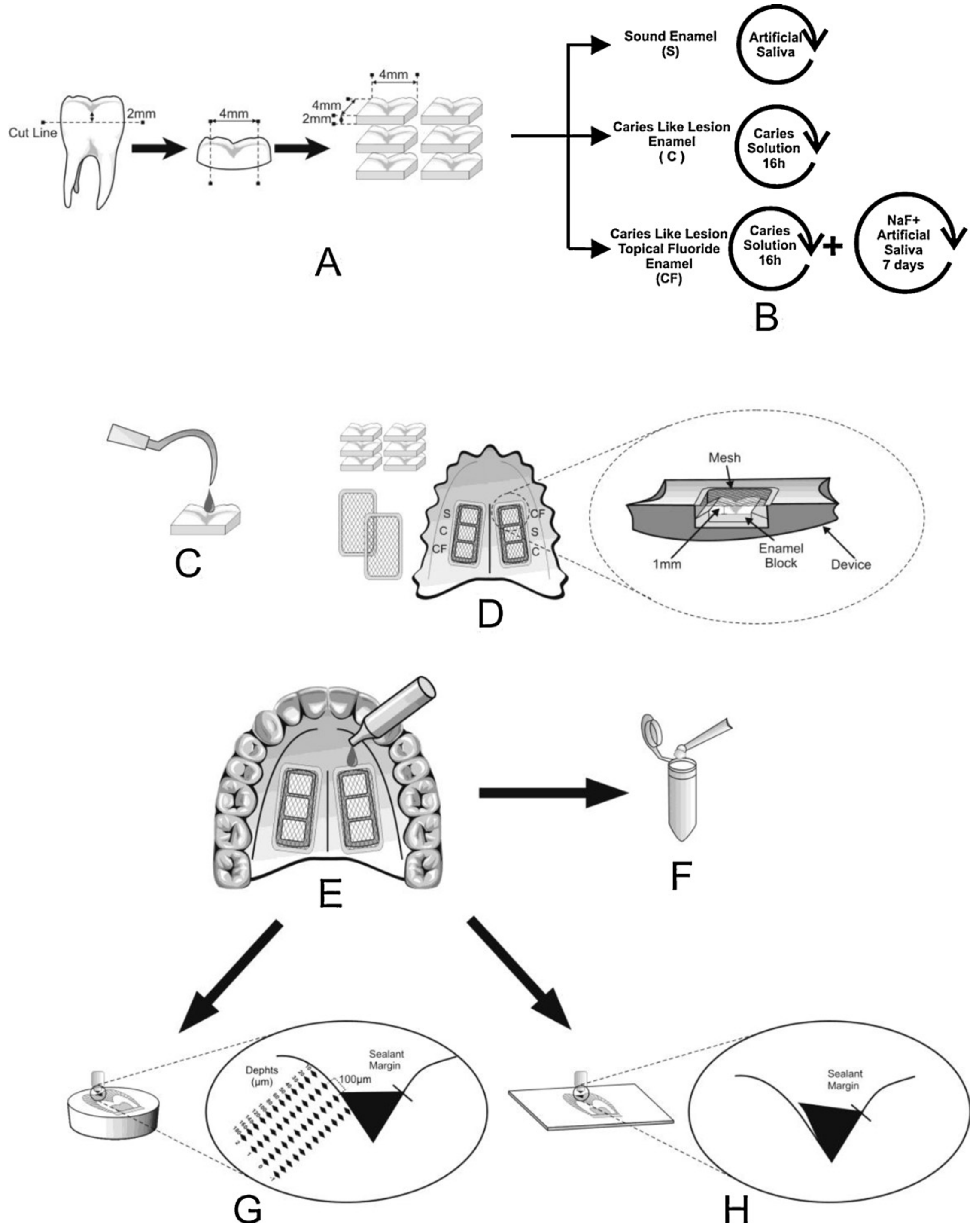

Fig. 1 – Representative scheme of methodology and experimental design: (A) root section 2 mm below the teeth central fissure; enamel block preparation (4 mm × 4 mm × 2 mm); (B) preparation of enamel conditions; (C) sealer material application on the total pit and fissure extension (FluroShield or Helioseal Clear Chroma); (D) laboratory *in situ* preparation; (E) clinical phase of *in situ* model; (F) analysis of dental biofilm; (G) enamel mineral loss analysis – cross-sectional microhardness test; (H) marginal adaptation analysis – polarized light microscope.

posterior teeth on each side of the device (anterior, central and posterior position on the left and right sides). Each of three blocks had different enamel substrates (Fig. 1D). For some volunteers, S blocks were placed in the anterior left and central right sides, while C blocks were placed in the central left and posterior right sides and CF blocks were placed in the posterior left and anterior right sides. These positions were randomly changed for each volunteer in accordance with the experimental phase (Fig. 1D). A 3-mm-deep space was created in the device for placing the enamel blocks, leaving a 1 mm

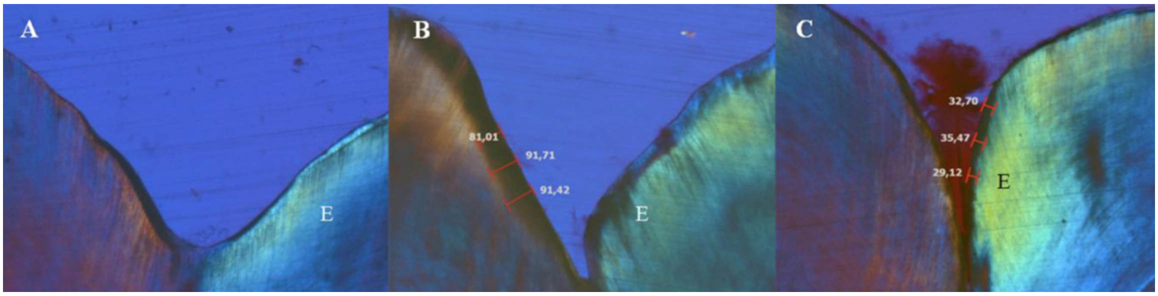

Fig. 2 – Polarized light microscopy baseline images of transverse section of sound enamel (A), artificial caries-like lesion (B) artificial caries-like lesion + fluoride application (C). (5×; 1280 mm × 1024 mm).

space for biofilm accumulation.[16] Dental biofilm was formed on the enamel blocks, which were protected from mechanical disturbance by a plastic mesh fixed in the acrylic surface (Fig. 1D). Before and after the initial 14 days of the experimental phase, a washout was applied over a 7-day period. Throughout the whole experiment, the volunteers brushed their natural teeth with fluoridated toothpaste (Colgate máxima proteção anticárie, with 1450 ppm F as monofluorophosphate; Colgate-Palmolive; São Bernardo dos Campos, SP, Brazil) and lived in a community with optimally fluoridated water supply (0.70 mg F/l). The 12 volunteers were required to wear the devices at all times, including during sleep, except during meals and oral hygiene. Additionally, the volunteers received oral and written instructions to refrain from using any antibacterial or fluoridated product, but no specific instructions regarding their daily diet. At the end of the entire experiment, all volunteers were submitted to the two different sealants under each of three different enamel conditions. After each 14-day phase, the biofilm formed on enamel slabs was evaluated for concentrations of calcium (Ca), phosphorus (P), fluoride (F), and insoluble extracellular polysaccharide (IEPS) (Fig. 1F). All blocks were assessed by cross-sectional microhardness analysis to evaluate the hardness loss as a function of lesion depth (ΔS) (Fig. 1G) as well as for marginal adaptation evaluation (Fig. 1H). For analytical purpose, each volunteer was considered as a statistical subject ($n = 12$). Sample size was determined by software (BioEstat program, version 5.0; Ayres; PA, Brazil), at a power value of 80% for each statistical test performed. The study was blind relative to the sealant. All reagents used in the present study were purchase from Sigma (Sigma–Aldrich; Saint Louis, MO, USA), otherwise stated.

2.2. *Preparation of enamel blocks*

One hundred and forty-four caries-free non-erupted human third molars, extracted for clinical and orthodontic reasons without any relation to this research project and free from caries, were selected. The teeth were cleaned and stored in 0.5% chloramine T solution for up to 2 months after extraction. The occlusal surfaces were cleaned with pumice/water slurry, and polished with a 5.0 μm alumina paste (Alpha Micropolish; Buehler; Lake Bluff, IL, USA). Their roots and part of the their crowns were sectioned off at 2 mm below the teeth central fissures using a double-faced diamond saw and discarded (KG Sorensen; Barueri, SP, Brazil). Each tooth was longitudinally sectioned perpendicular to the fissure orientation (Isomet; Buehler) in order to obtain occlusal sound enamel blocks, measuring 4 mm × 4 mm × 2 mm (Fig. 1A).

2.3. *Preparation of enamel conditions*

2.3.1. *Artificial caries-like lesion formation*

The surfaces of 96 occlusal sound enamel blocks were isolated with double coats of acid-resistant nail varnish (40 Graus; Colorama; São Paulo, SP, Brazil), except for the 4 mm × 4 mm top surface of the occlusal area. Artificial caries-like lesions were produced by immersing each enamel block in a solution containing 0.05 M sodium acetate (NaAc) buffer that was 50% saturated with enamel powder, pH 5.0, for 16 h at 37 °C, in a proportion of 2 ml/mm^2. To prepare this solution, enamel powder (particles of 74–105 μm) was agitated in 0.05 sodium acetate buffer, pH 5.0, for 96 h at 37 °C (0.50 g/l).[17] Lesion depths were 79.3 ± 33.9 μm (Fig. 2B).

2.3.2. *Artificial caries-like lesion remineralization*

Forty-eight blocks with artificial caries-like lesion were submitted to topical fluoride application. Fig. 2 illustrates baseline characteristics of the artificial caries-like lesion with and without remineralization. The enamel surfaces of these blocks were coated with 5% sodium fluoride (NaF) varnish (Duraphat; Colgate-Palmolive), using a microbrush. The varnished blocks were individually immersed in 20 ml of artificial saliva (1.5 mM Ca, 0.9 mM P, 150 mM potassium chloride [KCl] in 0.1 M tris(hydroxymethyl)aminomethane [Tris buffer], 0.05 μg F/ml, pH 7.0)[18] at 37 °C for 1 week.[19] The solution proportion was 1.25 ml/mm^2 of exposed enamel area to prevent solution saturation.[10] The varnished blocks were then removed from the artificial saliva solution and rinsed with distilled deionized water. Lesions depths were 61.3 ± 23.9 μm (Fig. 2C). All enamel blocks were sterilized in a gamma irradiation chamber (Gammacell 220 Excel; GC-220E, MDS; Nordion, Ottawa, Canada) for 24 h at 27 °C with a 14.5 kGy dose before the sealant application. The blocks were kept in a humid environment at 37 °C until the start of the experiment.

2.4. *Experimental groups*

All enamel blocks were then randomly divided into six groups in an interlocking arrangement with the three enamel

Table 1 – Brand, composition, manufacturers, and batch number of the sealer materials.

Materials	Composition	Manufacturers and batch #
FluroShield	Urethane modified Bis-GMA dimetacrylate; Barium aluminoborosilicate glass (30%), Polymerizabledimetacrylate resin, Bis-GMA, Sodium fluoride, Dipentaerythritolpentaacrylate phosphate, Titanium dioxide, Silica amorphous	DentsplyDeTrey Konstanz Germany # 317131
Helioseal Clear Chroma	Bis-GMA, Triethylene glycol dimethacrylate (>99 wt.%). Additional contents are stabilizers, catalyst and pigments (<1 wt.%)	Ivoclar/Vivadent Schaan Liechtenstein # F54463

substrates and the two sealer materials: SF = sound enamel + -FluroShield; CF = caries-like lesion + FluroShield; CFF = caries-like lesion + topical fluoride application + FluroShield; SH = sound enamel + Helioseal Clear Chroma; CH = caries-like lesion + Helioseal Clear Chroma; CFH = caries-like lesion + topical fluoride application + Helioseal Clear Chroma (Fig. 1B).

2.5. Sealant application

Sealants were applied on the total pit and fissure site following the manufacturer's instructions (Table 1) using a sharp explorer in order to avoid excessive spreading of materials (Fig. 1C). The enamel surface of each block was etched using a kit specific 37% phosphoric acid (H_3PO_4) gel for 30 s, rinsed for 10 s with water, and dried. FluroShield was applied and light cured for 40 s and the Helioseal Clear Chroma Group was applied and light cured for 20 s. Light curing was carried out using the Elipar Trilight unit (ESPE; America; Seefeld, Bavaria, Germany) with an 800 mW/cm^2 light intensity. The sealed samples were stored for 24 h at 37 °C at 100% humidity. The sealants' brand names, composition, manufacturers, and batch numbers are listed in Table 1.

2.6. Evaluation techniques

2.6.1. Dental biofilm analysis

At the end of each phase, the plastic meshes were removed and the dental biofilm formed on the two opposite enamel blocks was collected with plastic curettes 12 h after the last exposure to the sucrose solution (Fig. 1F). The biofilm sample was placed in a pre-weighted microcentrifuge tube and the wet weight of each sample was determined to ± 10 μg. To each tube, 0.5 M hydrochloric acid (HCl) was added in the proportion of 1.0 ml/10.0 mg biofilm wet weight. Once extracted after 3 h at room temperature under constant agitation, the same volume of total ionic strength adjuster (TISAB II, containing 20.0 g of sodium hydroxide [NaOH]/l – Fluka Chemie; Buchs, Switzerland) was added to the buffer.[20] The samples were centrifuged for 3 min at 14,000 rpm and the acid-soluble concentrations of F, P, and Ca were determined in the supernatant. For precipitation of IEPS, 1.0 N NaOH (0.1 ml/mg) was added. The samples were homogenized for 1 min and maintained under agitation for 3 h at room temperature. After centrifugation, the concentration of IEPS was determined in the supernatant. Fluoride was analysed using an ion-specific electrode (Orion 96-09; Orion Research; Boston, MA, USA) and an ion-analyser (Orion EA-940; Orion Research), which had been previously calibrated in triplicate with F standards (0.1–16.0 μg F/ml), in TISAB II. Inorganic phosphorus was determined using a colorimetric and a Beckman DU-65 spectrophotometer (Beckman Instruments; Fullerton, CA, USA).[21] Calcium was measured by atomic absorption using Varian AA140/240 (Varian Medical Systems; Palo Alto, CA, USA). Total carbohydrate was determined using the phenol-sulphuric method.[22]

2.6.2. Enamel hardness decrease analysis

Each enamel block was removed from the device and longitudinally sectioned through the center (Isomet; Buehler) in order to obtain a slab that included the occlusal-delimited area perpendicular to the fissure orientation. One side of the slab was randomly selected and embedded in polystyrene resin (Piraglass; Piracicaba, SP, Brazil). The specimens, cut from the slab, were polished with 400-, 600- and 1200-grit Al_2O_3 paper (Arotec; São Paulo, SP, Brazil), and cloth-polished with 1.0-μm diamond paste (Buehler Metadi II; Buehler). Cross-sectional microhardness tests were performed using a Knoop diamond tip under a 25-g load for 5 s (HMV 2000; Shimadzu, Tokyo, Japan). Four rows (-1, 0, 1 and 2) of 12 indentations each were made at the following depths: 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 180 μm from the enamel surface. These four rows were made at the sealant margin (row 0), 100 μm below the margin (row -1), 100 μm and 200 μm above the margin (rows 1 and 2, respectively) (Fig. 1G). The data at rows -1, 0, 1, and 2 were obtained and expressed as Knoop hardness number units (KHN – kg/mm^2). In order to determine enamel hardness decrease, indentations were independently assessed three times and the values averaged to represent KHN for each indentation.

Since there is a discrepancy in previous studies regarding the conversion of hardness to mineral concentration,[23,24] mineral loss was not calculated in the present study. Instead, it was obtained the variation of KHN (ΔS – integrated demineralization) from the difference between the areas of the KHN profile of the lesion and the KHN profile of the sound tooth area, in the same tooth. Hardness number was plotted against depth for each specimen and the integrated hardness number of the lesion was calculated. A mean average number of the hardness for depths greater at least than 100 μm was used as a measure of the integrated hardness of inner sound enamel. To compute ΔS parameters, the KHN content of the lesion was subtracted from the value obtained for the sound enamel, as previously described.[25] The hardness variation values (ΔS) were calculated for all groups (Table 2).

2.6.3. Marginal adaptation analysis

For evaluation of the sealant marginal adaptation on different enamel substrates, the other side of the enamel block slice was used (Fig. 1H). Non-decalcified sections were prepared as previously described.[26] Briefly, specimens were embedded in glycol-methacrylate resin (Technovit 7200; Heraeus Kulzer; Wehrheim, Germany) and 100 μm thick sections were

Table 2 – Mean average number of enamel hardness decrease (ΔS) and standard deviation (SD) of the experimental groups at distances from sealant margin.

Experimental groups	ΔS(mean average ± SD)			
	−1 (under sealant)	0 (sealant margin)	1 (100 μm outer sealant)	2 (200 μm outer sealant)
SF	6364 ± 3967	6682 ± 4127	7084 ± 5412	4901 ± 3822
SH	5584 ± 3788	8579 ± 5181	7239 ± 5495	7841 ± 5197
CF	3763 ± 2549	6022 ± 3669	6421 ± 3859	5443 ± 3813
CH	5408 ± 2657	10,856 ± 10,825	9662 ± 4331	8322 ± 3831
CFF	5033 ± 3448	6385 ± 4286	6533 ± 4246	6782 ± 4655
CFH	7474 ± 3455	8556 ± 3463	8631 ± 3404	8467 ± 2511

Enamel hardness number variation (ΔS) data are reported as mean average ± standard deviation (SD). There were no interactions among the three factors: enamel substrates and sealant materials ($p = 0.3857$); enamel substrates and distance from sealant margin ($p = 0.4840$); sealant materials and distance from sealant margin ($p = 0.4083$); and enamel substrates, sealant materials and distance from sealant margin ($p = 0.8091$).

obtained in the buccal-lingual direction as close to the mid portion of the crown as possible using a diamond saw (Exakt System; Exakt Apparatebau; Norderstedt, Germany). These sections were ground/polished to a thinner thickness ($\cong$50 μm), using a microgrinder system (Exakt System; Exakt Apparatebau). The tooth sections were examined under a polarized light microscope using lambda filter interface (5× magnification) to a high-resolution digital camera (DFC 280; Leica microsystems; Wetzlar, Germany) with LAS image analysis software (Leica microsystems), to verify sealant marginal adaptation. The images were acquired in real time. Image size was 1280 mm × 1024 mm with 24 bits per pixel colour resolution. A qualitative analysis was performed.

A blind calibrated examiner (KRK) evaluated the marginal adaptation in the fissure two times, with a 1-week interval between evaluations. Data were submitted to Spearman's correlation test and the intra-examiner coincidence level was 91%. The experimental groups were scored according to the ordinal scale, with marginal quality 0 = no sealant marginal adaptation or sealant marginal adaptation present on only one of the sides or total sealant loss (failure); 1 = sealant marginal adaptation present on both sides (success).

2.7. *Statistical analysis*

The mixed model analysis of data from a repeated measures design was applied to the enamel hardness decrease values and the concentration of Ca, P, F, and IEPS data was used to analyse the interactions among the three factors (enamel substrates, sealer materials and distance from the sealant margin). In order to assess significant differences within these factors, the Tukey test was applied. Volunteers were the statistical subjects. The assumption of equality of variances and the normal distribution of errors were checked with the selection of covariance structures by SAS software (SAS Institute Corporation, version 9.1.3; Cary, NC, USA). For sealant marginal adaptation, ANOVA-R analysis and Tukey test were used, with the significance level fixed at 5% ($p < 0.05$).

3. Results

For enamel hardness decrease (ΔS), there were no interactions among the three factors investigated: (1) enamel substrates and sealant materials ($p = 0.3857$); (2) enamel substrates and distance from sealant margin ($p = 0.4840$); (3) sealant materials and distance from the sealant margin ($p = 0.4083$); and (4) enamel substrates, sealant materials and distance from the sealant margin ($p = 0.8091$). However, significant differences were found for two factors when examined separately: sealant materials ($p = 0.0284$) and distance from the sealant margin ($p = 0.0160$). Table 2 shows the mean average of enamel hardness decrease and standard deviation in the experimental groups at the distance from the sealant margin. The mean values of ΔS for the materials and their 95% confidence intervals are shown in Fig. 3. Since there was no effect of enamel substrate and the distance from the sealant margin on enamel hardness decrease, their data were combined. The ΔS values of FluroShield were significantly lower than that of Helioseal Clear Chroma. Also, significant differences in enamel hardness decrease were observed according to the distances from the sealant margin. Fig. 4 shows the mean averages and confidence intervals of ΔS by the sealant materials at different distances from the sealant margin: −1 (under sealant) < 0 (sealant margin) = 1 = 2 (both outer margin).

With regard to the composition of dental biofilm, no interaction between enamel substrates and sealant materials was observed ($p > 0.05$). Table 3 shows the mean average and standard deviation of inorganic composition (Ca, F, and P) and IEPS concentration in the dental biofilm on different sealants formed in the presence of sucrose. The enamel substrates data were combined. With regard to Ca and F concentration,

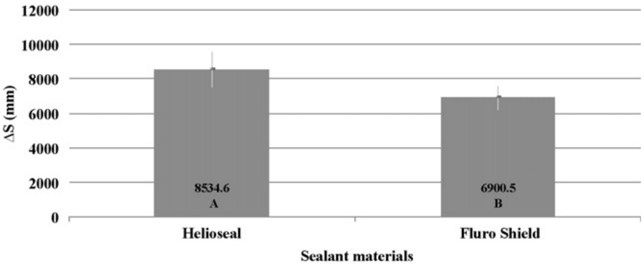

Fig. 3 – Microhardness variation values (ΔS) and 95% confidence intervals in enamel sealed with different materials. Different capital letters mean statistically significant difference for Tukey test ($p < 0.05$) with 95% of confidence interval ($n = 12$). The enamel substrate and the distance from the sealant margin data were combined.

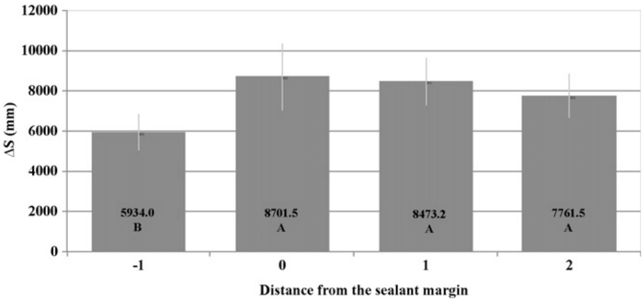

Fig. 4 – Microhardness variation values (ΔS) and 95% confidence intervals at different distances from the sealant margin. Different capital letters mean statistically significant difference for Tukey test ($p < 0.05$) with 95% of confidence interval ($n = 12$). The enamel substrate and sealant materials data were combined.

statistically significant differences were found between FluroShield and Helioseal materials ($p = 0.034$; $p = 0.062$, respectively), whereas the concentrations of P and IEPS showed no significant differences between sealant materials ($p > 0.05$). For marginal adaptation, ANOVA-R showed no statistically significant differences among the six experimental groups ($p > 0.05$) and no interaction between sealant materials and three enamel substrates ($p = 0.0948$).

4. Discussion

While fissure sealing acts as a diffusion barrier on the top of the lesion surface, the infiltration technique creates this barrier inside the lesion body, filling up the mineral loss with low-viscosity, light-curing resin.[27] The latter technique could be a promising alternative therapy in non-compliance individuals with approximal non-cavitated enamel lesions.[9,27] Despite such potential, the literature shows no studies using infiltrants for pit and fissures on occlusal surfaces of permanent molars. In such cases, it would be an ideal material, one with both characteristics of low-viscosity to fill the pores inside the lesion body, and of sufficient strength to obliterate the occlusal pit and fissure while supporting the mastigatory force.[9] Because there is no such ideal material, the fluoride containing sealants have been used effectively to lessen the enamel mineral loss.[11,28–30]

The first null hypothesis – fluoride and non-fluoride containing sealants would have no effect on enamel hardness decrease under different enamel conditions – was rejected. In this study, FluroShield had a significant effect on enamel hardness decrease in contrast to Helioseal Clear Chroma, regardless of enamel conditions. This difference most likely is due to the presence of fluoride in the material's composition, which is consistent with the dental literature, in which it has been established that the fluoride released from dental materials prevent caries initiation and progression.[28–30]

With respect to sealant efficacy on enamel hardness loss, ΔS values at different distances from the sealant margin, the under sealed hardness decrease value was significantly lower than those at other distances, regardless of sealant material used and enamel substrates (Fig. 4). This result is consistent with earlier reports showing that the cariostatic benefit of sound fissure sealing is due to the formation of an effective physical barrier that protects the underlying fissure enamel from biofilm formation and carious attack.[6,11]

A caries-risk situation was simulated by an *in situ* model that employed human subjects without actually causing caries in the natural dentition. The formation of biofilm and its metabolic activity were examined by exposing the biofilm regularly to a sucrose solution.[31] Literature reports indicate that a high exposure frequency to sucrose (the most cariogenic carbohydrates) can modify the biochemical characteristics of biofilm, such as more porosity,[32] high concentrations of insoluble polysaccharides and low concentrations of calcium, inorganic phosphorus and fluoride.[16] In addition, the presence of sucrose maintains a low pH value that may also diminish the time for saliva to replenish any lost tooth mineral.[16,33] In this environment, our study indicated that FluroShield had the potential to influence the biofilm structure, to reduce demineralization while encouraging remineralization. The fluoride released by the sealant may have acted as a catalyst accelerating remineralization with resulting formation of fluoride-rich apatite crystals and calcium fluoride, which are more mineralized and harder.[34,35] The present study is in agreement with an *in situ* study that evaluated the remineralizing potential of pit and fissure sealants in artificial carious lesions on smooth enamel surfaces.[36]

Our *in situ* results cannot be extrapolated directly to clinical practice since the chemical, thermal and mastication stresses that occur simultaneously in the human oral cavity are absent in *in situ* models. Future studies should be performed to establish whether the *in situ* results could be reproduced more accurately in the clinical arena. Additionally, future studies should also investigate whether FluroShield and Helioseal Clear Chroma could be used as infiltration materials to create a barrier inside the non-cavitated enamel lesion. A combination of sealing and infiltration may provide a better strategy to arrest initial enamel lesion on occlusal surfaces even in non-compliant patients.

The second null hypothesis – there would be no difference between the sealants with respect to marginal adaptation – was

Table 3 – Composition of dental biofilm formed in the presence of sucrose.

Sealant materials	F (μg/g)	Ca (mg/g)	P (mg/g)	IEPS (mg/g)
FluroShield	23.8 ± 28.1^a	5.0 ± 6.4^a	2.5 ± 1.4^a	518.5 ± 232.6^a
Helioseal	14.7 ± 9.8^b	3.1 ± 1.7^b	2.2 ± 0.8^a	510.5 ± 362.4^a

F, fluoride; Ca, calcium; P, phosphorus; IEPS, insoluble extracellular polysaccharide Data are reported as mean average ± standard deviation. Different small letters indicate statistically significant differences for Tukey test ($p < 0.05$) ($n = 12$) in column.
The enamel substrate data were combined.

not rejected. It should be noted that the sealing procedure was quite similar for both materials and that the enamel was etched with phosphoric acid, allowing the same pattern of substrate bonding. The integrity of the tooth-sealer interface plays an important role on sealing success. It depends on a number of factors, such as the mechanical and chemical properties of the sealant materials, the anatomy of the pits and fissures,[37,38] the physical-chemical conditions of the oral cavity, and the level of the clinician's skill.[39] However, in the present study, both FluroShield and Helioseal Clear Chroma resin sealants demonstrated a similar low percentage of success regarding gap formation (30 and 20%, respectively).

Artificially created enamel lesions are commonly used in *in vitro* studies to simulate *in vivo* caries behaviour in several tests.[6,17,40] The formation of artificial and natural caries is, however, different from each other. Natural caries, contrary to most artificial lesions, are produced by intermittent and prolonged demineralization periods. Furthermore, the presence of fluoride and other substances of the oral environment during natural caries formation, as well as the deposition of organic material in the porous microspaces may contribute to a higher acid resistance compared to sound enamel, which also can compromise resin material adhesion.[41] Although our study made an effort to mimic an *in vivo* caries process, artificial caries on the occlusal fissure were created in a caries solution where a long period of demineralization with no access to fluoride or other substances of the oral environment occurred during lesion formation. However, the enamel blocks did have access to a solution containing 0.05 M acetate buffer, 50% saturated with enamel powder (hydroxyapatite), which produces subsurface enamel lesions.[17]

Considering the limitations of this study, it was concluded that marginal adaptation was not affected by sealant type or substrate condition, whereas enamel hardness decrease was favourably impacted by the presence of fluoride in the sealant's composition. In addition, sealants were more effective as a physical barrier in reducing enamel hardness loss rather than their chemical potency. Finally, the results of this study again highlight the need for good dental hygiene practices and education, since sealants, by themselves, could not prevent enamel hardness decrease.

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