

In vitro effect of restorative, cementing and lining materials on *Streptococcus mutans*

Efeito in vitro de materiais restauradores, cimentantes e forradores sobre Streptococcus mutans

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Abstract

One of the principles of dental practice is the prevention of caries in the margins of restorations. Since *Streptococcus mutans* are the main cariogenic species, the antibacterial activity of dental materials is clinically relevant. The purpose of this paper was to verify the action of different dental materials on *S. mutans* by means of the agar diffusion test. Test specimens were made from eleven materials (Vidrion CTM, Vidrion FTM, Vidrion NTM, Vidrion RTM, VitremerTM, VitrebondTM, Maxxion RTM, zinc phosphate cement, IRMTM, Panavia FTM, and the Filtek Z-250TM) for microbiological testing both immediately (t0) and 24h (t1) after curing. Plates containing BHI agar were incubated at 35.5 °C for 24h, and the halos of bacterial growth inhibition around the test specimens were measured and analyzed. At t0, the inhibition halos were induced by VitremerTM, MaxxionRTM, IRMTM, Panavia FTM and VitrebondTM. VitrebondTM exhibited a statistically higher antibacterial activity than the others ($p < 0.001$), and was the only material that kept its activity even after 24h subsequent to curing (t1). The glass-ionomer cement VitrebondTM inhibits the bacterial growth and is potentially capable of decreasing the risk of secondary caries when used as a cavity liner; the other materials did not either inhibit or keep inhibiting the growth of *S. mutans* at 24h after curing.

Key words: *Streptococcus mutans*. Dental materials. Dental caries.

Introduction

One of the purposes of dentistry is to prevent the occurrence of dental caries, and another one, is to avoid the recurrence of marginal caries, because these are the main factors that influence the duration of dental restorations¹. For the prevention of secondary caries it is essential to consider their causes and the factors that may contribute to avoid them². Therefore, defective restorations associated with dental biofilms, containing cariogenic species such as *S. mutans*, are etiologic agents of secondary caries³. Thus, the antibacterial activity of restorative materials, during and after their curing, is an important clinical property¹. Glass-ionomer cements have been used for more than 20 years, and one of their greatest advantages is their anticariogenic potentiality due to their low pH while curing⁴, and to the release of fluorides and other ions, such as silver and calcium¹. Fluorides probably inhibit de-mineralization and enhance dental remineralization⁵. The efficacy of these materials in preventing bacterial *S. mutans* colonization of restorations is, however, controversial. Even the fluorides released by glass-ionomer cements were not effective in preventing the adherence and viability of *S. mutans* on the surface of restorations⁶. The retention and biofilm formation by *S. mutans* on the surface

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of conventional glass-ionomer cements, resin-modified glass-ionomer cements and hybrid composite resins were evaluated⁷, and the authors did not observe any significant difference between these materials. Although some dental adhesives can release fluorides, they do not prevent secondary caries as much as glass-ionomer cements do². Therefore, the purpose of this paper was to evaluate the *in vitro* effect of several restorative, cementing and lining dental materials on the growth of *S. mutans*, immediately, and after 24h subsequent to curing.

Materials and method

The restorative materials tested are listed in Table 1.

Table 1 - Restorative materials tested for the inhibition of *S. mutans* growth

Materials	Composition	Manufacturer
IRM™	Zinc oxide and eugenol reinforced by polymers	Dentsply Indústria e Comércio Ltda, Petrópolis, RJ, Brasil
Zinc Cement™	Zinc oxide; magnesium oxide; phosphoric acid; aluminum hydroxide; distilled water	S.S. White Artigos Dentários Ltda, Rio de Janeiro, RJ, Brasil
Panavia™	10-Methacryloyloxydecyl dihydrogen phosphate, hydrophobic aromatic dimethacrylate; hydrophobic aliphatic dimethacrylate; hydrophilic aliphatic dimethacrylate; sodium fluoride	Kuraray Medical Inc, Kurashiki, Okayama, Japan
Vidrion C™	Fluorsilicate of sodium; calcium and aluminum; polyacrylic acid; tartaric acid; distilled water	S.S. White Artigos Dentários Ltda, Rio de Janeiro, RJ, Brasil
Vidrion F™	Fluorsilicate of sodium; calcium and aluminum; barium sulphate; polyacrylic acid; ferrous oxide; tartaric acid; distilled water.	S.S. White Artigos Dentários Ltda, Rio de Janeiro, RJ, Brasil
Vidrion R™	Fluorsilicate of sodium; calcium and aluminum; polyacrylic acid; tartaric acid; distilled water.	S.S. White Artigos Dentários Ltda, Rio de Janeiro, RJ, Brasil
Vidrion N™	Fluorsilicate of sodium; calcium and aluminum; barium sulphate; polyacrylic acid; ferrous oxide; silver; copper; tin; zinc; tartaric acid; distilled water.	S.S. White Artigos Dentários Ltda, Rio de Janeiro, RJ, Brasil
Vitremer™	Fluoroaluminosilicate glass; microencapsulated potassium; persulfate and ascorbic acid; aqueous solution of a polycarboxylic acid modified; water; HEMA and photoinitiators.	3M ESPE, St. Paul, MN, USA
Vitrebond™	Fluoroaluminosilicate glass; modified polyacrylic acid with pendant methacrylate groups; HEMA, water and photoinitiator.	3M ESPE, St. Paul, MN, USA
Filtek Z-250™	Bis-GMA; Bis-EMA; UDMA Zirconium/silica, 60% in volume	3M ESPE, St. Paul, MN, USA
Maxxion R™	Fluoroaluminosilicate glass; polycarboxylic acid; calcium fluoride and water.	Dentscare Ltda, Joinville, SC, Brasil

Making of the test specimens

The manufacturers' instructions were followed for the making of test specimens. For the obtainment of samples with identical dimensions, each material was inserted into a teflon matrix with a diameter of 3 mm and thickness of 2 mm. Triplicates of test specimens of the eleven materials (Vidrion C™; Vidrion F™; Vidrion N™; Vidrion R™; Vitremer™; Vitrebond™; Maxxion R™; zinc phosphate cement; IRM™; Panavia F™, and the Filtek Z-250™) were then submitted to microbiological tests to verify the inhibitory activity of bacterial growth with the standard strain of *S. mutans* (ATCC 25175). The test specimens were divided into two groups for each material: Group I - samples submitted to tests immediately after curing; Group II - 24h after curing, stored in sterile deionized water until the testing time.

Microbiological tests

The inoculum of the *S. mutans* was prepared with turbidity equivalent to 0.5 McFarland scale (1.5×10^8 CFU/mL). Later, in a laminar flux chamber (VECO™, model VLSF 12, series EL 7838, Campinas, São Paulo, Brasil), plates containing BHI agar (Merck™, Darmstadt, Germany) were seeded with 100 µl of the inoculum. Wells 2 mm deep and a little over 3 mm in diameter were punched in the agar in order to receive the test specimens in close contact. Each Petri dish received three test specimens of different materials, besides the positive control (10 µg vancomycin disk), and a negative control (sterile filter paper disk), for the samples of Group I (t0) and Group II (t1). The test specimens and the control disks were placed on the surface of the inoculated and dried culture medium at a distance of approximately 3 cm between them and 1.5 cm from the dish border. The Petri dishes were then incubated in microaerophilia at 37 °C for 2 days⁸. The diameters of the halos of bacterial growth inhibition were measured and photographed. The mean values of the inhibition halos of each material were submitted to statistical tests (ANOVA, $p < 0.01$) to determine the difference between the materials and the two groups.

Results

Vitremer™, MaxxionR™, IRM™, Panavia F™ and Vitrebond™ exhibited inhibition halos in t0. Vitrebond™ showed statistically higher antibacterial activity than the other ones (Kruskal-Wallis, $p < 0,001$), and was the only material which kept its activity even after 24h post polymerization. In addition, its inhibition halo was similar to that of the positive control in both t0 and t1. The other materials (Vidrion™ C, F, R, N, zinc cement, Filtek Z-250™) showed no antibacterial effect in any of the times studied (Fig. 1 to 3; Tab. 2).

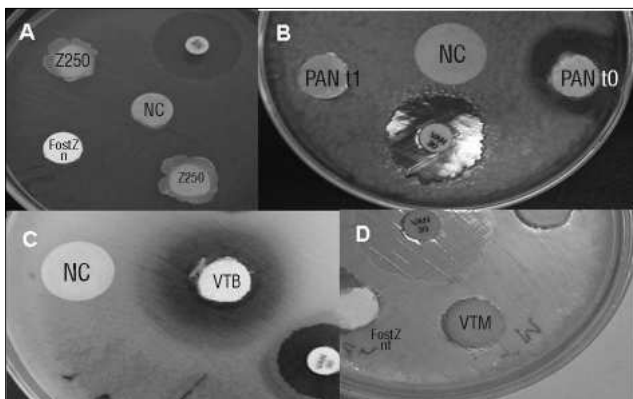


Figure 1 - Bacterial growth around dental materials. **A.** Filtek Z250™ (t1) shows a very high bacterial growth around the test specimens; **B.** Panavia™ (t0 e t1); **C.** Vitrebond™ (t1). **D.** Vitremer™ (t1)

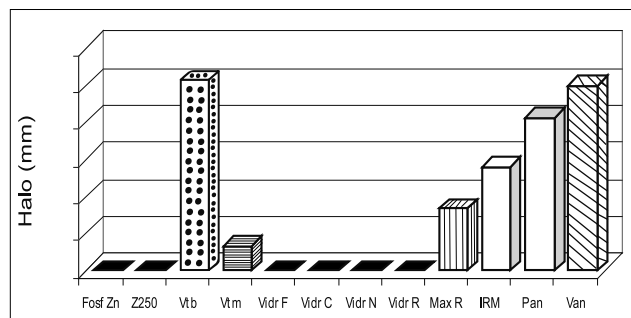


Figure 2 - Inhibition halos of *S. mutans* growth induced by dental materials (t0)

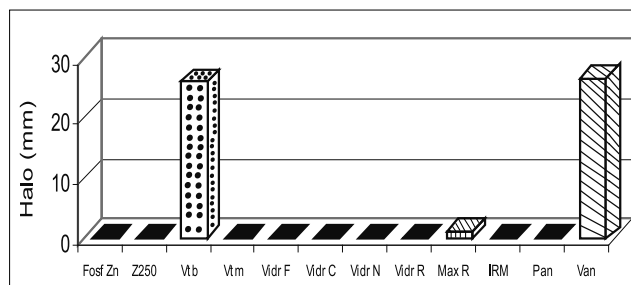


Figure 3 - Inhibition halos of *S. mutans* growth induced by dental materials (t1)

Fosf Zn (zinc cement); Z250™ (composite resin); Vtb (Vitrebond™); Vtm (Vitremer™); Vidr F (Vidrion™ F); Vidr C (Vidrion™ C); Vidr N (Vidrion™ N); Vidr R (Vidrion™ R); Max R (Maxxion™ R); IRM (cement IRM™); Pan (Panavia F™); Van (positive control).

Table 2 - Diameter (average) of the inhibition halos of *S. mutans* growth induced by dental materials (mm)

Materials	t0	t1
Zn phosphate™	Zero	Zero
Resin Z-250™	Zero	Zero
Vitrebond™	25.6	26
Vitremer™	3.3	Zero
Vidrion F™	Zero	Zero
Vidrion C™	Zero	Zero
Vidrion N™	Zero	Zero
Vidrion R™	Zero	Zero
Maxxion R™	8.3	1.3
IRM™	14	Zero
Panavia™	20.6	Zero
Negative control	Zero	Zero
Vancomycin	25	26.6

Discussion

Dental materials, that release fluoride, have been shown to be effective in caries inhibition around restorations, but adhesive materials would also be effective by sealing and protecting cavity margins from acidic demineralization⁹. However,

these authors did not observe the effect of marginal sealing of composite/dentin adhesive restorations in inhibit the formation and the progress of artificial root caries. They showed that the glass-ionomer cement (Chelon-Fill™) had a higher root caries preventive effect than the composite/dentin adhesive restorations (Z100™/SingleBond™). Therefore, it is possible that a good sealing between tooth/restoration is not sufficient to inhibit secondary caries, if the dental material does not release sufficient levels of fluorides.

Of the eleven materials tested in the present work, Vitremer™, Maxxion R™, IRM™, Panavia F™ and Vitrebond™ showed inhibition halos of *S. mutans* growth immediately after polymerization, but, statistically, Vitrebond™ exhibited a significantly higher activity than the others. In addition, Vitrebond™ was the only material that kept inhibitory activity at 24h after polymerization, and its inhibition halo was similar to that of the positive control. In the same manner, Vermeersch et al.¹ (2005) used the agar diffusion test to evaluate the action of different restorative materials on *S. mutans*, during and immediately after polymerization, and reported that Vitrebond™ showed a higher antibacterial activity than the others. According to these authors, the antibacterial effect of Vitrebond™ could be due not only to the release of fluorides and low pH, but mainly to the release of the DPICI initiator. A higher antibacterial activity of Vitrebond™, when compared to several restorative materials, on 6 strains of *S. mutans*, 6 of *S. sanguis* and 6 cultures of debris from caries lesions also was reported¹⁰. The antimicrobial activity *in vitro* of the glass-ionomer cement Vitrebond™ 3M against *S. mutans* and other microorganisms also was reported¹¹.

The ability of different adhesive materials to prevent microleakage in bonded amalgam restorations was analyzed¹², and the authors concluded that bonded amalgam was an effective technique, since all materials tested reduced microleakage compared to the control. Also, one material (Vitrebond™) provided total prevention of microleakage in all specimens. However, despite the absence of leakage in the Vitrebond™ group, one limitation of this material could be the higher solubility of the material in relation to other adhesive materials, which could reduce the effectiveness of the technique with aging. However, any procedure with the objective to offer resistance to oral microorganism attack will improve clinical performance of the restorations.

The inhibitory activity of photopolymerizing materials on *S. mutans* was tested¹ and an inhibitory halo around Vitremer™ immediately after their polymerization was observed, what supports our results. These authors did not test the inhibitory

effect of those materials at 24h after curing. Lins et al.¹³ (2005), however, used Vitremer™ after it has been stored for 24h in distilled water and observed its inhibitory activity on 16 clinic isolates of *S. mutans*. They also observed an inhibition halo (1 mm average diameter) in t0 induced by Vidrion R™. Likewise, others¹⁴ noticed inhibitory halos of Vidrion F™ (5.3 mm average diameter) against the standard strain GS-5 of *S. mutans*, immediately after the initial chemical polymerization, besides a marked decrease of the inhibitory action after the material was stored for 48h in distilled water.

The use of different lineages of *S. mutans* may contribute to clarify the possible differences of their sensitivity to fluoride, and also explain divergent results found with similar methods, besides problems with the standardization of the experiments. The *S. mutans* species used in most experiments with dental materials demonstrates the important role of strains of that microorganism in the etiology of caries. Further studies should be carried out to test the wide spectrum of oral microorganisms, including other bacteria, such as *Streptococcus*, *Lactobacillus*, *Actinomyces*, anaerobic gram-negatives, and mixed cultures¹⁵.

It is known that the diffusibility of an antimicrobial agent depends on its size, form of filler particles, and its concentration in the material¹⁶. In addition, the diffusibility of ions (F⁻, Ca⁺⁺, Al⁺⁺⁺, OH⁻) from glass-ionomer cements depends on the pH of the environment¹. To be effective, fluoride must be released above the values of minimal inhibitory concentration for each microorganism¹⁷. All these factors might affect the results of *in vitro* studies.

Several *in vitro* studies have confirmed the antibacterial potentiality of glass-ionomer cements^{14,18-20}, and glass-ionomers modified by resins seem to have better antibacterial properties when compared to the autopolymerizable cements¹⁸. Hara et al.⁹ (2000) suggest, for patients under high risk of caries, the tooth restoration with glass-ionomer when the esthetic or the mechanical resistance were not necessary. Amaral et al.²¹ (2006) also suggest that the glass ionomer cement is capable of interfering with the progression of the artificial caries lesion when used as pit and fissure sealant in a distance up to 125 µm. These authors observed a higher resistance to demineralization in fissures previously sealed with a glass-ionomer cement (Fuji IX™), and they concluded that these glass-ionomer cement was capable of remineralizing the margin of the fissure. However, a clear evidence of the inhibition of secondary caries by glass-ionomer cements has not yet been seen in the literature²². Vermeersch et al.¹ (2005) reported that the glass-ionomer cements Fuji II LC™ and Ketac-Fil™ showed an inhibition halo only during hardening, probably by the gene-

ration of a low pH around the test specimens. In the present study, the glass-ionomer cements Vidrion™ C, F, R, N, showed no bacterial inhibition halo neither in t0 or in t1, but the effect of these materials during hardening was not analyzed.

The zinc oxide-eugenol cement (IRM™) showed inhibitory activity, immediately after its polymerization, due to the release of eugenol; after 24h, such effect was not detected, probably because eugenol was not enough to prevent bacterial growth. However, the effect of various restorative materials against *S. mutans* was investigated¹⁵, and IRM™ showed the strongest antibacterial activity, which lasted 48h. Such divergences may be due to differences in the methods used in each study.

Composite resins have no significant antibacterial effect²⁰, according to our results, in which no inhibition halo was induced by Filtek Z250™. In other hand, a marked bacterial growth was evident around the resin specimen. Most often, the complete polymerization of composite resins does not occur, and its degree of conversion is in average from 50% to 70%²³. The monomer residues are known to cause many problems, such as a reduction in the mechanical properties and toxic effects for the pulp cells. In addition, the residual monomer may function as a scaffold for the formation of a bacterial biofilm⁸. The monomer may be used as a structure for biofilm formation⁸ and, besides, some bacteria contribute to the formation of polymers by producing peroxide, which may act as a barrier to protect the bacteria around the colonies, making them more tolerant to chemical substances or physical attacks. This may explain the marked growth of *S. mutans* around the composite resin test specimens that occurred in our study.

The extrapolation of the antimicrobial activity, using fluoride-releasing compounds to clinical situations, may be a problem¹³, once the microbiota of the bacterial plaque are resistant to the usual fluoride concentration. In addition, since the release of fluorides ionomer cements decreases with time²⁴, the effect of such materials on the microbiota may be proportional to that decrease, and the residual inhibitory activity of different materials from the same category may be very different. The absence of antibacterial activity in ionomers does not either restrict or counter indicate their use in daily clinical routine, once they exhibit characteristics such as resilience, thermal expansion near that of the tooth, and chemical adhesion to enamel and dentin. In addition, even small quantities of fluoride are efficient in the remineralization of the hard tissues of the tooth, a factor of great importance in the prevention of secondary caries. Therefore, further longitudinal studies are necessary to determine how much the antibacterial effects of dental materials

are really capable of decreasing the risk of secondary caries.

Conclusions

- The glass-ionomer cement Vitrebond™ inhibits bacterial growth, and can reduce the risk of secondary caries when used as a cavity liner.
- The other materials of this study did not either inhibit or keep the inhibition of the growth of *S. mutans* after 24h subsequent to curing.

Resumo

Um dos princípios da prática odontológica é evitar a ocorrência de cárie nas margens de restaurações, e, como Streptococcus mutans são a principal espécie cariogênica, a atividade dos materiais odontológicos contra esta espécie é clinicamente relevante. O objetivo deste trabalho foi verificar a ação de diferentes materiais odontológicos sobre S. mutans pelo teste de difusão em ágar. Foram confeccionados corpos-de-prova com onze materiais (Vidrion C®, Vidrion F®, Vidrion N®, Vidrion R®, Vitremer®, Vitrebond®, Maxxion R®, cimento de fosfato de zinco, IRM®, Panavia F®, e a resina Filtek Z-250®), para a realização dos testes microbiológicos imediatamente (t0) e 24h (t1) após a presa do material. Placas contendo ágar BHI foram incubadas a 35,5 °C, por 24h, e os halos de inibição do crescimento bacteriano em torno dos corpos-de-prova foram medidos e analisados. Apresentaram halos em t0 o Vitremer®, o MaxxionR®, o IRM®, o Panavia F® e o Vitrebond®; este último apresentou atividade antibacteriana estatisticamente maior que os demais (p < 0,001) e foi o único material que manteve esta atividade mesmo após 24h de sua presa (t1). O cimento de ionômero de vidro Vitrebond® inibe o crescimento bacteriano e tem potencial para diminuir o risco de cárie secundária, quando utilizado como forrador cavitário; os demais materiais estudados não inibiram ou não mantiveram a inibição do crescimento de S. mutans após 24h de sua presa.

Palavras-chave: Streptococcus mutans. Materiais odontológicos. Cárie dental.

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