MICROBIOLOGICAL EFFECT OF GLASS IONOMER CEMENT ADDED BY CHLORHEXIDINE DIACETATE

EFEITO MICROBIOLÓGICO DO CIMENTO DE IONÔMERO DE VIDRO ACRESCIDO DE DIACETATO DE CLOREXIDINA

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ABSTRACT

Objective: The objective of the present study was to evaluate the microbiological effectiveness of the glass ionomer cement added by 1% chlorhexidine diacetate. Methods: The antimicrobial activity was performed through the test of halo formation to inhibit *Streptococcus mutans* (*S. mutans*), *Staphylococcus aureus* (*S. aureus*) and *Candida albicans* (*C. albicans*) in the following groups: Group 1: glass ionomer cement (GIC), Group 2: GIC added by 1% chlorhexidine diacetate, Group 3: chlorhexidine digluconate solution at 0.12% and Group 4: chlorhexidine digluconate solution at 2%. The halo inhibition area was measured in square millimeter (mm²), at 24 and 48 hours after incubation (37º·C). Results: The chlorhexidine digluconate solution at 2% (group 4), used as a positive control had a significantly higher antimicrobial effect than the other groups (groups 1, 2 and 3) and against all the microorganisms tested ($p<0.01$, Kruskal-Wallis test). The glass ionomer cement added by 1.0% chorhexidine diacetate (group 2) showed a statistically higher antimicrobial effect against *S. aureus* and *S. mutans* species than groups 1 and 3 ($p<0.01$). Conclusion: It was concluded that the addition of 1% chlorhexidine diacetate enriched GIC functions demonstrated by the effectiveness in growth inhibition of *Streptococcus mutans* and *Staphylococcus aureus*.

KEYWORDS: Glass ionomer cements; Chlorhexidine; Microorgasnims; Dentistry.

INTRODUCTION

The glass ionomer cement (GIC) was launched in the early 1970s. It belongs to the class of dental biomaterials and it has been widely used in dentistry. In the powder there are 3 constituents based of silica, alumina and calcium fluoride. The liquid is an aqueous solution of polyalkenoic acids with setting accelerators. This dental material is resulting from an acid-base type reaction between ions released from the glass powder and a solution of organic acid, generally the polyacrylic acid[1]. With the development and improvement of glass ionomer cements, its use is proposed as an agent of sealing of fossulas and fissures, seeking to obtain additional preventive effects originated from the release of fluorine of these materials and because its low cost and effectiveness being so used in the public health system[2,3]. This material presents good biocompatibility, linear thermal expansion next to the tooth and ability to release fluoride. It also has good chemical adhesion with the dental structure, in which due to the greater number of minerals present in the accession of GIC it is stronger. Satisfactory color, adequate translucency, easy handling and stability in long-term oral environment are qualities this material, not requiring acid etching on enamel, because it chemically bonds in the dental structure. Arguably, its best properties are the continuous release of fluoride ions and the possibility of suffering refills, to be exposed again to this, acting as a reservoir of fluoride in the oral cavity[4,5].

A manner we have so that there is the release of fluoride and chlorhexidine simultaneously is through the incorporation of chlorhexidine to GIC[6]. The isolated use of fluorine shows insufficient to control the formation of visible plaque and reduce its acidogenicity, therefore it is recommended the use of fluorine concomitantly with chlorhexidine[7]. Scholars have shown that the addition of disinfectants substances to GIC improves the antibacterial effect without changing the physical properties of

the cement[8]. The addition of chlorhexidine in cement does not alter the GIC physical properties[9,10]. However, in the literature there are no studies that have stated that the properties of the glass ionomer cement can be changed when the chlorhexidine is added[6].

With respect to the release of fluoride and chlorhexidine simultaneously in the oral cavity, they occur initially at low pH during the reaction of the GIC tusk, therefore the greater quantity of chlorhexidine is released even in the early hours. After this time it tends to decline until reaching a minimum quantity in that entire chlorhexidine available is released[6]. The chlorhexidine presents a range of incompatibility with other materials, although in the case of GIC it does not react with any other substance present in cement and the greater amount of chlorhexidine released initially occurs due to molecules that are not chemically or physically stuck to GIC[11].

The chlorhexidine may be added in the most diverse ways, although in the form of chlorhexidine diacetate is the easiest way to be embedded into the cement and the concentrations may vary until reaching the value of 13%. Researchers tested various proportions of chlorhexidine diacetate (powder) and digluconate (liquid) in GIC in culture means for bacteria and fungi. After the microbiological analyzes performed, the authors stated that the modified GIC with chlorhexidine has antibacterial effect superior to conventional GIC and that the form of 2.5% chlorhexidine diacetate showed better results for *Streptococcus mutans*. In addition, the authors demonstrated null results for the inhibition of *Candida albicans*[9,12]. A study described *in vitro* microbiological tests with *Lactobacillus casei* and it was verified that the GIC containing 1% chlorhexidine diacetate was effective in the inhibition of bacteria associated with dental caries and it can be added without affecting the physical properties of the cement[13]. However, the authors suggest that future investigations are need to examine the effects in more complex biofilms. Another *in vitro* study described that the modified GIC by addition of chlorhexidine diacetate at 1% and 2% concentrations presented satisfactory antimicrobial results[8].

The objective of this *in vitro* study was to verify the microbiological effectiveness of GIC added by 1% chlorhexidine diacetateon the *Streptococcus mutans*, *Staphylococcus aureus* and *Candida albicans*.

MATERIAL AND METHODS

The antimicrobial activity of each material was performed through the test of formation of halo inhibition in agar medium established as standard by the NCCLS (National Committee for Clinical Laboratory Standards) for the antimicrobial study[14] for the following microorganisms: *Streptococcus mutans* (ATCC # 25175), *Staphylococcus aureus* (ATCC # 25923) and *Candida albicans* (ATCC # 562).

The materials used in this study were divided into four groups: Group 1 - glass ionomer cement (GIC) Maxxion R® (MGB, Joinville, SC, Brazil); Group 2 - GIC Maxxion R® (MGB) added by 1% chlorhexidine diacetate (dCHX) (Sigma Aldrich, Saint Louis, IL, USA); Group 3 - digluconate chlorhexidine solution (CHX) at 0.12% and Group 4 - CHX solution at 2%.

To achieve the microbiological analysis through the formation of halo inhibition test, a specific culture media for the growth of each microorganism was used. The microorganisms were inoculated into 5mL of Brain Heart Infusion (BHI) broth (Difco Laboratories, Detroit, MI, USA) and incubated into a bacteriological incubator at 37°C for 24h in agreement with the physiological characteristics of each microorganism to obtain a suspension called inoculum with approximate concentration of $1.5x10^8$ colony formation units per milliliters (CFU/mL) of culture medium.

In each sterile Petri dish, a layer-base containing 15mL of BHI agar culture medium for cultivation of *Streptococcus mutans* and *Staphylococcus aureus* was prepared. For cultivation of *Candida albicans* was used 15mL of culture medium *Sabouround Dextrose Agar* (SDA). After solidification of the culture medium, 200µL of each inoculum was seeded at Petri dishes/ plates.

On the plates two wells measuring 5mm in diameter each were made at equidistant points by plastic straws previously disinfected in 70% alcohol. The wells were completely fulfilled with one of the materials of groups 1 and 2, using a disposable insulin syringe. For the materials used as positive control, 5µL of each solution, CHX at 0.12% and CHX at 2% (groups 3 and 4, respectively) were applied in 4mm-diameter of paper filters discs each. The plates were incubated at 37°C for 24h and 48h. Microbial growth was evaluated after 24h and 48h of hatching in bacteriological incubator at 37°C, where the plates seeded with *Streptococcus mutans* were incubated in jug of microaerophilic conditions.

For each kind of cement six wells were made and for each solution six discs of paper filter were inserted for each strain analyzed. In table 1, we can verify the quantity of samples for each microorganism and groups.

Table 1 - Number of samples of the study

Groups	24h			48h		
	S. mutans	*S. aureus*	*C. albicans*	*S. mutans*	*S. aureus*	*C. albicans*
G1 (GIC)	6	6	6	6	6	6
G2 (GIC-dCHX)	6	6	6	6	6	6
G3 (0,12% CHX)	6	6	6	6	6	6
G4 (2% CHX)	6	6	6	6	6	6

After the incubation period, the halos inhibition was measured in millimeters using a vernier caliper (Digimess, São Paulo, SP, Brazil). All measurements were determined from two opposite points located in the most external limits of each halo formed around each well/disc. These measures were repeated three times and the average for each well/disc was obtained. The area of the halos was calculated by the formula used to estimate the area in circles and through the average of the radius obtained ($A= \pi \times R^2$).

The experimental data were submitted to the normality test of Shapiro-Wilk. The data were presented as median and interquartile range. Differences among groups were analysed by the Kruskal-Wallis test followed by Dunn's method and differences between periods in each group were performed by Tukey's test (a = 5%) (BioEstat 5.0, Civil Society Mamiraua/ MCT–CNPq, Belém, PA, Brazil).

RESULTS

The values of halos of different materials are presented in Table 2.

The chlorhexidine digluconate solution at 2% (group 4), used as a positive control, had significantly higher antimicrobial effect than the other groups (groups 1, 2 and 3) and against all analyzed microorganisms (*p<0.01*, Kruskal-Wallis' test). The glass ionomer cement added by 1.0% chorhexidine diacetate (group 2) showed statistically higher antibacterial effect against *S. aureus* and *S. mutans* species than groups 1 and 3 (*p<0.01*, Kruskal-Wallis' test). The glass ionomer cement (group 1) showed antibacterial effect against *S. mutans* in both analyzed periods, however the group 1 showed statistically lower antimicrobial effect than the other groups.

The group 2 showed a difference in the area of halo inhibition between the incubation periods of 24 and 48 hours against *C. albicans* (*p<0.05*, Tukey's test).

DISCUSSION

The aim of the present study was to evaluate the glass ionomer cement from a microbiological point of view. This restorative material used in dental practice has aroused the interest of researchers in recent years[15]. An important aspect will be in the field of prevention in Dentistry if such results obtained in the *in vitro* study were confirmed in the oral cavity of individuals with high risk of development of caries and periodontal diseases.

According to Deepalakshmi et al. (2010)[13], the concentration of chlorhexidine diacetate at 1% has been the most indicated for enrichment of GIC in function of *in vitro* inhibition of the growth of *Lactobacillus casei* and other bacteria involved in caries. This way the concentration of chlorhexidine diacetate used in this experiment is justified. Nevertheless, studies that used concentrations of chlorhexidine diacetate superior to 1% were performed with better antimicrobial results[12].

In addition to the qualities presented and scientifically known of chlorhexidine, we must consider that when it is used in the form of mouthrinse is possible to occur chemical interactions that decrease its inhibitor effect against microorganisms. For the action of bacterial inhibition, the minimum concentration of chlorhexidine capable of producing this effect is 0.12%.

Table 2 - Median (interquartile range) of the area ($mm^{2)}$ of halo inhibition of each group in cultures of *Staphylococcus aureus (Sa)*, *Streptococcus mutans (Sm)* and *Candida albicans (Ca)* within periods of 24 and 48h

Microorganisms	Periods	Groups			
		G1	G2	G3	G4
S. aureus	24h	0.0 (0.0)[a]	113 (14.8)[b]	60.5 (26.4)[c]	153.9 (15.9)[d]
	48 h	0.0 (9.5)[a]	122.1(19.4)[b]	47.2 (15.2)[c]	153.9 (5.9)[b]
S. mutans	24h	28,3 (6.5)[a]	78.5 (31.2)[b]	75.1 (29.9)[b]	233.7 (33.8)[c]
	48 h	26.0 (11.4)[a]	127.7 (20.3)[b]	75.1 (22.9)[c]	306.7 (116.3)[d]
C. albicans	24h	0.0 (0.0)[a]	0.0 (0.0)[a]*	28.3 (4.6)[b]	70.9 (44.8)[c]
	48 h	0.0 (0.0)[a]	12.6 (0.0)[b]	19.6 (4.1)[b]	70.8 (29.8)[c]

[a, b, c, d] Different letters, in line, means statistical differences among groups by Kruskal--Wallis test, *p<0.05*. *Statistical differences between periods in each group by Tukey's test, *p<0.05*. Conventional GIC (Group 1), GIC added by 1% chlorhexidine diacetate (Group 2), chlorhexidine digluconate at 0.12% (Group 3) and chlorhexidine digluconate at 2% (Group 4).

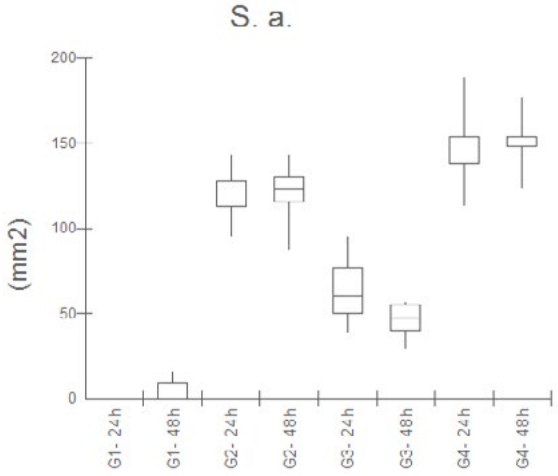

Figure 1 - *Box-plot* (maximum and minimum values, median and interquartile range) of the halo of inhibition of *Staphylococcus aureus* from different experimental groups, in periods of 24h and 48h. Identical letters represent statistically significant difference (Kruskal-Wallis test, p< 0.001).

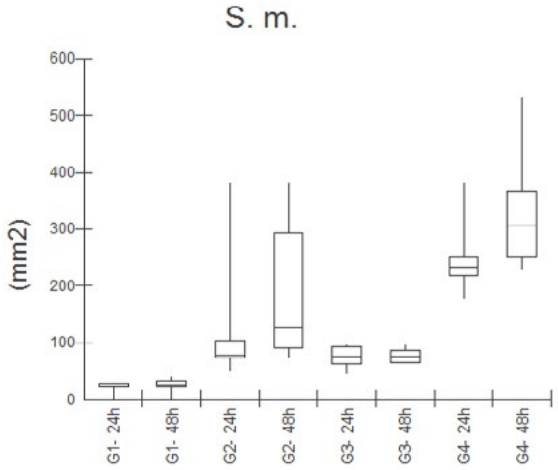

Figure 2 - *Box-plot* (maximum and minimum values, median and interquartile range) of the halo of inhibition of *Streptococcus mutans* of different experimental groups, in periods of 24h and 48h. Identical letters represent statistically significant difference (Kruskal-Wallis test, p< 0.001).

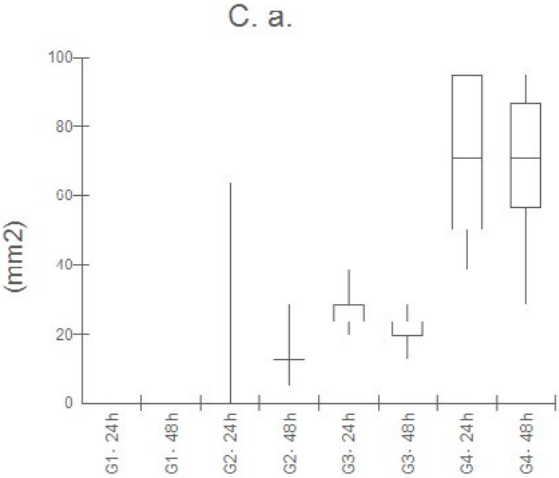

Figure 3 - *Box-plot* (maximum and minimum values, median and interquartile range) of the halo of inhibition of Candida albicans from the different experimental groups, in periods of 24h and 48h. Identical letters represent statistically significant difference (Kruskal-Wallis test, p< 0.001).

However, when used immediately after brushing with dentifrice containing sodium monofluorophosphate, it interacts with the chlorhexidine forming salts of low solubility decreasing the action of both agents. The same situation with respect to chemical interaction occurs with the sodium lauryl sulphate, which is a detergent very used in toothpaste[16]. This substance binds to chlorhexidine forming salts of low solubility and, thus, reduces the action of chemotherapeutic agent[17]. Due to these inconsistencies, the patient must comply with an interval of 30 minutes to 2 hours, preferably, to perform the mouthrinse with the solution of chlorhexidine after brushing[18]. Due to this mismatch between the substances present in fluoridated toothpaste and mouthrinse solutions, it would be of great importance for the population that there was a material that could liberate simultaneously fluoride and chlorhexidine without compromising the physical-chemical properties of both agents[11]. Like this, the idealization of a GIC that is also capable of releasing continuously chlorhexidine is justified.

According to the research held with the modified GIC by chlorhexidine, whether it is in the form of digluconate or diacetate, both offer anti-microbial effect greater than the conventional GIC, and no studies were found in literature that disagreed on the antimicrobial effect of GIC enriched with chlorhexidine[8,10,12,13,19-23].

Independent of the concentration and form of chlorhexidine added, all the researches, who had microbiological analysis are concordant in relation to inhibit the growth of microorganisms related to caries and periodontal diseases[12,21,24]. The search result of Frencken et al. (2007)[21] was similar to that obtained in this research with regard to *Streptococcus mutans*. It can be explained by the similarity of the chlorhexidine form and used concentration, and microbiological analyzes performed, even that in the mentioned research there have been performed atraumatic restorations. In the study conducted by Türkün et al. (2008)[12], the result was very close to that obtained by this research, since the authors observed inhibition of growth of *Streptococcus mutans* colonies and invalidity in the inhibition of *Candida albicans* even at higher doses of chlorhexidine. According to Reche (2005)[17], the chlorhexidine also has inhibitory effect on fungi and yeasts. However, the results obtained by Türkün et al. (2008)[12], and also in this study, contradict the previous statement regarding *Candida albicans*, suggesting that the addition of chlorhexidine to GIC can alter the effectiveness of cationic detergent.

The present study suggested that the greatest antimicrobial effects are attributed to chlorhexidine digluconate solution at 2.0% whether the analyzed microorganism. Except for *Candida albicans*, the GIC added by dCHX was shown to be able to inhibit microbial growth in a superior manner to CHX solution at 0.12%. It is still possible to say that the dCHX has greater ability to inhibit microbial growth compared to GIC independently of the evaluated microorganism.

CONCLUSION

It was concluded that glass ionomer cemente added by 1% chlorhexidine diacetate enriched GIC functions demonstrated by the effectiveness in growth inhibition of *Streptococcus mutans* and *Staphylococcus aureus*.

REFERENCES

01. Padovani GC. Avaliação *in vitro*, da atividade antibacteriana, liberação de fluoreto e capacidade de recarga de cimento de ionômero de vidro para base forradora [Dissertação de Mestrado]. Araraquara: UNESP; 2008.
02. Bernardo PC, Rodrigues CRMD, Souza Paiva JÁ, Singer JM, Sañudo A. Avaliação clínica de um cimento de ionômero de vidro utilizado como selante oclusal. Pesq Odont Bras. 2000; 14(1): 53-57.
03. Hoshi AT. Efeitos de um cimento de ionômero de vidro e um selante resinoso e esmalte bovino desmineralizado, submetido ou não a desafio cariogênico- estudo *in situ* [Tese de Doutorado]. Bauru: USP; 2006.
04. Vieira ALF. Comportamento clínico de cimento de ionômero de vidro utilizados como selante oclusal por meio da técnica do tratamento restaurador atraumático (ART) [Tese de Doutorado]. Bauru: USP; 2004.
05. Oliveira TMMG. Efeito das Variáveis Microestruturais nas Propriedades Mecânicas de Cimento Ionômero de Vidro Convencional [Dissertação de Mestrado]. Rio de Janeiro: UFRJ; 2005.
06. Sacramento PA, Papa AMC, Carvalho FG, Puppin-Pontani RM. Propriedades antibacterianas de materiais forradores - Revisão de literatura. Rev Odontol UNESP. 2008; 37(1): 59-64.
07. Amorim RG, Leal SC, Bezerra ACB, Amorim FPLG, Toledo AO. Association of chlorhexidine and fluoride for plaque control and White spot lesion remineralization in primary dentition. Int J Pediatr Dent. 2008; 18(6): 446-51.
08. Tüzüner T, Ulusu T. Effect of antibacterial agents on the surface hardness of a conventional glass-ionomer cement. J Appl Oral Sci. 2012; 20(1): 45-49.
09. Palmer G, Jones FH, Billingto RW, Pearson GJ. Chlorhexidine release from an experimental glass ionomer cement. Biomaterials. 2004; 25(23): 5423-31.
10. Ferreira HC. Avaliação *in vitro* de propriedades físico-quimicas de cimento de ionômero de vidro convencionais, após adição de própolis e antibiótico [Dissertação de Mestrado]. Taubaté: Universidade de Taubaté; 2005.
11. Ferrarini M, Baby AR, Pinto CASO, Velasco MVR, Pinto TJA, Kaneko TM. Influência do Kollidon® 90F e do Polyox® WSR301NF na força de adesão de comprimidos bucais de clorexidina. Lat Amer J Pharm. 2007; 26(4): 541-7.
12. Türkün LS, Türkün M, Ertugrul F, Ates M, Brugger J. Long-term antibacterial effects and physical properties of a chlorhexidine-containing glass ionomer cement. J Esthet Restor Dent. 2008; 20(1): 29-44.
13. Deepalakshmi M, Pooni S, Miglani R, Indira R, Ramachandran S. Evolution of the antibacterial and physicalproperties of glass ionomer cements containig chlorhexidine and cetrimide: An *in vitro* study. Indian J Dent Res. 2010; 21(4): 552-6.
14. Barry AL, Thornsberry C. Susceptibility tests: Diffusion Test Procedures. In: Balows A, Hauser WJ, Hermann KL, Isenberg HD, Shamody HJ. Manual of clinical microbiology. 5ed. Washington, DC: American Society for Microbiology; 1991. p. 1117-25.
15. Łuczaj-Cepowicz E, Marczuk-Kolada G, Zalewska A, Pawińska M, Leszczyńska K. Antibacterial activity of selected glass ionomer cements. Postepy Hig Med Dosw. 2014; 68:23-8.
16. Herrera BS, Mendes GIAC, Porto RM, Rigato HM, Moreira LD, Musacará MN, Magalhães JCA, Mendes GD. O papel da clorexidina no tratamento de pacientes com gengivite no distrito de São Carlos do Jamari-RO. Rev Periodontia. 2007; 17(3): 60-4

17. Reche NSG. Controle de placa em deficientes mentais com uso da clorexidina [Dissertação de Mestrado]. Marilia: Unimar; 2005.

18. Ramacciato JC. Atividade antimicrobiana de soluções à base de alho (*Allium sativum*), óleo de malaleuca (*Melaleuca alternifólia*) e clorexidina sobre microrganismos totais e estreptococos do grupo *mutans*. Estudo *in vivo*. [Dissertação de Mestrado] Piracicaba: UNICAMP; 2000.

19. Millett DT, Doubleday B, Alatsaris M, Love J, Wood D Luther F, Devine D. Chlorhexidine-modified glass ionomer for band cementation? An *in vitro* study. J Orthod. 2005; 32(1): 36–42.

20. Takahashi Y, Imazato S, Kaneshirro AV, Ebisu S, Frenken JE, Tay FR. Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach. Dent Mater. 2006; 22(7): 647-52.

21. Frencken JE, Imazato S, Toi C, Mulder J, Mickenautsch S, Takahashi Y, Ebisu S. Antibacterial effect of chlorhexidine- containing glass ionomer cement *in vivo*: A pilot study. Caries Res. 2007; 41(2): 102-7.

22. Farret MM, Lima EM, Mota EG, Oshima HMS, Barth V, Oliveira SD. Can we add chlorhexidine into glass ionomer cements for band cementation? Angle Orthod. 2011; 81(3): 496-502.

23. Ahluwalia P, Chopra S, Thomas AM. Strength characteristics and marginal sealing ability of chorhexidine-modified glass ionomer cement: An *in vitro* study. J Indian Soc Pedod Prevent Dent. 2012; 30(1): 41-6.

24. Du X, Huang X, HuangC, Frencken JE, Yang T. Inhibition of early biofilm formation by glass-ionomer incorporated with chlorhexidine in vivo: a pilot study. Aust Dent J. 2012; 57(1): 58-64.

RESUMO

Objetivo: O objetivo do presente estudo foi avaliar o efeito antimicrobiano do cimento de ionômero de vidro acrescido de diacetato de clorexidina a 1%. Métodos: A atividade antimicrobiana foi realizada por meio do teste de formação de halo de inibição de *Streptococcus mutans* (*S. mutans*), *Staphylococcus aureus* (*S. aureus*) e *Candida albicans* (*C. albicans*) nos seguintes grupos: Grupo 1: cimento de ionômero de vidro (CIV), Grupo 2: CIV acrescido de 1% de diacetato de clorexidina, Grupo 3: solução de digluconato de clorexidina a 0,12% e Grupo 4: solução de digluconato de clorexidina a 2%. A área do halo de inibição foi medida em milímetros quadrados (mm²),nos períodos de 24 e 48 horas após incubação (37ºC). Resultados: A solução de digluconato de clorexidina a 2% (grupo 4), utilizada como um controle positivo apresentou efeito antimicrobiano significativamente maior do que os outros grupos (grupos 1, 2 e 3), contra todos os microrganismos testados ($p < 0,01$, teste de Kruskal-Wallis). O cimento de ionômero de vidro acrescido de 1% de diacetato de clorexidina (grupo 2) demonstrou efeito antimicrobiano estatisticamente maior do que os grupos 1 e 3, contra as espécies *S. aureus* e *S. mutans* ($p < 0,01$). Conclusão: Conclui-se que a adição de 1% de diacetato de clorexidina enriqueceu as funções do cimento de ionômero de vidro demonstradas pela efetividade em inibir o crescimento de *Streptococcus mutans* e *Staphylococcus aureus*.

PALAVRAS-CHAVE: Cimentos de ionômero de vidro; Clorexidina; Microrganismos; Odontologia.

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