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INCORPORATION OF CHLORHEXIDINE IN DIFFERENT GLASS IONOMER CEMENTS: SURFACE MICROHARDNESS AND SCANNING ELECTRON MICROSCOPY

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ARTICLE INFO

ABSTRACT

Objective: To evaluate the effect of adding chlorhexidine gluconate (CHX) to resin-modified glass ionomer cement (RMGIC) liquid on surface microhardness and scanning electron microscopy (SEM). *Materials and methods:* Two RMGIC (Riva and Vitremer) were selected and subdivided according to CHX incorporation and storage time. CHX was incorporated into the liquid of each material at concentrations of 05%, 1% and 2%. Specimens (SP) were made with 4 mm in diameter and 2 mm in thickness and kept in saline solution at 37ºC for 2, 7 and 30 days with n=10. Then subjected to Knoop microhardness at three equidistant points on the top surface. SEM microscopy was performed after 7 and 30 days. *Results:* Regarding the material, Riva was the most susceptible to the action of CHX in relation to microhardness. For the CHX concentration, the Riva Light Cure 2% had its surface values significantly increased. Analysis by SEM indicated more cohesive surface for Vitremer for all conditions evaluated. *Conclusion:* CHX did not impair the surface microhardness properties of the studied glass ionomer cement. Vitremer showed superior behavior at most concentrations compared to Riva Light Cure. SEM showed that immersion time caused more surface changes than addition of CHX.

INTRODUCTION

Childhood caries, especially in developing countries, is the most prevalent chronic disease and, consequently, a public health problem (CHAFFE; CHENG, 2014). Depending on the severity of the disease and the number of dental infection foci, it can cause functional, aesthetic and psychosocial disorders that reduce the quality of life of children and their families (CHAFFE; CHENG, 2014).

In these cases, this is still extremely worrying, making restorative treatments with materials capable of releasing fluoride into the oral environment a viable alternative for disease control. In this sense, the glass ionomer cement, as it presents fluoride release with a high initial release pattern, presents itself as a promising restorative material in cases of patients with chronic caries and needs for oral and nutritional adjustment (TERADA *et al*., 1998) . Their main physical propertiesare: fluoride release, adhesiveness, linear thermal expansion coefficient, biological compatibility (WIEGAND *et al*., 2007; FOOK *et al*, 2008), low solubility, good long-term clinical performance and

bacterial reduction (WIEGAND *et al*., 2007). Glass ionomer cements (GIC) can be classified as conventional and reinforced by resin. Conventional ones basically consist of powder and liquid. The powder is composed by the fusion of its main components: silica (SiO_2), alumina (Al_2O_3) and calcium fluoride (CaF_2). The first two components are responsible for the material's strength, while calcium fluoride participates in the setting reaction, but together with other fluorides it is responsible for releasing fluoride into the medium. The liquid, on the other hand, is usually composed of polyacrylic acid, with an R radical that complements the molecule in the polyalkenoic acid, and the carboxylic group (-COOH) is responsible for the union with the glass particles and the tooth structure, water is an essential component for the ionization of polyacrylic acid to occur (SIDHU; NICHOLSON, 2016).O CIV reforçado com resina, foi introduzido para melhorar as propriedades mecânicas e estéticas dos convencionais por meio da incorporação de monômeros resinosos. As propriedades como biocompatibilidade, liberação de flúor, atividade antimicrobiana, coeficiente de expansão semelhante ao do dente e ligação físico-química com a estrutura do dente foram mantidas, e propriedades como resistência mecânica, sensibilidade reduzida á umidade, foram realçadas, aumentando suas indicações clínicas, como restaurações sanduíche aberta, túnel e classes V (SEKHAR *et al*., 2017; SIDHU; NICHOLSON, 2016).

To improve its antibacterial properties, some researchers have suggested the incorporation of antimicrobial agents (CASTILHO *et al*., 2012; CASTILHO *et al*., 2013; CHAFFEE; CHENG, 2014). Chlorhexidine is an effective antimicrobial agent against gram negative and gram-positive bacteria and yeasts (BARBOUR *et al*., 2013), has proven efficacy in the chemical removal of dental biofilm (FERREIRA *et al*., 2012). The most common commercial form of chlorhexidine is digluconate, which has greater activity, due to its solubility, and which allows combination with alcohol. Chemically, CHX is a bis-biguanide, composed of a hexamethylene bridge, having at the end a ring with the 4-chlorophenyl group, being a positively charged molecule (two positive charges, one on each side of the hexamethylene bridge). It is a strong, bi-cationic base at pH above 3.5. Its bi-cationic nature makes the molecule very interactive with ions, which influences its efficacy, safety, local side effects and difficulties in commercial availability (MATHUR *et al*., 2011; MOHAMMADI, 2008). Based on the properties of CHX, it can be a therapeutic agent in the management of caries disease, due to its antimicrobial characteristics, in addition to improving inhibitory action on residual microorganisms, and presenting a broad spectrum against bacteria (CASTILHO *et al*., 2013). Its addition to the GIC could significantly improve the mechanical properties and antibacterial effect of these materials (HOSZEK; ERICSON, 2008; FARRET *et al*., 2011; KORKMAZ *et al*., 2013; GULCE *et al*., 2013; YADIKI *et al*., 2016). Its use in restorative dentistry previously or incorporated into adhesive systems is well documented in the literature (CARRILHO *et al*., 2007; BRESCHI *et al*., 2010; STANISLAWCZUK *et al*., 2014). However, the ideal concentration is still contradictory (SILVA *et al*., 2019). Therefore, the null hypothesis of this study is that the addition of different concentrations of CHX to the GIC liquid will not harm its microhardness properties. This work aimed to evaluate the effect of adding chlorhexidine gluconate to resin-modified glass ionomer cement liquid on surface microhardness and SEM.

METHODOLOGY

Sample Calculation: The sample size calculation was based on probability distributions of the F family, with a design of repeated families, with interaction within and between the factors. The effect size used was 0.15, type 1 error (α) of 0.05, power of analysis of 0.80 guaranteed a minimum of sample units of 80 (body of evidence), with 10 samples per experimental group. Sample calculation was performed using the GPower program (version 3.1.9.2 - University of Düsseldorf, Düsseldorf - Germany). Two groups were performed with RMGIC, according to the flowchart (Figure 1).
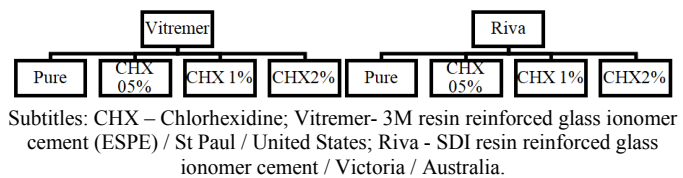

Subtitles: CHX – Chlorhexidine; Vitremer- 3M resin reinforced glass ionomer cement (ESPE) / St Paul / United States; Riva - SDI resin reinforced glass ionomer cement / Victoria / Australia.

Figure 1. Flowchart of group distribution for Vitremer and Riva (n=10)

Materials used: In the research resin-modified ionomer cements (RMGIC), as shown in Table 1, were used.

Table 1. Composition of Resin-Modified Glass Ionomer Cements

Resin Modified Glass Ionomer Cements	Manufacturer / City / Country	Composition
Vitremer	3M(ESPE) / St Paul / USA	Powder: Fluoroaluminum silicate crystals, potassium persulfate, ascorbic acid and pigments Liquid: Polyalkenoic acid, methacrylate groups, water, HEMA, camphorquinone. Finishing gloss (glaze): Bis GMA, TEGDMA and hotoinitiator(camphorquinone).
Riva Light Cure	SDI/ Victoria/ Australia	Powder: Aluminum Silicate Fluoride Liquid: Polyacrylic acid, tartaric acid, hydroxyethylmethacrylate, dimethacrylate, acidified monomer.

Preparation of specimens (SP): All materials used were provided and handled by a single operator, strictly following the recommendations of each manufacturer (Table 1). In order to standardize the portion of powder and liquid used, 5 consecutive measurements of a portion of the powder for each material were performed on an analytical balance (Mettler Toledo AB-204 - Switzerland), from which the measurements were obtained. an average, used as the default value, corresponding to a portion of the material. The same procedure was performed with the liquid and subsequently the incorporation of CHX at the concentrations (05%, 1% and 2%). A silicone matrix (additive silicone, Adsil, Coltene., Araraquara – São Paulo, Brazil) 2mm thick and 4mm in diameter was positioned on a polyester strip (Airon, Maquira Dental Products Industry LTDA., Maringá - Paraná, Brazil) and this on a 10 mm high glass plate. With the aid of a No. 1 resin spatula (Golgran Dental Products Industry LTDA., São Caetano do Sul - São Paulo, Brazil), each GIC was inserted into the matrix with the aid of a Centrix syringe (Nova DFL, Curicica - Rio de Janeiro, Brazil). Then the matrix cavities were covered with another polyester matrix strip, followed by a 10 mm high glass plate to press this set against the upper portion of the matrix and keep it in position. Then, it was photoactivated for 20s with a Radii-Cal wireless Led light device (SDI, Bayswater, Victoria, Australia) with a light intensity equal to 1200 mW/cm². After preparation, the SPs were stored in 37% saline solution (± 1°C).

Surface microhardness test: Surface microhardness was performed at 2, 7 and 30 days after making the SP. These were subjected to the Knoop Hardness Penetration Resistance Test (HK). For each SP, three indentations were performed, in the center of each SP, equidistant from each other, totaling thirty measurements for each analyzed group. At the end of the measurements, the arithmetic mean of the microhardness values for each group was obtained. Microhardness measurements were performed with a digital microhardness meter (FM 800 Future Tech Corp., Equilam, Tokyo, Japan), under a load of 10 g for 20 s.

Scanning Electron Microscopy (SEM) Analysis: SEM was performed after 2 and 30 days, in a sample from each group. The samples were fixed with conductive colloidal silver glue (Ted Pella, Redding, California, USA). The top surface was coated with gold-palladium alloy (Polaron SC 7620 Sputter Coater, Quorum

Technologies, Newhaven, UK) (time: 130 s; current 10-15 mA; vacuum 130 mTorr; plating rate: 3, 5 nm / min; Pd - Au layer: about 80 A). The SEM was operated at 20 kV. The visualization was performed at 5000X magnification.

Statistical analysis: The mean values obtained for each SP were organized in tables and later submitted to the Shapiro-Wilk test, in order to verify the adherence of the data to the normality curve. Considering the positive result to the Shapiro-Wilk test, the data were then submitted to the Analysis of Variance (ANOVA) test for two criteria, followed by the Tukey post test, $p < 0.05$, Bioestat 5.3, (Mamirauá, Belém, Pará , Brazil, 2007).

RESULTS

The results of the statistical analysis of the surface-to-top microhardness test for each group are shown in tables 1 to 5. In table 1, regarding the Riva Light Cure RMGIC, the time analysis shows significant differences only in the 2% concentration in the 30-day period. The analysis of the concentration of incorporation of CHX revealed a significant increase in microhardness values at the concentration of 2% in the period of 2 and 30 days.

Table 1. Mean and standard deviation of the mean values obtained for Knoop microhardness (KHN) for the experimental groups according to time and concentration for the Riva Light Cure material

[%]	2 days		7 days		30 days	
0%	23,42($\pm$2,63)	ABa	21,08($\pm$3,74)	Aa	19,11($\pm$1,80)	Aa
05%	18,70($\pm$4,46)	Aa	21,53($\pm$3,89)	Aa	21,82($\pm$3,72)	Aa
1%	19,60($\pm$ 2,73)	Aa	20,71($\pm$3,67)	Aa	22,54($\pm$3,29)	Aa
2%	28,15($\pm$ 2,46)	Ba	22,25($\pm$2,27)	Ab	28,81($\pm$4,68)	Ba

Different letters mean statistically significant differences, $p < 0.05$. Intracolumn analysis – capital letters. Intraline analysis – lowercase letters.

Table 2 refers to the statistical analysis of Vitremer. The effect of time shows that within 30 days the surface microhardness values significantly increased. For the CHX concentration factor, it showed a significant increase to 2% concentration within 2 days.

Table 2. Mean and standard deviation of the mean values obtained for Knoop microhardness (KHN) for the experimental groups according to time and concentration for the Vitremer material

[%]	2 days		7 days		30 days	
0%	29,57($\pm$4,79)	ABa	30,83($\pm$2,60)	Aa	27,00($\pm$3,23)	ABa
05%	29,37($\pm$5,82)	ABa	28,93($\pm$4,35)	Aa	32,13($\pm$1,99)	Aa
1%	22,76($\pm$1,77)	Aa	19,60($\pm$1,16)	Ba	23,13($\pm$2,71)	Ba
2%	34,25($\pm$10,13)	Ba	30,16($\pm$4,71)	Aa	32,14($\pm$4,40)	Aa

Different letters mean statistically significant differences, $p < 0.05$. Intracolumn analysis – capital letters. Intraline analysis – lowercase letters.

The comparisons between the materials are shown in tables 3, 4 and 5. Table 3 shows that in 2 days of storage the variable concentration for Riva Light Cure 2% presented higher values than the others (Pure, 05% and 1%). In the analysis between materials, Vitremer showed higher values for all concentrations, but statistically significant for the 05% concentration.

Table 3 Mean and standard deviation of the mean values obtained for Knoop microhardness (KHN) for the experimental groups according to material and concentration for the time 2 days

[%]	0%	05%	1%	2%
Riva	23,42 ($\pm$ 2,63) Aa	18,70 ($\pm$4,46) Aa	19,60($\pm$2,73) Aa	28,15($\pm$2,46) Aa
Vitremer	29,57($\pm$4,79) Aa	29,37 ($\pm$5,82)Ba	22,76($\pm$1,77)Aa	34,25($\pm$10,13)Ab

Different letters mean statistically significant differences, $p < 0.05$. Intracolumn analysis – capital letters. Intraline analysis - lowercase letters.

Table 4. Mean and standard deviation of the mean values obtained for Knoop microhardness (KHN) for the experimental groups according to material and concentration for the time 7 days

[ ]	0		0.05		0.01		0.02	
Riva	21,08 ($\pm$3,74)	Aa	21,53 ($\pm$3,69)	Aa	20,71 ($\pm$3,67)	Aa	22,25 ($\pm$2,27)	Aa
Vitremer	30,83 ($\pm$2,60)	Ba	28,93 ($\pm$4,35)	Ba	19,60 ($\pm$1,16)	Ab	30,16 ($\pm$4,71)	Ba

Different letters mean statistically significant differences, $p < 0.05$. Intracolumn analysis – capital letters. Intraline analysis – lowercase letters.

Table 4 shows the comparison between material and concentration for the time of 7 days. Riva Cure Light showed a statistically similar behavior for all concentrations. Vitremer, on the other hand, had statistically lower values for the 1% concentration. The comparison between material shows a statistically similar behavior for the 1% concentration. Table 5 presents the comparison between material and concentration for a period of 30 days. Comparison between material reveals statistically similar behavior for concentrations of 1 and 2%. The influence of concentration shows that for Riva Light Cure the concentration of 2% presented significantly higher values, for Vitremer this behavior was for (05% and 2%).

Scanning Electron Microscopy (SEM): The SEM analysis are shown in figures 3 to 10. It was possible to observe changes in the micromorphological pattern of the surface of Riva Light Cure and Vitremer, after storage of both in saline solutions.
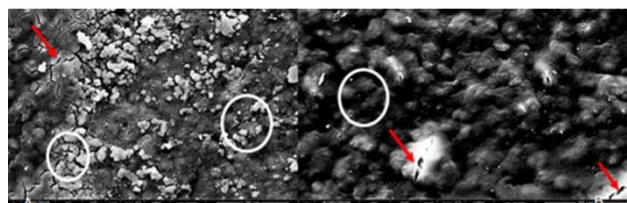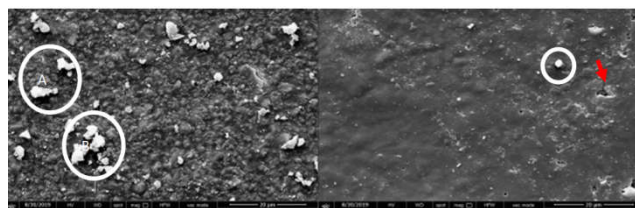

Figure 03. SEM Riva Light Cure without addition of CHX in 2 days (A) and 30 days (B)

The circle in Figure 03 indicates the presence of charge particles weakly bonded to the matrix on the surface of the material. The arrow shows the presence of surface cracks.

In 2 days, there is a greater presence of charge particles on the surface of the material, and few cracks. The circle in Figure 04, of the two images, represents the particles loosely bound to the matrix, this fact is more striking in 2 days (A). Arrows show the presence of cracks.
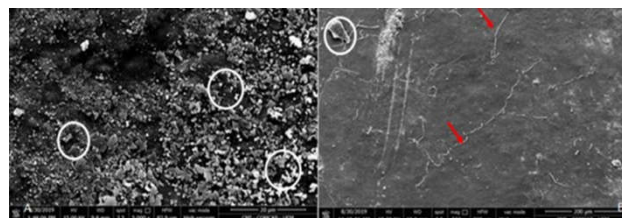

Figure 05. SEM Riva Light Cure with 1% CHX in 2 days (A) and 30 days (B)

The circle of the two images (Figure 05) represents the particles loosely bound to the matrix, which became more evident in 2 days (A). In 30 days (B) the surface is smoother and has small cracks represented by the arrows.
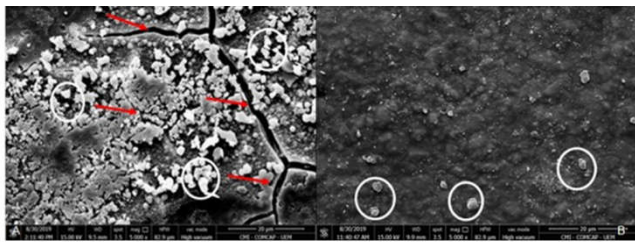
Figura 06. MEVRiva Light Cure com 2% de CHX em 2 dias (A) e 30 dias (B)

The circles of the two images (Figure 06) represent the particles weakly joined to the matrix, where it can be observed in both times, but the arrows show the presence of cracks, where this fact became remarkable in 2 days.
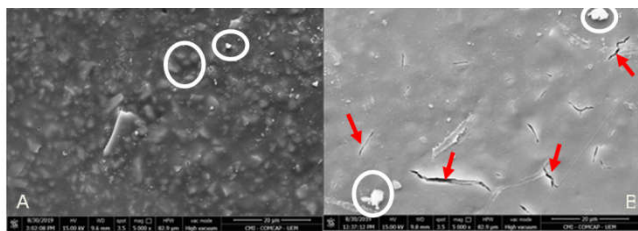

Figure 07. SEM Vitremer without addition of CHX in 2 days (A) and 30 days (B)

The circle in Figure 07, image (A) represents the loosely joined particles. The arrows show the presence of cracks, where this fact became more evident in 30 days.
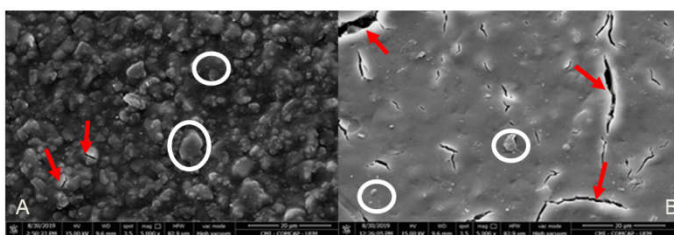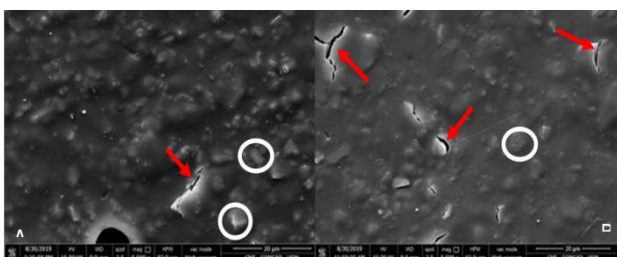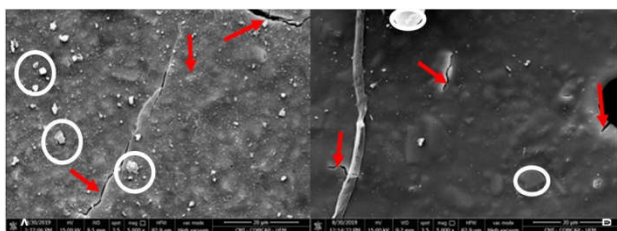

Figure 08. SEM Vitremer with 05% addition of CHX in 2 days (A) and 30 days (B)

The circle in Figure 08 of the image (A) shows a rough surface with the particles involved in the material matrix. In (B) the circles show loose particles on the surface and the marked presence of cracks (arrows).

DISCUSSION

The main change over time in glass ionomer cements was the addition of resin in their composition to improve their physical and mechanical properties, as well as the handling characteristics, effectiveness and longevity of restorations, without harming their release of fluorine (SPAJIC *et al.*, 2019). The incorporation of CHX was suggested in the present study to improve the antibacterial action of MRGIC, since its antimicrobial properties promote membrane rupture and it is effective against a wide variety of microorganisms, including those involved in the carious process. In this sense, Bellis *et al.* (2018) and Duque *et al.* (2017) state that the inclusion of CHX to the GIC improves the antimicrobial/antibiofilm action, without causing harmful effects on the cytotoxicity, mechanical properties and fluoride release of the material. Reinforcing these findings Mathew *et al.* (2013) found that the inclusion of antibacterial compounds eliminates the recurrence of caries in the margins of restorations, inhibits the formation of bacterial plaque on the restored surfaces, in addition to reducing the number of microorganisms in salivary fluids and in the oral cavity. The incorporation of CHX can be performed in different ways: as diacetate (SILVA *et al.*, 2019; MATHEW *et al.*, 2013), in a concentrated chlorhexidine hexametaphosphate paste (BELLIS *et al.*, 2018), or as a digluconate of chlorhexidine as in the present study SHANMUGAAVEL *et al.*, 2015; HOSZEK; ERICSON, 2008; FARRET *et al.*, 2011; KORKMAZ *et al.*, 2013; GULCE *et al.*, 2013; YADIKI *et al.*, 2016).

All forms are intended to significantly improve the mechanical properties and antibacterial effect of the GICS. The use of CHX, in the form of digluconate and diacetate was chosen due to its solubility, allowing better dissipation in the aqueous medium. It is important to consider that the higher the concentration of material, the greater the probability of an adverse effect on mechanical properties and surface degradation after addition of CHX at concentrations above 5% (BELLIS *et al.*, 2018), justifying the concentration of 2 % used in this study was 2%. It was found in this research that the incorporation of CHX at the different concentrations used did not harm the surface microhardness values for both materials, accepting the null hypothesis of this work. Corroborating our findings, SILVA., *et al* 2019, found that the addition of CHX at a concentration of 1.25% did not influence the mechanical properties of compressive strength and surface microhardness. The work by Marti *et al.* (2014) showed that concentrations above 1% cause a reduction in the microhardness of GIC, perhaps the use of conventional GIC can justify the contradictory results in relation to our research. Another decisive factor in incorporating CHX into GICs is the chemical composition of each material, which can determine its behavior against solvent sorption to monomers. Resin-based materials present different patterns of water absorption, depending on the chemical structure of the resin, which involves the hydrophilic nature of the monomers and the differences between the solubility parameter of the monomers and the solvent (BROMBATTI *et al.*, 2018). MRGIC Riva Light Cure does not contain HEMA, which is hydrophobic, and was more susceptible to the action of CHX with the increase of its hardness at concentrations of 1% and 2%. Perhaps the most hydrophilic characteristic of the Riva Light Cure, which allowed for a better interaction of CHX with the water present in this material, this was reflected in the SEM images for the period and 2 days with greater upwelling of particles and in a more uniform surface.

The action of CHX on MRGIC can be explained by the presence of several NH groups that allow the formation of intra and intermolecular hydrogen bonds, this characteristic may be associated with greater rigidity and strength. It also has non-polar portions in the molecule that tolerate interaction with both polar substances (water) and non-polar substances, that is, it has hydrophilic and hydrophobic properties. In the present study, Vitremer had less influence on its surface microhardness property due to the addition of CHX, which may be due to the presence of HEMA. The presence of this monomer can improve the properties of compressive strength, hardness, higher modulus of elasticity, greater resistance to solubility and resistance to bacterial adhesion (Spajic *et al.* 2019).

Table 5. Mean and standard deviation of the mean values obtained for Knoop microhardness (KHN) for the experimental groups according to material and concentration for the time 30 days

[ ]	0%		05%		1%		2%	
Riva	19,11($\pm$1,80)	Aa	21,82($\pm$3,72)	Aa	22,54($\pm$3,39)	Aa	28,81($\pm$4,68)	Ab
Vitremer	27,00($\pm$3,23)	Ba	32,13($\pm$7,99)	Bb	23,13($\pm$2,71)	Aa	32,14($\pm$4,40)	Ab

Different letters mean statistically significant differences, $p < 0.05$. Intracolumn analysis – capital letters. Intraline analysis – lowercase letters.

What could justify the more cohesive surface of Vitremer when analyzed in SEM any concentration of CHX when compared to Riva Light Cure. Another factor that may have contributed to the results for Vitremer is the triple polymerization gelling reaction (Spajic *et al.* 2019). The fundamental acid-base reaction begins as soon as the powder and liquid are agglutinated, forming a network of polysalts. This reaction takes approximately 48 hours to reach its entirety, in the MRGIC it is supplemented by the free radical-mediated polymerization of methacrylate monomers, while the two reactions take place simultaneously. Polymerization of monomers can be chemically or photochemically induced, depending on the initiator system used. Thus, the commercially available MRGIC are double cure in the case of Riva Light Cure (acid-base reaction + monomer light reaction or acid-base reaction + monomer self-cure) or triple cure (acid-based reaction + cure in monomer light + monomer autopolymerization) for Vitremer. Possibly, the triple polymerization could increase the mechanical properties of the material and have contributed to the high microhardness values found by Vitremer. This fact can be seen in the SEM images, evidencing the presence of a more cohesive organic matrix. It was also observed in the present study, an increase in surface microhardness values for Riva Light Cure at concentrations of 2% and 1% while Vitremer maintained the values for these concentrations. This finding is likely due to the better polymerization and greater amount of charge particles present in Vitremer (Spajic *et al.* 2019). Similar results were found by Duque *et al.* (2017), that the mechanical properties of the GICS were not negatively affected by the addition of CHX. Within the limitations of the present study, it was possible to verify the incorporation of CHX in MRGIC are dependent on the composition of the material, storage time and concentration of the antimicrobial agent. More studies are essential to ensure the results found.

CONCLUSION

The addition of CHX did not impair the surface microhardness properties of the studied MRGIC. Vitremer showed superior behavior at most concentrations compared to Riva Light Cure. Scanning electron microscopy showed that the immersion time caused more surface changes than the addition of CHX.

ACKNOWLEDGMENT

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