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Original

Fluoride-releasing capacity and cariostatic effect provided by sealants

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Abstract: This study evaluated the effect of sealants on enamel demineralization, focusing on physical protection of the sealed enamel and fluoride protection of the adjacent unsealed enamel. Occlusal fissures with areas measuring 12 mm^2 were delimited in 48 extracted molars, randomly divided into 4 groups (n =12): 1) no sealing; 2) sealing with a resin-modified glass-ionomer (Vitremer™, 3M ESPE); 3) sealing with a fluoride-releasing composite sealant (Clinpro Sealant™, 3M ESPE); and 4) sealing with a non-fluoridated composite sealant (Concise™, 3M ESPE). A 4-mm^2 window was outlined on the buccal enamel for analysis of fluoride uptake. Following treatment, groups 2, 3 and 4 were subjected to 5-days of pH-cycling, while group 1 was kept in a moist environment at 37°C. Fluoride uptake was assessed by dental biopsy, and the amount of fluoride released to the cycling solutions was determined by ion analysis. Enamel demineralization around the sealants was evaluated by cross-sectional micro-hardness analysis. Group 2 showed higher levels of fluoride release ($P < 0.01$) and uptake by enamel ($P < 0.05$), and lower levels of demineralization ($P < 0.05$) than groups 3 and 4. Group 3 exhibited reduced demineralization on unsealed enamel and provided fluoride uptake in a distant enamel area, while group 4 did not. (J. Oral Sci. 47, 35-41, 2005)

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Introduction

Over the last three decades, caries prevalence has declined worldwide (1). However, the decline has not occurred uniformly on all dental surfaces, and most new carious lesions in children and adolescents are now located on occlusal surfaces (2,3).

Non-invasive sealants have been recommended for children and adolescents at risk of caries (4). Some studies have suggested that the benefit provided by protecting pits and fissures is based on good retention and the integrity of the sealant material (5-8). However, since the retention of the sealant is not permanent, this physical effect could be enhanced if the material simultaneously released fluoride (9,10).

Some reports comparing caries development around composite sealants and ionomeric sealants have shown the superiority of the resinous material (11-13) because of its better retention (6,14). Other authors, however, found no significant difference between these kinds of sealants (6,15), or found a greater potential for caries inhibition in ionomeric sealants (16). Thus, the development of composite sealants containing fluoride and resin-modified glass-ionomer sealants would combine the benefits of enamel adhesion and fluoride-release in one material (17).

Previous studies have demonstrated that fluoride release by glass-ionomer cement restorations ensures an anticariogenic effect around the enamel (18,19) and on the adjacent tooth (20,21). However, further research is still necessary concerning fluoride release by occlusal sealants, particularly its effect on fluoride uptake on buccal surfaces.

Therefore, the aim of the present study was to evaluate the cariostatic effect provided by three different occlusal sealants; a resin-modified glass-ionomer (Vitremer™), a fluoride-releasing composite sealant (Clinpro™Sealant) and a non-fluoridated composite sealant (Concise™), focusing on the benefits of the physical barrier formed over the sealed enamel, the fluoride protection for the unsealed enamel adjacent to sealant, and the uptake of fluoride into distant enamel.

Materials and Methods

Ethical Aspects

This *in vitro* study using human teeth was deemed to be ethical according to the Brazilian Guidelines (Resolution 196 of the National Health Council, 1996), and the protocol was approved by the Institutional Ethics Committee of Piracicaba School of Dentistry, University of Campinas. Three commercial restorative materials were tested: a resin-modified glass-ionomer (RMGI), a fluoride-releasing composite sealant (FRCS) and a non-fluoridated composite sealant (NFCS). The authors have no connection with the manufacturer of these products.

Tooth Selection and Preparation

Forty-eight impacted human third molars, extracted for orthodontic reasons and free from macroscopic defects or staining on the occlusal and buccal surfaces, were selected for this study. After pumicing, their roots were sectioned (Isomet, Buheler, Lake Bluff, IL, USA) and their crowns were stored in a 0.1% aqueous thymol solution (pH 7.0) at 4°C for at least a month (22). Then, teeth were randomly divided into four groups (n = 12), according to the sealant materials used (Fig. 1): Group 1; teeth were not sealed; Group 2; teeth were sealed with a RMGI (Vitremer™, 3M ESPE Dental Products, St. Paul, MN, USA); Group 3; teeth were sealed with a FRCS (Clinpro™Sealant, 3M ESPE); and Group 4; teeth were sealed with a NFCS (Concise, 3M ESPE).

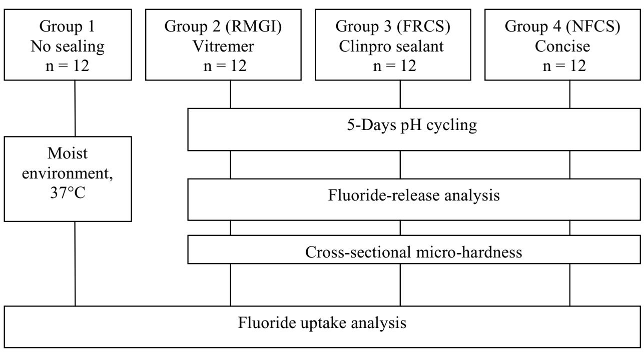


Fig. 1 Schematic presentation of the study design.

Using a digital caliper (Mahr Federal, Germany, UK), the following areas were outlined on each tooth: an occlusal area 3 mm long and 4 mm wide (12 mm^2), with the main occlusal fissure at its center, and a square window measuring 4 mm^2, positioned on the buccal surface. The reasons for delimitating the occlusal and buccal areas were, respectively, to standardize the volume of sealant material applied (3 mm^3) and to create an area of distant enamel for posterior analysis of fluoride uptake. The tooth crowns were fixed with orthodontic wires to facilitate manipulation of specimens. They were then identified and isolated with an acid-resistant nail varnish (Colorama, São Paulo, Brazil), excepting for the occlusal and buccal delimited areas.

Sealing Procedures

Sealants were applied according to the manufacturer's instructions, except for the RMGI samples, which were treated with a 1:2 powder/liquid ratio (23). Tooth fissures of groups 2, 3 and 4 were etched with 35% phosphoric acid (3M ESPE) for 15 seconds and washed for the same time. After slight air-drying, sealants were applied with a sharp explorer under 10 × magnification (Meiji 2000, Meiji, China) to avoid excessive spreading of the sealant and to leave a bilateral rim 1 mm wide and 3 mm long of unsealed adjacent enamel. Sealants were photopolymerized within the recommended time (Optilux 500, Demetron/Kerr, Danbury, CT, USA).

pH-Cycling Regimen

Group 1 was kept in a moist environment at 37°C, while the sealed groups (2, 3 and 4) were subjected to a 5-day pH-cycling model, simulating a high caries challenge, essentially according to Featherstone et al. (24). Teeth were individually immersed in 0.5 ml of a demineralizing solution (De) (2 mM calcium, 2 mM phosphate in 0.075 M acetate buffer, pH 4.3) for 6 h, at 37°C. After this, they were washed in distilled water for 10 s, dried with absorbent paper and individually immersed in 0.5 ml of a remineralizing solution (Re) (1.5 mM calcium, 0.9 mM phosphate, 150 mM of KCl in 0.1 M Tris buffer, pH 7.0) for 18 h, at 37°C (25). The solutions were changed daily. At the end of each cycle, De and Re solutions were mixed and stored at 4°C for posterior analysis of fluoride release.

Fluoride Uptake Determination

Immediately after the pH-cycling phase, the occlusal surfaces of all teeth (including Group 1) were protected with wax, leaving exposed only the buccal enamel window (4 mm^2). Three layers of enamel were sequentially removed from each specimen by immersion in 0.5 ml of an aqueous solution of 0.5 M hydrochloric acid for 15, 30 and 60 s under agitation. An equal volume of TISAB II (Orion Research, Boston, MA, USA) pH 5.0, modified with 20 g NaOH/L, was added to each solution containing the dissolved enamel layer (4,26).

Fluoride measurements were made in duplicate, using an ion-specific electrode (Orion 96-09) and an ion-analyzer (Orion EA-940) (Orion Research, Boston, MA, USA) which had been calibrated previously with triplicate fluoride standards (0.025 to 4.0 μg F/ml), prepared in 50% TISAB II, containing 0.25 M/L HCl. Group 1 served as a control for fluoride uptake, expressed per layer of removed enamel rather than as a function of depth (27).

Analysis of Fluoride Release

The amount of fluoride released by the sealants during the pH-cycling regimen was analyzed daily, pooling the demineralizing and remineralizing solutions after each cycle. Measurement was made as described for fluoride uptake, except different fluoride standards were used (0.025 to 2.0 μg F/ml), in TISAB III. The cumulative fluoride release in the De and Re solutions during the 5 days was used in the statistical analysis to estimate the difference among the sealants.

Cross-sectional Micro-hardness

After fluoride analysis, teeth of groups 2, 3 and 4 were tested for enamel cross-sectional micro-hardness (CSMH). Teeth from group 1 were not tested, because there was no sealing of the occlusal surface, and thus no reference of sealed or unsealed enamel areas. The other teeth were sectioned through the center of the occlusal sealed area, with the cut being perpendicular to the fissure orientation. One of the tooth halves was randomly selected and embedded in epoxy resin, with the outer enamel surface perpendicular to the resin surface. The samples were serially polished with Al_2O_3 papers of 400, 600 and 1200-grit (Carborundum, São Paulo, Brazil) and then cloth polished with 1.0-μm diamond paste (Buehler Metadi, Buheler, Lake Buff, ILL, USA). Cross-sectional micro-hardness tests were performed using a Knoop diamond under a 25-g load for 5 s (28,29) (FM-ARS, Future-Tech, Tokyo, Japan). Two rows of five indentations each (at depths 10, 20, 30, 40 and 50 μm from surface enamel) were made at distances of 100 μm below and 100 μm above the sealant margin, on sealed and unsealed enamel, respectively (Fig. 2). The Knoop hardness units data (KHN) were converted to mineral content (volume %) using the equation: mineral content = 4.3 ($\sqrt{KHN}$) + 11.3 according to Featherstone *et al*. (28).

Statistical Analysis

For fluoride uptake, the third layer was transformed (square root) before applying ANOVA and Newman-Keuls multiple-comparison tests (SAS/STAT Guide for personal computers, SAS Institute, Cary, NC, USA, 2001) because variance was not homogeneous. The sum of fluoride released in De and Re solutions during pH-cycling was analyzed using the Krushkal-Wallis and Dunn test (α = 0.05). A multi-factor ANOVA with a *split-split-plot* design was applied to the CSMH data to analyze the interactions among the factors (sealants, position from the margin of the sealant and depth from the enamel surface). A multiple comparison Tukey test (α = 0.05) was chosen to check differences in means within these factors.

Results

There was no significant difference in the level of fluoride released during pH-cycling between FRCS and NFCS ($P < 0.01$). However RMGI released more fluoride than all the other groups ($P < 0.01$) (Table 1).

There were statistically significant differences in fluoride uptake (Table 1) between sealants ($P = 0.0009$) for the first enamel layer, with the RMGI group (Vitremer™) showing the highest amount of incorporated fluoride compared with FRCS, NFCS and the control. In the second and third layers, however, these differences were not statistically significant ($P > 0.05$).

Regarding the mineral content data, multi-factor ANOVA (Table 2) revealed an important interaction among the factors 'sealant' and 'position from the sealant margin' (P = 0.00016). The Tukey test showed no statistically significant differences between all the groups on the sealed enamel ($P > 0.05$) (Fig. 3). However, on the unsealed enamel area at depths 10 and 20 μm from the enamel

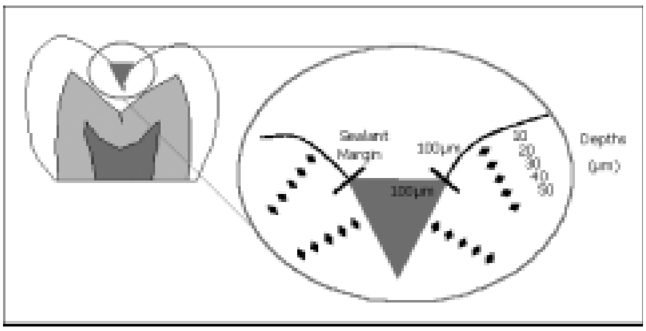


Fig. 2 Diagrammatic representation of the cross-sectional micro-hardness assay. Two rows of five indentations each (at depths 10, 20, 30, 40 and 50 μm from surface enamel) were made at distances of 100 μm below (sealed enamel) and 100 μm above (unsealed enamel) the sealant margin.

Table 1 Fluoride concentration in enamel (μg F/cm^2) and sums of fluoride released during pH-cycling (μg F/ml) according to groups, Means (SD; n = 12)

Groups	**(μg F/cm^2)**			**μg F/ml**
	Layer 1	Layer 2	Layer 3	
Group 1	3.08 (0.83) ^{b}	3.84 (1.24) ^{a}	3.96 (1.34) ^{a}	-
Group 2	5.47 (1.17) ^{a}	5.26 (1.39) ^{a}	4.98 (1.33) ^{a}	1.91 (0.53) ^{a}
Group 3	3.89 (1.50) ^{b}	4.45 (1.83) ^{a}	5.52 (2.59) ^{a}	0.12 (0.17) ^{b}
Group 4	4.05 (1.56) ^{b}	3.98 (0.99) ^{a}	4.39 (1.15) ^{a}	0.07 (0.17) ^{b}

a, b; Different letters in columns show statistical significance among groups ($P < 0.05$).

Table 2 Multi-factor analysis of variance for cross-sectional micro-hardness data

Source	**Degree of freedom**	**Sum of squares**	**Mean square**	***F* value**	***P* value**
Sealant	2	183264.49	91632.24	11.97	0.00027
Error (A)	33	252722.38	7658.25		
Plots	35	435986.87			
Position (sealed/unsealed)	1	1154589.43	1154589.43	236.48	0.00001
Sealant × Position	2	129853.30	64926.65	13.30	0.00016
Error (B)	33	161118.58	4882.38		
Subplots	71	1881548.17			
Depth	4	1545033.02	386258.26	318.21	0.00001
Sealant × Depth	8	13482.25	1685.28	1.39	0.20087
Position × Depth	4	103214.87	25803.72	21.26	0.00001
Sealant × Position × Depth	8	26657.30	3332.16	2.75	0.00655
Error (C)	264	320454.67	1213.84		
Total	359	3890390.28			

General mean = 235.23

Coefficient of variation: (A) = 11.765%; (B) = 13.284%; (C) = 14.811%

surface, group 2 showed less demineralization ($P < 0.01$) than groups 3 and 4 (Fig. 3). At the other depths, group 2 and 3 did not differ from each other ($P > 0.05$), but were statistically significantly different from group 4 ($P < 0.01$).

Discussion

A synergistic cariostatic effect would be expected to occur as a function of integrating retention and fluoride-releasing properties in sealant materials. Fluoride is a world-wide recognized anticariogenic substance (18,30), and its release from a dental material can be effectively estimated in simulated caries procedures (31). In the present study, the RMGI samples released the highest amounts of fluoride, thus confirming previous results (32-35). However, the FRCS group and the NFCS group performed similarly. These results could be explained by the differences in the composition between ionomeric and resinous materials, resulting in subsequent differences in fluoride releasing profiles (35). According to Asmussen and Peutzfeldt (36), 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 enhances fluoride diffusion and release (36). On the other hand, the matrix of resinous sealants is much less hydrophilic, making fluoride release more difficult (37).

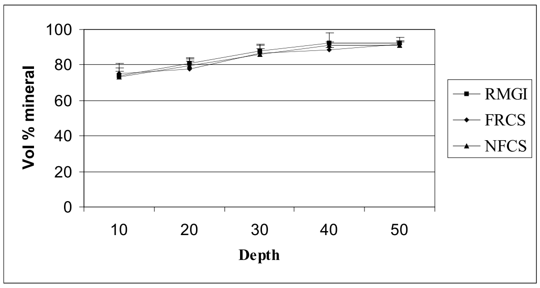
With regard to mineral content values, no demineralization could be demonstrated on sealed enamel in all materials tested (Fig. 3). This result is consistent with earlier reports (7,38,39) showing that the cariostatic benefit of fissure sealing is provided by the formation of a physical barrier that protects the fissure from plaque stagnation and carious attack. However, in the present study, there was demineralization on the enamel that was immediately adjacent to the sealant and directly exposed to the pH-cycling solutions (Fig. 3). As expected, the NFCS showed no cariostatic effect at any of the depths evaluated for the unsealed enamel. Méjare and Mjör (40) noted the same results when comparing Concise™ and glass-ionomer as fissure sealants.

On the other hand, resin-modified glass-ionomer showed significantly reduced demineralization in the shallower enamel depths (10 and 20 µm), where the acid attack seemed to be stronger. The behavior of this material is consistent with previous results in deciduous (39) and permanent teeth (32). Reduction of demineralization around resin-modified glass-ionomer restorations seems to be a result of its fluoride-releasing capacity, since the presence of fluoride in the fluid around the enamel crystals during the caries challenge reduces demineralization and enhances remineralization (31).

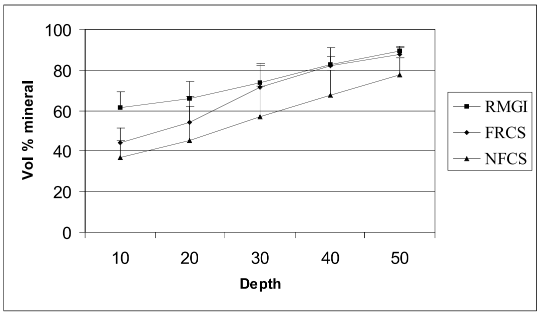
Fluoride uptake by buccal enamel confirmed the superior fluoride-releasing capacity of the resin-modified glass-ionomer, in relation to the other materials. The incorporation of fluoride into the enamel adjacent to a fluoride-releasing restorative material is a useful way to estimate its cariostatic effect (41), since fluoride reduces enamel solubility (42). The higher levels of fluoride uptake in the enamel adjacent to glass-ionomers and resin-modified glass-ionomers can be explained by the enamel mineral being continuously lost after acid attacks and regained during the dynamics of the caries process (18). Glass-ionomers and resin-modified glass-ionomers release more fluoride and thus provide a cariostatic effect when used for fissure sealing. Their protective effect relies on the continuous fluoride release from the remaining particles confined to fissure sites, even in cases of significant loss of the material (6,9,40).

These results for fluoride uptake on buccal enamel confirm previous studies that demonstrated fluoride uptake at areas located up to 7.5 mm from the restoration margins (34) or on the adjacent tooth (43). Our results confirm that fluoride released from resin-modified glass-ionomer is capable of affecting not only enamel adjacent to the sealant margin, but also enamel located distant from it.

In conclusion, the present study shows that the fluoride-releasing capacity of resin-modified glass-ionomer provides



A
Sealed enamel



B
Unsealed enamel

Fig. 3 Means (SD; n = 12) of mineral content (vol %) at different distances from enamel surface (depth) for each sealant. (A) Sealed enamel: 100 µm below the margin of the sealant; (B) Unsealed enamel: 100 µm above the margin of the sealant.

cariostatic benefits in areas both close to the sealant and distant from it. These properties would be especially beneficial for patients with high caries risk.

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