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Clinical Report

In Vitro-evaluation of Secondary Caries Formation around Restoration

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Abstract

The objective of this *in vitro* study was to evaluate demineralization around restorations. Class V preparations were made on the buccal and lingual surfaces of each tooth. TPH (Group 1), Fuji II LC (Group 2), Tetric (Group 3), Dyract (Group 4), GS 80 (Group 5) and Chelon Fil (Group 6) were randomly placed in equal numbers of teeth. The teeth were submitted to a pH-cycling model associated with a thermocycling model. Sections were made and the specimens were examined for the presence of demineralization under polarized light microscopy. Demineralization was significantly reduced with Chelon Fil (Group 6). Furthermore, a similar inhibitory effect on the development of demineralization was observed in Groups 2, 4 and 5.

Key words: Fluoride—Demineralization—Polarization microscopy

Introduction

The development of marginal alterations and secondary caries around composite resin restorations has been documented[9,10,25] and is considered a major cause of restorative failure and replacement[7,20,25]. The key factors in preventing secondary caries around restorations are marginal integrity of the restoration[14], durable adhesion[15], the physical properties of the restorative materials themselves, and oral hygiene[13]. However, replacement of restorations due to secondary caries is still a problem in restorative dentistry[1,6].

Fractures, insufficient marginal adaptation and excess restorative material, failures that are frequently observed in restorations, result in microleakage. Openings permit penetration of oral fluids and microbiological agents along the interface between dental tissue and restoration, which may lead to the development of secondary caries[5].

It is currently believed that the cariostatic effect of fluoride is enhanced in lower, yet permanent, concentrations in the oral environment, so incorporating fluoride into

restorative materials is of special interest[19,22]. Fluoridated materials offer a potential source of this element, which would expand the spectrum of prevention in restorative dentistry. The use of fluoride to demineralize and remineralize early enamel carious lesions directly interferes with the evolution of caries[1,6,19]. It is accepted that part of the fluoride present in restorative materials may be directly released onto areas of risk, such as restoration margins, where secondary caries may develop[1,19,22]. Thus, the use of fluoride may be considered an additional method of preventing caries.

Glass ionomer cement, initially described in the early 1970s, is regarded as the material of choice in cases where cavity sealing and prevention of secondary caries are desirable, due to its properties of adhesion to dental structures and its high rate of fluoride release[16,17,22].

However, the predominance of resin composites among esthetic restorative materials is evident, especially due to their highly satisfactory esthetics and easy manipulation. The greatest difficulty with this material is the occurrence of secondary carious lesions adjacent to the restoration, which is observed less frequently in teeth restored with glass ionomer[1,13,17,22].

In an attempt to obtain an ideal material that would combine the good properties of both resin composites and glass ionomer cements, new materials have been developed, such as resin-modified glass ionomers and polyacid-modified resin composites.

Fluoride-releasing restorative materials are important in inhibiting secondary carious lesions, especially in patients at high risk[1,22] and those with high caries activity. Therefore, a comparative evaluation of the cariostatic action of such materials together with that of conventional resin composites is required to enhance our knowledge of their behavior. The aim of this study was to evaluate marginal demineralization around restorations using polarized light microscopy.

Materials and Methods

Thirty extracted human third permanent molars, which had been cleaned and stored in 2% formaldehyde solution (pH 7.0) at room temperature, were utilized.

Class V preparations were made in the middle third of both buccal and lingual surfaces of each tooth with a water-cooled high-speed handpiece and a # 16F diamond bur (KG Sorensen, Barueri, SP, Brazil). The approximate dimensions of the 60 formed cavities were: 1.5 mm in depth and 1.2 mm in diameter. The bur was discharged after every 5 cavities. No bevel was made on the cavo-surface angle.

The tested restorative materials placed in the prepared teeth are shown in Table 1.

Prior to restoration placement, the teeth were individually immersed in deionized water and randomized into 6 treatment groups of 10 specimens each (Table 2). The technique for application of all materials followed the manufacturers' instructions. Teeth restored with composite resin (TPH) and Polyacid modified composite resin (Dyract) after acid

Table 1 Materials used in each group

Material	Group		Manufacturer	Lot #
Composite resin (TPH)	1	Light-cured	Dentsply	C830475
Resin modified glass-ionomer (Fuji II LC)	2	Light-cured	G.C. American	310351
Fluoride-containing composite resin (Tetric)	3	Light-cured	Vivadent	580356
Polyacid modified composite resin (Dyract)	4	Light-cured	Dentsply	C940756
GS 80	5	—	SDI	091316
Glass-ionomer (Chelon Fil)	6	Self-cured	ESPE	Z098

etching (37% phosphoric acid), received 2 applications of adhesive system Prime & Bond (Dentsply Caulk, Milford, DE, USA). The incremental technique was used for these restorative materials. Only specific pre-treatment was applied prior to the restorative procedure (Table 2).

All restored teeth were stored in a humid environment for 48 hrs to allow completion of reactions such as polymerization, crystallization and gelation.

Restorations in groups 1, 2, 3, 4 and 6 were polished with Sof-Lex Pop On (3M/ESPE Dental Products St. Paul, USA) disks; amalgams were finished with a low-speed handpiece and flame-shaped burs, followed by abrasive rubber (KG Sorensen, Barueri, SP, Brazil).

Round segments (4 mm in diameter) of adhesive tape (3M/ESPE Dental Products, St. Paul, USA) were placed on the restorations. Then, all tooth surfaces were coated with acid-resistant varnish. A nylon wire was fixed at each tooth to facilitate its handling. The tape was removed from the tooth as soon as the varnish dried, leading to exposure of the restoration as well as of 1 mm of dental tissue around the restorative material.

Each group of teeth was immersed in 100 ml synthetic acid demineralizing solution (2.0 mmol l^{-1} Ca, 2.0 mmol l^{-1} P, in 75 mmol l^{-1} acetate buffer, pH 4.3) for 4 hrs. The teeth were then immersed in 20 ml remineralizing solution (1.5 mmol l^{-1} Ca, 0.9 mmol l^{-1} P, 0.1 buffer, pH 7.0) for 20 hrs. All teeth were rinsed thoroughly with deionised water and dried with absorbent paper before and after the dematerializing period. Continuous cycles of demineralization and remineralization were carried out for 28 days. During the *in vitro* demineralization/remineralization cycling model, the teeth in each group were subjected to thermocycling for 100 cycles. A complete cycle consisted of 90 sec at 37°C, 90 sec at 55°C and 90 sec at 5°C.

After 28 days, the teeth were individually fixed in acrylic resin blocks and sectioned to a thickness of about 500 μm using a diamond sectioning saw (Isomet 2000-Buehler UK LTD, Lake Bluff, USA). The sections were then ground to a thickness of approximately 100 μm. After 48 hours of imbibition in deionised water, the sections were examined and photographed using polarized light microscopy (Axiophot-ZEISS DSM-940 A, Oberkochen, Germany).

Readings were taken at the R1 and R2 regions, around the occlusal and cervical margins of the restoration, along the interface between the dental tissue and the restoration (P1) and at 100 μm (P2), 200 μm (P3) and 300 μm (P4) from the interface between the

Table 2 Groups according to treatment, time and materials

Group	Number of specimens	Pre-treatment	Time	Material
1	10	Enamel/Dentin 37% Phosphoric Acid (gel)	15 sec	Prime&Bond TPH
2	10	Enamel/Dentin G.C. Conditioner/Poliacrilic Acid	15 sec	Fuji II LC
3	10	Enamel/Dentin 37% Phosphoric Acid (gel)	15 sec	Tetric
4	10	Enamel/Dentin Dyract Primer PSA	30 sec	Prime & Bond Dyract
5	10	Enamel/Dentin 1.23% APF	60 sec	GS-80
6	10	Enamel/Dentin 25% Poliacrilic Acid	15 sec	Chelon Fil

dental tissue and the restoration. Demineralization was determined by depth (Fig. 1).

Results

Mean depths of demineralization were calculated (Fig. 1) for each group and position (Table 3).

Application of the Tukey test at the 5% level, as shown in Table 3, revealed that interaction between group 6 and any of the positions analyzed clearly demonstrated a higher resistance to the development of demineralization, since the interactions observed presented the lowest mean depths of demineralization on the enamel surface for all positions investigated. Groups 2 and 4, where fluoride release materials were used, showed the strongest effect in the P1 position. However, no effect was observed in Group 1 in any position, with this group exhibiting the highest mean depth of demineralization. Furthermore, P1 was deeper than P4 in Group 1, which was not the case in the other groups.

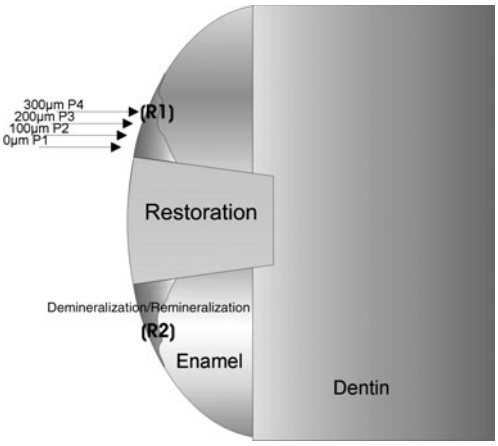

Fig. 1 Positions P1, P2, P3, P4 in terms of demineralization/remineralization development in regions R1 and R2

Discussion

Control of carious lesions is mainly related to the presence of fluoride in the oral cavity, and may not be considered as directly dependent on the amount incorporated by the tooth[6,17], since the main mechanism of action of fluoride is dynamic, inhibiting demineralization and enhancing remineralization[4,19]. Therefore, the constant presence of fluoride in the oral cavity, in saliva or oral fluids, dental plaque and enamel, may control or even inhibit the appearance of secondary carious lesions, as well as lead to arrest of existing lesions, preventing progression of incipient lesions to formation of cavity[6,22].

It should be noted that the model of caries development adopted in this *in vitro* study is similar to that advocated by Featherstone *et al.*[4], which assumes a correlation with the onset and progression of carious lesions *in vivo* where there is a high risk of caries. However, in terms of the cariogenic challenge employed in the present study, besides utilization of pH cycling, as suggested by Featherstone *et al.*[4], thermal cycling was also

Table 3 Mean values (standard deviation) of demineralization depth (μm) according each positions

Groups	0 μm (P1)	100 μm (P2)	200 μm (P3)	300 μm (P4)
1	190.5 (65.21)^{a}	176.5 (43.72)^{a}	173.0 (40.84)^{a}	158.0 (31.02)^{a}
2	44.5 (36.76)^{c}	77.5 (34.99)^{d}	95.5 (25.19)^{c}	96.5 (23.95)^{b}
3	126.0 (21.32)^{b}	132.0 (24.06)^{bc}	136.0 (24.59)^{ab}	136.0 (24.36)^{a}
4	69.5 (31.04)^{c}	102.0 (20.17)^{cd}	115.5 (14.62)^{bc}	121.0 (13.08)^{ab}
5	147.0 (39.33)^{b}	154.5 (37.66)^{ab}	158.0 (25.76)^{a}	157.5 (21.99)^{a}
6	18.5 (30.37)^{d}	32.5 (38.17)^{e}	38.6 (39.98)^{d}	40.1 (41.41)^{c}

Minimum significant difference at the 5% level. Means with different letters indicate difference between means.

conducted to approach the real conditions of the oral cavity.

Polarized light microscopy revealed the depth of demineralization in the dental enamel. Table 3 shows the mean values for group and position. The values presented in Table 1 represent interaction at the P1 position (interface) in each group, and show that the material employed in Group 6 (CHELON FIL) performed best in control of demineralization, followed by the materials in Groups 2 (FUJI II LC), 4 (DYRACT), 3 (TETRIC) and 5 (fluoride solution was applied as pretreatment before amalgam restoration), and finally Group 1 (TPH), which exhibited the worst performance.

Similarly, as shown in Table 3, interaction in each group at positions P2, P3 and P4 demonstrated the same performance as the material employed in Group 6 (CHELON FIL) for control of demineralization. However, the other groups revealed a tendency toward similar outcomes in terms of cariostatic potential, proportional to the distance from the tooth/restoration interface[25]. These findings further reinforce the belief that the amount of fluoride present in the material, as well as its concentration and release, are important aspects in the reduction of demineralization.

This leads to the assumption that the cariostatic property of CHELON FIL (Group 6) may be explained by the intensive fluoride release of this material[17,19,25], and the amount of fluoride released depends on its concentration in the material; in addition, ionic fluoride is present in this material, which favors its release[17].

This is corroborated by the mean values observed in this study, which demonstrated that the material employed in Group 6 (CHELON FIL), with high fluoride release and bonding to the tooth structure, presented a better performance for control of demineralization. Furthermore, these results for Group 6 (CHELON FIL) (Fig. 2) are in agreement with the findings of Hicks *et al.*[8]

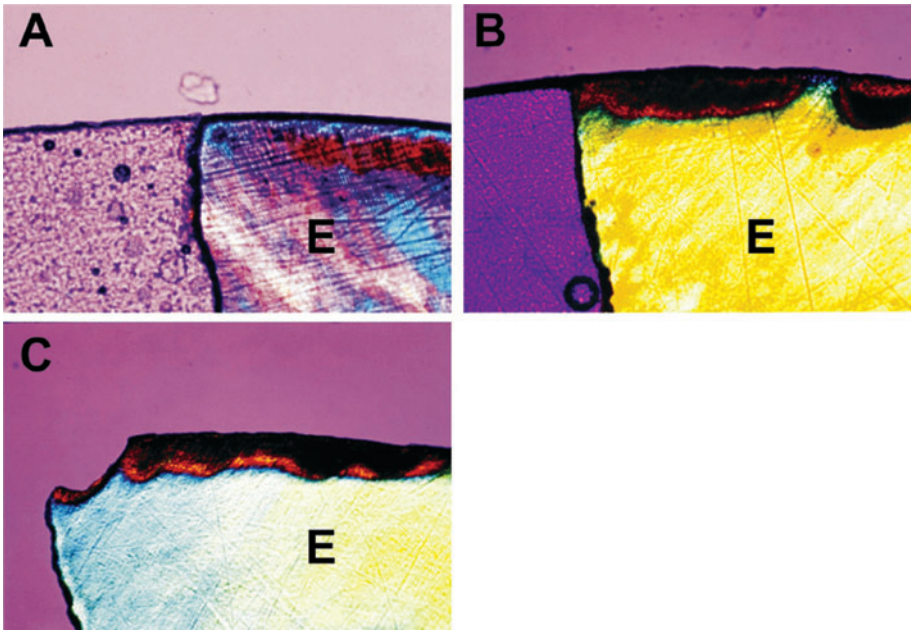

Fig. 2 Longitudinal sections of enamel-resin interface

(A) Photomicrograph (40×) of interface of one specimen from Chelon Fil restoration illustrating integrity of restoration surface. (B) Photomicrograph (40×) of Dyract restoration illustrating marginal caries formation (P2). (C) Photomicrograph (40×) of GS-80 restoration illustrating marginal caries formation. (E) Enamel.

and Purton & Rodda[21] in studies using polarized light microscopy which revealed formation of mild demineralization and that the establishment and progression of demineralization in these cases was reduced, possibly due to the precipitation of calcium and phosphate triggered by the high fluoride release of these materials. This may be explained by the intensive fluoride release of this material, which depends on its concentration in the material and especially on the presence of ionic fluoride, which enhances its release.

In Group 2 (FUJI II LC), its performance was inferior to that in Group 6 (CHELON FIL), thereby demonstrating that resin-modified glass ionomers also present an anticariogenic action, but are inferior when compared to conventional glass ionomer. Therefore, the results obtained in this study for Group 2 (FUJI II LC) are in agreement with the findings of Croll *et al.*[2] and Pin *et al.*[19], who achieved similar results, observing a significant reduction in demineralization, assigned to the significant fluoride release of this material[11], because of the spontaneous acid-base reaction, which leaves free fluoride ions in the bulk to be released.

The performance presented by the polyacid-modified resin composite in Group 4 (DYRACT-Fig. 2) also demonstrated a moderate ability to inhibit demineralization, which may be explained by the different fluoride release of polyacid-modified resin composite compared to conventional glass ionomers, or even to resin-modified glass ionomers[3,22,23]. According to the manufacturer, curing of the material with posterior water absorption is required for the occurrence of fluoride release, since it favors an acid-base reaction and fluoride ion release[23]. However, this late acid-base reaction may considerably limit fluoride release, even in the demineralization process.

The material TETRIC in Group 3 demonstrated that composites are not effective in caries inhibition[19]. Even though the fluoridated resins currently available present fluoride release, they do not maintain this pattern to favor a considerable incorporation of fluoride by the tooth or even reduce the mineral loss close to restorations; thus the present outcomes are in agreement with those of Pin *et al.*[19].

Group 5 (GS 80-Fig. 2) revealed that the amalgam did not totally inhibit demineralization, being similar to the performance presented by FUJI II LC (Group 2), DYRACT (Group 4) and TETRIC (Group 3). Similarly, the results demonstrated that the ability of topical fluoride application before insertion of amalgam was not significant enough to prevent the occurrence of caries when compared to CHELON FIL (Group 6).

Within this context, Pimenta *et al.*[18] (1998) also evaluated the effectiveness of application of acidulated phosphate fluoride before accomplishment of amalgam restorations compared to the bonded amalgam technique and observed that application of this solution in the cavity was unable to effectively inhibit demineralization, yet reduced its formation at the tooth/restoration interface.

Group 1 (TPH) revealed higher values of depth of demineralization. Since it represented the control group, its performance is in agreement with the findings of Purton and Rodda[21], who observed that non-fluoridated composite resins do not present any potential to inhibit demineralization.

Another factor, besides fluoride release, should be considered. Thermal cycling can induce interface degradation in materials that use adhesive technique[12]. This process can increase microleakage with some factors. The principal factor is the difference in the thermal expansion coefficient between restorative material and tooth. This difference can overload the interface during thermal cycling and lead to gap formation[12]. Therefore, Groups 1, 3 and 4 may have been influenced by thermal cycling, principally with composite resin, which showed a deeper P1 than P4 (Table 3). Possibly, the fluoride release capacity contributed to reduced demineralization in Groups 3 and 4 on P1 distance when they are compared to the Group 1 (Table 3).

The present findings agree with Ten Cate[24] (1990), who stated that enamel remineraliza-

tion requires the presence of partially demineralized hydroxyapatite crystals and is dependent on the degree of saturation of the area. This also corroborates the results found for material CHELON FIL (Group 6), which may be related to its higher fluoride release compared to the other materials, presenting a more effective action in the process of inhibition and/or progression of demineralization. It should be noted that materials presenting low fluoride release, such as FUJI II LC and DYRACT (Groups 2 and 4), were not effective at distant sites, but were effective on the tooth/restoration interface, confirming that efficiency for control of distant lesions requires topical fluoride application and utilization of fluoridated mouth rinses and dentifrices, which allow the constant presence of low concentrations of fluoride in the oral cavity, which is more effective than fluoride release by materials.

These results demonstrated that control of demineralization depends on the ability of materials to release fluoride ions; however, the clinical individuality of each patient should be considered for indication and application of a material or restorative technique.

Conclusion

The present results indicate the following:

(1) Conventional glass ionomer was more effective against progression of demineralization on the enamel surface at all distances and depths analyzed when compared to fluoridated resin materials such as resin modified glass ionomer and polyacid modified composite resin, which were only effective in the initial position (P1).
(2) The highest mean depths of demineralization were observed after utilization of non-fluoridated resin material, demonstrating the inefficiency of its potential for inhibition of demineralization.
(3) Application of acidulated phosphate fluoride solution on the cavity before insertion of amalgam was unable to effectively inhibit the occurrence of demineralization.

References

1) Attar N, Onen A (2002) Fluoride release and uptake characteristics of aesthetic restorative materials. J Oral Rehabil 29:791–798.
2) Croll TP, Helpin ML, Donly KJ (2000) Vitremer restorative cement for children: three clinicians' observations in three pediatric dental practices. ASDC J Dent Child 67: 374, 391–398.
3) de Araujo FB, Garcia-Godoy F, Cury JA, Conceicao EN (1996) Fluoride release from fluoride-containing materials. Oper Dent 21: 185–190.
4) Featherstone JDB, O'Really MM, Shariati M, Brugler S (1986) Enhancement of remineralization *in vitro* and *in vivo*; Factors Relating to Demineralization and Remineralization of the Teeth, Leach SA, 1st ed., pp.23–34, Oxford.
5) Fontana M, Dunipace AJ, Gregory RL, Noblitt TW, Li Y, Park KK, Stookey GK (1996) An *in vitro* microbial model for studying secondary caries formation. Caries Res 30:112–118.
6) Fontana M, González-Cabezas C, Haider A, Stookey GK (2002) Inhibition of secondary caries lesion progression using fluoride varnish. Caries Res 36:129–135.
7) Hickel R, Manhart J (2001) Longevity of restorations in posterior teeth and reasons for failure. J Adhes Dent 3:45–64.
8) Hicks MJ, Flaitz CM, Silverstone LM (1986) Secondary caries formation *in vitro* around glass ionomer restorations. Quintessence Int 17:527–532.
9) Kidd EA, Beighton D (1996) Prediction of secondary caries around tooth-colored restorations: a clinical and microbiological study. J Dent Res 75:1942–1946.
10) Kidd EA, Toffenetti F, Mjor IA (1992) Secondary caries. Int Dent J 42:127–138.
11) Kotsanos N (2001) An intraoral study of caries induced on enamel in contact with fluoride-releasing restorative materials. Caries Res 35: 200–204.
12) Mitsui FH, Peris AR, Cavalcanti AN, Marchi GM, Pimenta LA (2006) Influence of thermal and mechanical load cycling on microtensile bond strengths of total and self-etching adhesive systems. Oper Dent 31:240–247.
13) Mjor IA, Toffenetti F (2000) Secondary caries: a literature review with case reports. Quintessence Int 31:165–179.
14) Nakabayashi N (1985) Bonding of restorative materials to dentine: the present status in Japan. Int Dent J 35:145–154.
15) Okuda M, Pereira PN, Nakajima M, Tagami J, Pashley DH (2002) Long-term durability of resin dentin interface: nanoleakage vs. micro-

tensile bond strength. Oper Dent 27:289–296.
16) Okuda M, Pereira PN, Nikaido T, Tagami J (2003) Evaluation of *in vitro* secondary caries using confocal laser scanning microscope and X-ray analytical microscope. Am J Dent 16: 191–196.
17) Pereira PN, Inokoshi S, Tagami J (1998) *In vitro* secondary caries inhibition around fluoride releasing materials. J Dent 26:505–510.
18) Pimenta LA, Fontana UF, Cury JA, Serra MC, Elderton RJ (1998) Inhibition of demineralization *in vitro* around amalgam restorations. Quintessence Int 29:363–367.
19) Pin ML, Abdo RC, Machado MA, da Silva SM, Pavarini A, Marta SN (2005) *In vitro* evaluation of the cariostatic action of esthetic restorative materials in bovine teeth under severe cariogenic challenge. Oper Dent 30:368–375.
20) Prati C, Chersoni S, Suppa P, Breschi L (2003) Resistance of marginal enamel to acid solubility is influenced by restorative systems: an *in vitro* scanning electron microscopic study. Clin Oral Investig 7:86–91.
21) Purton DG, Rodda JC (1988) Artificial caries around restorations in roots. J Dent Res 67: 817–821.
22) Serra MC, Cury JA (1992) The *in vitro* effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. Quintessence Int 23:143–147.
23) Suljak JP, Hatibovic-Kofman S (1996) A fluoride release-adsorption-release system applied to fluoride-releasing restorative materials. Quintessence Int 27:635–638.
24) Ten Cate JM (1990) *In vitro* studies on the effects of fluoride on de- and remineralization. J Dent Res 69. Spec No:614–619; discussion 634–636.
25) Tsuchiya S, Nikaido T, Sonoda H, Foxton RM, Tagami J (2004) Ultrastructure of the dentin-adhesive interface after acid-base challenge. J Adhes Dent 6:183–190.

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