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Antimicrobial and Anti-Caries Effect of New Glass Ionomer Cement on Enamel Under Microcosm Biofilm Model

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The occurrence of caries lesions adjacent to restorations is a serious problem in Dentistry. Therefore, new antimicrobial restorative materials could help to prevent recurrent carious lesions. This study evaluated the effect of a new glass ionomer cement (Ion Z) on the viability of a microcosm biofilm and on the development of enamel demineralization. Enamel samples were filled with the following materials (n=9): A) Ion-Z (FGM Ltda); B) Maxxion R (FGM Ltda); C) Ketac Fil Plus (3M ESPE) and D) no restoration (control). The samples were then exposed to human saliva mixed with McBain saliva (1:50) containing 0.2% sucrose for 14 days. The live and dead bacteria were quantified by fluorescence using a confocal laser-scanning microscope. The enamel demineralization was analyzed using transverse microradiography (TMR). The data were submitted to ANOVA/Tukey or Kruskal-Wallis/Dunn test ($p<0.05$). Ion Z induced a higher percentage of dead bacteria (60.96±12.0%) compared to the other groups (Maxxion R: 39.8±6.7%, Ketac Fil Plus: 43.7±9.71% and control 46.3±9.5%). All materials significantly reduced the average mineral loss compared to control (Ion-Z 25.0±4.2%vol, Maxxion R 23.4±8.0%vol, Ketac Fil Plus 30.7±7.7 and control 41.2±6.6%vol). Ion-Z was the only material able to significantly improve the mineral content at the surface layer (Zmax: 63.5±18.2%vol) compared to control (38.9±11.3%vol). Ion-Z shows antimicrobial potential, but its anti-caries effect was similar to the other materials, under this model.

Key Words: biofilm model, enamel demineralization, glass ionomer cement, zinc

Introduction

Development of caries lesions adjacent to restorations represents a serious problem in Dentistry (1). The occurrence of this undesirable condition is more common close to composite restorations since resins allow a greater biofilm accumulation compared to other restorative materials (2).

Dental caries is a multifactorial disease induced by the interaction between diet, host and microorganism (biofilm) over time (3). Some environment factors can negatively affect the relationship between host and microorganisms in biofilm such as a diet rich in sugar (especially sucrose, the substrate for the formation of extracellular polysaccharides) (3), low salivary flow and bad oral hygiene. Therefore, positive changes in patients' behavior during the treatment are essential to avoid recurrent caries and failure of restorations. On the other hand, fluoride and antimicrobial (4) treatments can positively interfere in the homeostasis between host and microorganisms.

Accordingly, the development of new antimicrobial restorative materials could help preventing recurrent carious lesions. Glass ionomer cement (GIC) is known as an anticariogenic material able to release fluoride (5). Fluoride in turn controls de-remineralization processes and has some antimicrobial effect due to the enolase enzyme inhibition, which indirectly controls the phosphotransferase system, responsible for sugar input into bacteria and energy achievement (6).

GIC sealants have shown potential to prevent caries 3.1 to 4.5-fold than resin sealants, after 5 years of monitoring (7). GIC applied in ART also shows 4 times more chance to avoid recurrence of carious lesions compared to composite resin after a follow-up of 4 years (7). However, according to a recent systematic review, the clinical evidence suggests similar caries-preventive efficacy of GIC and resin-based sealants after a period of 4 years (8). Furthermore, the evidence concerning a possible superiority of GIC compared to resin-based sealants after 5 years is still poor, due to the high level of studies' bias (8). On the other hand, the mechanical resistance and the esthetic appearance of the GIC are not as good as composite resin (9).

Recently, a new GIC was released to the market, with a promise of improving antimicrobial capacity and mechanical resistance. Due to the presence of zinc in its composition, this new GIC may have some antimicrobial effect against *Streptococcus mutans* as shown by zinc oxide (10). This modified GIC also may inhibit the activity of osteoclasts, which are responsible for the bone reabsorption (11). Furthermore, it can increase the mechanical resistance and chemical adhesion (depending on the Zn concentration) and improve biomineralization (12).

Generally, the antimicrobial and anti-caries effect of GIC has been tested mostly by applying monospecies (*S. mutans*) biofilm and abiotic models to induce tooth demineralization or *in situ* biofilm models (2,10,12,13). The use of a microcosm biofilm, produced from microorganisms present in human saliva, can bring advantages over *in vitro* studies with monospecies biofilm, once this model allows the presence of high number of microorganisms and the interactions between them and the tooth in the presence of fluoride or antimicrobial agents (14,15).

Considering the need of first studying this new GIC modified by zinc under experimental *in vitro* models closer to the clinical condition, this work compared the antimicrobial and anti-caries effect of this new GIC with two commercial conventional GICs using a microcosm biofilm model. Since several studies have compared GIC with composite resin with respect to anti-caries potential (5,7,8), only the conventional GICs were included in the study. The tested hypotheses are: 1) The new GIC (Ion Z) significantly decreases the bacteria viability compared to the commercial ones (Maxxion R and Ketac Fil Plus); 2) The new GIC (Ion Z) significantly protects against enamel demineralization compared to the commercial ones (Maxxion R and Ketac Fil Plus).

Material and Methods

Saliva Collection

The study was firstly approved by the local Ethical Committee of Bauru School of Dentistry, University of São Paulo, Brazil (CAAE number 48102115.3.0000.5417 and approval document: 1.235.560). Total saliva needed for the experiment was collected from 2 healthy donors, who matched the following inclusion criteria: 1) normal salivary flow (stimulated saliva flow > 1 mL/min and non-stimulated saliva flow > 0.3 mL/min), 2) with previous history of caries, but no caries active (no active white spot and/or cavitated lesions), 3) with no gingivitis/periodontitis (gum bleeding or tooth mobility) and 4) with no ingestion of antibiotics in the last 3 months. A day before the saliva collection, the donors did not brush their teeth. They were further not allowed to ingest food or drinks 2 h prior to the saliva collection. The saliva was collected under stimulation by chewing a rubber material for 10 min. during the morning. After collection, the pool of saliva was diluted in glycerol (70% saliva and 30% glycerol). Aliquots of 1 mL were stored in -80 °C (16). Before the biofilm formation, *Streptococcus mutans* and *Streptococcus sobrinus* were identified in the saliva of the donors by using SB-20 M medium containing 15 g of bacto-casitone (Difco), 5 g of yeast extract (Kasvi, Curitiba, PR, Brazil), 0.2 g of L-cysteine hydrochloride (Sigma, Steinheim, Germany), 0.1 g of sodium sulphite (Sigma), 20 g of sodium acetate (Synth), 200 g of coarse granular cane sugar, 15 g of agar (Kasvi) and 0.2 U mL^{-1} of bacitracin (Sigma, Steinheim, Germany) in 1 l distilled water (autoclaved). The plates were then incubated at 5% CO_2 and 37°C, for 48 h. The CFU numbers were counted and transformed to $\log_{10}$ CFU ml^{-1} (17).

Tooth Sample Preparation and Treatment

Enamel samples (surface area: 16 mm^2, 3 mm height) were prepared from bovine teeth (4-5 years old cattle, Mondelli Frigorífico, Bauru, Brazil), using a semi-precision cutting machine (Buehler, Lake Bluff, IL, USA). The samples were fixed in acrylic discs with wax and polished in a metallographic polishing machine (Arotec, Cotia, SP, Brazil) using water-cooled silicon-carbide disc (600-grit papers ANSI grit; Buehler, Enfield, CT, USA) to achieve a standardize enamel surface roughness of approximately 0.131 ± 0.043 µm. Enamel samples with roughness lower than 0.1 or higher than 0.2 µm were excluded. The average surface roughness (Ra) was assessed using profilometer and Mahr Surf XCR 20 software (5 readings of 3 mm length, 250 µm apart from each other, Mahr, Gottingen, Lower Saxony, Germany). The samples were then sterilized using ethylene oxide [Gas exposure time (30% ETO / 70% CO_2) for 4 h under a pressure of 0.5 ± 0.1 kgF/cm^2].

Enamel samples were randomly distributed to the groups according to their *Ra* means, in order to standardize similar enamel *Ra* values between the groups. The groups were (n=9): A) Ion-Z (FGM Ltda, Joinville, SC, Brazil); B) Maxxion R (FGM Ltda, Brazil); C) Ketac Fil Plus (3M ESPE, Sumaré, SP, Brazil) and D) no cavity and restoration (control).

For groups A, B and C, cavities (2 mm diameter and 1.5 mm depth) were prepared using diamond bur #1094 (KG Sorensen, Cotia, SP, Brazil) under high rotation and refrigeration (Fig. 1). One bur was used for each 9 samples. The filling materials were manipulated according to the manufacture's instruction and placed into the enamel cavities. Table 1 shows the composition of the materials.

Table 1. The composition of the glass ionomer cements tested in the present study

Material	Composition
Ion-Z (FGM)	Powder: micronized glass ionomer (calcium-aluminum-zinc-fluoride silicate glass) and pigment (titanium dioxide and iron oxide). Liquid: polycarboxylic and tartaric acids and deionized water
Maxxion R (FGM)	Powder: fluoro- aluminum silicate glass, calcium fluoride and radiopacifiers. Liquid: Polycarboxylic and tartaric acids and deionized water
Ketac Fill Plus (3M ESPE)	Powder: fluoro- aluminum silicate glass. Liquid: Copolymer of acrylic acid, maleic acid, water and tartaric acid

After restoration and polishing, the samples were kept in deionized water for 24 h, at 37 °C. The roughness of the materials was also measured after the restoration: Ion-Z (Ra: 0.488 ± 0.07µm), Maxxion R (Ra: 0.571 ± 0.05µm) and Ketac Fil Plus (Ra: 0.501 ± 0.03µm).

Prior to the biofilm formation, two parts of the enamel surfaces (0.5 mm from the borders in case of groups A, B and C; 1 mm from the borders for group D) were protected using cosmetic nail polish to obtain control areas for the transverse microradiography-TMR analysis. Figure 1 shows the samples dimension and the experimental design.

Microcosm Biofilm Formation

The human saliva was defrosted and mixed with McBain artificial saliva in a proportion of 1:50 (17,18). The McBain saliva contained 2.5 g/L type II mucin from porcine stomach, 2.0 g/L bacteriological peptone, 2.0 g/L tryptone, 1.0 g/L yeast extract, 0.35 g/L NaCl, 0.2 g/L KCl, 0.2 g/L $CaCl_2$, 0.1 g/L cysteine hydrochloride, 0.001 g/L hemin, 0.0002 g/L vitamin K1, at pH 7.0. All reagents were from Sigma-Aldrich (St. Louis, MO, USA).

The samples were placed in a 24-well plate and the solution of human saliva and McBain saliva was added to each well (v=1.5 mL/well), which was incubated at 5% CO_2 and 37˚C, for 8 h. The enamel samples were then transferred using tweezers to new wells containing fresh McBain saliva with 0.2% sucrose and incubated at the same conditions. After 16 h, the samples were again transferred to new wells containing fresh McBain saliva with 0.2% sucrose and incubated for 24 h at the same conditions (19). This procedure was repeated each 24 h, for a total time of 14 days.

Bacteria Viability analysis

After 14 days, the samples were immersed in phosphate-buffered saline (PBS) solution under stirring to remove unattached bacteria. The biofilm was stained using the nucleic acid markers diluted in PBS (1 mL PBS + 1 µL SYTO9 + 1 µL propidium iodide, 10 µL/well) (Kit Live & Dead® cells viability assay, Thermo Fisher Scientific, Waltham, MS, USA) for 15 min in a dark environment. Live bacteria were stained with SYTO 9 producing a green fluorescence, and dead lysed bacteria were stained with propidium iodide/SYTO9 producing a red fluorescence (20). Biofilm was examined using confocal laser scanning microscope (Leica TCS SPE, Leica Mannheim, Mannheim, Baden-Wurttemberg, Germany) and Leica Application Suite-Advanced Fluorescence software (LAS AF, Leica Mannheim). The excitation and emission fields were 480/500 nm for SYTO 9 and 490/635 nm for propidium iodide. Three images (275 µm²) were captured from the surface of the enamel adjacent to restoration of each sample (same region analyzed in the Transverse microradiography -TMR) and analyzed using BioImage L 2.0 software, to quantify the live and dead bacteria (%). This assay was done in biological triplicate (n=3/each experiment).

Transverse Microradiography (TMR)

After cleaning the teeth with acetone solution 1:1, the enamel samples were transversally sectioned and polished to obtain slices with 80-100 µm of thickness (Fig. 1). The enamel slices were fixed in a sample-holder together with an aluminium calibration step wedge with 14 steps. A microradiograph was taken using an x-ray generator

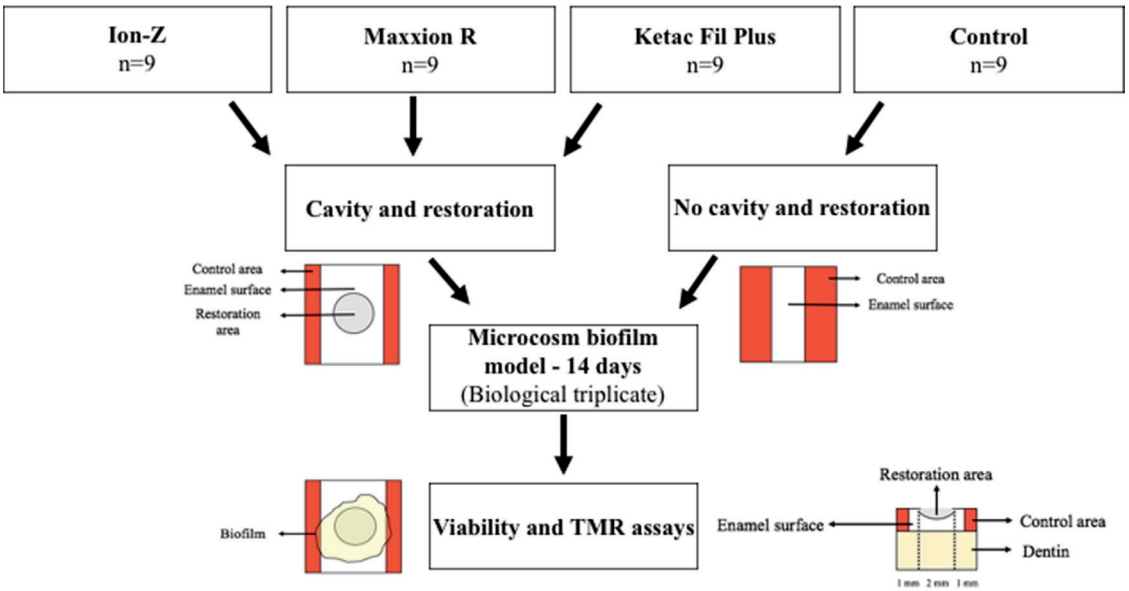

Figure 1. Samples dimension and experimental design.

(Softex, Tokyo, Japan) on the glass plate at a distance of 42 cm, under 20 kV and 20 mA for 13 min. The glass plates were developed for 7 min, rinsed in deionized water, fixed for 7 min in a dark environment, and then rinsed in running water for 10 min and air-dried (all procedures were done at 20 °C). The developed plate was analyzed using a transmitted light microscope fitted with a 20x objective (Zeiss, Oberkochen, Baden-Wurttember, Germany), a CCD camera (Canon, Tokyo, Japan), and a computer. Two images per specimen were taken on each side of the restoration using data-acquisition (version 2012) and interpreted using calculation (version 2006) softwares from Inspektor Research System bv (Amsterdam, The Netherlands). The mineral content was calculated, assuming the density of the mineral to be 3.15 kg l^{-1} and 87 %vol of mineral content for the sound enamel. The lesion depth (LD, µm), the integrated mineral loss (ΔZ, %vol. µm), the average mineral loss over the lesion depth (R, %vol) and the maximum mineral content at surface layer (Zmax, %vol) were calculated (17).

Statistical Analysis

Data were statistically analyzed using the software Graph Pad Instat for Windows (GraphPad Software Inc., San Diego, CA, USA). The normal distribution and homogeneity were checked using Kolmogorov & Smirnov and Bartlett's tests, respectively. Ordinary ANOVA followed by Tukey's test were applied to compare the different materials with respect to the bacteria viability. The differences between live and dead bacteria within the same material were also compared using t test. For TMR parameters, Kruskal-Wallis followed by Dunn's test was applied (except for R data). A significance level of 5% was set. The statistical power was calculated based on the mean and SD of % live bacteria and ΔZ from Ion Z and Ketac Fill plus.

Results

S. mutans and *S. sobrinus* were identified in the human saliva before the experiment (2.87 ± 0.09 $\log_{10}$ CFU/mL and 3.42 ± 0.08 $\log_{10}$ CFU/mL, respectively), showing the potential of the source to produce dental caries lesions.

Table 2 shows the results of the viability assay. Ion-Z showed the highest percentage of dead bacteria (the lowest percentage of live bacteria either) in biofilm compared to the other groups, which in turn did not differ from each other (statistical power of 87.20%). The percentage of dead bacteria was significantly higher than the percentage of live bacteria for Ion Z (p=0.0005), while for the other groups the opposite happened (Maxxion R p=0.0001; Ketac Molar p=0.0003 and control p<0.0001). Therefore, Ion-Z presented a higher antimicrobial capacity compared to the other GIC materials.

Our biofilm model was able to produce a subsurface enamel caries lesion as seen in the TMR pictures (Fig. 2). All materials significantly reduced the average mineral loss (R) compared to control. Ion-Z was the only material able to improve the mineral content at the enamel surface layer (Z max) compared to control (p<0.05), but it did not significantly differ from the other restorative materials. Despite the lowest value of integrated mineral loss (ΔZ) was seen in enamel restored with Ion-Z, no significant differences were found for both ΔZ and lesion depth (LD) among the groups (Table 3, statistical power of 80.12%). Therefore, the data shows that materials were able to decrease mineral loss at the surface but not in deep, which justify the lack effect on LD and ΔZ.

Figure 2 shows a representative TMR picture and the lesion profile of one representative sample from each group, highlighting the differences between Ion-Z (Fig. 2A) and the other GICs materials and control (Fig. 2B-D) with respect to enamel lesion profile. Figure 2A shows a less demineralized and not so deep lesion compared to the other Figures (2B-D).

Discussion

It is known that GIC has potential to release fluoride and may present anti-caries effect. Most of the studies that tested the antimicrobial activity of GIC have applied monospecies (10,12,13) or *in situ* (6,13) biofilm models. Despite monospecies biofilm allows to standardize the number of bacteria and to see a specific effect of the material, microcosm biofilm better represents the variety of microorganisms present in oral cavity (14), which could be involved in caries etiology. Furthermore, more important than the type of bacteria is what they are doing on the tooth and how the material could interfere in the most important outcome, which is the dental caries development. TMR is considered the gold standard method to quantify tooth demineralization (caries lesions), however, it is a destructive assay and, therefore, some samples loss is expected.

In addition to fluoride, other chemical components may be added to GICs in order to enhance their antimicrobial

Table 2. Mean and SD of the percentage of live and dead bacteria (%)

Treatment	% live bacteria	% dead bacteria
Ion-Z (FGM)	38.59±9.87[a]	60.96±9.71[a]
Maxxion R (FGM)	59.59±7.23[b]	39.81±6.86[b]
Ketac Fill Plus (3M ESPE)	56.07±16.11[b]	43.67±15.93[b]
Control	53.55±9.50[b]	46.31±9.48[b]

Different letters show significant differences among the groups (ANOVA, p<0.0020 for live and p<0.0017 for dead bacteria, n=9).

activity, such as zinc sulphate, zinc oxide and silver nanoparticles (21). In our study, Ion-Z (GIC modified by Zn) presented a higher antimicrobial capacity compared to the other GIC materials. Therefore, the first hypothesis of the study can be accepted. Spencer et al. (10) showed that zinc oxide has significant dose-dependent antimicrobial effect, using disc diffusion test. The ZnO concentrations of 13% and 23.1% showed significant antimicrobial activity compared to the negative control. According to the manufacture, Ion Z contains around 0.25-0.55% Zn. We speculate that this amount of Zn associated with F content could be responsible by its antimicrobial effect under the microcosm biofilm model. Other possible hypothesis is that Ion Z has a smoother surface compared to the other GICs as shown in the methods, which in turn could reduce biofilm formation on this material and, consequently, at the interface with enamel.

On the other hand, a recent study (12) demonstrated that low concentrations of zinc oxide (1% and 2%), added to conventional and resin modified GIC, did not improve the antimicrobial capacity of GICs on *S. mutans* CFU counting. We speculate that Zn released from GIC could affect other type of microorganisms when using microcosm biofilm, justifying its better antimicrobial effect, but not the anti-caries effect. It would be interesting to check if this experimental material (Ion Z) could affect some specific microorganisms, besides *S. mutans*, also involved in caries etiology, such as *Lactobacillus*, *Bifidobacteria*, fungi by using CFU counting, or more sophisticated assay as qPCR.

The mechanism of action of zinc is based on blocking the electron transport chain or inhibiting ATP formation in a dose-dependent relationship (10). In addition, Zinc can act as a "reservoir", responding with a rapid mobilization of ions to the sites where there is zinc-consuming reaction or to some receptor site from microorganism (22). At free concentrations above 0.05%, zinc may interfere with membrane transport mechanisms, causing conformational changes in transporter proteins (21), while

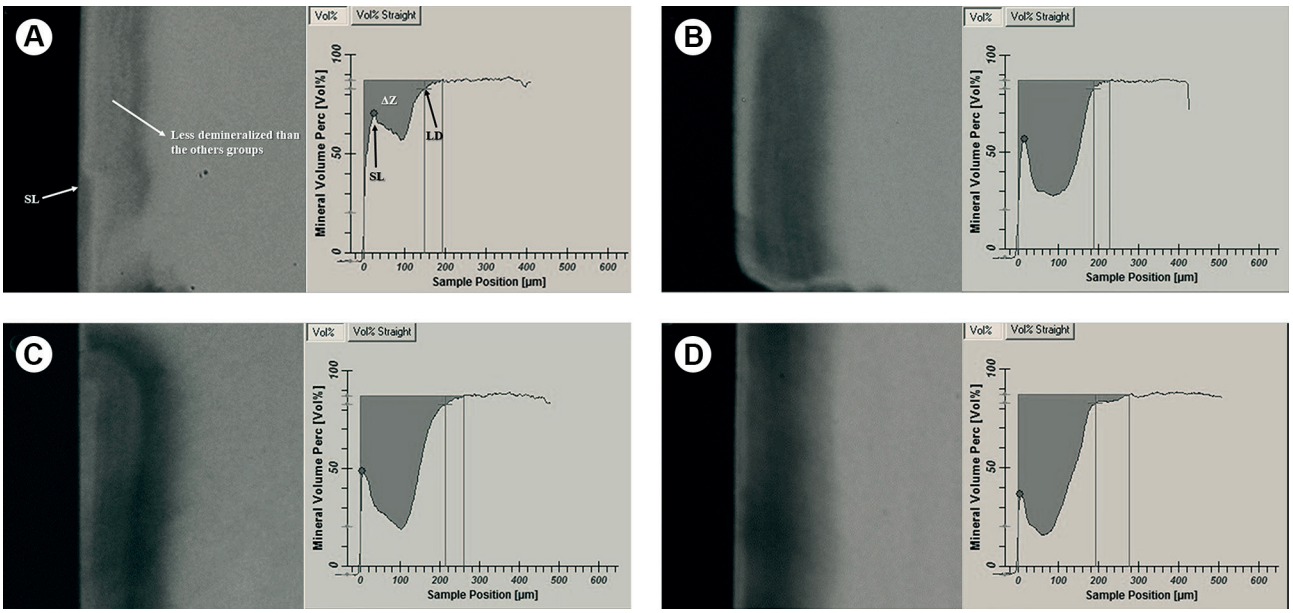

Figure 2. Representative TMR image (20×) and lesion profile of an enamel sample from: A: Ion-Z; B: Maxxion R; C: Ketac Fill Plus; D: control. The ΔZ was calculated based on the grey area of the graphic. SL, surface layer; ΔZ, integrated mineral loss; LD, lesion depth.

Table 3. Median; minimum-maximum (average±standard deviation) values of the TMR parameters

	ΔZ* (%vol.µm)	LD* (µm)	R** (%vol)	Z max* (%vol)
Ion-Z (FGM)	3006; 2650-5010[a]	140.2; 117.5-149.9[a]	25.0±4.2[a]	69; 28-80[b]
Maxxion R (FGM)	3735; 1170-7860[a]	116.2; 73.7-185.7[a]	23.4±8.0[a]	56; 51-59[ab]
Ketac Fill Plus (3M ESPE)	6178; 2020-9460[a]	161.4; 80.1-213.1[a]	30.8±7.7[a]	60; 49-75[ab]
Control	5774; 3620-9570[a]	148.9; 104.0-193.3[a]	41.2±6.6[b]	37; 23-55[a]

*Kruskal-Wallis/Dunn; **ANOVA/Tukey ($p<0.0001$ for R and $p=0.016$ for Z max. For the other parameters $p>0.05$). Different letters show significant differences among the groups (n=8 Ion-Z, control and Maxxion R and n=7 Ketac Fill Plus). Some samples were lost during TMR preparation.

at free concentrations lower than 0.009% no cell damage is observed (22). However, we should keep in mind that the above studies tested the mechanism of free Zn but not of materials modified by the addition of Zn, in which the amount of released Zn may be much lower than the content incorporated into the material. The release of Zn from the material and its relationship with the antimicrobial effect shall be better explored in the future.

It is already known that the anti-caries mechanism of GIC materials occurs through the release of fluoride ions to the environment, especially during the first few days after restoration (13), however, they are not able to completely prevent the development of the carious lesion (13). We do not know if the effect of Zn from GIC occurs by direct contact with tooth surface or if its release pattern happens in similar way as Fluoride. Fluoride released from the material has the ability to be incorporated into tooth apatite, increasing its acid resistance especially at the surface lesion (23). Accordingly, all materials were similarly able to partially reduce the caries lesion development at the surface, by reducing the average mineral loss, without interfering in the lesion depth, compared to control. Therefore, this study showed that the effect of fluoride from GICs is more superficial and that Zn may not enhance its anti-caries protection. Considering the similarity between the anti-caries effects of the GICs, the second hypothesis can be rejected.

Mayer et al. (24) have shown that when carbonated apatite is precipitated in the presence of Zn, Zn can replace some Ca, being incorporated into the carbonated apatite structure. Although Zn can reduce the solubility of both enamel and apatite, inhibiting demineralization, no anti-caries effect has been proved so far (25). Our study was able to show some anti-caries effect of Ion Z, but not superior compared to other GICs. The limited anti-caries effect of the GICs in this study may be due to the high cariogenic challenge provoked by the constant presence of sucrose in the medium, simulating patients at high risk for caries. If a lower cariogenic challenge should have been applied under this model, a better anti-caries effect of GICs could be expected.

Interesting finding was that Ion-Z had an additive effect increasing the mineral content at the surface lesion (which might be rich in Zn-modified apatite), once it was the only material that significantly differed from control with respect to Z max values. We expected that this effect could indirectly help reducing the progression of demineralization at the subsurface, which was not the case. Under *S. mutans* biofilm, Lobo et al. (13) found 53% of mineral content at the surface layer (30 µm from the surface) for conventional GIC (Fuji II), similar what we found in the present study. Further studies shall attempt to check if Zn is incorporated into the enamel surface and, if so, the depth of its incorporation by using EDX-SEM assay.

This was the first study that attempted to test the antimicrobial and anti-caries effect of Ion-Z using microcosm biofilm. The results of the present study shall be confirmed by using other methods of analysis of biofilm (CFU counting, qPCR, acid and extracellular polysaccharide production assays). Other points to be studied are the effect of ageing on the antimicrobial properties as well as the mechanical properties of this new material. If the antimicrobial effect of Ion-Z is proved, efforts shall be done to understand its mechanism based on the Zn action.

In conclusion, ion-Z shows antimicrobial potential, but its anti-caries effect was similar to the other GICs, under this model.

Resumo

A ocorrência de lesões de cárie adjacentes a restaurações é um sério problema na Odontologia. Portanto, novos materiais restauradores antimicrobianos poderiam ajudar a prevenir as lesões cariosas recorrentes. Este estudo avaliou o efeito de um novo cimento de ionômero de vidro (Ion Z) sobre a viabilidade de um biofilme microcosmo e o desenvolvimento da desmineralização do esmalte. Amostras de esmalte foram restauradas com os seguintes materiais (n=9): A) Ion-Z (FGM Ltda); B) Maxxion R (FGM Ltda); C) Ketac Fil Plus (3M ESPE) e D) sem restauração (controle). As amostras foram submetidas a uma mistura de saliva humana com saliva de McBain (1:50) contendo sacarose a 0,2% por 14 dias. As bactérias vivas e mortas foram quantificadas por fluorescência usando um microscópio confocal de varredura à laser. A desmineralização do esmalte foi analisada usando microradiografia transversal (TMR). Os dados foram submetidos aos testes ANOVA/Tukey ou Kruskal-Wallis/Dunn ($p<0,05$). O Ion Z induziu uma porcentagem mais elevada de bactérias mortas (60,96 ± 12,0%) comparado aos outros grupos (Maxxion R: 39,8 ± 6,7%, Ketac Fil Plus: 43,7 ± 9,71% e controle 46,3 ± 9,5%). Todos os materiais reduziram significativamente a perda mineral média em relação ao controle (Ion-Z 25,0 ± 4,2% vol, Maxxion R 23,4 ± 8,0% vol, Ketac Fil Plus 30,7 ± 7,7% vol e controle 41,2 ± 6,6% vol). O Ion-Z foi o único material capaz de melhorar significativamente o conteúdo mineral na camada superficial (Zmax: 63,5 ± 18,2% vol) em comparação com o controle (38,9 ± 11,3% vol). Ion-Z mostrou potencial antimicrobiano, mas seu efeito anti-cárie foi semelhante aos outros materiais, sob este modelo.

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