Mineral content of ionomer cements and preventive effect of these cements against white spot lesions around restorations

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Abstract: This study evaluated the ion exchange at the material/enamel interface and the preventive effect of restorative materials submitted to cariogenic challenge against white spot. Restorations in enamel/dentin of bovine teeth were performed with composite resin (Filtek™ Z250 – control group) and glass-ionomers cements - GICs (Ionomaster R™ and Fuji IX™ - experimental groups). Samples were grouped and submitted to neutral saliva (n = 15) or pH-cycling regimen (n = 15). After eight days of pH cycling, material/enamel interfaces were analyzed by EDX in order to determine the differences ($p < 0.05$) in ionic exchange (Ca, P, F, Al, Sr, and Si) between restorative materials and teeth. In addition, enamel white spot lesion formation was evaluated macroscopically ($p < 0.05$). Sr content was higher in the enamel of the control group ($p > 0.05$) versus the experimental groups. Ca and P content were higher in enamel than in restorative materials. After pH cycling, the GIC enamel bulk showed a significantly higher Sr content compared with the composite resin ($p < 0.05$). Filtek™ Z250 was not able to prevent white spot formation around restorations in comparison with Ionomaster R™ ($p < 0.001$) and Fuji IX™ ($p = 0.004$). GICs reduced white spot formation and presented a preventive effect ($p = 0.051$). GICs presented a greater percentage of fluoride, aluminum, and strontium, and proved effective in white spot lesion prevention around restorations.

Keywords: Tooth; Ion Exchange; Hydrogen-Ion Concentration.

Introduction

The development of reliable materials to complement sparing approaches and minimal intervention dentistry is an ongoing pursuit.[1,2,3,4] Glass-ionomer cements (GICs) have been used for over 20 years in dentistry as important tools in caries prevention.[1,4,5,6] Certain GIC properties indicate that it is a "smart material"; these include ion exchange and recharge between conventional GICs and the external environment, a thermal expansion coefficient similar to teeth, and chemical adhesion to tooth enamel and dentin.[3,7]

High-viscosity restorative glass ionomers were introduced into dentistry because of their promising attribute of enhancing mechanical properties of cements,[8] and their ability to remineralize carious tissue remaining after hand excavation[9] and to prevent secondary caries.[10] Restorative GIC is a rich reservoir of apatite-forming ions, such as fluoride (F), calcium

(Ca), strontium (Sr) and phosphate (P), and both F and Ca ions were found to migrate from GIC to the softened dentin left at the base of cavities.[9,10,11] This exchange was found to harden the surface of the GIC.[7,12] According to Ab-Ghani *et al.*,[13] the depth of exchange between the GIC and the area surrounding the tooth does not exceed 50 µm. For this reason, this zone of the teeth is of major importance in determining ion incorporation in cases of remineralization, in studies evaluating ionic exchange.

The fluoride release of GIC is dependent on the composition of glass powder and liquid.[14,15,16,17] Some changes have also been made in the glass composition to improve anticariogenic properties[10] and to promote the formation of a chemical bond with tooth apatite,[13] by substituting Ca with the Sr content. However, the role of other GIC ions in preventing caries when submitted to a cariogenic challenge is still unknown. Acidic conditions can be expected to promote both demineralization around the restoration and white spot lesions in superficial enamel. Otherwise, the release of ions from GICs is known to be higher at low pHs.[5] However, incorporating Ca and Sr into the area surrounding the teeth, in acidic conditions, has not been correlated to the formation of white spot lesions.

The aim of this study was to evaluate the relationship between the presence and incorporation of the mineral content (Ca, P, F, Al, Sr, and Si) of ionomer cements and enamel white spot formation.

Methodology
Cavity Preparation and Placement of Restorative Materials

Groups of 15 bovine teeth slabs (6 x 6 x 3 mm) were submerged in acrylic resin mounting blocks (Jet, São Paulo, Brazil) with the vestibular surface facing the glass plate. Silicon carbide abrasive paper of successive grits 600 and 800 – (3M Espe, Monrovia, USA) was used on a polishing machine (Arotec, São Paulo, Brazil) to remove excess resin, thus exposing the enamel area.

The specimens were divided into three groups (n = 30) according to Table 1, as follows: a universal restorative resin composite, Filtek™ Z250 (3M Espe, Monrovia, USA), and two restorative hand-mixed glass-ionomer cements, Ionomaster R™ (Wilcos, Petropolis, Brazil) and Fuji IX™ (GC Corporation, Tokyo, Japan), respectively. Cavities were prepared with 3 mm diameter spherical diamond burs (KG Sorensen, Rio de Janeiro, Brazil) on the surface of the enamel, 1.5 mm deep, thus ensuring exposure of the dentin. Cavity preparations for both glass ionomer and composite restorations were etched and dried according to the manufacturer's instructions. The adhesive system used for the resin composite was Adper Single Bond™ 2 (3M Espe, Monrovia, USA) according to the manufacturer's instructions.

Table 1. Samples of composite resins (CR) and glass-ionomer cement (GIC) in control (C) and experimental (E) conditions

Groups (n = 15)	Restorative materials	Condition
Filtek-C (control group)	Filtek™ Z250	Remineralizing saliva*
Filtek-E (experimental group)	Filtek™ Z250	pH Cycling
Ionomaster-C (control group)	Ionomaster R™	Remineralizing saliva*
Ionomaster-E (experimental group)	Ionomaster R ™	pH Cycling
Fuji-C (control group)	Fuji IX™	Remineralizing saliva*
Fuji-E (experimental group)	Fuji IX™	pH Cycling

*Groups submitted to neutral remineralizing artificial saliva

Demineralizing/Remineralizing Cariogenic Challenge

A 25 mm^2 area of the restoration and surrounding enamel of each slab was left exposed, and the entire residual area of the blocks was coated with red nail varnish (Risqué, São Paulo, Brazil). Negative post-cycling control groups (n = 45) were kept in artificial remineralizing saliva, whereas the experimental group was submitted to pH cycling, alternating for 8 hours in demineralizing saliva and then 16 hours in remineralizing saliva, for 8 days.[18]

The experimental groups were submitted daily to demineralizing saliva adjusted to pH 5.0 with sodium hydroxide in 400 mL 0.1 M acetate buffer - pH 5.0 - containing 1.28 mM Ca, 0.74 mM P_i, and 0.03 µg F/mL (Vetec, Rio de Janeiro, Brazil)[18,19] for 8 hours. After the samples were washed with deion-

ized water, they were kept in remineralizing saliva adjusted to pH 7.0 in 200 mL solution containing 1.5 mM Ca, 0.9 mM P_i, 150 mM KCL, 0.05 µg F/mL, and 0.1 M Tris buffer – (Vetec, Rio de Janeiro, Brazil)[17,18] for 16 hours, completing a cycle of 24 hours.

During pH cycling periods, specimens were kept in an incubator (Fanem Ltda., São Paulo, Brazil), at 37°C, to simulate the oral environment. This procedure was reproduced for a period of 8 days. The procedure used by Moi *et al.*[18] was modified as follows: the proportion of demineralizing and remineralizing solutions per area of block was maintained (2:1) with fixed volumes of solution (400 and 200 mL, respectively), and both solutions were replaced by fresh ones on the 4th day. After the 8th day, the blocks were kept in the remineralizing solution for an additional 24 h.

Evaluation of the Caries-Preventive Effect – Occurrence of White Spot Lesion Inhibition Zone

The caries-preventive effect was evaluated by direct macroscopic observation of the presence of white spot lesions. All enamel surfaces of each group (n = 15) were classified by two trained examiners, according to the levels of presence or absence of white spot lesions around the restoration, using four scores ranging from 0 to 3, as follows: score 0 - 0% of white spot lesion; score 1 - area of white spot lesion < 50%; score 2 - area of white spot lesion > 50%; and score 3 - area of white spot lesion = 100%.

Ion Exchange Evaluation - Scanning Electron Microscopy with X-ray Microanalysis (SEM/EDS)

Hemisectioned samples transverse to the prepared surface were obtained by means of a low-speed diamond saw (Buehler, Lake Bluff, USA) and prepared for SEM, in the same way as biological material. Sectioned surfaces were analyzed on a scanning electron microscope by back-scattered electrons (BSE) at 20kV voltage, low vacuum mode (45 Pa) and magnification of 500 X (Jeol Ltd., Tokyo, Japan).

Chemical analysis for the assessment of relative mineral content of enamel and restorative materials was performed by energy-dispersive X-ray spectroscopy (Jeol Ltd., Tokyo, Japan) with EDX Link. The

energy calibration was performed using the internal standards for the equipment. The content of the Ca, P, F, Al, Sr, and Si structural elements was assessed, and the Ca/P ratio was calculated.

Analyses were performed about 1 mm from the dentin/enamel interface, and four points were considered for EDX analysis: (MB) material bulk, (MI) material interface, (EI) enamel interface, and (EB) enamel bulk (Figure 1). The interface areas were chosen to evaluate whether any alteration in ionic exchange could be expected in response to a cariogenic challenge. The bulks of material and teeth (depth < 50 µm)[13] were also analyzed to investigate ion exchange and incorporation in the area surrounding the teeth.

Statistical analysis

Score values of white spot formation and EDX semi-quantitative analysis were entered in the statistical program SPSS 16.0 (SPSS Inc., Chicago, USA). The white spot scores from the restorative materials were submitted to the Kruskal-Wallis and the Mann-Whitney tests. The EDX values from the four points (MB, MI, EI, and EB) were submitted to ANOVA and post hoc Tukey tests for each restorative material, before and after pH cycling. The correlation between white spot scores and ions, at the two enamel points (EI and EB), was evaluated by the Pearson correlation test. Statistical tests were performed with a confidence interval of 95% ($p < 0.05$).
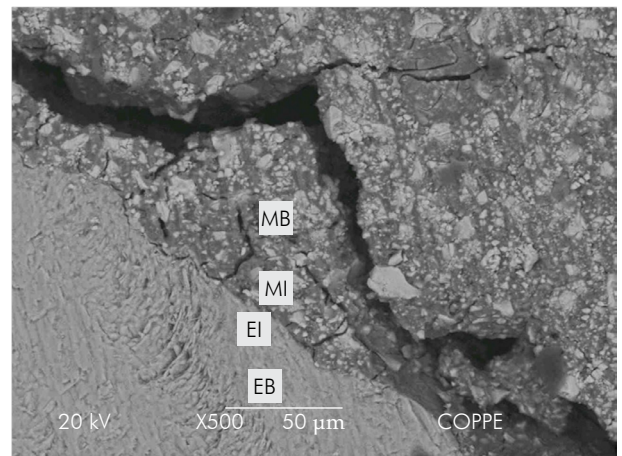
Figure 1. Fuji IX™ group SEM image with representative EDX points: (MB) material bulk; (MI) material interface; (EI) enamel interface; (EB) enamel bulk.

Results

Occurrence of White Spot Lesions

Score values of white spot lesions are shown in Table 2. Both Ionomaster R™ and Fuji IX™ glass ionomers showed relatively minor mineral loss when submitted to pH cycling, in comparison with Filtek™ Z250 composite resin.

Statistical significance was observed between the control and the pH cycling groups for Filtek™ Z250 ($p < 0.001$), Ionomaster R™ ($p = 0.016$), and Fuji IX™ ($p = 0.001$). The Filtek™ Z250 group presented the highest scores, i.e., 2 (46.7%) and 3 (26.7%). Filtek™ Z250 presented a less preventive effect than Ionomaster R™ ($p < 0.001$) and Fuji IX™ ($p = 0.004$). However, the glass ionomers presented a similar preventive effect ($p = 0.051$).

Scanning Electron Microscopy with X-ray microanalysis (SEM/EDX)

Cracks were observed in the glass ionomers, caused by the vacuum required to perform the SEM and EDX analysis; however, the inorganic content remained intact. Since the presence of cracks may influence the mineral measurements, especially in ionomeric materials, in which this situation occurs more frequently, the cracked areas were excluded from analysis and only continuous regions were assessed, to avoid any bias. Table 3 presents a descriptive analysis of the relative mineral content of Ionomaster R™ and Fuji IX™, measured by EDX analysis. Fuji IX™ inorganic content presented a reduced percentage weight of F, Na, P, K, and Ca, compared with Ionomaster R™.

Filtek™ Z250 (Table 4) was used as a control for ionomeric materials. It was possible to identify lower Ca content in the enamel interface than in the enamel bulk (Figure 1), probably due to the enamel etch conditioning step of the restoration procedure. Furthermore, after pH cycling, Ca content in enamel bulk decreased, indicating demineralization, and Sr, F, and Al were not identified in this composite group. All of these situations together could explain the greater enamel white spot formation in this group (Table 2). In Fuji IX™, Ca content in the enamel bulk apparently decreased after pH cycling, and F content also decreased. In terms of the cariogenic challenge, the Ca content in both EI and EB was lower than in the control group; however F, Al, and Sr content was detected, as shown in Figure 2A-F. It should be pointed out that Sr, F, and Al content was already greater in the enamel structure of the control groups. Nevertheless, Sr content was higher for the control groups of all the materials at EI and EB ($p > 0.05$).

In terms of the pH cycling groups, Sr content in the enamel interface was significantly higher than in the composite group only for Fuji IX™ ($p = 0.016$), whereas both Ionomaster R™ and Fuji IX™ showed a significantly higher Sr content compared with the Filtek™ Z250 composite ($p = 0.001$ and $p = 0.010$, respectively) in the enamel bulk. The Ionomaster R pH cycling group showed a significantly higher F content in enamel bulk ($p = 0.013$), compared with Filtek™ Z250 and Fuji IX™.

Both ionomeric materials presented similar mineral content (Table 3). In general, a decrease in the

Table 2. pH cycling score values of white spot lesions for experimental groups with median scores and interquartiles of restorative materials

	Material		
Scores	FiltekTM Z250a	Ionomaster R™b	Fuji IX™c
0	0.0 %	66.7 %	33.3 %
1	26.7 %	33.3 %	26.7 %
2	46.6 %	0.0 %	33.3 %
3	26.7 %	0.0 %	6.7 %
Median scores (interquartile)	2 (1)	0 (1)	1 (2)
p-value (before x after pH cycling)	< 0.001	0.016	0.001
p-value (experimental conditions)	a x b = < 0.001	a x b = < 0.001	a x c = 0.004
	a x c = 0.004	b x c = 0.051	b x c = 0.051

Kruskal-Wallis and Mann-Whitney test ($p < 0.05$).
All groups before pH cycling presented 100% of score 0.
The letters indicate the restorative materials in the statistical analysis.
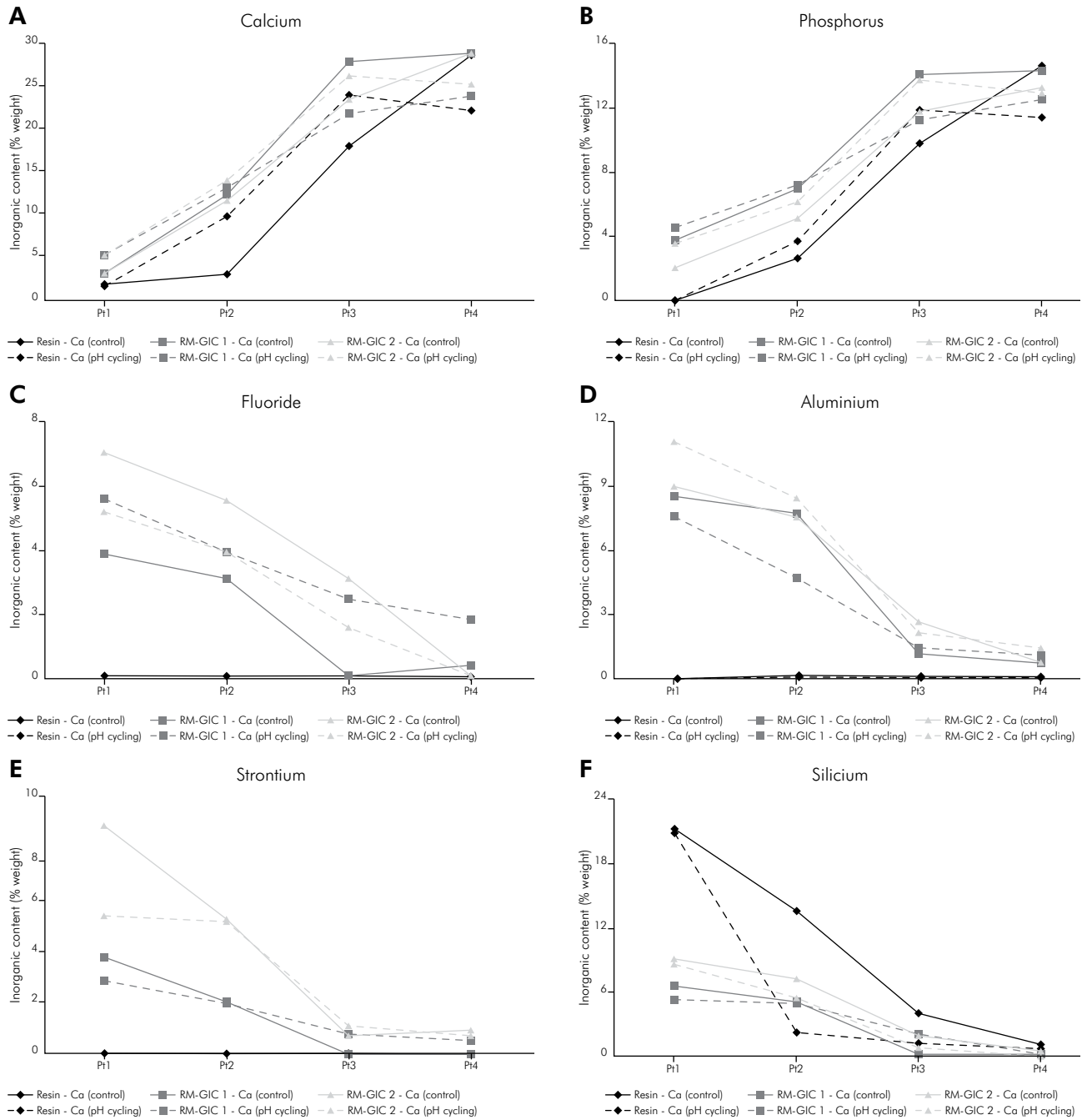
Figure 2. Ca (A), P (B), F (C), Al (D), Sr (E), and Si (F) content within each material group for control and samples submitted to pH cycling among points: (MB) material bulk; (MI) material interface; (EI) enamel interface; (EB) enamel bulk.

Table 3. Descriptive compositions of glass (% weight) of GICs evaluated by EDX

Glass ionomer composition (% wt)	O	F	Na	Al	Si	P	K	Ca	Sr	Ba
Ionomaster R™	37.8	11.9	3.3	16.8	12.4	4.2	0.1	0.7	12.6	-
Fuji IX™	28.3	9.5	1.3	20.2	22.0	-	-	-	18.4	0.3

Table 4. Inorganic content of samples (% weight) at different points: material bulk; material interface; enamel interface; and enamel bulk.

| Point | Material | | | | | | | | | | | | p-value |
| | FiltekTM Z250 | | | | Ionomaster RTM | | | | Fuji IXTM | | | | |
	MB	MI	EI	EB	MB	MI	EI	EB	MB	MI	EI	EB	
Ca Control	1.81a (±0.1)	2.94 (±1.3)	17.99b (±6.4)	28.55 (±2.5)	3.07 (±0.6)	12.29 (±6.1)	27.87c (±1.3)	28.87 (±1.8)	3.28a (±0.8)	11.59 (±6.8)	23.40 (±4.8)	28.70 (±2.9)	a=0.033 / b=0.042
Ca pH cycling	1.57c (±0.2)	3.48 (±1.0)	23.92 (±4.9)	22.09 (±5.4)	5.20c (±2.1)	13.16 (±10.1)	21.79 (±7.4)	23.82 (±6.1)	5.14 (±1.1)	14.01 (±6.6)	26.14 (±3.1)	25.25 (±1.4)	c=0.003
P Control	0.00ab (±0.0)	2.56cd (±2.5)	9.83e (±2.9)	14.64 (±1.1)	3.75a (±1.0)	6.99c (±1.1)	14.12e (±0.3)	14.37 (±0.7)	3.43b (±0.7)	6.23d (±1.2)	11.74 (±1.1)	14.48 (±1.0)	a,b< 0.001 / c=0.026 / d=0.037 / e=0.015
P pH cycling	0.00ab (±0.0)	3.69 (±0.5)	11.86 (±2.3)	11.45 (±2.5)	4.54ac (±1.0)	7.26 (±3.2)	11.30 (±3.4)	12.56 (±2.4)	3.57bc (±0.4)	6.18 (±1.3)	13.79 (±1.3)	12.91 (±0.7)	a,b<0.001 / c=0.016
Ca/P Control	NE	1.11 (±0.4)	1.83 (±0.1)	1.95 (±0.1)	0.82 (±0.1)	1.76 (±0.7)	1.97 (±0.1)	2.01 (±0.1)	0.96 (±0.1)	1.86 (±0.9)	1.99 (±0.3)	1.98 (±0.1)	...
Ca/P pH cycling	NE	2.64 (±0.7)	2.02 (±0.1)	1.93 (±0.1)	1.14 (±0.3)	1.81 (±0.7)	1.93 (±0.3)	1.90 (±0.2)	1.44 (±0.3)	2.27 (±0.8)	1.90 (±0.1)	1.96 (±0.1)	...
F Control	0.00ab (±0.0)	0.00c (±0.0)	0.00d (±0.0)	0.00 (±0.0)	6.86a (±1.7)	3.49 (±2.0)	0.00e (±0.0)	0.00 (±0.0)	6.95b (±1.4)	5.46c (±2.3)	3.03de (±1.5)	0.00 (±0.0)	a b,e<0.001 / c=0.010 / d=0.006
F pH cycling	0.00ab (±0.0)	0.00cd (±0.0)	0.00 (±0.0)	0.00e (±0.0)	5.51a (±0.8)	3.86d (±1.2)	2.39 (±2.3)	1.76ef (±1.4)	5.13b (±1.7)	3.88d (±1.8)	1.49 (±1.0)	0.00f (±0.0)	a,b,c,d<0.001 e,f=0.013
Al Control	0.00ab (±0.0)	0.12cd (±0.2)	0.10e (±0.2)	0.00gh (±0.0)	8.54a (±1.8)	7.72c (±4.5)	1.09f (±0.5)	0.75g (±0.1)	8.95b (±1.1)	7.53d (±2.2)	2.60ef (±0.9)	0.75h (±0.1)	a,b,g,h<0.001 d=0.014 / e=0.002 f=0.026
Al pH cycling	0.03ab (±0.1)	0.03cd (±0.0)	0.04fg (±0.0)	0.07hi (±0.0)	7.58a (±3.0)	4.66ce (±2.8)	1.44f (±0.9)	1.12h (±0.6)	11.12b (±1.6)	8.47de (±1.5)	2.12g (±0.6)	1.141 (±0.7)	a,b,d<0.001 c=0.005 / d=0.050 e=0.011 / f=0.001 g=0.023 / h=0.016
Sr Control	0.00 (±0.0)	0.00 (±0.0)	0.00 (±0.0)	0.00ab (±0.0)	3.80 (±1.6)	3.04 (±2.4)	0.00 (±0.0)	0.32ac (±0.1)	8.89 (±1.7)	5.28 (±3.6)	0.71 (±1.6)	0.93bc (±0.2)	a=0.010 / b,c<0.001
Sr pH cycling	0.00 (±0.0)	0.00a (±0.0)	0.00d (±0.0)	0.00ef (±0.0)	2.85 (±1.6)	1.97b (±1.4)	0.78 (±0.3)	0.51e (±0.3)	5.40 (±0.9)	5.16ab (±1.9)	1.12d (±0.9)	0.71f (±0.3)	a<0.001 / c=0.008 d=0.016 / e=0.010 f=0.001
Si Control	21.21ab (±0.5)	13.61 (±4.7)	4.01c (±3.5)	1.07de (±0.3)	6.53a (±1.6)	4.92 (±3.2)	0.31c (±0.2)	0.05df (±0.1)	9.02b (±2.5)	7.17 (±2.3)	1.84 (±0.8)	0.33ef (±0.1)	a,b,d,e<0.001 c=0.050 / f=0.046
Si pH cycling	20.82ab (±1.5)	2.13 (±1.9)	1.14d (±0.4)	0.67ef (±0.2)	5.30ac (±2.8)	3.01 (±2.8)	0.31d (±0.4)	0.05e (±0.1)	8.58bc (±1.3)	5.44 (±2.0)	0.67f (±0.3)	0.00f (±0.0)	a,b,e,f<0.001 c=0.050 / d=0.08

minerals at MB, MI, EI, and EB was observed after pH cycling. Even after the cariogenic challenge, EI and EB still showed signs of these minerals. The fact that these ions subsisted explains the reduced mineral loss observed in these groups, justifying the lower white spot formation (Table 2).

Statistical differences among restorative materials are represented by letters

The Pearson's correlation test demonstrated a negative correlation ($p < 0.05$) between the white spot lesions scores and the Sr, Al, F, and Si ions (Table 5). This result demonstrated that white spot lesion formation was inversely proportional to Sr, Al, F, and Si concentrations in enamel interface and bulk.

Table 5. Pearson correlation between white spot scores and ionic content in enamel

Ion/Localization	R Pearson correlation	p-value
Ca/EI	0.107	0.48
Ca/EB	- 0.152	0.32
P/EI	0.056	0.71
P/EB	- 0.206	0.18
Sr/EI	- 0.402	0.01
Sr/EB	- 0.431	< 0.01
Al/EI	- 0.391	0.01
Al/EB	- 0.406	0.01
F/EI	- 0.419	< 0.01
F/EB	- 0.433	< 0.01
Si/EI	- 0.465	0.01
Si/EB	- 0.536	< 0.01

Discussion

It has been widely reported in the literature that GICs show less enamel demineralization caused by cariogenic challenge, in comparison with resin composite material.[20,21,22] This preventive effect has been vastly discussed in the literature.[3,4,11,13] It is commonly associated with the different amounts of fluoride content between these materials and the fluoride exchange among glass-ionomer cements and teeth. The current study showed that other inorganic exchanges occur in addition to that of fluoride between glass-ionomer cements and the mineral content of teeth. Furthermore, it seems that these exchanges are also important for enamel structure reinforcement. The

formation of more insoluble apatites, such as strontium-apatite, fluorapatite, and hydroxy-fluorapatite, enhances protection against caries. In addition, under acidic conditions, a much greater number of ions are released from the glass-ionomer cement, and the high release rates are sustained.[23,24] This dynamic is crucial for caries prevention.

The difference in the ionic dynamics of both GIC groups may be related to the inorganic glass composition. The basicity of the glasses ensured by sufficient replacement of silicon with aluminum in the cement matrix could play an important role in the bioactivity of cement and ion incorporation in the teeth. The apparent exchange of Ca and Sr at the restoration interface can be expected as a result of an equilibrium-driven diffusion.[13] The relatively high diffusion of Sr content in the enamel bulk under pH cycling is probably related to the bioactivity of Fuji IX glasses. Their composition is based on glasses with extensive substitution of calcium by strontium, and their advantage is that strontium has potential anti-cariogenic properties, as demonstrated in the current study, where strontium had a preventive effect in reducing white spot formation. The explanation for this substitution is the slightly larger ionic radius of strontium, in comparison with calcium (113 pm compared with 99 pm).[25,26]

In addition to Sr uptake, Fuji IX™ maintained greater amounts of Ca content in the enamel interface and bulk during neutral (control) and acidic conditions (pH cycling). Ionomaster R™ had a greater amount of F content in the bulk material, and showed a general decrease in the inorganic content of enamel (EI and EB) in the pH cycling group. Despite this occurrence, the decrease in Ca content was not significant in the GIC groups (Table 2). The only significant loss of Ca content was observed in the Filtek™ Z250 control group ($p < 0.042$), presumably due to a phosphoric acid conditioning process, which could explain the low correlation with white spot prevention. In regard to the pH cycling groups, the F content of Ionomaster R™, at both EI and EB, was higher than that of Fuji IX™. However, the Ca, P, and Sr content of Ionomaster R was lower than that of Fuji IX™, at both EI and EB. Considering the better preventive effect against caries of F, and its correlation with white spot pre-

vention, one can suppose that F was an important ion involved in caries prevention (Table 5).

Surfaces were examined using a visual macroscopic scale to evaluate the caries-preventive effect. Despite the limitation of this method in showing quantitative mineral loss values, it allowed a qualitative distinction of the caries-preventive effect among different materials, as in previous studies.[19,20] A visual method was chosen because the aim of this study was not to quantify the mineral loss, but rather, to make a qualitative macroscopic evaluation of the enamel, by analyzing white spot lesion formation under a cariogenic challenge, and to identify the possible influence of restorative material minerals on this formation. The presence of Al in the enamel structure of GIC groups may be explained by the Wilson[27] postulate of complex species occurrence of complex ions AlF^{2-} and AlF_2^- among the ions leached from glass ionomers. The similarity in the release and absorption behavior of aluminum and fluoride may be significant. Whether this complex occurrence is significant in influencing the clinical effectiveness of fluoride is not known, and is an important topic for further research.

Conclusion

The ionomer cements presenting a greater percentage of fluoride, aluminum, and strontium incorporated in the enamel structure proved effective in white spot lesion prevention around restorations.

Acknowledgments

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