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Evaluation of the Antibacterial Action of Two Resin-Modified Glass Ionomercements After Incorporation of Chlorhexidine: An *In vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RSV and PFC were responsible for the practical research and writing of the article. Authors JKU and FCH managed the bibliographic research, Author GB managed the study analyzes and Author VC performed the statistical analysis and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The purpose of this study was to evaluate the antibacterial effectiveness of chlorhexidine digluconate (DCHX) when incorporated into two different types of resin-modified glass ionomer cements (CIVMR): Riva Light Cure and Vitremer.

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Study Design: This was an experimental *In vitro* study.
Place and Duration of Study: The study was conducted in a controlled laboratory setting during a 24-hour incubation period.
Methodology: Eighty specimens, each 4 mm in diameter and 2 mm thick, were fabricated from two types of CIVMR, each subdivided into four groups based on DCHX concentration (0%, 0.5%, 1%, and 2%). After 24 hours of storage in saline at 37°C, the specimens were tested for antibacterial activity against Streptococcus mutans. The data were analyzed using two-way ANOVA followed by Tukey's test ($p < 0.05$).
Results: The Riva Light Cure specimens exhibited a significant reduction in Colony Forming Units (CFU/mL) with the 1% and 2% DCHX concentrations, reducing bacterial counts by over 90%. In contrast, Vitremer showed a statistically significant reduction only at the 2% concentration, with lesser effects at lower concentrations**.**
Conclusion: The study demonstrated that the incorporation of DCHX at higher concentrations significantly enhances the antibacterial properties of Riva Light Cure glass ionomer cement. However, the effectiveness of DCHX in Vitremer requires higher concentrations to achieve similar antibacterial outcomes. These findings suggest the potential for targeted antimicrobial enhancements in dental restorative materials.

Keywords: Glass ionomer cement; antibacterial; chlorhexidine; streptococcus mutans; Childhood caries; chronic caries.

1. INTRODUCTION

Childhood caries, especially in developing countries, is the most prevalent chronic disease and consequently a public health problem. Depending on the severity of the disease and the number of dental foci of infection, it can cause functional, aesthetic, and psychosocial disorders that reduce the quality of life of children and their families [1]. In these cases, it is still an extremely worrisome fact, because despite significant advances in preventive dentistry, caries continues to affect many children [2], making restorative treatments with materials capable of releasing fluoride into the oral environment a viable alternative for disease control [3].

The prevention of dental caries is very important because it has a considerable impact on self-esteem, mastication, nutrition and health. In this sense, the glass ionomer cement (GIC) for presenting fluoride release with high initial release pattern presents itself as a promising restorative material in cases of patients with chronic caries and the need for oral and alimentary readjustment [4]. GIC's main properties are fluoride release, adhesiveness, linear thermal expansion coefficient, biological compatibility, low solubility, and bacterial reduction [4].

GIC can be classified into conventional, and resin reinforced. The conventional ones are basically composed of powder and liquid. The powder is composed of the fusion of its main components: silica (SiO_2), alumina (Al_2O_3) and calcium fluoride (CaF_2). While the liquid, it is usually composed of polyacrylic acid [5]. Glass ionomers adhere to tooth structure by chelating the carboxylic groups of polyacrylic acid with the calcium present in enamel and dentin [6].

Resin-modified GIC was introduced to improve the mechanical and aesthetic properties of conventional ones by incorporating resin monomers [5] .Properties such as biocompatibility, fluoride release, antimicrobial activity, tooth-like expansion coefficient, and physicochemical bonding with the tooth structure were maintained, and properties such as mechanical strength and reduced moisture sensitivity were enhanced, increasing their clinical indications (Shekar et al., 2017).

Kohno [7] have discussed the advantages of adding antimicrobial agents to the composition of resin materials to prevent biofilm formation, and the use of chlorhexidine (CHX) may be an option. According to studies by Barbour et al. [8], chlorhexidine has been found to effectively combat both gram negative and gram-positive bacteria as well as yeasts. It has also been proven to be successful in chemically removing dental biofilm, as demonstrated by Borompiyasawat et al. [9]. Chemically, chlorhexidine is a bis-biguanide with a hexamethylene bridge and a ring with a 4-chlorophenyl group at one end. Due to its positive charge, chlorhexidine exhibits a high degree of interaction with ions, which plays a role in its effectiveness [10].

The antibacterial action is explained by the fact that the cationic molecule of chlorhexidine is strongly attracted by the negative charge of the bacterial cell wall, being adsorbed to the cell membrane by electrostatic interactions. Because this adsorption is concentration-dependent, at high dosages it causes precipitation and coagulation of cytoplasmic proteins and bacterial death, and at lower doses it alters the integrity of the cell membrane, resulting in an extravasation of low molecular weight bacterial components [11].

Based on the properties of chlorhexidine, it can be a therapeutic agent in the management of caries disease, due to its antimicrobial characteristics, besides improving inhibitory action on residual microorganisms and presenting a broad spectrum against bacteria [12]. Studies show that its addition to GIC can significantly improve its mechanical properties and the antibacterial effect of these materials [13]. However, the optimal concentration is still contradictory [14].

Therefore, the hypothesis of this research is that adding chlorhexidine digluconate (DCHX) to resin-modified glass ionomer cement (CIVMR) will significantly increase its antibacterial effect in vitro.

2. MATERIALS AND METHODS

2.1 Sample Calculation

According to the sample calculation performed using an entirely randomized design, with the aid of the BioStat 5.3 program (User-friendly biology and medicine oriented statistical software | AnalystSoft | StatPlus:mac | StatPlus | BioStat | StatFi – open access), an n equal to 10 per group was defined, with an analysis power equivalent to 08. Thus, 80 specimens were made and divided into 8 experimental groups (n=10), as shown in Fig. 1 and Fig. 2.

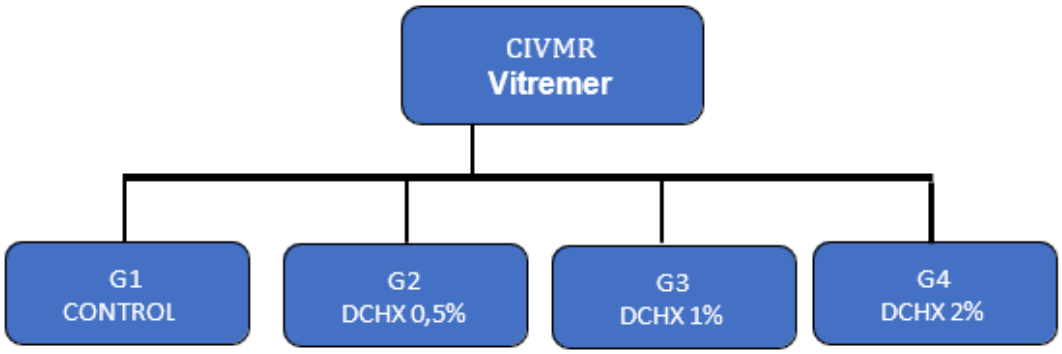

Fig. 1. Flowchart of the distribution of the resin-modified glass ionomer cements (CIVMR), Vitremer® experimental groups. G1 – group control and G2, G3 and G4 with incorporation of chlorhexidine digluconato

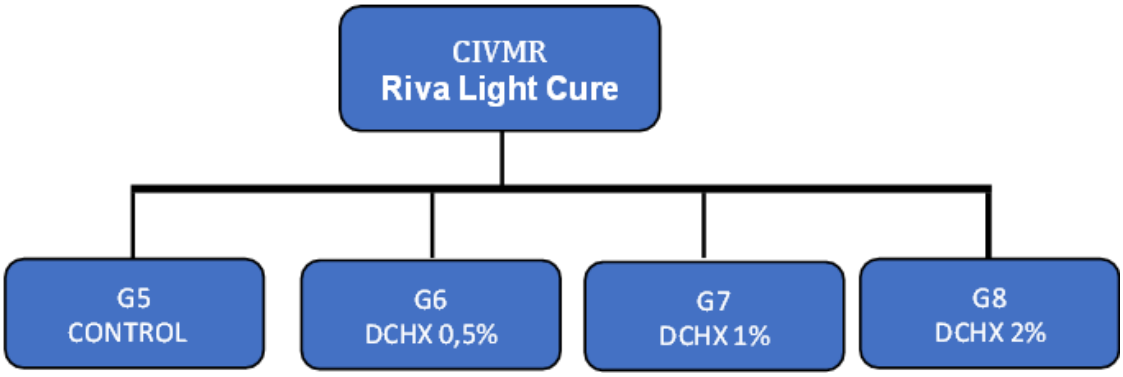

Fig. 2. Flowchart of the distribution of the experimental groups for resin-modified glass ionomer cements (CIVMR), Riva Light Cure®. G5 - group control and G6, G7 and G8 with incorporation of chlorhexidine digluconato

2.2 Materials Used

In this research the resin-modified glass ionomer cements Vitremer® (3M ESPE, Saint Paul, Minnesota, USA) Riva Light Cure (SDI/Victoria/Australia) were used and chlorhexidine digluconate was manipulated pharmaceutically together with the liquid of the material, as described in Table 1 and Table 2. The manipulation was performed in such a way that the amount of chlorhexidine to be added had a final concentration of 0.5%, 1%, and 2% of chlorhexidine digluconate in the sample.

2.3 Confection of Specimes (CP)

The CIVMR was proportioned and manipulated by a single operator, strictly following the manufacturer's recommendations, a proportion of powder for two proportions of liquid, according to the meter that comes with the material. To standardize the portion of powder and liquid used, five consecutive measurements of a portion of the powder, for each material, were performed in an analytical balance (Mettler Toledo AB-204, Switzerland), where from the measurements an average was obtained, used as a standard value, corresponding to a portion of the material. The same procedure was performed with the liquid, after the incorporation of DCHX in concentrations of 0.5%, 1% and 2%. The material was spatulated with the aid of a metal spatula and glass plate. A silicone matrix in the shape of a disc (2x4 mm) was employed as a mold to standardize the specimens. Each silicone matrix was positioned on a polyester strip (Maquira, Maringá, Paraná, Brazil) to prevent adherence to the workbench surface. A glass plate with a height of 10 mm was utilized to ensure the extrusion of any excess material, thereby guaranteeing uniform volume and quantity across all samples. The CIVMR was inserted into the matrix with the aid of a Centrix syringe (Nova DFL, Curicica, Rio de Janeiro, BR) to prevent bubble formation. Then the matrix cavities were covered with another polyester matrix strip, followed by another glass plate, to press this set into position. Photoactivation was performed for 20 seconds with a Radii-Cal Led appliance (SDI, Bayswater, Victoria, AUS) with light intensity equal to 1200 mW/cm². After polymerization, the PC were removed from the silicone matrix and divided into experimental groups according to the concentration of DCHX.

Table 1. Composition of the materials used in the research

Product	Manufacturer	Composition
Vitremer	3M (ESPE)/ Saint Paul/USA	**Powder:** fluor aluminosilicate crystals, potassium persulfate, corbic acidas and pigments. **Liquid:** Polyalkenoic acid, methacrylate groups, water, HEMA, camphorquinone**.** Finishing gloss: Bis GMA, TEGDMA and camphoroquinone.
Product	**Manufacturer**	**Composition**
Riva Light Cure	SDI/Victoria/Australia	**Powder:** Aluminem silicate fluoride, stontium, fluoride ions and bioactive glass (Ionglass TM). **Liquid:** Polyacrylic acid, tartaric acid, hydroxyethyl methacrylate, dimethacrylate, acidified monomer

Table 2. Mean quantification of the number of CFU/mL of S. mutans after addition of chlorhexidine digluconate to the resin-modified glass ionomer cement liquid to make the specimens

Chlorhexidine concentration	Ionomer type	CFU/mL		
0.0	RIVA	279700	±	18166 A
	VITREMER	987	±	103 B
0.5	RIVA	270700	±	9900 A
	VITREMER	1086	±	81.9 B
1.0	RIVA	21280	±	637 C
	VITREMER	1708	±	170 B
2.0	RIVA	2496	±	383 B
	VITREMER	817	±	88.2 B

2.4 Antibacterial Activity

For this study, Streptococcus mutans strain CCT 7086 provided by the André Tosello Tropical Research and Technology Foundation (Campinas, São Paulo, BR) was used.

2.5 Reactivation of the Species

Streptococcus mutans, was reactivated in 5 mL of Brain Heart Infusion (BHI) culture medium and maintained in a microaerophilic environment at 37°C ±1 for 18 hours. After growth, the suspension was centrifuged at 3000 rpm for 15 min and the cells washed twice with sterile saline. The product was suspended in BHI broth and the turbidity of the material adjusted to the absorbance of 0.15 read in a spectrophotometer at 600nm which corresponds to a suspension to stock solution of 108 cells/mL-1.

2.6 Formation of the Bacterial Biofilm

Previously, the PCs were distributed in microtiter plates (10 wells) containing 1000µL of BHI and Streptococcus mutans bacterial suspension. All CPs were submerged in the culture medium and incubated at 37°C ±1 for 24 hours.

2.7 Antibacterial Test

After bacterial biofilm formation, the suspension from each well was aspirated and the PCs were washed with 1000 µL of sterile PBS (Alkaline Phosphate Buffer), and this procedure was repeated 3 times to remove non-adhered bacterial cells. After washing, the PCs were removed from the wells and placed in Falcon tubes with 5 mL of PBS. The tubes were agitated with the aid of a Vortex for 1 minute and immersed in water in an ultrasonic cleaner for 8 minutes. This procedure was performed to disintegrate the biofilm and release the bacterial cells that were adhered to the PC for further quantification of Colony Forming Units (CFU). The method used for counting was serial dilution, where from the initial solution, from which the number of cells was desired, three dilutions were produced, here were 10^{-1},10^{-2} and 10^{-3}, which represent the concentration of the bacterial sample, for each well of each group investigated, followed by plating of the dilutions. Subsequently, the plates were incubated in an incubator at 37°C ± 1°C for 24 hours for subsequent CFU counting.

2.8 Statistical Analysis

The results were tabulated and submitted to statistical analysis using the JAMOVI 2.3.26 software. To verify normality and homogeneity of variances the Shapiro-Wilk test was applied. In turn, for the analysis of the data related to the amount of CFU/mL, because they are categorical parametric data, the test performed was the two-criteria ANOVA, followed by Tukey's follow-up test ($p < 0.05$).

3. RESULTS AND DISCUSSION

The results obtained were submitted to statistical analysis using the Shapiro-Wilk normality test, then the two-criteria ANOVA parametric test ($p < 0.05$), followed by Tukey's follow-up test. Overall, the addition of chlorhexidine to Vitremer® did not result in statistically significant differences, while for Riva Light Cure there was a statistically significant difference at the 1 and 2% concentrations.

The quantification of CFU/mL also showed a correlation between the addition of chlorhexidine and the type of glass ionomer cement tested. In general, it was possible to observe a statistically significant difference between the groups in which RIVA was used in comparison to VITREMER®, except for the 2% concentration. The data are shown in Table 2.

Agreeing with the hypothesis that the addition of chlorhexidine digluconate (DCHX) to resin-modified glass ionomer cement (CIVMR) enhances its antibacterial effect in vitro, the results of this study confirmed this expectation. Evaluating the colony-forming units per milliliter (CFU/mL) of S. mutans, it was observed that increasing concentrations of DCHX significantly reduced the bacterial load in both types of ionomers tested (RIVA and VITREMER). These findings underscore the potential of DCHX in enhancing the antibacterial activity of CIVMR, thereby supporting the study's initial hypothesis.

The Resin Modified Glass Ionomer Cement (RIMC) is a material that presents antibacterial activity attributed to the release of fluoride, responsible for modifying the biofilm through changes in enzymes and interference in its metabolism, thus leading to cell apoptosis, in addition to the reduction of pH of the medium in which it is found, resulting from its acid-base reaction [15]. The incorporation of antimicrobial substances, such as chlorhexidine, in restorative materials has the main objective of reducing the incidence of secondary caries. In this study, an evaluation of the antimicrobial behavior, after 24

hours, of adding different concentrations of DCHX to Vitremer® CIVMR was performed.

According to the results, it was observed that there were no significant differences regarding the antimicrobial property of the material (without the addition of DCHX), in the evaluation of 24h at concentrations of 0.5% and 1%, being only the addition of 2% of DCHX more effective when compared to the control, with a reduction of colonies established in 17.22%, but not statistically significant. The data found are compatible with those also found by Marti and collaborators [16] who evaluated the antibacterial activity of a IVC associated with chlorhexidine gluconate at concentrations of 0.5% 1% and 2% and observed that the antibacterial activity was unchanged.

An evaluation at longer times after the incorporation of DCHX into Vitremer® CIVMR is justified, because according to Palmer [17] in the first 24 hours there is a release of less than 10% of the total mass of chlorhexidine incorporated into the specimens. According to the authors, the highest concentration release occurred up to 20 days after the beginning of the experiment. This is clinically important, as one should consider the importance of controlling mature biofilms, those formed after 14 days of development, which occur in places that are difficult to clean, leading to the development of caries lesions [18].

Streptococcus mutans adheres to the tooth structure by means of adhesins, which are specific molecules present on its surface, promoting the interruption of enamel mineral homeostasis. In the presence of sucrose, glycolysis occurs, producing byproducts that potentiate the pathogenesis of the dental biofilm and transform it from the planktonic phase into mature biofilm, providing inter-rein interactions with other pathogens, such as Candida albicans, as well as increasing the progression of other microorganisms, such as Veillonela parvula and Streptococcus salivarius, favoring carious relapse and the development of severe caries [19].

The human body's immune system responds to this tissue damage by recognizing microbial components through pattern recognition receptors (PPRs), which are present on macrophages and dendritic cells; this reaction triggers the release of inflammatory mediators such as cytokines and chemokines. However, in chronic contact with these microorganisms, without the elimination of the mature biofilm, invasion to other dental tissues, such as dentin and even pulp, occurs, compromising the healthy structure (Liu et al., 2023). Thus, the incorporation of DCHX to the material seeks to assist in the host response, decreasing the expression of adhesins to the dental element and the consequent formation of biofilm.

Regarding the number of colonies formed, with the incorporation of other percentages of DCHX in Vitremer® CIVMR, it was possible to evidence an increase established in 11% in the CFU count when added 0.5% of DCHX (G2) and 73% when added 1% (G3). The increase in the number of colonies in groups G2 and G3 compared to the control, can be explained by the interaction between fluoride and the cationic molecule of chlorhexidine, which, according to Horsek & Ericson (2008), results in the precipitation of salts with lower solubility, leaving less fluoride accessible, reducing its antibacterial effect.

In addition, during the agglutination of the material, ion displacement occurs, where the aqueous phase of the acids moistens and dissolves the outer layer of the glass particles of the powder by the attack of the hydrogen ion on the glass particles, releasing the metal ions Al3+ and Ca2+ that migrate to the aqueous phase of the cement. The calcium reacts with the anionic chains of the polyacid, forming calcium polyacrylate [20]. It is also known that the mechanism of action of chlorhexidine is because its cationic molecule is strongly attracted by the negative charge of the bacterial cell wall, being adsorbed to the cell membrane by electrostatic interactions [11].

As the acid present in the liquid of the material is an anionic compound, that is, it has a negative charge in its polar part, it is suggested that there is a dispute for sites of the cationic molecule of chlorhexidine between binding to the acid or the bacterial cell wall, which may explain the fact that with the use of the concentration of 0.5% and 1% an increase in colonies was observed, respectively 11% and 73%, in relation to the control in CIVMR Vitremer®. In contrast to this, in the 2% concentration, there is a greater number of cationic molecules available, with no dispute for sites between cement and bacterial cell wall, resulting in a lower quantification of CFU/mL, a reduction of 17.22% of established colonies.

Furthermore, the study analyzed the effectiveness of incorporating DCHX in CIVMR

Riva Light Cure at the same concentrations (0.5%, 1% and 2%), and a statistical relevance was obtained in the addition of 1% and 2%, with percentage reductions of CFU/mL in 92% and 99% respectively. The data are compatible with the findings by Marti and collaborators [16] who evaluated the antimicrobial effectiveness by the technique of halo of inhibition in the concentrations of 1% and 2%, observing a reduction in bacterial growth in the S. mutans strain and, did not obtain statistical relevance in the concentration of 0.5%. Moreover, the findings of the authors make a relationship between the addition of CHX and the resistance of the material, pointing out that concentrations above 5% drastically affect the porosity, changing its mechanical properties and reducing its resistance. The 0.5% incorporation reduced the number of pores, but increased their diameter, facilitating the establishment and development of bacterial colonies [21].

In this context, a comparative analysis between CIVMR Vitremer® and Riva Light indicates that, overall, Riva at concentrations of 1% and 2% demonstrated greater antibacterial activity than Vitremer® at the same DCHX concentrations. Additionally, there is a noticeable reduction in the mean CFU/mL count as the concentration of chlorhexidine increases, reflecting enhanced antibacterial efficiency of the tested materials. The greatest variability was observed at the 2% concentration, with Riva Light Cure showing superior results at the 1% concentration, reducing CFU/mL by 92% compared to Vitremer®, which at the same DCHX concentration increased bacterial proliferation by 73%.

However, it is important to consider that the release and antibacterial activity of chlorhexidine may vary over time, justifying the need for evaluations over longer periods. In addition, the interaction between fluoride and chlorhexidine, along with competition for binding sites, may influence the results. Therefore, future studies are needed to better understand the antimicrobial efficacy of these materials and optimize chlorhexidine concentrations to reduce the incidence of secondary caries and promote oral health.

4. CONCLUSION

Within the limitations of the present study, it was possible to observe that the incorporation of DCHX into Vitremer® CIMVR did not bring statistically significant benefits to its antimicrobial property in the evaluation of 24 hours, the addition ended up increasing the formation of CFU/mL at the 1% and 2% concentrations. In contrast, the CIVMR Riva Light Cure material showed better performance with the incorporation of DCHX at 1% and 2% concentrations, reducing the CFU/mL by more than 90%. However, further studies, evaluating different times and micro-hardness analyses are fundamental to justify the results and ensure or not the clinical use of modified material.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gupta K, Muthu MS, Saikia A, Sriram S, Nirmal L, Wadgave U, Dhar V. Association of exposures to environmental chemicals estimated through primary teeth biomatrix and health outcomes in children and adolescents - A systematic review. The Science of the total environment. 2024;928:172032.
2. Seow WK. Early Childhood Caries. Pediatric Clinics of North America. 2018; 65:941-954.
3. Alsabek L, Al-Narabieah Z, Bshra N, Comisi JC. Retention and remineralization effect of moisture tolerant resin-based sealant and glass ionomer sealant on non-cavitated pit and fissure caries: Randomized controlled clinical trial. Journal of Dentistry. 2019;86:69-74.
4. Luo H, Wu B, Wu Y, Moss ME. Dental Caries and Preventive Dental Visits Among Children in the U.S.: The Impact of Race/Ethnicity and Immigration. AJPM focus. 2024;3(4):100230.
5. Sidhu SK, Nicholson JW. A review of glass-ionomer cements for clinical dentistry. Journal of Functional Biomaterials. 2016;7:1-15.
6. Hegde D, Suprabha BS, Rao A. Organic antibacterial modifications of high-viscosity glass ionomer cement for atraumatic restorative treatment: A review. The Japanese dental science review. 2024; 60:22–31.

7. Kohno T, Kitagawa H, Tsuboi R, Deng F, Sakai H, Wu T, Fan YS, Xiao L, Imazato S. Development of Antibacterial Resin Composites Incorporating Poly(METAC) Clusters. Materials (Basel, Switzerland). 2024;17(4):896.
8. Barbour ME, Maddocks SE, Grady HJ, Roper JA, Bass MD, Collins AM, Dommett RM, Saunders M. Chlorhexidine hexametaphosphate as a wound care material coating: antimicrobial efficacy, toxicity and effect on healing. Nanomedicine. 2016;11(16):2049–2057.
9. Borompiyasawat P, Putraphan B, Luangworakhun S, Sukarawan W, Techatharatip O. Chlorhexidine gluconate enhances the remineralization effect of high viscosity glass ionomer cement on dentin carious lesions in vitro. BMC oral health. 2022;22(1):60.
10. Mathur S, Srivastava R, Mathur T, Khatri R. Chlorhexidine: The gold standard in chemical plaque control. National Journal of Physiology, Pharmacy, and Pharmacology. 2011;1:45-50.
11. Bernardi A, Teixeira CS. The properties of chlorhexidine and undesired effects of its use in endodontics. Quintessence International. 2015;46:575-582.
12. Castilho AR, Duque C, Negrini TV, Sacono NT, Paula AB, Costa CAS, Spolidorio DM, Puppin-Rontani RM. *In vitro* and *in vivo* investigation of the biological and mechanical behaviour of resin modified glass-ionomer cement containing chlorhexidine. Journal of Dentistry. 2013; 41:155-163.
13. Yadiki JV, Jampanapalli SR, Konda S, Inguva HC, Chimata VK. Comparative evaluation of the antimicrobial properties of glass ionomer cements with and without chlorhexidine gluconate. International Journal of Clinical Pediatric Dentistry. 2016;9:99-103.
14. Silva MER, Danelon M, Souza JAS, Silva DF, Pereira JA, Pedrini D, Camargo ER, Delbem, ACB, Duque C. Incorporation of chlorhexidine and nano-sized sodium trimetaphosphate into a glass-ionomer cement: Effect on mechanical and microbiological properties and inhibition of enamel demineralization. Journal of Dentistry. 2019;84:81-88.
15. Martins RA, Becci ACO, Giro EMA, Rastelli ANS, Marti LM, Zuanon ACC. Glass ionomer cement associated with chlorhexidine diacetate or zinc oxide nanoparticles: Microbiological evaluation. Journal of Dentistry of UNESP. 2019; 48:99-107.
16. Marti LM, Becci AC, Spolidorio DM, Brighenti FL, Giro EM, Zuanon AC. Incorporation of chlorhexidine gluconate or diacetate into a glass-ionomer cement: porosity, surface roughness, and anti-biofilm activity. American Journal of Dentistry. 2014;27:318-322.
17. Palmer G, Jones FH, Billington RW, Pearson GJ. Chlorhexidine release from an experimental glass ionomer cement. Biomaterials. 2004;25:5423-5431.
18. Exterkate RA, Crielaard W, Ten Cate JM. Different response to amine fluoride by Streptococcus mutans and polymicrobial biofilms in a novel high-throughput active attachment model. Caries Research. 2010;44:372-379.
19. Ellepola K, Truong T, Liu Y, Lin Q, Lim TK, Lee MY, Cao T, Koo H, Seneviratne CJ. Multi-omics analyses reveal synergistic carbohydrate metabolism in Streptococcus mutans-Candida albicans mixed-species biofilms. Infect Immun. 2019;87:e00339-19.
20. Alebady MH, Hamama HH, Mahmoud SH. Effect of various surface coating methods on surface roughness, micromorphological analysis and fluoride release from contemporary glass ionomer restorations. BMC oral health. 2024;24(1):504.
21. Raghimi EC, Biglar N, Sadighian S, Karamitanha F, Nouri A, Nourian A. Compressive strength and fluoride release profile of a glass ionomer cement reinforced with silver-hydroxyapatite-silica hybrid nanoparticles: An in vitro study. International orthodontics. 2024;22 (2): 100871.

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