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Original Research Article

Morphological, chemical and antimicrobial analysis of glass ionomer cements used for ART in posterior primary teeth

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Abstract

Introduction: Glass ionomer cements (GICs) have been gaining prominence as material for atraumatic restorative treatment (ART) due to their acceptable physicochemical and biological properties. **Objective:** To analyze the surface morphology, chemical constitution, and antimicrobial action of GICs used for ART in posterior primary teeth. **Material and methods:** The tested materials were Vitro Molar®, Ketac Cem Easymix® and Riva Self Cure®. For the structural and chemical analysis, polyethylene tubes with an internal diameter of 3 mm and 3 mm in length were prepared, filled, and then transferred to a chamber with 95% relative humidity and a temperature of 37°C. The surface morphology of the tested materials was examined by scanning electron microscopy (SEM) and main components were investigated by energy-dispersive X-ray microanalysis. For the antimicrobial efficacy analyses, strains of *Streptococcus mutans* (ATCC 27853) were used. Petri dishes with brain heart infusion agar (BHIA) were inoculated with the microbial suspensions and three cavities were made in each agar plate and filled with one of the GICs. The plates were pre-incubated for 1 hour at room temperature and then incubated at 37°C for 24 to 48 hours. The inhibition zone around each well was recorded in mm.

Results: SEM revealed irregular and rough external surface. Cracking was not observed. The main constituents were found to be aluminum, silicon, sodium, and fluoride. Barium was only observed in Vitro Molar®, while lanthanum was only observed in Ketac Cem Easymix®. Elemental mapping of the outer surface revealed high concentration of aluminum and silicon. Inhibition halos were only observed in Riva Self Cure®. **Conclusion:** The GICs presented irregular outer surfaces and similar chemical elements. Only Riva Self Cure® showed antibacterial action against the *S. mutans*.

Introduction

Dental caries is an infectious and transmissible disease, characterized by the demineralization of the inorganic and destruction of the organic substance of the tooth [16]. This is a serious problem that concerns professionals who work with children in both primary and permanent dentition [12].

The reduction in the prevalence of dental caries observed in last decades is related to the extensive use of fluoride [22]. When it is present, it can make the calcified tissues of the tooth more resistant to acid dissolution [23, 24]. In addition, fluoride can interfere with the mechanism of dental biofilm formation [23, 24], by the inhibition of glycolytic enzymes and ATPase, as well as intracellular enzymes such as acid phosphatase, pyrophosphatase, peroxidase and catalase [9, 17].

Among the restorative materials currently available, glass ionomer cements (GICs) are highlighted, as they can release fluoride, adhere to dental structures (mainly dentin), have antimicrobial activity, and serve as fluoride reservoirs [13, 25, 27]. GICs have been the materials of choice for atraumatic restorative treatment (ART) [12, 34], a technique of managing dental caries based on the sealants for preventing carious lesions in pits and fissures, and restorations for cavitated dentin carious lesions [12, 20].

The preventive action obtained with the release of fluoride from GICs has encouraged manufacturers to improve existing materials and to launch other new ones [7, 18]. Unfortunately, most professional deals with commercial products of unknown composition, microstructure, and properties [14, 15]. Sound knowledge about the chemical composition of restorative materials, whose elements are distributed on the surface of their structure, may facilitate the understanding of their properties and their interaction with the tissues that are in contact [6]. One of the GICs' characteristics, the antimicrobial action, may be directly affected by their chemical composition [9, 23, 24]. The study of outer surface and chemical compounds of GICs in association with their bacterial property may bring news perspectives for preventive dental practice. Thus, the present study aimed to analyze the surface morphology, chemical constitution, and antimicrobial action of GICs used for ART in posterior primary teeth.

Material and methods

Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) microanalysis

Three commercially available GIC were used: Vitro Molar® (Nova DFL, Rio de Janeiro, RJ, Brazil), Ketac Cem Easymix® (3M ESPE, Sumaré, SP, Brazil) and Riva Self Cure® (SDI, São Paulo, SP, Brazil). The GICs were mixed according to the manufacturers' instructions and placed in standard polyethylene tubes with an internal diameter of 3 mm and a thickness of 3 mm. The tubes were placed on glass slab (75 x 25 x 1 mm), slightly overfilled with the freshly prepared materials, and then transferred to a chamber with 95% relative humidity and temperature of 37°C for a period corresponding to 3 times the manufacturer's recommended setting time. Three homogeneous specimens of each material were made.

Morphological analysis of outer surface of GIC was performed using a scanning electron microscope (JSM-6610; Jeol Ltd., Tokyo, Japan) at 500 X magnification, using an accelerating voltage of 10 kV and a working distance of 15 mm. The samples were sprinkled on carbon double-side tape over a metallic stub, critical point dried and sputter-coated with gold palladium (Bal-Tec AG, Balzers, Germany) at 20 mA. The morphologies of the external surface were qualitatively analyzed according to criteria used by Carvalho *et al.* [4].

EDX was performed with detection-analysis-system NSS Spectral Analysis System 2.3 (Thermo Fischer Scientific, San Jose, CA, USA) to determine the constituent elements of the tested materials. One EDX spectrum was collected from the central region of each specimen under the following conditions:

25 kV accelerating voltage, 110 μA beam current, 10^{-6} Torr pressure (high vacuum), 130 x 130 μm area of analysis at 1000 X magnification, 100 s acquisition time and 30-35% detector dead time. The elemental analysis [weight% (wt.%) and atomic% (at.%)] of samples was performed in nonstandard analysis mode, applying PROZA (Phi-Rho-Z) correction method. The elemental maps were archived by NETCOUNTS method, with high resolution, using the same detection-analysis-system (NSS Spectral Analysis System 2.3).

Agar diffusion test

A reference strain of *Streptococcus mutans*, obtained from the American Type Culture Collection, was used (ATCC 27853). The bacterial strain was inoculated in 7 ml of brain heart infusion (BHI) (Difco Laboratories, Detroit, MI, USA) and incubated at 37°C for 24 hours. The experimental suspensions were prepared by cultivating the biological marker on the surface of brain heart infusion agar (BHIA) (Difco Laboratories, Detroit, MI, USA), following the same incubation conditions. Bacterial cells were resuspended in saline to achieve the final concentration of about 3 x 10^8 cells ml^{-1}, adjusted to #1 MacFarland turbidity standard.

For the agar diffusion test, Petri dishes with 20 ml of BHIA (Difco Laboratories, Detroit, MI, USA) were inoculated with 0.1 ml of microbial suspension. The inoculum was spread on the surface of the culture medium, to obtain a confluent growth. Three cavities (4 mm in depth and 4 mm in diameter) were made in each agar plate with a copper coil and filled with the GICs. The plates were pre-incubated for 1 hour at room temperature, and then incubated at 37°C for 24-48 hours. Microbial inhibition diameters were measured with digital caliper. Positive and negative controls were done, keeping the plates inoculated and without inoculum, for the same periods and under identical incubation conditions. All experiment was carried out under aseptic conditions and in triplicate.

Results

MEV and EDX analysis

The results obtained from SEM analysis are shown in figure 1. It was noted that all GIC had an irregular external surface. Cracking was not observed.

A quantitative result of the main components of the tested materials is presented in table I. Similar chemical elements were found in all materials and there was a small variation between then. Essentially, the materials were composed of elements namely aluminum (Al), silicon (Si), sodium (Na) and fluoride (F). Barium (Ba) was only observed in Vitro Molar®, while lanthanum (La) was only observed in Ketac Cem Easymix®. EDX wide spectrums are presented in figure 2. Elemental mapping revealed the elements distributed throughout the outer surface. Aluminum and silicon were strongly detected by such mapping (figure 3).

Agar diffusion test

The results of the agar diffusion test are presented in table II. Inhibition halos were only observed in Riva Self Cure® (figure 4).

Table I – Main components of glass ionomer cements analyzed with energy dispersive X-ray microanalysis

Element	Vitro Molar®		Ketac Cem Easymix®		Riva Self Cure®	
	wp.%	at.%	wp.%	at.%	wp.%	at.%
Oxygen	31.68	46.27	32.73	50.24	44.24	55.39
Aluminum	15.25	13.21	11.97	10.89	16.11	11.96
Barium	12.06	2.05	–	–	–	–
Calcium	11.73	6.84	11.64	7.13	–	–
Fluoride	17.36	21.35	11.05	14.28	11.65	12.98
Sodium	1.97	2.00	4.15	4.43	2.51	2.18
Lanthanum	–	–	17.01	3.01	–	–
Silicon	9.95	8.28	11.45	10.02	25.49	18.18

Table II – Mean diameter (mm) of the microbial inhibition halos of the tested materials

Biological marker	Tested materials					
	Vitro Molar®		Ketac Cem Easymix®		Riva Self Cure®	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
Streptococcus mutans	0.0	0.0	0.0	0.0	13.0	11.0

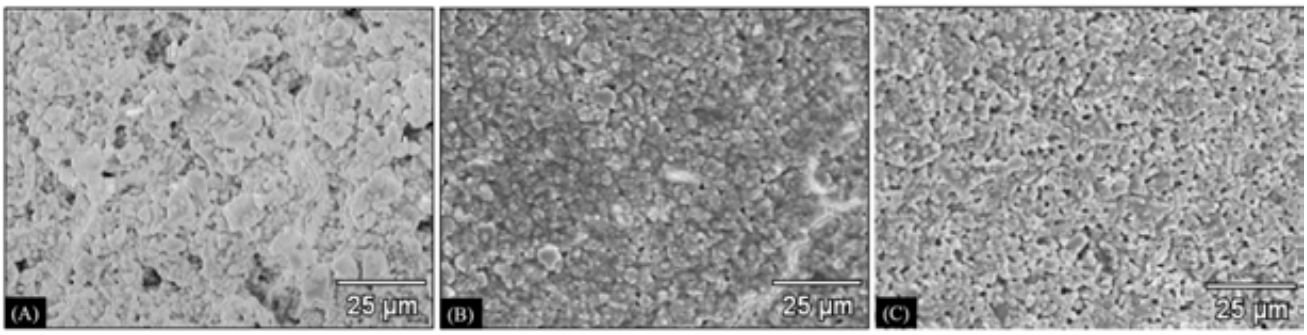

Figure 1 – SEM images of the external surface of the tested glass ionomer cements. (A) Vitro Molar®; (B) Ketac Cem Easymix®; (C) Riva Self Cure®

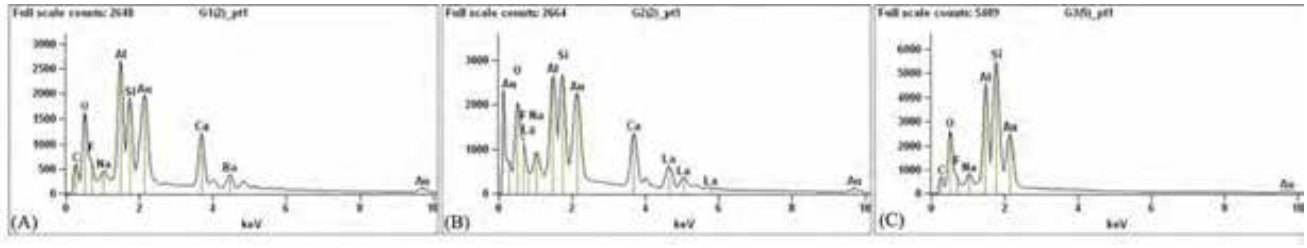

Figure 2 – Representative EDX spectrum of the tested glass ionomer cements. (A) Vitro Molar®; (B) Ketac Cem Easymix®; (C) Riva Self Cure®

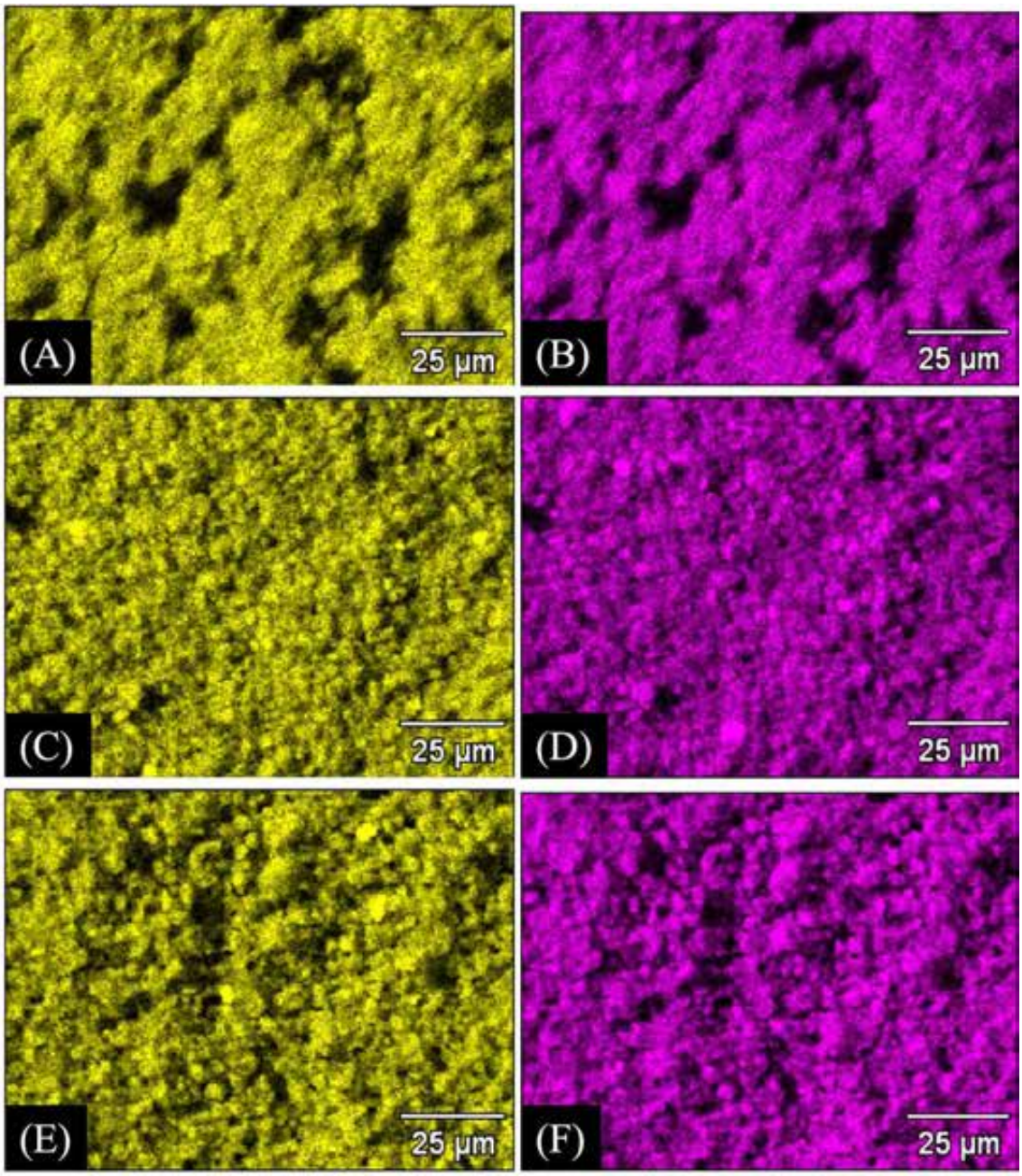

Figure 3 – EDX elemental map of aluminum (yellow) and silicon (purple) distribution throughout the external surface of tested glass ionomer cements. (A and B) Vitro Molar®, (C and D) Ketac Cem Easymix®, and (E and F) Riva Self Cure®

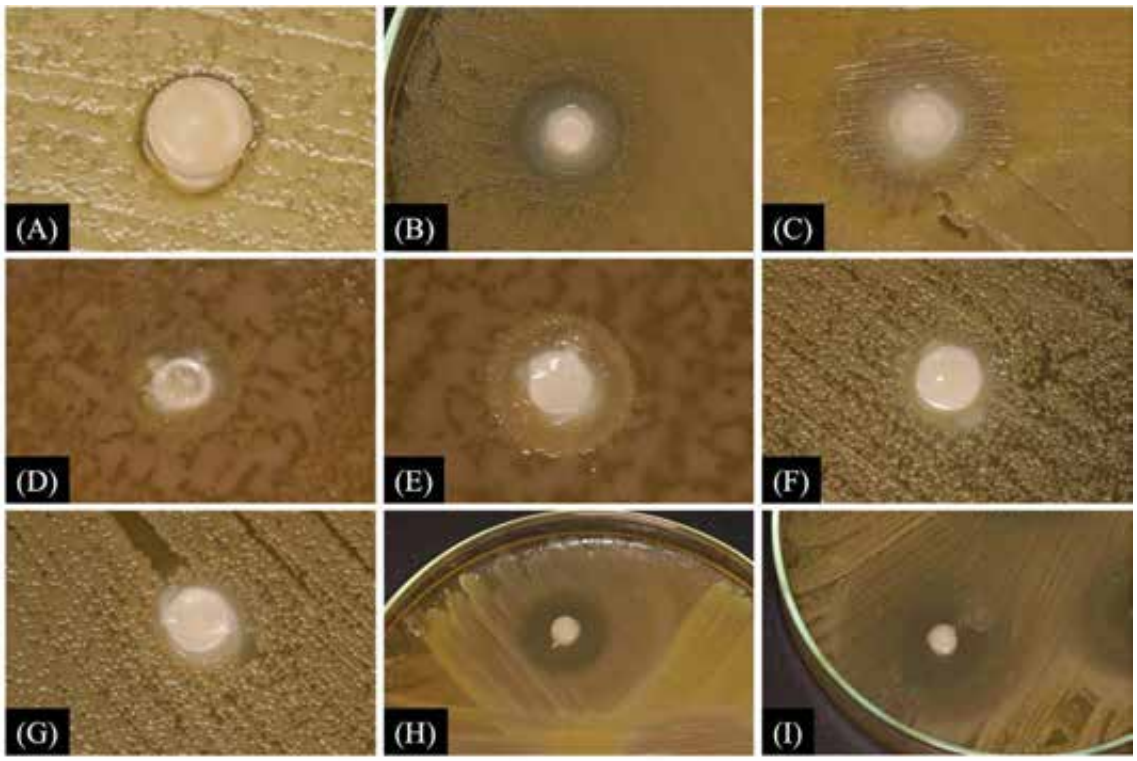

Figure 4 – Antimicrobial action of tested glass ionomer cements. (A-C) Vitro Molar®, (D-F) Ketac Cem Easymix®, and (G-H) Riva Self Cure®

Discussion

The selection of a restorative material imposes strict quality control. The verification of physicochemical and antimicrobial properties is essential. In this sense, all material for clinical use needs to be constantly evaluated. The present study analyzed the surface morphology, chemical constitution, and antimicrobial action of GICs used for ART in posterior primary teeth. Vitro Molar®, Ketac Cem Easymix® and Riva Self Cure® presented similar chemical compositions, whose main elements were Al, Si, Na and F. Only the Riva Self Cure® showed antibacterial activity.

Among the requirements of an ideal restorative material, a regular-looking surface is included [28]. Materials that have a high surface irregularity are likely to cause greater bacterial adhesion and, consequently, increase the risk of dental caries [2]. In addition, to check the properties of materials and interactions with biological systems, it is necessary to know the surface of the materials [6, 27]. Scanning electron microscopy (SEM) images have been shown to be useful research tools to investigate the particle size or granulation present on the surfaces of materials [11, 33]. In the present study, the outer surfaces of the materials were qualitatively analyzed and classified according to a previous study [4]. The analysis revealed that all GICs showed outer surface with irregular aspect, which was also observed in previous studies [2, 4, 15, 33]. Factors such as shape, distribution and number of particles, interfacial bonding between particles, interfacial bonding between the particles and matrix, storage media of GIC specimens, GIC's liquid component, and powder: liquid ratio may affect the GICs' surface roughness and hardness [2, 32].

Energy dispersive X-ray (EDX) microanalysis represents a reliable, accurate and reproducible method to quantify the main constituents or compounds present in a material or mixture [6, 11, 15, 32]. However, this method has limitations regarding the detection of low molecular weight elements. The proportion of ionizing events that result in X-ray emission decreases as the atomic number of the element decreases. Thus, the quantification of organic compounds, which contain carbon, hydrogen, and oxygen, cannot be accurately performed [30].

All the evaluated GICs had similar chemical components. Although the values have varied, the GICs were composed mainly for Al, Si, Na and F (table I). Yap *et al.* [32] studied the glass powder of

the Fuji IX GP Fast and Fuji XP GP and observed that in both GICs the three main elements presented were oxygen, silicon, and aluminum. Zanata *et al.* [34] evaluated the chemical composition of a high-viscous GIC and noted that after 10 years of clinical use the main chemical compounds were F, Al, Si, P, K Ca and strontium (Sr). Guedes *et al.* [15] analyzed the chemical constitution of Maxxion R, VitroFill, Vidrion R and Vitremer and registered high values of Al, Si, Ca, Na and F.

S. mutans was chosen for this study because it is considered the most cariogenic microorganism found in dental biofilm due to its ability to use a carbohydrate-based diet to synthesize extracellular polysaccharides, in addition to its aciduric and acidogenic capacity. Extracellular polysaccharides are important virulence factors of *S. mutans* for promoting bacterial adherence to the tooth surface [26], contributing to the structural integrity of the dental biofilm [19, 31] and, consequently, induces an increase in enamel demineralization [5].

The analysis of the antimicrobial potential (agar diffusion test) of GICs adopted in the present study was based on previous studies [1, 10] and its choice was due to its simplicity, reproducibility, and effectiveness [1]. It is worth noting that this technique is not without limitations. The size of the microbial inhibition zone depends on the solubility and diffusibility of the test substance in the agar diffusion method and, therefore, may not express its full potential. Also, the agar diffusion test does not distinguish between bacteriostatic and bactericidal properties of dental materials neither does it provide any information about the viability of the test microorganism [10]. Furthermore, factors such as pre-incubation, dried culture medium and maintenance for periods that exceed the ideal time for analysis can yield doubtful results [1]. All these factors were controlled for in this study.

The results of the agar diffusion test showed that only the Riva Self Cure® was effective on *S. Mutans*, with inhibition halo values between 11- and 13-mm. Tobias [29] evaluated the antimicrobial action of different GICs. The cements, after setting, showed decreased or lost antimicrobial activity. The author stated that a material soon after its manipulation is more soluble than after its setting, as well as presenting the action of other constituent elements that may also reflect in the variation of the antimicrobial action. Bengtson *et al.* [3] evaluated the antimicrobial capacity of three glass ionomer cements: Vidrion R, Ketac Molar and Meron R, on mixed bacterial culture from the oral cavity, using the agar diffusion test and observed that all materials tested showed antimicrobial action, and the greatest antimicrobial power was demonstrated by the Meron R. The antimicrobial activity verified in agar diffusion tests has been attributed, in the case of GICs, to the low pH during the setting reaction and the high fluorine content [8, 21]. Depending on the fluoride concentration to which they are subjected, all bacteria are subject to different inhibitory effects. These effects can range from inhibition of a single step of metabolism to cell death [17].

Future studies, *in vitro* and *in vivo*, are necessary to determine the components of GICs responsible for the inhibition of cariogenic bacteria, also evaluating the concentrations and time in which they are released.

Conclusion

The GICs presented irregular outer surfaces and similar chemical elements. Only Riva Self Cure® showed antibacterial action against the *S. mutans*.

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