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The short-term in situ model to evaluate the anticariogenic potential of ionomeric materials

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Summary *Objectives*. Aiming to contribute to the study of mechanisms involved in the anticariogenic properties of dental materials, this study assessed the suitability of a short-term in situ model to evaluate the anticariogenic potential of ionomeric materials.

Methods. The study used a 3-phase crossover, double blind design, and in each phase eight volunteers wore palatal appliances containing four enamel blocks restored with one of the following materials: composite resin (CR-Z250) (negative control), a conventional glass ionomer cement (GIC-Ketac-Fil) or a resin-modified GIC (RM-GIC-Vitremer). The restored blocks were covered with a 'test plaque' of *S. mutans*, placed in palatal appliances and a cariogenic challenge was made during 1 min with 20% sucrose solution. After 45 min, test plaque was collected for fluoride (F) analysis. Enamel surface microhardness was previously determined at one side of the restoration and the percentage of surface microhardness change (%SMC) in relation to baseline (other side) was calculated. F concentration in enamel was also evaluated.

Results. Split-plot ANOVA showed a statistically lower %SMC on enamel around the ionomeric materials than around the CR ($p<0.05$). This result was supported by a statistically higher F concentration in test plaque ($P<0.001$) and in enamel ($P<0.001$) restored with the ionomeric materials when compared to the CR.

Conclusions. The results suggest that the short-term in situ model tested is useful for studying the anticariogenic potential of dental materials that release fluoride.

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Introduction

Glass-ionomer cements present anticariogenic potential, which has been related to its fluoride (F) release.[1] The effect of F released has been strongly associated to the physico-chemical process involved in inhibition of enamel demineralization and enhancement of remineralization,[2] although some antibacterial action has also been demonstrated.[3-5]

Several in vitro models[2,4-6] and also long-term in situ models[3,7,8] have been successfully used to evaluate the effects of F released from glass-ionomer cements. Nevertheless, these models present limitations.[9] In vitro models do not take into account several factors involved in the caries development, such as the role of saliva and the effect of substances on bacterial metabolism. Also, long-term in situ models are subjected to biological variation among volunteers and rely on their compliance.[10]

The short-term in situ model (or intra-oral enamel demineralization test, IEDT), developed by Brudevold et al.[11] and improved by Zero et al.[12] evaluates enamel demineralization under a 'test plaque' prepared from *Streptococcus mutans*. Although the model does not rely on naturally formed dental plaque, the test plaque presents biofilm response to antibacterial agents.[13] This model is fast and accurate, and allows the study of the relationship among carbohydrate challenge, bacterial virulence, and enamel demineralization.[12] The model has been successfully used to assess the role of intra and extracellular polysaccharides in enamel demineralization.[12,14,15] Also, reduction of enamel demineralization by components of fluoridated toothpastes[16] and F rinses[13] were successfully investigated using it, and the model was recently used to evaluate enamel remineralization in the presence or absence of the test plaque.[17] The model would be useful to study the anticariogenic potential of dental materials, allowing a rapid assessment of their effect on enamel or dentine demineralization/remineralization process, as well as the mechanisms involved, such as the release of F or other ions. However, the anticariogenic potential of restorative dental materials has never been tested using this model.

Thus, the aim of this study was to assess the suitability of a short-term in situ model to evaluate the anticariogenic potential of glass-ionomer cements.

Materials and methods

Experimental design

The study involved a cross-over, double-blind design conducted in three distinct phases. Ethical approval was obtained from the Research and Ethics Committee of Faculty of Dentistry of Piracicaba. Eight healthy volunteers [mean age (SD) 27.5 (4.2) years], one male and seven females, wore a palatal appliance containing four bovine enamel blocks, with a slot crossing the surface restored with one of the materials to be tested: composite resin (CR) as a negative control, conventional glass-ionomer cement (GIC) or resin-modified glass-ionomer cement (RM-GIC) (Fig. 1). At each phase, a different material was used. Enamel surface microhardness (SMH) was assessed at one side of the restoration previously to each experimental phase. The blocks were covered with a layer of bacteria (test plaque) obtained from a culture of *S. mutans* and fixed on the palatal appliance using acrylic holders (Fig. 1).[12,16] The appliances were kept inside the mouth of volunteers for 30 min, after which volunteers gently rinsed during 1 min with a 20% sucrose solution. After 45 min, enamel blocks and test plaque were collected. The inhibition of enamel demineralization by the restorative materials was assessed by change of SMH. F concentration in test plaque and in enamel around the restorations was also determined. Seven days of lead-in and wash-out periods were followed, during which volunteers used a non-fluoridated dentifrice.

Preparation and restoration of enamel blocks and baseline SMH determination

Enamel blocks of 5×5×2 mm were prepared from bovine incisors, which were stored in 2% formaldehyde solution, pH 7.0, for at least one month.[16] The enamel surface of each block was polished plane as described by Zero et al.[18] Bovine enamel was used because it has a large and relatively flat surface, adequate for this model.[10] Furthermore, it presents a composition less variable than human enamel, and it decreases the time of the study, without qualitative differences to the lesions produced in comparison with human enamel.[10,19]

A slot, 0.9 mm wide and 1.0 mm deep, was made crossing the surface of the enamel block, using a #1342 diamond bur (KG Sorensen, São Paulo, SP, Brazil). Blocks were randomly assigned to be restored with one of the materials to be tested: Z250™ (3M ESPE, St. Paul, MN, USA) (negative control), Ketac-Fil Plus™ (3M ESPE) or Vitremer™

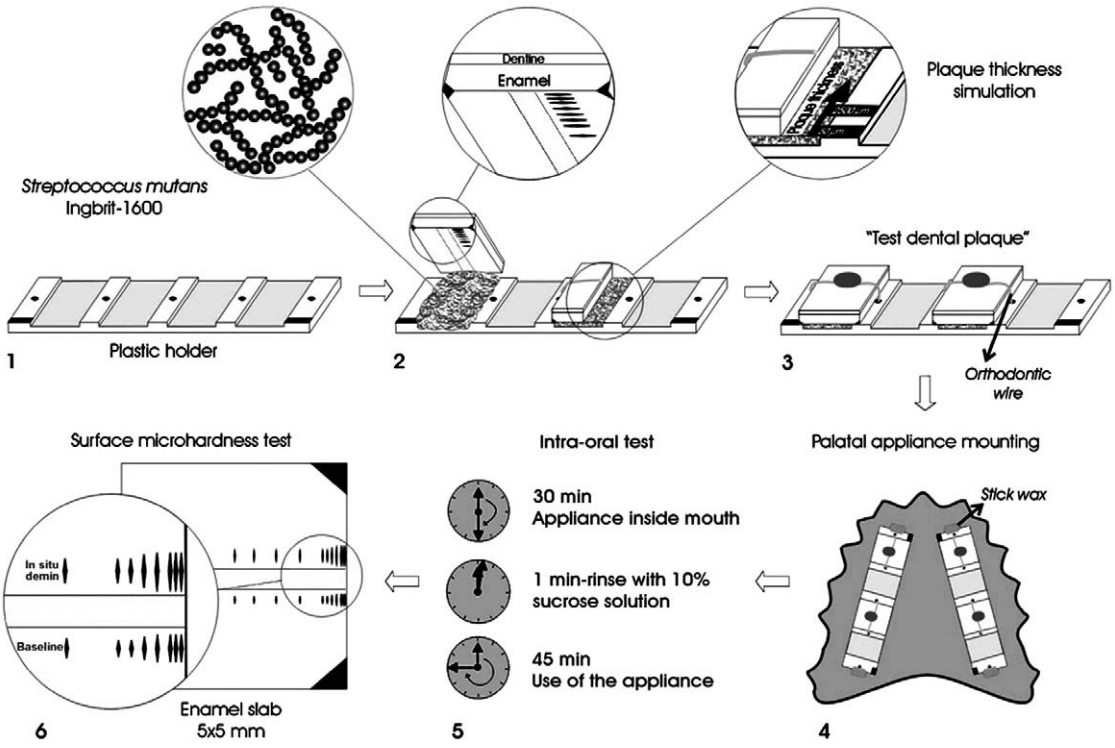

Figure 1 Schematic illustration of the short-term in situ model used. (1) Plastic holder used to carry enamel blocks. (2) Restored enamel blocks, with known surface microhardness, placed in the plastic holders with restoration facing the 'test plaque'. Sucrose solution diffuses through the plaque, simulating increased thickness. (3) Restored enamel blocks fixed with orthodontic wire. (4) Stick wax is used to keep the plastic holders in the palatal appliance. (5) Description of the intra-oral test. (6) Surface microhardness test after the in situ cariogenic challenge.

(3M ESPE). All restorations were made according to the manufacturers' instructions, using materials' kits. For Z250™, the adhesive Single Bond™ (3M ESPE) was used. Cavities to be restored with Ketac-Fil Plus™ were etched for 10 s with 40% polyacrylic acid (Durelon™ liquid, 3M ESPE) and restorations were protected using non-colored nail varnish. All blocks were kept in a 100% humidity environment at 37 °C for 24 h after restorations were made, in order to allow proper setting of the glass-ionomer cements. After this period, enamel blocks were serially polished again in order to remove excess of restorative material, and SMH was assessed 150 μm away from the right side of the restoration at distances of 50, 75, 100, 200, 300, 400, 500, 1000, 1500, 2000 and 2500 μm from one edge of the enamel block, which was marked for future positioning of the block on the appliance (Fig. 2(a)). The distance of 150 μm was chosen because it allowed microhardness determination at a very flat surface at the side of the restoration, avoiding the effect of any marginal shrinkage on the hardness values. For SMH measurements, a Knoop indenter was used with a 50-g load for 5 s in a Future-Tech FM microhardness tester coupled to FM-ARS software. Until the following day, blocks were kept in a humid environment at 8 °C.

Palatal appliance mounting

Test plaque was prepared from *S. mutans* Ingbritt-1600 (kindly donated by the Eastman Department of Dentistry, Rochester, NY, USA), as described by Cury et al.[16] Palatal appliances capable of carrying two plastic holders were constructed for each volunteer. Two enamel blocks of the same material were mounted in each holder, with enamel surface in contact with the test plaque. The plastic holders were fixed to the palatal appliance, with the marked side of the enamel block where the baseline measurements were made facing the center of the palatal appliance (Fig. 1). Further details can be found in Cury et al.[16]

Intra-oral test

Intra-oral tests were performed 48 h after restorations were made. Volunteers kept the palatal appliance holding the restored blocks and test plaque inside mouth for 30 min. After this period,

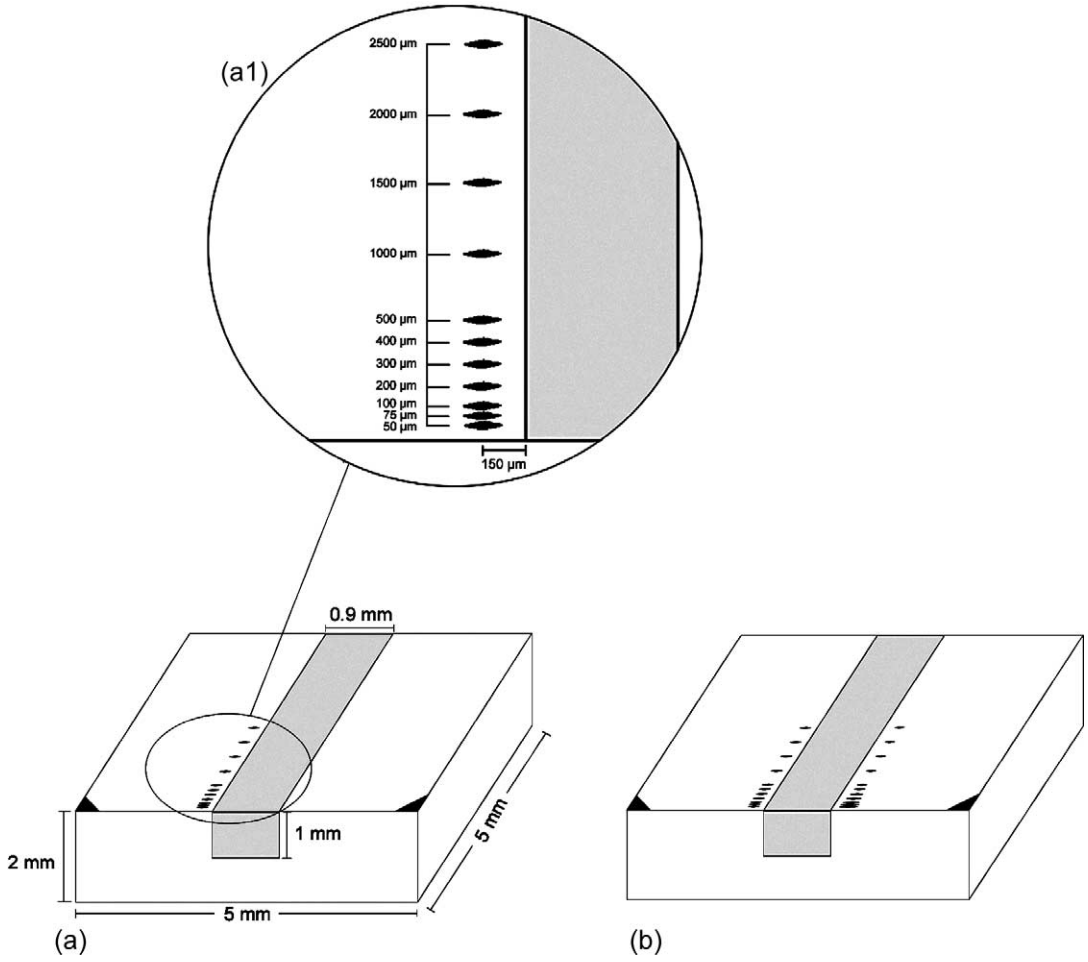

Figure 2 Schematic illustration of the enamel blocks, showing the restoration and the microhardness indentations. (a) Baseline indentations at different distances (a1) from the marked edge of the enamel block. (b) Enamel block after final microhardness analysis.

each volunteer gently rinsed with 15 mL of a 20% sucrose solution during 1 min, without removing the devices. Volunteers wore the appliances during a subsequent 45-min period. During the entire intraoral test, subjects were instructed to refrain from talking, drinking or eating.

After the 45-min period, test plaque was collected for analysis of F concentration, and the enamel was analyzed for SMH and F uptake.

Analysis of fluoride in 'Test plaque'

The bacteria on all enamel blocks were recovered, pooled, and dried for 24 h in vacuum over P_2O_5, after which the dry weight was obtained using an analytical balance (± 10 µg). F was extracted by the hexamethyldisiloxane (HMDS)-diffusion technique, as described by Taves.[20] F analyses were conducted using an ion-specific electrode (Orion, model 96-09, Orion research Incorporated, Cambridge, MA, USA) and an ion analyzer (Orion, model EA 940), previously calibrated with standard solutions (0.025–6.40 µg F/mL) prepared like the samples. The results were expressed in µg F/g of test plaque dry weight.

Surface microhardness analysis

Enamel blocks removed from the holders were washed with deionized water and SMH was measured again, as already described. The indentations were made at the left side of the restoration, at 150 µm from it and at 50, 75, 100, 200, 300, 400, 500, 1000, 1500, 2000, 2500 from the block edge (Fig. 2(b)). From this block edge, sucrose solution and saliva had access to the enamel surface covered by test plaque, simulating dental plaque thickness of up to 2.5 mm.[10,12] The percentage of surface microhardness change (%SMC) was calculated [%SMC = (SMH after demineralization − baseline SMH) × 100/baseline SMH]. The results found in

the four blocks at each distance for each volunteer were averaged and submitted to statistical analysis.

Analysis of fluoride in enamel

After SMH measurements, each restored enamel block was covered with an acid-resistant nail varnish, with exception of an area of enamel at each side of the restoration. This area was measured ($\pm$0.01 mm) and corresponded to the area of enamel that was in contact with the test plaque. A layer of this enamel was removed by immersion in 0.25 mL of 0.5 M HCl for 15 s under agitation and acid-extracted F was determined.[21] F concentration in enamel of the four blocks used by each volunteer was averaged and expressed as μg F/cm^2 of enamel.

Statistical analysis

For %SMC, data were analyzed using split-plot ANOVA, considering the three restorative materials as plots and the different distances from the block edge (simulating plaque thickness) as subplots. As the interaction material $\times$ thickness was found to be significant ($p<0.001$), the materials were compared at each distance using Tukey test. The effect of distances (plaque thickness) within each material was compared using regression analysis. For F in test plaque and in enamel, data were transformed, respectively, to the inverse of the square root and the log 10, in order to fit the assumption of equality of variances and normal distribution of errors. These data were then analyzed using ANOVA and Tukey test. For all statistical analyses, SAS System 6.11 software (SAS Institute Inc.) was used and the significance limit was set at 5%.

Results

Both ionomeric materials were able to significantly ($p<0.05$) decrease enamel demineralization around the restoration when compared to the control material (Fig. 3). This effect was observed at all distances from the edge of the block, which simulates plaque thickness, but the difference between the ionomeric materials was not statistically significant at any distance ($p>0.05$).

The %SMC according to the distance from the edge of the block (plaque thickness) varied linearly only for the control material ($p<0.001$), with a decrease in enamel demineralization associated with the increase of plaque thickness. For the two ionomeric materials, no effect of plaque thickness was observed.

F concentration in test plaque was significantly increased around both ionomeric materials in comparison with the control material ($p<0.001$) (Table 1). Also, F concentration in enamel after the in situ cariogenic challenge was significantly higher around both glass-ionomer restorations, when compared to the control ($p<0.001$). Furthermore, F concentration in enamel around the conventional glass ionomer (Table 1) was significantly higher than in enamel around the resin-modified glass ionomer ($p<0.01$).

Discussion

The short-term in situ model used in the present study demonstrated that the two ionomeric materials tested were able to significantly inhibit enamel demineralization when compared to the composite resin. This finding is in accordance with

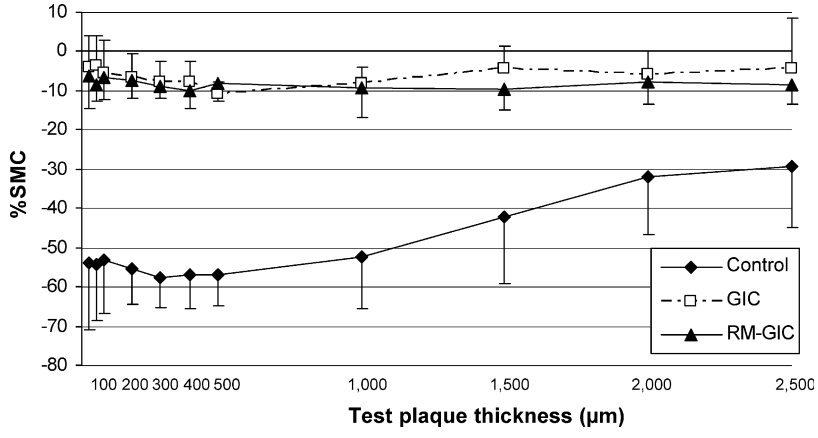

Figure 3 Means ($n=8$) of percentage of enamel surface microhardness change (%SMC) according to the restorative materials used and 'test plaque' thickness (μm). Groups GIC and RM-GIC differed statistically from control group at all distances ($p<0.05$). Bars indicate standard deviation.

Table 1 Means (SD) of fluoride concentrations in the 'test plaque' (μg F/g dry weight) and in enamel (μg F/cm^2) according to the treatment/groups ($n=8$).

Treatment/ groups	F in the 'test plaque'	F in enamel
Control	3.6 (0.4) A	0.35 (0.06) A
GIC	291.9 (340.9) B	1.85 (0.44) B
RM-GIC	150.9 (217.5) B	1.26 (0.13) C

Treatment/groups whose means are followed by distinct letters differ statistically ($p<0.05$).

the anticariogenic effect of glass-ionomer cements demonstrated either in vitro[2,4,5] or in long-term in situ studies.[3,7,8]

Furthermore, no statistically significant differences were observed between the two ionomeric materials with regard to reduction of enamel demineralization. These data are in accordance with Kotsanos,[8] who did not find significant differences in enamel demineralization around conventional and resin-modified glass-ionomer cements. A superior effect of Vitremer on the reduction of enamel demineralization would be expected due to the simultaneous release of fluoride and aluminum by this material.[5,22] However, Tabchoury et al.[23] also did not find any difference between the same ionomeric materials used in the present study with respect to the reduction of enamel demineralization when using a long-term in situ model.

The data of reduction of enamel demineralization are supported by the analysis of F in test plaque and in enamel adjacent to the ionomeric restorations (Table 1). It is known that F release from ionomeric materials is time dependent and the highest amount is released from fresh samples. F found in test plaque was probably released during the 30 min that the apparatus was kept into the volunteers mouth, and mainly during the cariogenic challenge with sucrose, since F release is also pH dependent.[24,25]

The F concentration found in test plaque (Table 1) covering the ionomeric materials may be responsible for the reduction of enamel demineralization observed (Fig. 3), either by reducing the ability of *S. mutans* to ferment sucrose in acids or by a physicochemical mechanism. The inhibition of acidogenicity is reasonable, since the F concentrations found are superior to the minimum concentration known to interfere with oral bacteria acidogenesis.[26] The physicochemical effect is supported by the higher F concentration in enamel restored with the ionomeric materials when compared with the control (Table 1). The uptake of F by enamel can be explained by the fact that, during the dynamic of caries process, there is loss of more soluble minerals, but in presence of F part of these minerals are rebuilt as fluorapatite.[27,28] As consequence, there is a reduction of demineralization and an increase of F in enamel. The findings of F in test plaque and in enamel of the present study, using the IEDT model, are in agreement with long-term in situ study evaluating the anticariogenic effect of GIC.[3] The highest F concentration in enamel restored with GIC is coherent with the F concentration in test plaque on this material (Table 1).

The reduction of enamel demineralization around the ionomeric materials was observed throughout the plaque thickness simulation (Fig. 3). This could be explained by the F released from the materials, which was probably homogeneous around all the extension of the restoration (Fig. 2). However, around the composite resin, the degree of enamel demineralization depended on plaque thickness and the higher demineralization close to the surface is in accordance to Zero.[10]

It should be emphasized that the findings were found with fresh ionomers. Since the effect of long-term F release from ionomeric materials on the inhibition of secondary caries has been questioned,[29] this model could also be used to evaluate if aged materials retain their anticariogenic potential.

Conclusions

The findings showed that the short-term model is able to evaluate the anticariogenic effect of fresh-prepared restorative ionomeric materials. It may also be suitable to evaluate aged restorative material and this is the aim of further research. Furthermore, it could be used to explore the mechanisms involved in the effect of dental materials on bacteria metabolism, such as the antibacterial properties of resin composites and dentin bonding agents.[30]

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