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ORIGINAL ARTICLE

Inhibition of mineral loss at the enamel/sealant interface of fissures sealed with fluoride- and non-fluoride containing dental materials *in vitro*

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Abstract

Objective. In this *in vitro* study we evaluated the enamel mineral loss effect of fluoride-containing and non-fluoride-containing materials at different distances from the sealant margin, and verified the fluoride-releasing capability of these materials. ***Material and methods.*** Extracted molars were randomly assigned into nine groups ($n = 12$): Concise (C), FluroShield (F), Helioseal Clear Chroma (H), Vitremer (V), Fuji II-LC (FII), Ketac Molar (KM), Fuji IX (FIX), Single Bond (SB), and Clearfil Protect Bond (CF). All groups were subjected to thermo and pH cycling. Enamel mineral loss was evaluated by cross-section micro-hardness analysis at distances: −100 μm, 0 μm, 100 μm, 200 μm. The mineral loss data were analyzed using a multi-factor ANOVA with split-plot design, and fluoride-released data were submitted to ANOVA and Tukey tests. ***Results.*** FIX demonstrated a lower mineral loss than C, F, and H, but did not differ from the SB, CF, V, FII, and KM groups, which also demonstrated no difference among them. C, F, H, and V presented the highest mineral loss, with no difference among them. V did not differ from the other groups ($p > 0.05$). Regarding the different distances from the sealant margin, −100 μm presented the lowest mineral loss. FIX showed the highest fluoride release on the 7th and 14th days of evaluation, while CF showed high fluoride release only on the 7th day. ***Conclusion.*** Resin sealant did not prevent enamel mineral loss, contrary to glass-ionomer cement, which showed the highest capacity for fluoride release. It is not exclusively the presence of fluoride in a material's composition that indicates its capability to interfere with the development of enamel caries-like lesions.

Key Words: *Demineralization, fissure sealing, fluoride, micro-hardness, prevention*

Introduction

Although there has been a considerable reduction in caries in children and adolescent populations in developed nations [1,2] over the past several decades, subgroups of overall populations continue to experience a high incidence of dental caries [3]. Studies have demonstrated that the occlusal surfaces of 1st and 2nd permanent molars are the sites most frequently struck by dental caries in 6- to 17-year-old patients [3,4]. More importantly, it has been shown that, in fluoridated communities, over 90% of dental caries comprise pit and fissure caries exclusively [1,5]. This high prevalence of occlusal caries is due to the ready accumulation of bacteria and nutrients in pits and fissures, mainly in the central fossa of mandibular and mesial fossa of maxillary molars, and to the difficulty or inability of mechanical cleaning of these areas [6,7]. Thus, the development of effective preventive measures against pit and fissure caries is necessary.

Pit and fissure dental sealants are recognized as an important adjunct approach to the prevention of caries in the occlusal surface. Their safety and effectiveness have been demonstrated in more than 30 years of research as a physical barrier formation that prevents the metabolic exchange between the fissure micro-organisms and the oral environment

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[8–11]. In order to obtain long-term success with sealants, the first and perhaps the most important condition is maintenance of a satisfactory retention of the material to enamel [12].

In addition to the fact that sealants act by physically protecting vulnerable areas, the introduction of fluoride-releasing sealants has added another dimension to the prevention of pit and fissure caries [13]. While resin-based sealants (Bis-GMA) act only by isolating enamel from a cariogenic challenge, depending on sealant retention on the occlusal surface, the fluoride-releasing sealants seem to provide another caries inhibition effect, since the fluoride inhibits demineralization and favors the remineralization processes [3,14,15].

Among different fluoride-releasing materials that have been used as fissure sealants are glass ionomer cements (GIC) [16], resin-modified GICs (RMGIC) [17], fluoride-releasing composite sealants (FRCS) [18], and adhesive systems [10]. GICs provide some protection against a carious attack even after visible loss of material [19,20]. The introduction of RMGICs improved retention, adherence, esthetics, and physical properties [16,21], but some concerns have been raised regarding the fluoride release from the resin matrix of RMGICs [22].

In vitro studies have investigated the effect of fluoride release by dental materials such as GICs, RMGICs, FRCS and adhesive systems in demineralization enamel inhibition [4,20,23]. However, these studies employed qualitative methods in evaluating demineralization on the enamel, since they considered the depth of artificial caries lesions and demineralization zone extension. Moreover, with the exception of the study performed by Seppa & Forss [20], these studies evaluated mineral loss on flat buccal or lingual enamel surfaces, which are less susceptible to enamel demineralization than pit and fissure surfaces. Based on this information, an evaluation focused on the quantitative measurement of the mineral loss of the enamel adjacent to the sealant should be performed on pits and fissures.

The aims of this *in vitro* study were therefore to quantitatively evaluate the effect of fluoride-containing and non-fluoride-containing occlusal sealants on the enamel mineral loss of permanent teeth at different distances from the sealant margin and to verify the fluoride-releasing capability of these materials. It was first hypothesized that the fluoride-containing materials would allow lower mineral loss than the non-fluoride-containing materials. Additionally, the hypothesis that fluoride-containing materials would release higher fluoride levels and, consequently, would promote a greater demineralization inhibition effect was tested.

Material and methods

The study was conducted after the approval of the Ethics Committee of the Piracicaba Dental School, University of Campinas (protocol no. 089/2004) had been obtained. One-hundred-and-eight impacted human 3rd molars were selected. These were extracted for clinical and orthodontic reasons and were free of apparent caries, macroscopic cracks, abrasions, and staining on the occlusal surface, and were assessed by visual examination. The teeth were cleaned and stored in 0.5% Chloramine T solution for up to 2 months after extraction. Their roots were sectioned off 1 mm below the enamel cement junction using a double-face diamond saw (K. G. Sorensen, São Paulo, SP, Brazil) and the pulpal chambers of all teeth were filled with resin composite. The occlusal surface was cleaned with pumice/water slurry, and polished with a 5.0 μm alumina paste (Alpha Micropolish, Buehler, Lake Bluff, Ill., USA).

Nine materials indicated for sealing procedures were tested. Their brand names, type, composition, manufacturers, and batch numbers are listed in Table I.

Teeth were randomly distributed into nine groups ($n = 12$) according to the sealant materials used as follows: Concise Group, FluroShield Group, Helioseal Clear Chroma Group, Vitremer Group, Fuji II-LC Group, Ketac Molar Group, Fuji IX Group, Clearfil Protect Bond Group, and Single Bond Group.

The materials were applied on the total pit and fissure extension in accordance with the manufacturer's instructions and are described as follows. *Concise Group – C*: The enamel surface was etched using a 35% phosphoric acid (H_3PO_4) gel for 15 s, rinsed for 10 s, and dried; the material was applied to the pit and fissure using a probe. *FluroShield Group – F*: The enamel surface was etched using 37% phosphoric acid (H_3PO_4) gel for 30 s, rinsed for 10 s, and dried. The material was applied and light-cured for 40 s. *Helioseal Clear Chroma Group – H*: The enamel surface was etched using 37% phosphoric acid (H_3PO_4) gel for 30 s, rinsed for 10 s, and dried. The material was applied and light-cured for 20 s. *Vitremer Group – V*: The enamel surface was treated with Vitremer Primer applied using a brush for 30 s, air-dried, and light-cured for 20 s. The material was applied, light-cured for 40 s, and the surface was protected with Vitremer Finish Gloss and light-cured for 20 s. *Fuji II-LC Group – FII*: The enamel surface was treated with GC cavity conditioner for 10 s, rinsed thoroughly in water, and dried. The material was applied and light-cured for 20 s. *Ketac Molar Group – KM*: The enamel surface was treated with polyacrylic acid conditioner for 30 s, rinsed for 10 s, dried, and the material was applied. The surface was protected with appropriate

Table I. Brand, composition, and batch number of the materials used in the present study

Materials	Types	Composition	Manufacturer and batch no.
Concise	Resin sealant	Bis-GMA, TEGDMA (78wt.%), benzoyl peroxide, tertiary amine of dimethyl-paratoluidine, titaninum dioxide and stable iron oxides	3M/ESPE St. Paul, Minn., USA no. 18094
FluroShield	Resin sealant	Urethane modified Bis-GMA dimethacrylate; barium aluminoborosilicate glass (30%), polymerizable dimethacrylate resin, Bis-GMA, sodium fluoride, dipentaerythritol pentaacrylate phosphate, titanium dioxide, silica amorphous	Dentsply, Germany no. 317131
Helioseal Clear Chroma	Resin sealant	Bis-GMA, triethylene glycol dimethacrylate (>99wt.%) Additional contents are stabilizers, catalysts and pigments (<1wt.%)	Ivoclar/Vivadent Schaan, Liechtenstein no. F54463
Vitremer	Resin-modified glass ionomer	Powder: fluoraluminosilicate glass, redox catalyst system, pigments Liquid: aqueous solution of a polycarboxylic acid modified with pedant methacrylate groups, vitrebond copolymer, water, HEMA, photoinitiators Primer: Vitrebond copolymer, HEMA, ethanol, photoinitiators	3M/ESPE St. Paul, Minn., USA no. 20020612
Fuji II-LC	Resin-modified glass ionomer	Powder: Alumino-silicate glass Liquid: Polyacrylic acid, 2-Hydroxyethyl methacrylate, proprietary ingredient, and trimethyl hexamethylene dicarbonate	GC Co Tokyo, Japan no. 0405281
Ketac Molar	Glass ionomer	Powder: Aluminium-calcium-lanthanum-fluorisilicate glass, 5% polycarbonate acid Liquid: Polycarbonic acid and tartaric acid	3M/ESPE St. Paul, Minn., USA no. 159323
Fuji IX	Glass ionomer	Powder: polyacrylic acid and aluminosilicate glass Liquid: polyacrylic acid and proprietary ingredient	GC Co Tokyo, Japan no. 209271
Single Bond	Adhesive system	BisGMA, HEMA, dimethacrylates, ethanol, water, photoinitiator system, methacrylate functional copolymer of polyacrilic and polyitaconic acids	3M/ESPE St. Paul, Minn., USA no. 4BM
Clearfil Protect Bond	Adhesive system	Primer: MDP, MDPB, HEMA, Hydrophobic methacrylate, water Bond: MDP, Bis-GMA, HEMA, hydrophobic methacrylate, dI-Camphorquinone, N,N-Diethanol-p-toluidine, silanated colloidal silica, surface-treated sodium fluoride	Kuraray Okayama, Japan no. 61113

varnish. *Fuji IX Group – FIX*: The enamel surface was treated with polyacrylic acid conditioner for 30 s, rinsed for 10 s, dried, and the material was applied. The surface was protected with appropriate varnish. *Single Bond Group – SB*: The enamel surface was etched using 35% phosphoric acid (H_3PO_4) gel for 15 s, rinsed for 10 s, and dried. The material was applied and light-cured for 10s. *Clearfil Protect Bond Group – CF*: The enamel surface was etched using primer for 20 s, dried with mild air flow, bond was applied, and light-cured for 10 s.

Sealants were applied with a sharp explorer, in order to avoid excessive spreading, and light-cured within the recommended time using a Elipar tri-light unit (ESPE – America Co., Seefeld 82229, Germany). Light intensity was periodically checked in the unit and was set at 580 mW/cm^2. The specimens were stored for 24 h at 37°C and 100% humidity.

Using a digital caliper (Mitutoyo, Suzano, SP, Brazil) and a double layer of acid-resistant nail varnish (Colorama, São Paulo, SP, Brazil), an occlusal area of 4×4 mm (16 mm^2), with the main occlusal fissure at the center, was delimited on each tooth.

The teeth were subjected to thermocycling (500 cycles of 5°C and 55°C with a dwell time of 30 s) in distilled water. Afterwards, an acid-resistant varnish coating was re-applied to the previously varnished surfaces of the remaining occlusal delimited area. The samples were then subjected to a 14-day pH-cycling model simulating a high cariogenic challenge according to Featherstone et al. [14]. Each cycle consisted of a 6-h immersion in demineralizing solution followed by an 18-h immersion in remineralizing solution. Teeth were individually immersed in 40 ml of a demineralizing solution (2 mM calcium, 2 mM phosphate in 0.075 M

acetate buffer, 0.03 μg F/ml, pH 4.3, 37°C). The solution was applied in the proportion of 2.5 ml/mm^2 of exposed enamel area. Teeth were then washed in deionized water for 30 s, dried with absorbent paper, and individually immersed in 20 ml of a remineralizing solution (1.5 mM calcium, 0.9 mM phosphate, 150 mM of KCl in 0.1 M Tris buffer, 0.05 μg F/ml, pH 7.0, 37°C) applied in the proportion of 1.25 ml/mm^2. Both solutions contained thymol crystals to avoid microbial growth. The solutions (demineralizing and remineralizing) were changed on the 7th day.

The total amount of fluoride released by the dental materials during the pH-cycling regimens was analyzed on the 7th and 14th days. Fluoride measurements in demineralizing and remineralizing solutions were taken in duplicate using an ion-specific electrode (Orion 96-09) and an ion-analyzer (Orion EA-940, Orion Research, Boston, Mass., USA), which had been previously calibrated in triplicate with fluoride standards (0.015 to 0.5 μg F/ml) in TISAB III.

Each tooth was longitudinally sectioned (Isomet, Buheler, Lake Bluff, Ill., USA) in order to obtain a slice 3 mm wide, to include the occlusal-delimited area, perpendicular to the fissure orientation. One side of the slice was randomly selected and embedded in polystyrene resin (Piraglass, Piracicaba, SP, Brazil). The specimens were polished with 400-, 600-, and 1200-grit Al_2O_3 paper (Arotec S.A. Ind. and Com., São Paulo, Brazil), and cloth-polished with 1.0-μm diamond paste (Buheler Metadi II, Buheler, Lake Buff, Ill., USA). Cross-sectional micro-hardness 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 depths: 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 180 μm. Four rows were made at the sealant margin (row 0), at a distance of 100 μm below the margin (row -1), 100 μm and 200 μm above the margin (rows 1 and 2, respectively) (Figure 1). The Knoop hardness number units data (KHN) at rows -1, 0, 1, and 2 were obtained and converted into volume percentage mineral according to Featherstone et al. [24] using the equation: volume% mineral $= 4.3\ \mathrm{KHN}^{1/2} + 11.3$. After calculating volume percentage mineral profiles, the mineral loss values (ΔZ) were obtained for all groups [25].

Mineral profiles were obtained by linear function passing through the 12 coordinated points of volume percentage mineral (Y axis) and depth under the outer surface (X axis). The area under the curve was calculated by the integral of the third-order equation. Enamel mineral loss values (ΔZ) were obtained from the difference between the areas of the mineral profile of the lesion and the mineral profile of sound teeth. Volume percentage mineral was plotted against depth for each specimen and the integrated mineral content of the lesion was calculated. A mean of volume percentage mineral for depths >100 μm was used as a measure of the integrated mineral

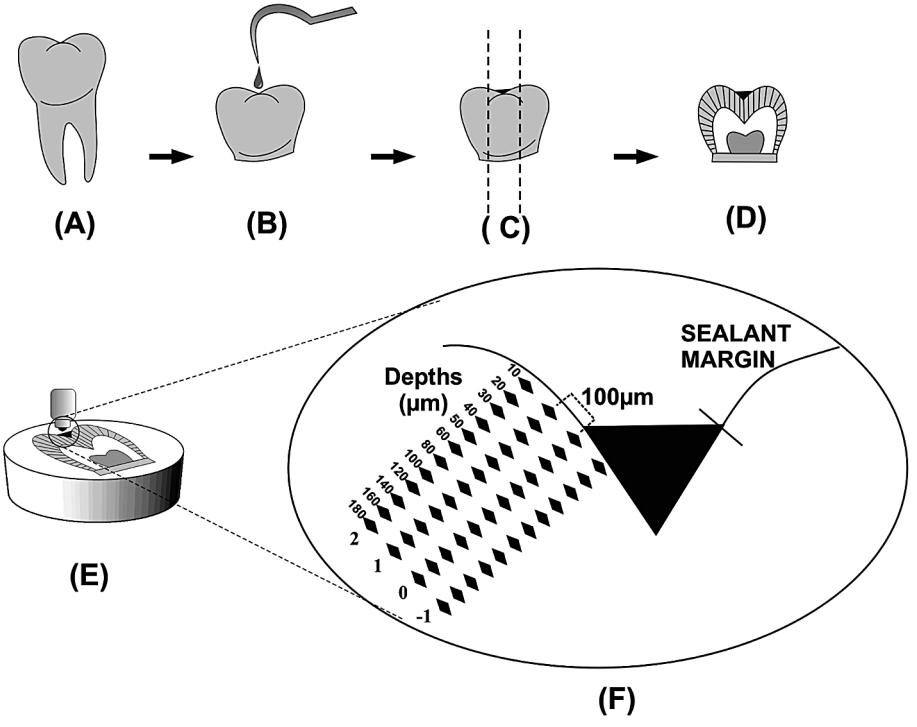


Figure 1. Diagrammatic representation of cross-sectional micro-hardness assay. A. Impacted human 3rd molars. B. Sealant application. C. Tooth longitudinally sectioned to obtain a slice 3 mm wide. D. Obtained slice – mesial view. E. Knoop-hardness indenter on the embedded slice. F. Scheme of cross-sectional micro-hardness measurements. Four rows (-1, 0, 1, and 2) of 12 indentations each at depths 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 180 μm from surface enamel.

Table II. Mean, SD, and confidence interval of ΔZ calculated based on the original data, Tukey's test ($p < 0.05$) for multiple comparisons from material means, and contrast test for treatment blocks

Material	Mean	SD	Confidence mean interval (95%) limits		Tukey test*	Treatment blocks**
			Superior	Inferior		
Helioseal	1873.83	1028.35	2172.43	1575.23	A	Resinous sealant
FluroShield	1790.26	856.07	2038.84	1541.69	A	AB
Concise	1474.24	779.20	1705.63	1242.84	AB	Control A
Vitremer	1460.28	860.56	1710.16	1210.40	ABC	RMGIC AC
Fuji II LC	875.18	514.27	1024.51	725.85	BC	
Clearfill	1002.03	702.21	1205.93	798.13	BC	Adhesive systems
Single Bond	971.50	757.32	1191.41	751.60	BC	AC
Ketac Molar	795.51	548.19	954.69	636.33	BC	CIV A
Fuji IX	702.20	461.18	837.61	566.79	C	

Different capital letters means statistically significant difference for Turkey* and contrast** tests ($p < 0.05$) with 95% confidence interval.

content of inner sound enamel. To compute ΔZ parameters, the integrated mineral content of the lesion was subtracted from the value obtained for sound enamel [26].

Original data from enamel mineral loss means (ΔZ) were transformed (0.6 exponential) before applying ANOVA and Tukey tests, because variance was not homogeneous. A multi-factor ANOVA with split-plot design was applied to the cross-sectional micro-hardness data to analyze the interactions between the factors (materials and distance from the sealant margin). In order to assess significant differences within these factors, the Tukey test was applied. In addition, a contrast test was performed to verify the difference among the types of materials. For fluoride release to de-remineralizing solutions on the 7th and 14th days, ANOVA and Tukey tests were applied to verify the difference among the dental materials in their fluoride-releasing capability. The software SAS system (version 8.02, SAS Institute Inc., Cary, NC, 1999) was used and the significance limit was set at 5%.

Results

The original averages and 95% confidence interval of the enamel mineral loss (ΔZ) for the 9 groups are shown in Table II. According to the statistical analysis, multi-factor ANOVA showed no interaction between materials and distance from sealant margin ($p = 0.0775$). There were significant differences among the materials and distance from the sealant margin when the Tukey test was applied ($p < 0.0001$). The enamel mineral loss of FIX was significantly inferior to that of C, F, and H, but not from groups SB, CF, V, FII, and KM (Table II). Materials such as SB, CF, FII, and KM presented ΔZ values with no difference among them. C, F, H, and V presented the highest enamel mineral losses, with no difference among them ($p > 0.05$). V was not statistically different from any group ($p > 0.05$). When types of materials were compared, statistical analysis showed significant differences among resin sealants versus RMGICs ($p = 0.002$), resin sealants versus GICs ($p < 0.001$), resin sealants versus adhesive systems ($p < 0.001$), and RMGICs versus GICs ($p = 0.01$), as indicated in Table II.

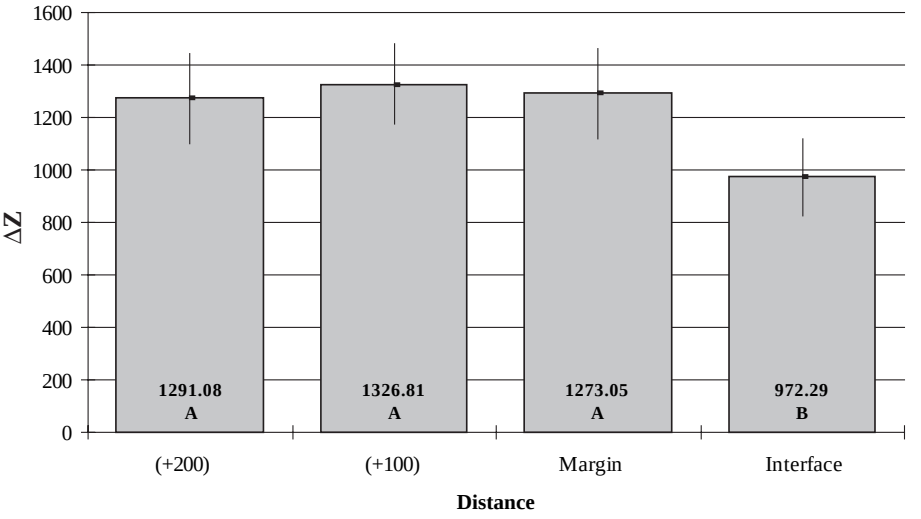
Figure 2 shows the averages and confidence intervals of the enamel mineral loss at different distances from the sealant margin (-1, 0, 1, 2), regardless of material groups, since there was no statistically significant interaction between different distances and materials. Statistical analyses showed significant differences among interfaces (-1) and the other distances from the sealant margin (0, 1, 2).

FIX and CF were significantly superior to the materials with regard to fluoride release during the pH cycling on the 7th day. FII and KM presented intermediate levels of fluoride release and superior to C, F, H, V, and SB ($p < 0.01$). Additionally, these materials presented no difference from each other ($p > 0.05$); nor on the 14th day was there any significant difference between C, F, H, V, and SB. However, only FIX showed the highest fluoride release level. FII, KM, and CF presented intermediate levels of fluoride release (Figure 3).

Discussion

The hypothesis that the enamel of the occlusal surface sealed with any of the fluoride-containing materials would show lower enamel mineral loss than that sealed with any of the non-fluoride-containing materials was not accepted. Fluoride-containing materials, including Fuji IX, Ketac Molar, Fuji II LC, and Clearfil Protect Bond, had a significant effect on enamel mineral loss inhibition, in contrast to fluoride-containing materials such as FluroShield and Vitremer. This finding suggests that the presence of fluoride in the material's composition alone does not indicate the material's behavior regarding its capability to interfere with the development of like-caries lesions in permanent enamel.

With respect to the effect of material at different distances from the sealant margin on mineral loss, it



Similar capital letters mean no statistically significant difference for Tukey test ($p < 0.05$) with 95% confidence interval, regardless of the materials used.

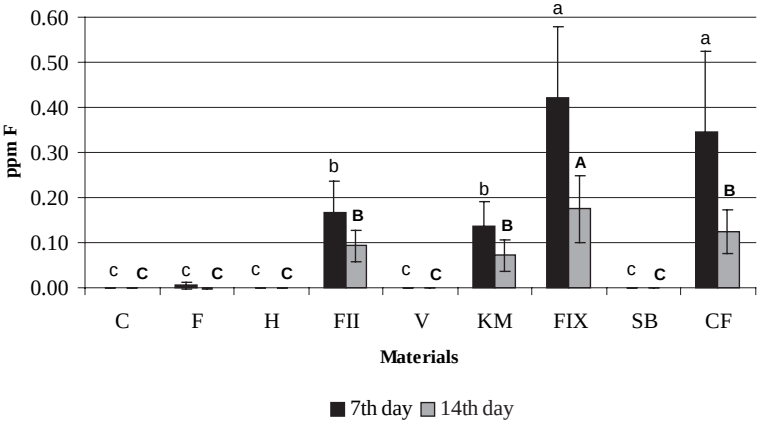
Figure 2. Mean original mineral losses (ΔZ) and 95% confidence intervals at different distances from sealant margin.

was observed that the enamel/sealant interface showed enamel mineral loss of significantly lower values than those at the other distances. This could have been related to the physical barrier properties of the materials used in this study, as shown by the absence of significant interaction between material/distance.

As expected, Concise and Helioseal Clear Chroma, which did not contain fluoride in their composition, did not affect the development of enamel mineral loss on permanent teeth enamel. This finding is in agreement with those of other studies [17,27] evaluating the effect of sealants on enamel demineralization. Surprisingly, FluroShield presented high enamel mineral loss and low levels of fluoride released during the pH-cycling regimen compared to the other fluoride-containing materials (Figure 2,4). Other studies too showed low levels of fluoride release for FluroShield after 2 weeks of analysis [28,29]. These results may be explained by the characteristics of resin sealant matrix, which is much less hydrophilic, making fluoride release more difficult [30] and slower after polymerization [29].

In this study, Single Bond and Clearfil Protect Bond showed similar ability to reduce the enamel mineral loss despite the fact that the Clearfil Protect Bond released significantly more fluoride into the cycling solutions than Single Bond (Figures 2,4). This may be attributed to the fact that adhesive systems are performed as a physical barrier which isolates enamel from a cariogenic challenge. The success of pit and fissure sealant is thus dependent on maintaining an intact seal and suggests possibly that the sealing ability of the materials may be more relevant in inhibiting mineral loss than the fluoride-releasing capacity.

In this study, RMGICs showed intermediate values of enamel mineral loss. Vitremer and Fuji II LC did not differ from each other, but showed a different profile. It was observed that different



Similar small letters at 7th day mean no significant statistical difference ($p > 0.05$); ANOVA and Tukey tests.
Similar capital letters at 14th day mean no significant statistical difference ($p > 0.05$); ANOVA and Tukey tests.

Figure 3. Total fluoride released during pH cycling (ppm F) according to type of materials. Means (SD; $n = 12$).

material brands from the same material classes, such as RMGICs, performed in a different manner. While Vitremer showed similar results for mineral loss inhibition to those of resin sealants, Fuji II-LC performed similar to GICs. It has been suggested that the fluoride release mechanisms of Fuji II-LC are similar to those of the GICs [31]. Similar fluoride-releasing capacities were observed for both Fuji II-LC and Ketac Molar in this study. On the other hand, Vitremer showed no fluoride release at all (Figure 4); this may have occurred due to the type and amount of resin used for the light-curing reaction of Vitremer, and to the finishing gloss used to coat the sealant, as recommended by the manufacturer, that might have had some influence on the fluoride-releasing process [32]. Mathis & Ferracane [33] assumed that in the set resin materials, fluoride ions might be firmly encapsulated by the resin matrix and consequently its fluoride release rate into an aqueous environment could be smaller and lower than that of conventional GICs.

The GICs (Fuji IX and Ketac Molar) produced the lowest enamel mineral loss values (ΔZ). In previous studies [34,35], GICs were able to interfere with the development of artificial caries lesions on the adjacent enamel to sealant because of the action of fluoride release, and the continual presence of low concentration of fluoride, which appears to inhibit demineralization and enhance remineralization [14]. Moreover, the differences in the composition of ionomeric and resinous materials can result in subsequent differences in fluoride-releasing levels [36] and in enamel mineral loss. According to Asmussen & Peutzfeldt [37], diffusion of water into the material is necessary for the formation of hydrogen ions, which attack the fluoride-containing glass particles, releasing fluoride. Ionomeric materials are more permeable to water, and this aspect would be expected to enhance fluoride diffusion and release.

Fluoride released from GICs concentrates on the enamel surface, can reduce enamel solubility [38] and acid production by bacteria that initiate caries lesions [39]. These results are in line with those of Serra & Cury [40], who used micro-hardness in a situation that simulated high caries risk around GIC restorations, and other studies [15,19,23,41] that observed a significant reduction of lesions on enamel adjacent to the GIC. With regard to fluoride release, Fuji IX released 2.7 $\times$ more fluoride than Ketac Molar (Figure 4). In spite of different fluoride-releasing characteristics, both materials could be recommended for children with high caries risk.

These research data suggest that further evidence of the importance of fluoride release by sealant materials should be supplied by *in situ* and *in vivo* studies. These studies might be designed to analyze the effect of fluoride release on biofilm and also determine the minimum level of fluoride release required to obtain an anticariogenic action in the pit and fissure occlusal.

Within the limits of the present study, it can be concluded that resin sealant fluoride- containing or non-fluoride-containing did not prevent enamel mineral loss, suggesting the need to adopt additional preventive measures. On the other hand, glass-ionomer cement sealant demonstrated the lowest values of enamel mineral loss, even in a situation that simulated a high cariogenic challenge. The fluoride release level of the material was able to prevent enamel mineral loss when Fuji IX was used. Moreover, the presence of fluoride in the material's composition should not be used as an indication of the material's behavior with regard to its capability to interfere with enamel mineral loss from permanent teeth.

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