Chemical or Microbiological Models of Secondary Caries Development Around Different Dental Restorative Materials

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Abstract: This study evaluated artificial secondary caries around restorative materials, induced by means of chemical or microbiological models. The following materials were used randomly to restore 130 dental blocks: (1) zinc-oxide eugenol-free temporary filling: Coltosol (Coltène/Whaledent Inc.; $n = 30$), (2) silver amalgam: Permite C (SDI Limited, $n = 20$), (3) composite resin: Filtek™ Z250 (3M ESPE; $n = 20$), (4) glass-ionomer cement: Fuji II (GC America Inc.; $n = 20$), (5) resin-modified glass ionomer: Vitremer™ (3M ESPE; $n = 20$), and (6) polyacid modified resin: Dyract AP (Dentsply; $n = 20$). Ten specimens of Group 1 were kept in humidity, and had no carious formation (NC). Ten specimens of each group were submitted to pH cycling (CG, $n = 60$), and the others were immersed in a medium containing *Streptococcus mutans* and sucrose (BG, $n = 60$). Mineral content was determined by microhardness assessment, and lesion depth was measured in polarized light photomicrographs. In the chemical model (CG), mineral content values in the vicinities of restoration were high for Groups 5 (75.7 ± 11.9), 4 (70.8 ± 14.2), and NC (95.4 ± 3.8); intermediate for Groups 1 (55.8 ± 18.5), 6 (45.6 ± 11.0), and 2 (44.3 ± 11.2); and reduced for Group 3 (34.7 ± 9.7). In the microbiological model (BG), results were similar to CG, although there was less demineralization. The highest lesion depths were found for Groups 3 (182.3 ± 33.2) in CG and 6 (126.5 ± 42.8) in BG, when compared to Group 5 (114.6 ± 26.0 and 56.2 ± 33.2, respectively). In both models of caries induction, ionomeric materials showed a superior cariostatic effect when compared to the other restorative materials. © 2005 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 74B: 725–731, 2005

Keywords: secondary caries; caries models; fluoride; pH cycling; *Streptococcus mutans*

INTRODUCTION

Individuals with caries-risk and active caries are prone to developing secondary caries, identified as the major cause for the failure of dental restorations.[1,2] Restorative materials with cariostatic properties have been indicated for these patients, as it can help caries control in a localized manner.[3,4]

The cariostatic effects of several restorative materials have been widely studied *in vitro* with models that simulate high caries challenge and aim to develop artificial lesions comparable to those that take place *in vivo*.[5,6] Chemical systems focus on physicochemical aspects of dental caries, being static (acid gelatins or solutions[7,8]) or dynamic (pH regimens.[9–11] Biological models use microorganisms as acid producers, in simple cultures,[12–15] consortia,[16] or artificial mouths.[17,18]

Although all these artificial caries models present limitations, pH-cycling regimens are considered more similar to the natural situation, as they simulate demineralization and remineralization cycles,[19] producing lesions with the same histological features of *in vivo* caries.[10,11] However, the inclusion of microorganisms in the caries model may lend some information about the cariostatic effects of restorative materials over bacteria.[20,21]

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Information given by these two models about cariostatic effects of restorative materials could be complementary, providing physicochemical and antibacterial aspects.[22] Nevertheless, there are few studies using different artificial caries models in order to evaluate cariostatic effects of restorative materials.[21] Therefore, this study aimed to evaluate, *in vitro*, secondary caries-like lesion development around different restorative materials, using chemical or biological models of caries induction.

MATERIALS AND METHODS

Tooth Preparation

One hundred thirty inferior bovine incisors free from structural defects were selected for this study. After being pumiced, they were stored in 0.1% thymol solution at 4°C for 30 days.[23] The labial surface of each tooth was sectioned into blocks of approximately 6.0 mm in width, 6.0 mm in length, and 5.0 mm in thickness, with the use of double-faced diamond discs (7020-KG Sorensen, SP, Brazil). Each tooth block was sanded with a water-cooled mechanical grinder (Maxigrind-Solotest, SP, Brazil), with 600- and 1200-grit Al_2O_3 abrasive papers (Waterproof, Carborundum, Brazil), in order to remove a surface-enamel layer of approximately 100 μm.[24] Cylindric cavities measuring 2.0 mm in diameter and 2.0 mm in depth were prepared in the center of the labial surface area of the tooth blocks, with a high-speed diamond bur (2294 KG Sorensen, SP, Brazil) under constant water refrigeration.

Restoration and Polishing Procedures

The prepared tooth blocks were randomly divided into six groups according to the restorative material used: (1) zinc-oxide eugenol-free temporary filling (Coltosol, Coltène/Whaledent Inc.; $n = 30$), (2) silver amalgam (Permite C, SDI Limited, Victoria, Australia; $n = 20$), (3) composite resin (Filtek™ Z250, 3M ESPE Dental Products, St. Paul, MN; $n = 20$), (4) glass-ionomer cement (Fuji II, GC America Inc.; $n = 20$), (5) resin-modified glass ionomer (Vitremer™, 3M ESPE; $n = 20$), and (6) polyacid modified resin (Dyract AP, Dentsply Int. Inc.; $n = 20$). All materials were used according to manufacturer's instructions. After 24 h in a moist environment (37°C), restored surfaces were polished with aluminum oxide abrasive disks for plastic materials (Sof-Lex, 3M, Brazil, Lot 04032), and abrasive rubber points for amalgam (KG Sorensen, SP, Brazil). Each specimen was analyzed in a stereomicroscope at 40x magnification to ensure that there was no overhang on the restoration/tooth interface.

After this phase, 10 specimens of each group were randomly selected and assigned to a chemical-caries model group (CG, $n = 60$) consisting of a 10-day pH-cycling regimen. Another ten specimens of the same groups were assigned to a bacterial-caries model group (BG, $n = 60$), consisting of the immersion of specimens in a medium with sucrose and *Streptococcus mutans* (ATCC 25175), for 5 days. The last 10 specimens of Group 1 were not submitted to any carious challenge, and thus they were considered negative control for caries formation (NC, $n = 10$). They were kept in a moist environment at 37°C during the experimental phase. Tooth block surfaces were covered with acid-resistant varnish (Colorama-CEIL, SP, Brazil), except for an area of 12.56 mm^2 for the chemical group (the restoration area and an enamel rim of 1.0 mm), or an area of 36.0 mm^2 for bacterial group and control (the total labial area). All blocks were sterilized in a gamma irradiation chamber (Gammacell 220 Excel, GC-220E).[25]

pH Cycling Model

Tooth blocks were individually immersed on demineralizing solution (DE) (2.0 m*M* Ca, 2.0 m*M* P, and 0.03 ppm F in a buffer solution of 74.0 m*M* of acetate and pH 4.3) and remineralizing solution (RE) (1.5 m*M* Ca, 0.9 m*M* P, and 0.05 ppm F in a buffer solution of 20.0 m*M* Tris (hydroxymethyl)-aminomethane at pH 7.0) in a regimen similar to that proposed by Featherstone et al.[10] and modified by Serra and Cury,[26] and Argenta et al.[27] Because bovine enamel is more porous than human,[24] it was necessary to predetermine the number of cycles so that the Knoop cross-sectional microhardness test could be made after the artificial carious challenge. This explains the choice of ten 24-h cycles: 18 h in RE solution and 6 h in DE solution, and a weekend (48 h) in RE solution. Solutions were renewed daily for RE and after the end of the fifth cycle for DE.

Bacterial Caries Model

Subgroups of 10 sterile tooth blocks were suspended with orthodontic wires in test recipients containing 25.0 mL of sterile brain–heart infusion broth (Merck KGaA, Germany, Lot 811893) with 5% sucrose (w/v). The recipients were inoculated with 100 μL of a cell suspension of S*treptococcus mutans* (ATCC 25175) prepared from 18-h growth pure cultures, yielding $1–2 \times 10^8$ CFU/mL. The concentration of this bacterial suspension was determined by measuring absorption at 660 nm (A_{660}).[28] To adjust the number of viable bacteria to A_{660}, the number of colony-forming units (CFUs) per milliliter bacterial suspension was determined with the use of standard spreading techniques at various optical densities.[28] Inoculation occurred only in the first day of experiment, but renewal of culture media was made every 24 h during the 5 days.[29] Contamination was verified in the media for each day by means of Gram coloration.

Microhardness Assessment

At the end of the artificial caries induction, the tooth blocks were longitudinally sectioned through the center of the restorations. One of its halves was randomly selected and imbibed in epoxy resin so that the cut section was exposed and serially polished with 400-, 600-, and 1200-grade Al_2O_3 papers and 1.0-μm diamond paste over polishing cloths (Metadi® Buehler, Lot 20063). In all samples, four rows of

TABLE I. Mineral Content (%, Means ± SD) Regarding the Interaction Between the Factors Group and Distance from Restoration Margin on Chemical Caries Model (CG)

	Distance (μm)			
Group	50	150	250	350
NC—control	95.4 ± 3.8 aA	95.5 ± 4.4 aA	95.7 ± 3.6 aA	95.7 ± 3.9 aA
G5—resin-modified glass ionomer	75.7 ± 11.9 abA	63.3 ± 13.8 abAB	60.5 ± 15.9 abAB	57.6 ± 15.1 abB
G4—glass-ionomer cement	70.8 ± 14.2 abcA	57.8 ± 18.4 abAB	52.3 ± 17.1 bcB	43.7 ± 12.4 bcB
G1—temporary filling	55.8 ± 18.5 bcdA	51.6 ± 15.6 bcA	44.8 ± 11.8 bcA	48.6 ± 11.9 bcA
G6—polyacid-modified resin	45.6 ± 11.0 bcdA	39.3 ± 11.2 bcA	38.8 ± 10.4 bcA	39.9 ± 11.4 bcA
G2—silver amalgam	44.3 ± 11.2 cdA	32.8 ± 6.9 cAB	31.5 ± 4.2 cB	31.1 ± 4.7 cB
G3—composite resin	34.7 ± 9.7 dA	30.2 ± 5.9 cA	32.8 ± 8.9 cA	35.0 ± 8.6 bcA

Note: $\alpha = 0.05$. Lower-case letters indicate statistical differences among Groups, and capital letters indicate statistical differences among Distances.

indentations were made at distances of 50, 150, 250, and 350 μm from the restoration margin at both sides. Each row had three indentations made at 30-μm intervals from the outer enamel surface across the demineralized area, by means of a Knoop diamond under a 25-g load for 5 s (Future-Tech FM-ARS). Indentation lengths were converted to volume mineral (%) with the use of a published formula.[30]

Polarized Light Photomicrographs

Tooth sections of 400 μm were removed from the other tooth halves with the use of a cut machine (Buehler Isomet). The sections were manually polished with 600- and 1200-grade Al_2O_3 papers, until they achieved a thickness of 100 ± 20 μm.[6] Polished tooth sections were imbibed in deionized water and photographed under polarized light microscopy (Leica, MLST). Standard 35.0-mm photomicrographs were taken and carious lesion depth was analyzed with the use of specific software (Image Pro-Plus®).[31] Linear distances between enamel surface and the lower limit of the lesion were measured at distances of 50, 150, 250, and 350 μm from the restoration margin. Results were obtained in micrometers.

Statistical Analysis

Statistical analysis for groups CG and BG was conducted separately. Microhardness results (mineral volume) were analyzed with the use of ANOVA ($\alpha = 0.05$) with a split–split-plot design, considering the factors of restorative material (Groups 1–6), distance from restoration margin (50, 150, 250, and 350 μm), and depth from enamel surface (30, 60, and 90 μm). For polarized light microscopy results (lesion depths), the same ANOVA ($\alpha = 0.05$) was applied with the use of a split-plot design, considering the factors Restorative Material and Distance. A study of interaction among these factors was made and a multiple comparison Tukey test ($\alpha = 0.05$) was applied to check differences in means within the factors of each variable.

RESULTS

Microhardness Assessment

For the chemical group (CG), the interactions of interest were Group × Distance ($p = 0.00008$) and Groups × Depth ($p = 0.00001$). Tables I and II show that demineralization occurred for all groups, and was more severe at 60 μm from enamel surface. However, ionomeric materials (Vitremer™ and Fuji II) did not differ from control at any distance evaluated, showing less demineralization at 30 μm from enamel surface when compared to the other materials. Also, when demineralization for each material within all distances was compared, a greater cariostatic effect of these ionomeric materials at the vicinities of the restoration could be observed. Curiously,

TABLE II. Mineral Content (%, Means ± SD) regarding the interaction between the factors Group and Depth from Enamel Surface on Chemical Caries Model (CG)

	Depth (μm)		
Group	30	60	90
NC—control	95.4 ± 3.8 aA	95.5 ± 4.4 aA	95.7 ± 3.6 aA
G5—resin-modified glass ionomer	63.7 ± 9.8 bB	56.2 ± 17.8 bC	74.7 ± 16.1 abA
G4—glass-ionomer cement	53.6 ± 13.5 bB	50.8 ± 19.2 bcC	64.7 ± 20.1 bcA
G1—temporary filling	38.0 ± 6.9 cC	51.6 ± 11.5 bcB	68.1 ± 15.0 bcA
G6—polyacid-modified resin	38.5 ± 9.3 cB	37.2 ± 9.6 cdB	51.2 ± 16.9 cA
G2—silver amalgam	35.9 ± 6.9 cA	30.5 ± 5.3 dB	36.5 ± 9.5 dA
G3—composite resin	32.6 ± 6.7 cA	29.0 ± 6.4 dB	34.3 ± 10.5 dA

Note: $\alpha = 0.05$. Lower-case letters indicate statistical differences among Groups, and capital letters indicate statistical differences among Depths.

TABLE III. Mineral Content (%, Means ± SD) Regarding the Interaction Between the Factors Group and Distance from Restoration Margin on Bacterial Caries Model (BG)

	Distance (μm)			
Group	50	150	250	350
NC—control	95.4 ± 3.8 aA	95.5 ± 4.4 aA	95.7 ± 3.6 aA	95.7 ± 3.9 aA
G5—resin-modified glass ionomer	84.6 ± 13.8 abA	78.6 ± 14.3 abA	78.5 ± 13.8 abA	79.7 ± 14.0 abA
G4—glass-ionomer cement	81.3 ± 14.0 abA	78.3 ± 13.5 abA	76.4 ± 14.9 abA	75.1 ± 14.5 abA
G1—temporary filling	68.0 ± 14.8 bA	69.2 ± 15.5 bA	66.8 ± 15.1 bA	66.2 ± 15.3 bcA
G6—polyacid-modified resin	67.0 ± 15.1 bA	65.2 ± 14.5 bA	64.2 ± 14.5 bA	64.6 ± 16.9 bcA
G2—silver amalgam	65.1 ± 14.3 bA	64.5 ± 14.8 bA	66.0 ± 16.1 bA	64.2 ± 15.9 bcA
G3—composite resin	73.8 ± 14.8 abA	58.0 ± 17.0 bB	58.8 ± 15.5 bB	58.2 ± 15.4 cB

Note: $\alpha = 0.05$. Lower-case letters indicate statistical differences among Groups, and capital letters indicate statistical differences among Distances.

silver amalgam had reduced carious lesion formation at the distance of 50 μm in relation to the other distances, regardless of showing severe demineralization when compared to control. In addition, Vitremer presented less caries at the vicinity of the restoration when compared to Coltosol.

For the bacterial group (BG), as well as the CG, interactions between Restorative Material and Distance ($p = 0.0004$) and Restorative Material and Depth ($p = 0.00001$) were observed. The challenge was more severe at the depth of 30 μm, where the carious lesion could be clearly observed (Tables III and IV). Considering the distances from the margin, the ionomeric group showed high levels of mineral content in enamel, not differing from control. However, these materials had moderate demineralization at the depth of 30 μm, differing from control and from the other groups. Composite resin showed severe demineralization at distances of 150, 250, and 350 μm, but showed less demineralization at immediate vicinity of the restoration.

Polarized Light Microscopy

For the CG group, there were significant differences among restorative materials ($p = 0.0047$), but no effect of these materials over carious lesion formation at the distances evaluated ($p = 0.2004$) was seen (Table V). At all distances, ionomeric materials presented reduced lesions when compared to composite resin. Silver amalgam, polyacid modified resin, and temporary cement showed intermediate levels of lesion depth (Figure 1). The same analysis for the BG group detected differences between restorative materials ($p = 0.0134$) and distances ($p = 0.0465$). Interaction between these factors ($p = 0.0169$) showed that the restorative materials influenced carious lesion depth at the distances evaluated. Table VI shows that at 50 μm from the restoration margin, ionomeric materials showed shallower carious lesions when compared with temporary cement. Vitremer™ presented fewer caries at the vicinity of the restoration, in despite of Coltosol.

Figure 1 illustrates carious lesions around the different restorative materials, in both models of caries induction.

DISCUSSION

The present study used a pH-cycling regimen or a simple culture to evaluate cariostatic potential of restorative materials, and both chemical and biological models were capable of inducing caries around restorations. With the same goal and similar results, Francci et al.[15] and Grossman and Matejka[6] applied simple cultures and acid solutions. In the present study, restorative materials had similar behaviors in both caries models, even though they have offered different levels of severity. However, it was not possible to compare these experimental models, because they have distinct methodolo-

TABLE IV. Mineral Content (%, Means ± SD) Regarding the Interaction Between the Factors Group and Depth from Enamel Surface on Bacterial Caries Model (BG)

	DEPTH (μm)		
Group	30	60	90
NC—control	95.4 ± 3.8 aA	95.5 ± 4.4 aA	95.7 ± 3.6 aA
G5—resin-modified glass ionomer	70.2 ± 15.0 bB	83.5 ± 12.1 abA	89.9 ± 9.0 abA
G4—glass-ionomer cement	67.1 ± 14.1 bB	83.1 ± 13.0 bcA	87.2 ± 8.5 abA
G6—polyacid-modified Resin	52.6 ± 10.2 cC	72.7 ± 16.0 cdB	83.7 ± 10.9 abA
G1—temporary filling	52.1 ± 8.3 cC	69.7 ± 10.5 cdB	81.4 ± 8.1 bA
G2—silver amalgam	47.6 ± 6.1 cC	68.1 ± 8.7 dB	83.1 ± 8.5 bA
G3—composite resin	47.0 ± 11.9 cC	67.0 ± 11.5 dB	80.9 ± 13.9 bA

Note: $\alpha = 0.05$. Lower-case letters indicate statistical differences among Groups, and capital letters indicate statistical differences among Depths.

TABLE V. Carious Lesion Depths (μm, Means ± SD) Regarding the Interaction Between the Factors Group and Distance from Restoration Margin on Chemical Caries Model (CG)

	Distance (μm)			
Group	50	150	250	350
G3—composite resin	182.3 ± 33.2 aA	179.1 ± 34.1 aA	188.2 ± 41.0 aA	180.7 ± 32.1 aA
G2—silver amalgam	175.0 ± 64.0 abA	157.3 ± 50.0 abA	159.4 ± 48.2 abA	154.5 ± 45.4 abA
G6—polyacid-modified composite resin	154.1 ± 34.0 abA	156.2 ± 32.5 abA	155.9 ± 29.5 abA	153.9 ± 30.4 abA
G1—temporary filling	130.8 ± 41.4 abA	149.9 ± 56.5 abA	145.0 ± 53.8 abA	141.5 ± 55.4 abA
G4—glass-ionomer cement	127.8 ± 20.2 bA	121.1 ± 15.0 bA	130.8 ± 26.0 bA	124.7 ± 26.4 bA
G5—resin-modified glass-ionomer	114.6 ± 26.0 bA	124.5 ± 22.1 bA	121.5 ± 38.7 bA	133.7 ± 46.6 bA

Note: $\alpha = 0.05$. Lower-case letters indicate statistical differences among Groups, and capital letters indicate statistical differences among Distances.

gies. Therefore, this study focused on the analysis of carious lesion features.

The cariostatic effect was more evident for ionomeric materials in both models, because they maintained higher values of mineral content and lower depths of carious lesions. Nevertheless, these materials were not able to completely prevent caries development, in agreement with several *in vitro* studies.[7–15,18] Cariostatic properties of ionomeric materials could be explained by the high levels of fluoride releasing in the first days after the restoration procedure.[32,33] Released fluoride would act to inhibit demineralization and to activate remineralization at the tooth/restoration interface, and at the surface of adjacent enamel. In this context, ionomeric materials have demonstrated relevant clinical implications, particularly over caries-prone patients, because they help caries control in a localized manner.[3]

The amount of fluoride that is released from a restorative material is directly related to its cariostatic effect,[7] and is influenced by its physicochemical features.[34] This fact could explain the differences often found between cariostatic ef-

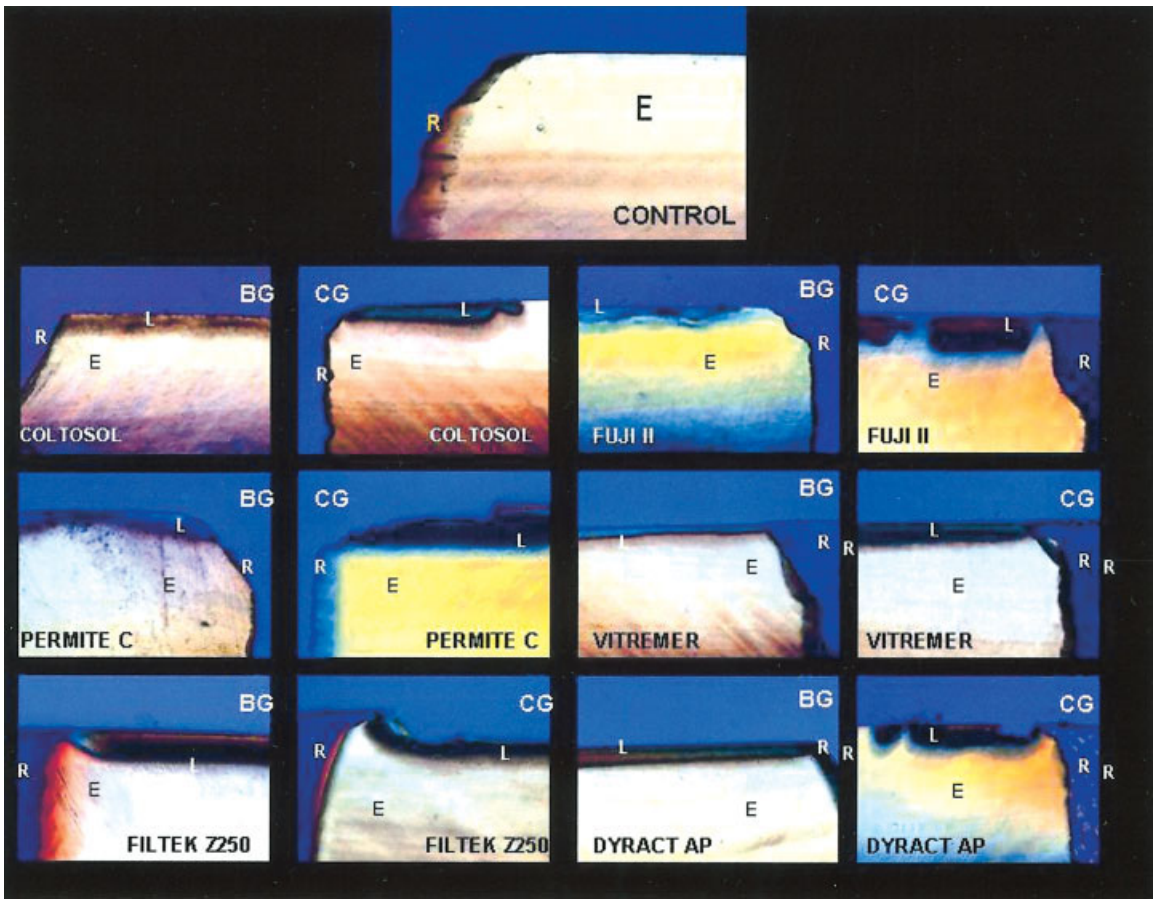

Figure 1. Standard 35-mm polarized light photomicrographs. (BG) bacterial caries model, (CG) chemical caries model, (R) restoration, (E) enamel, (L) carious lesion. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE VI. Carious Lesion Depths (μm, Means ± SD) Regarding the Interaction Between the Factors Group and Distance from Restoration Margin on Bacterial Caries Model (BG)

	Distance (μm)			
Group	50	150	250	350
G1—Temporary filling	126.5 ± 42.8 Aa	111.2 ± 43.4 aAB	105.9 ± 50.6 aB	120.1 ± 39.7 aAB
G3—Composite Resin	101.7 ± 23.4 abA	96.2 ± 24.0 abA	108.1 ± 28.9 aA	98.9 ± 20.6 aA
G2—Silver Amalgam	101.5 ± 40.6 abA	102.1 ± 47.8 abA	102.4 ± 29.4 aA	104.9 ± 45.5 aA
G6—Polyacid-modified composite resin	85.9 ± 22.2 abA	88.3 ± 21.9 abA	91.5 ± 28.9 aA	91.9 ± 28.6 aA
G4—Glass-ionomer cement	74.2 ± 41.6 bA	73.2 ± 44.0 abA	70.8 ± 44.3 aA	75.9 ± 48.4 aA
G5—Resin-modified glass-ionomer	56.2 ± 33.2 bB	60.9 ± 27.4 bAB	69.1 ± 21.2 aAB	79.1 ± 25.2 aA

Note: $\alpha = 0.05$. Lower-case letters indicate statistical differences among Groups, and capital letters indicate statistical differences among Distances.

fects of glass-ionomer cements (GIC) and fluoridated composite resins (FCR). The former release 10 to 50 times more fluoride than the latter,[32] because the fluoride on FCR remains retained on the resin matrix, inhibiting water sorption and consequent fluoride release.[34] Materials such as resin-modified glass ionomers (RMGI) and polyacid modified resins (PMR) could offer intermediate effects.[7,8,35] However, some authors report similarities on fluoride release between GIC and RMGI.

The current results show that RMGI had better cariostatic effects when compared to PMR and GIC, and agree with Tantbirojn et al.,[36] Torii et al.,[18] and Hara et al.[37] It confirms the observation that the presence of fluoride in the composition of a restorative material does not guarantee cariostatic effects over the adjacent enamel.[5] According to Carvalho and Cury[32] and Peng et al.,[33] recent PMR restorations release less fluoride than ionomeric materials (GIC and RMGI), because they are chemically similar to composite resins and FCRs.[33]

Secondary caries can occur as a result of two types of acid attack: on enamel surface and on cavity walls.[1,38] Preventing microleakage—which reduces acid diffusion over the tooth/restoration interface—is one of the ways of reducing secondary caries formation, in addition to fluoride release.[11] Therefore, the sealing ability of a material is also a desired property. Hsu et al.[39] had observed an inhibition zone on cavity walls adjacent to fluoridated restorative materials. However, thin inhibition zones were also observed around nonfluoridated adhesive materials, probably associated with the hybrid layer, which is acid resistant.[18]

In the present study, two restorative materials showed reduction of demineralization in the vicinity of the restoration: silver amalgam Permite C in CG (Table II) and composite resin Filtek Z250 in BG (Table III). Both results could be explained by the occurrence of an efficient marginal sealing as a function of silver amalgam corrosion[40] and acid resistance of the hybrid layer, respectively.[18] In regard to BG, although the literature supports that zinc ion can act as an antibacterial agent,[41] Coltosol, which has zinc in its content, did not show any cariostatic effect that could be indirectly related to an antibacterial action. However, in CG, this cement showed less demineralization when compared to Filtek Z250 and Permite C at 60 and 90 μm from the enamel surface (Table II). Chemical elements of this cement might have interfered on the physicochemical aspects of carious development *in vitro.* Mayer et al.[42] and Davey et al.[43] studied incorporation of zinc on apatite and concluded that zinc ions could or might substitute calcium in hydroxyapatite. Shinkai et al.[44] did not find differences of cariostatic effects provided by ionomeric adhesive cement or zinc phosphate cement on enamel or dentin adjacent to metallic restorations. More studies are necessary to clarify the interference of zinc over carious-lesion formation *in vitro*.

Subsuperficial caries-like lesions were visualized under polarized light microscopy, in a pattern similar to that found by Gilmour et al.,[12] Gilmour and Edmunds[5] and Torii et al.[18] Measurement of lesion depth has confirmed some cariostatic effect provided by GIC (Fuji II) and RMGI (Vitremer), in both caries models. In CG, composite resin (Filtek Z250) had the highest depth values (Table V) and this result agrees with that of Hicks et al.,[35] who used the same material, but induced caries in a static chemical model for 8 weeks. In the bacterial model (BG), RMGI showed higher cariostatic effect in the vicinities of the restoration, in contrast with the temporary cement (Coltosol) (Table VI).

Considering the current results, it can be assumed that marginal sealing and fluoride release are important to reduce or inhibit secondary caries formation. Ideally, a unique material should gather these capacities and help prevent and control disease development in caries-prone individuals.

CONCLUSIONS

From the present results it can be concluded that (a) both chemical and biological models of caries induction were able to produce secondary caries, and (b) ionomeric materials—Vitremer and Fuji II—showed a superior cariostatic effect when compared to the other restorative materials, in both caries models.

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