Inhibition of Demineralization Adjacent to Tooth-colored Restorations in Primary Teeth after 2 In Vitro Challenges

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ABSTRACT

Purpose: As clinical diagnosis of secondary caries is the most common reason for restoration replacement, fluoride-releasing restorative materials have been developed to address this problem. The purposes of this study were to verify demineralization inhibition produced by 5 restorative materials submitted to two methods of in vitro cariogenic challenge and verify whether these methods influence material behavior by means of polarized light microscopy and microhardness.

Methods: Class V cavities were prepared on buccal surfaces of 100 extracted primary molars and randomly restored with 1 of the 4 fluoride-releasing materials, Fuji IX, Vitremer, Dyract, Tetric Ceram, and Filtek Z250 as control material (N=10). Specimens were submitted to in vitro caries induction by two different methods, acid gel immersion, and pH cycling. Teeth submitted to gel were then sectioned and prepared for polarized light microscopy in water, while teeth cycled were prepared for microhardness evaluation.

Results: Polarized light microscopy: Means of demineralization areas (μm^2) differed significantly, depending on the restorative material. Tukey's test revealed the smallest demineralization areas adjacent to Fuji IX and Vitremer restorations, with no difference between them ($P>.05$). The greatest demineralization area mean values were verified using Dyract and Filtek Z250, without differences between them ($P>.05$). Microhardness: Glass ionomer cements (GICs) performed better on the area of great cariogenic challenge, closer to the surface, than other materials indicating minor mineral loss during pH cycling. The compomer Dyract presented similar performance to GICs and composite resin Tetric Ceram, but it was better than Filtek Z250.

Conclusion: The experimental model of caries lesion induction may influence material performance. GICs, however, are superior in preventing in vitro demineralization independently of the method. (J Dent Child 2007;74:209-14)

KEYWORDS: PRIMARY TEETH, RESTORATIONS, SECONDARY CARIES

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Restoration success is determined by several factors, including patient caries activity, restoration quality, and restorative material used.[1] While clinical diagnosis of secondary caries is the most common reason for restoration replacement,[2] fluoride-releasing restorative materials have been suggested to reduce the frequency and severity of this problem.[3] Factors related to the restorative material that can affect secondary caries lesion formation are material type, retention mode (mechanical or adhesion), and the ability to release fluoride.[4]

Based on clinical observations that caries reappeared less frequently in silicate cement restorations despite microleak-

age, it has been suggested that fluoride release by restorative materials may have an anticariogenic effect.[5] Therefore, the effect of fluoride released by restorative materials over the demineralization-remineralization process has been the focus of various studies.[6-14]

Even after the introduction of glass ionomer cements (GICs), studies have been conducted to improve material properties and enamel strengthening facing demineralization process. Resin-modified glass ionomer cements (RMGICs) and polyacid modified resins, or compomers, have been developed to improve their wear-resistance and esthetic character, maintaining their fluoride-releasing ability[15-19] and serving as a fluoride reservoir.[5,19-22]

Different methodologies and experimental designs seek to simulate the real conditions of the mouth. A technique proposed by Silverstone[23] using an acidified gel with a pH around 4.5 is still favored due to its simplicity, ease of use, and ability to produce lesions histologically similar to that naturally developed in enamel under light polarized microscopy.

This model has been criticized as being strictly a demineralizing model, lacking saliva and biofilm. Also, saturation in the gel can occur due to liberation of components by restorations such as fluoride[24]. The in vitro model of pH cycling, which uses demineralizing (DE) and remineralizing (RE) solutions, addresses these drawbacks since it provides alternate periods of demineralization and remineralization and constant changes inhibiting the solution saturation. This model has been criticized because neither the duration of DE/RE periods are known nor does it contain saliva and biofilm. Thus, these models are capable of simulating chemical changes that occur during lesion development and its reversion, but they do not simulate the caries process.[25]

The benefits of fluoride-releasing restorative materials and their performance in the restoration-tooth interface during the demineralization process has been widely studied.[3,6-14,26-34] Few investigations, however, have focused on the primary dentition in which these materials are widely recommended.

The purposes of this study were to verify demineralization inhibition produced by 5 restorative materials submitted to 2 methods of in vitro cariogenic challenge, and verify if these methods influence material behavior by means of polarized light microscopy and microhardness.

METHODS

One hundred caries- or enamel defect-free mandibular first primary molars were selected from the human teeth bank of the University of São Paulo, São Paulo, Brazil. Standardized Class V-like cavities (3 mm x 2 mm x 1.5 mm) with enamel margins were prepared on the teeth's buccal surfaces with a high-speed diamond bur (no. 1092). Teeth were then randomly restored with:

a. 1 of the 4 fluoride-releasing materials:
   1. Fuji IX (GC Corp, Tokyo, Japan);
   2. Vitremer (3M ESPE, St. Paul, MN, USA);
   3. Dyract (Dentsply Ind. Com. Ltd, Petrópolis, RJ, Brazil); and
   4. Tetric Ceram (Ivoclar Vivadent, Schaan, Liechtenstein); and
b. 1 control material: Filtek Z250 (3M ESPE, St. Paul, MN, USA).

All procedures followed the manufacturers' instructions.

After 24 hours, the teeth's pulp chambers were filled with epoxy resin and sealed with 2 coats of cosmetic nail varnish, so that the restoration and a 1-mm enamel margin remained exposed.

ACID GEL CARIES INDUCTION AND INHIBITION AREA ANALYSIS

Fifty specimens were then submitted to in vitro caries induction by immersion for 14 days in an acid gel renewed at the seventh day under room temperature. Demineralized teeth were included in an orthophtalic resin and sectioned into 200 µm slices that were reduced by manual wear with sandpapers of increasing grit (600, 1,000, 1,200, and 1,400 grit). Slices were immersed in water and analyzed via polarized light microscope (Zeiss) with a quartz accessory under X25 and X100 magnification.

Demineralization area images from the occlusal and cervical region of each restoration were analyzed and measured by image software (Leica QWin, Bannockburn, IL, USA). The values of the demineralization area (μm^2) were also compared statistically by analysis of variance and posthoc Tukey's tests at the 95% significance level using GMC software v. 8.1 (Ribeirão Preto, SP, Brazil).

CARIES INDUCTION BY PH CYCLING AND MICROHARDNESS ANALYSIS

Fifty other specimens were submitted to the pH-cycling procedure to create artificial incipient caries lesions. The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 50 mM acetic acid adjusted to pH 4.8. The remineralizing solution contained 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCl adjusted to pH 7.0. Each specimen was cycled in 10 mL for 3 hours in the demineralizing solution and 21 hours in the remineralizing solution and was kept 30 minutes in artificial saliva between them during 10 days. This procedure was carried out at room temperature and without shaking.

Cycled teeth were sectioned with a diamond saw perpendicular to the occlusal surface by the center of the restoration and embedded in orthophtalic resin, keeping exposed the internal portion to be analyzed. Specimens were polished with 600-, 1200-, and 4000-grit sandpapers and with 3 µm and 1 µm diamond abrasive paste (Büehler, Lake Bluff, IL, USA) on polishing cloths. Microhardness measurements were performed using a microhardness tester (Shimadzu Corp, Tokyo, Japan) with a Knoop diamond under a 50g load for 30 seconds. Nine indentations were made on the incisal portion of each specimen, divided into 3 lines and 3 columns. The first indentation was located 50 µm away from the enamel margin and 100 µm away from the preparation

Table 1. Microhardness Mean Values ± (SD; kgf/mm2) for Restorative Material and Position Interaction *

Material position	Fuji Ix	Vitremer	Dyract	Tetric Ceram	Filtek Z250
A1	189.9±27.5	206.6±49.0	115.9±67.0	79.2±46.5	48.4±19.14
A2	198.0±43.2	189.7±56.2	124.6±64.5	73.9±48.4	53.7±18.6
A3	205.3±39.9	192.2±52.3	120.4±70.0	71.5±43.1	51.6±17.0
B1	236.2±29.9	215.3±35.3	229.9±48.1	221.1±33.3	184.2±79.6
B2	224.4±19.7	219.5±27.4	250.9±36.7	215.3±50.7	194.7±85.5
B3	234.3±19.0	226.6±37.0	234.0±26.1	225.4±39.8	181.3±84.2
C1	229.2±15.9	222.3±26.9	257.8±37.4	224.1±25.6	194.5±79.2
C2	235.1±27.2	237.4±30.0	243.8±38.0	228.8±55.3	198.6±81.8
C3	240.4±26.3	231.3±23.1	246.5±21.8	242.6±35.3	216.4±88.1
Control A	266.9±36.7	258.4±32.4	281.5±34.8	278.4±28.4	257.4±36.0
Control B	272.5±33.4	275.9±43.9	277.6±22.8	269.4±33.0	239.9±29.1
Control C	283.8±24.4	269.1±37.1	257.8±24,7	294.5±25.9	243.7±19.0

** F= 66.4*

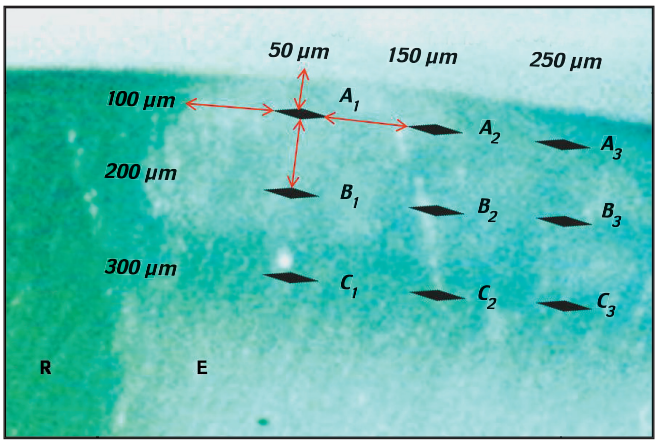

Figure 1. Microhardness evaluation positions in relation to a restoration.

margin (A1; Table 1). The following indentations were 100 µm distant each other in both direction (Figure 1). The palatal portion of each tooth was prepared for the microhardness test and used as a control for demineralization.

RESULTS

AREA OF INHIBITION ASSESSED BY POLARIZED LIGHT MICROSCOPY

All specimen images revealed caries lesions adjacent to restorations with parallel contour to the enamel surface. It was also possible to see a nearly intact superficial zone with negative birefringence (yellow), which is characteristic of sound enamel and a positive birefringence zone (brown), which better characterizes the lesion body internally. Lesion extensions varied according to restorative material used (Figures 2 and 3). The means of demineralization areas (μm^2) according to each restorative material are expressed in Figure 4. Tukey's test revealed the smallest demineralization areas adjacent to GIC Fuji IX and RMGIC Vitremer, with no difference between them. The greatest demineralization areas were observed in the compomer Dyract and composite resin Filtek Z250, with no statistical difference between them.

MICROHARDNESS AFTER PH CYCLING

Results of microhardness are expressed in Table 1. All materials presented differences concerning microhardness values from the respective control (A) at a depth of 50 µm in any distance of the restoration, except Vitremer in the position A1, which was similar to its control. Fuji IX and Vitremer showed no differences among the 3 evaluated depths (A, B, and C). Dyract, Tetric Ceram, and Filtek Z250, however, which were different from their controls, also presented microhardness values statistically lower in the depth A than in the depths B and C. Compomer Dyract was similar in cariostatic effect to the composite resin Tetric Ceram but still better than Filtek Z250. Between the resins, there was no difference. From positions B and C to any distance of the restoration, all microhardness values found were similar to controls that suffered no cariogenic challenge.

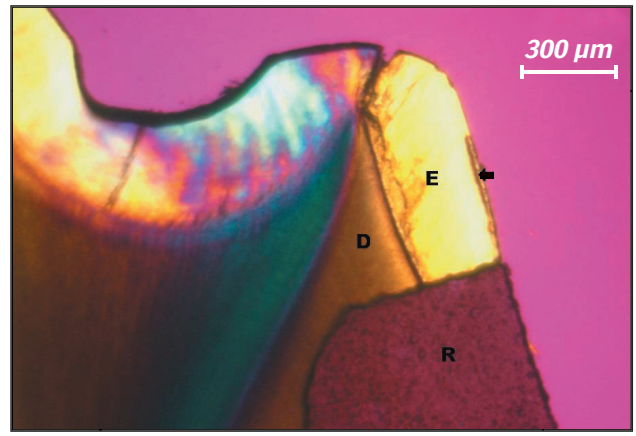

Figure 2. Image of a specimen from the Fuji IX group showing demineralization on the enamel surface adjacent to a restoration. An almost intact superficial zone with negative birefringence (yellow) can be seen, characteristic of sound enamel, while a positive birefringence zone (brown) characterizes the lesion body. (D) Dentine; (E) enamel; (R) composite resin. Note the smallest demineralization area (arrow).

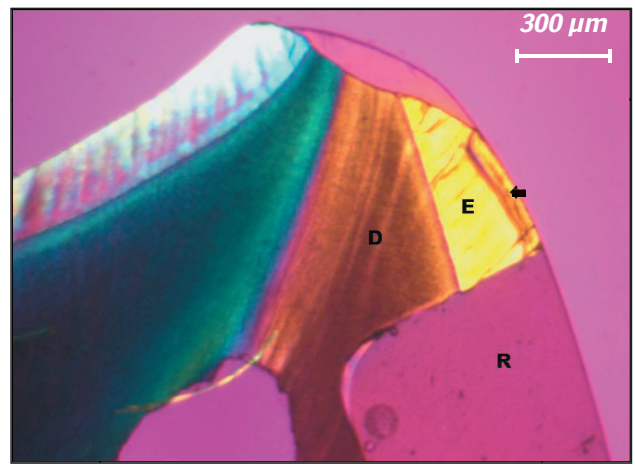

Figure 3. Sample from Filtek Z250 group. Note the greatest demineralization area (arrow). (D) Dentine; (E) enamel; (R) composite resin.

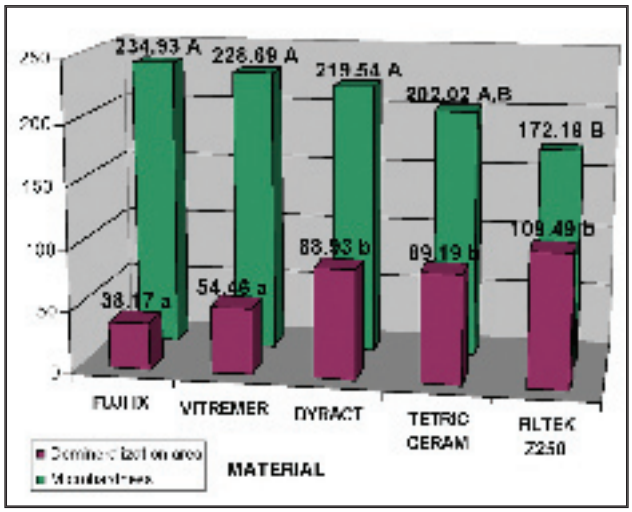

Figure 4. Distribution of demineralization area mean values (µm2) and microhardness mean values (kgf/mm2) according to restorative material. F=16.15 for area, and F=34.23 for microhardness. Different letters indicate a statistically significant difference (p<.05).

DISCUSSION

This study intended to not only verify the demineralization area and microhardness values, but also rank the materials regarding inhibition of the artificial caries development, verifying whether it differs in function of the models used for caries induction.

In this study, conventional and resin-modified GICs (Fuji IX and Vitremer) demonstrated the greatest capacity for inhibiting artificial caries lesions adjacent to restorations. This finding was consistent with the results of previous studies using the acidified gel method,[3,14,29,] probably due to its superior capacity to release fluoride.[18,35-37] In the pH cycling model, microhardness results confirmed the behavior of the ionomeric materials in the acidified gel method, since only glass ionomer cements Fuji IX and Vitremer presented greater microhardness values on the surface (position A) than other studied materials. It is noteworthy that, despite the intense cariogenic challenge provided by the pH cycling, Vitremer was the only one in the A1 position with values similar to the respective control, probably because that position should receive more fluoride since it is the nearest to the restoration.

The compomer Dyract presented demineralization areas as large as composite resins, contrasting with the findings of some in vitro studies,[4,33,38-42] which can be explained by Dyract's smaller capability of releasing fluoride.[43] Facing pH cycling, Dyract had a behavior similar to ionomeric material and Tetric Ceram, but still better than Filtek Z250 in its cariostatic effect. The greatest difference between the two in vitro methods occurred with Dyract, since this material had a moderate effect on mineral loss when submitted to pH cycling, presenting an intermediate hardness between ionomers and composite resins—being inferior to the first ones and superior to Filtek Z250. This contrasted with the acidified gel model—where Dyract had the worst performance—similar to composite resins but still also similar to the Tetric Ceram.

In both methods, composite resins presented similar behavior, obtaining the greatest demineralization area mean values and the least microhardness values. These findings agree with Hicks et al,[4] who verified that Filtek Z250 did not increase enamel resistance undergoing an intense demineralization. Tetric Ceram, however, contains fluoride and has the capability to release fluoride ions.[36,44] Therefore, a caries inhibition effect could be expected from Tetric Ceram.

This study intended to verify the influence of the in vitro method of caries lesion induction on the materials' ability to inhibit demineralization. The qualitative evaluation by means of polarized light microscopy demonstrated that glass ionomer cements Fuji IX and Vitremer were superior to other materials facing both models. The behavior of composite resins Tetric Ceram and Filtek Z250 also were similar in both methods. Although there were no significant differences between them, Tetric Ceram formed smaller areas of demineralization than Filtek Z250. After pH cycling, areas adjacent to Tetric Ceram had minor mineral loss (bigger microhardness), although they were statistically similar to Filtek Z250.

It is important to highlight that extrapolating in vitro results to clinical situations can be highly misleading, mainly due to differences on the absence of biofilm, salivary proteins, gingival or dentinal fluids on tooth surfaces, and gaps. While in vivo caries results from complex interactions between biofilm and teeth, in vitro caries-like lesions are basically a demineralization process.[24] Results of an in vivo study[32] showed no secondary caries lesion adjacent to GIC restorations at gap-free regions. In gap regions, however, no preventive effect was exerted by GICs to protect the adjacent enamel wall from demineralization attack. The number of in vivo studies is limited, and most of them show that fluoride release is temporary and insufficient in suppressing recurrent caries. A systematic review of 52 clinical trials found no conclusive evidence of a GIC caries inhibitory therapeutic effect.[45]

Restorative materials with a demineralization inhibitory effect due to fluoride release have been developed to prevent or delay secondary caries lesion development. The restoration undoubtedly plays an important role in re-establishing and maintaining oral health. It must be understood, however, that secondary caries results from demineralization imbalance continuity occurring in the oral environment, and the cure cannot be attributed to restoration.

CONCLUSION

Based on this study's results, it is possible to conclude that the experimental model of caries induction influences material behavior. It is unanimous, however, that for both methods glass ionomer cements are superior in preventing in vitro demineralization.

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